MORPHOLOGICAL, REPRODUCTIVE, GENETIC, AND DISEASE AND INSECT RESISTANCE CHARACTERIZATION IN NASCENT ALLOTETRAPLOIDS CROSS-COMPATIBLE TO CULTIVATED PEANUT (*ARACHIS HYPOGAEA* L.)

by

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(Under the Direction of Peggy Ozias-Akins)

ABSTRACT

About 49 million tons of peanut (*Arachis hypogaea* L.) are produced globally each year; however, pathogen and insect pests plague global production. Average peanut yields in developing countries are only a fraction of the average yield achieved in the United States primarily because of pathogen and insect pressures. High yields in the United States reflect accessibility to effective chemicals for pathogen control, yet these chemicals are costly to apply. Genetically improved cultivars with stronger, more durable resistance are needed to feed the world's growing population in a sustainable manner. Yet, the narrow genetic base of cultivated peanut limits crop improvement.

There are more than 80 wild *Arachis* species with a wide range of genetic and phenotypic diversity, including strong, diverse resistances, that can be deployed for peanut cultivar improvement. The most efficient way to introgress genetic variability from wild *Arachis* species into cultivated peanut is to produce allotetraploid interspecific hybrids that are cross-compatible to peanut. To enable effective utilization of allotetraploids, morphological, reproductive, and pathogen and insect resistance characterization of novel allotetraploids and allotetraploid-derived

breeding materials was performed. In this study, allotetraploids were found to generally have greater production of flowers during the growing season, larger flowers, larger and hairier leaves, taller main stems, longer primary laterals, longer internodes, lower percentage of reproductive nodes, heavier plant body masses, and smaller seeds and pods than cultivated peanut. This diversity will likely need to be selected against while desirable traits such as pathogen and insect resistance are maintained. In addition, the weak peg strength of wild *Arachis* species was thought to be potentially detrimental to yield; however, peg strength comparable to that of peanut breeding lines was recovered in F_1 hybrids. Therefore, weak peg strength is a minor concern to plant breeders when using these materials. Resistance to fall armyworm and rust were identified in allotetraploids, and five, novel putative QTL for tomato spotted wilt orthotospovirus resistance were discovered that can be utilized for marker-assisted selection once validated. This work will contribute to producing high-yielding, resistant cultivars that will protect peanut production in the United States and promote global food security.

INDEX WORDS: Allotetraploids, *Arachis,* breeding, fall armyworm, host plant resistance, introgression, peanut, phenotypic diversity, peg anatomy, peg strength, rust, *Spodoptera frugiperda*, tomato spotted wilt orthotospovirus

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DEDICATION

For Micah, the love of my life. Thank you for your endless encouragement and for supporting me so thoroughly that you joined our lab as a technician and shelled more peanuts than Mr. Peanut himself (not many spouses can say they would go so far). For my parents, whose love allowed me to dream big. For my sisters and family-in-law, who filled me with love and encouragement and for editing my proposals and manuscripts (especially Ethan, who continued to do so without ever receiving that promised gift basket). L'chaim!

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Peanut, also known as groundnut (*Arachis hypogaea* L.), is grown worldwide as an oil, cash, food, and feed crop, and is a key source for protein, calories, vitamins, and minerals (Suchoszek-Lukaniuk et al., 2011; Guimón and Guimón, 2012). It is cultivated in tropical and subtropical environments and about 49 million metric tons of peanut are produced globally each year (FAOSTAT, 2019). Peanut is most important in developing countries, which account for about 94% of peanut world production. Asia, Africa, and the Americas account for 56%, 34%, and 10% of peanut world production, respectively (FAOSTAT, 2019). In these regions, peanut provides a similar amount of calories as soybean, and in Africa, peanut production exceeds all other grain legumes combined (Bertioli et al., 2011). The United States accounts for 5% of world peanut production (FAOSTAT, 2019), and peanut as a crop became popular in the Unites States largely due to the research efforts of George Washington Carver. He invented more than 300 products from peanut, aiding economic growth in the rural South in the early 1900's. However, South America, the region of origin of peanut and where the *Arachis* genus is endemic, only accounts for 4% of world peanut production (FAOSTAT, 2019).

Despite the importance of peanut in the developing world, peanut yields achieved there are only a fraction of that achieved in the United States primarily because of insect and pathogen pressure (FAOSTAT, 2019; Kalule et al., 2010; Kemerait et al., 2004). High yields in the United States reflect accessibility to effective chemicals for pathogen managment, yet these chemicals are expensive to apply (Kemerait et al., 2004). Cultivars with genetic resistance to certain pests

have already been shown to have economic and environmental benefits by increasing peanut yield gains and decreasing pesticide use in the United States (Holbrook et al., 2015) and by improving yield gains in Uganda (Fermont and Benson, 2011) and in India (Bantilan et al., 2003). Genetically improved cultivars with stronger resistance, with more genetically diverse sources of resistance, and with resistance to a greater number of pests are needed to feed the world's growing population in a sustainable manner (Bantilan et al., 2003; Holbrook et al., 2015). Yet, the narrow genetic base of cultivated peanut limits crop improvement (Stalker et al., 2016).

Like many of the world's most important crops, peanut has a narrow genetic base due to a domestication bottleneck (Cuc et al., 1973; Grabiele et al., 2012; Halward et al., 1992; Hyten et al., 2006; Kochert et al., 1996; Subramanian et al., 2000). These bottlenecks have resulted in limited allelic diversity among peanut landraces and commercial cultivars; however, wild *Arachis* species have promising allelic diversity for use in peanut genetic improvement programs. There are 80 wild peanut relatives in the *Arachis* genus with diverse insect and pathogen resistance, abiotic and biotic stress tolerance, and other traits such as dormancy that can be introgressed into cultivated peanut (Moretzsohn et al., 2013; Stalker et al., 2016). So far, wild species have been underutilized in peanut breeding programs, and only a few wild introgressions have been incorporated into commercial cultivars in the United States (Holbrook et al., 2008; Simpson and Starr, 2001). Understanding wild species genetic relationships to each other and peanut can hasten their implementation in breeding programs, since transferring genes from wild species to cultivated crops is easier when the wild species is genetically closely related to the crop species (Moretzsohn et al., 2013).

Evolution of the *Arachis* genus

The genus *Arachis* L., in the family Fabaceae, is considered to have originated in the Sierra de Amambay, on the border between Mato Grosso do Sul in Brazil and Paraguay, where *A. guaranitica* grows (Gregory et al., 1980; Krapovickas and Gregory, 1994). *Arachis* originated in this woodland-savannah and subsequently adapted to the dry lowlands of South America (Krapovickas and Gregory, 1994). *Arachis* species developed in vastly diverse environments, and both annual and perennial types exist (Stalker et al., 2016). All *Arachis* species developed their fruit underground, which may have protected the seeds from predators, and rhizomes and tuberous roots may have helped *Arachis* spread to new environments (Simpson et al., 2001). Geocarpic fruit limited the rate in which the range of *Arachis* spread; however, tectonic upheaval of the Brazilian Shield and water flow increased the range of *Arachis* (Simpson et al., 2001).

The *Arachis* genus includes 81 named species that are grouped into nine sections distinguished by morphology, cross-compatibility and geographic location (Stalker et al., 2016; Valls and Simpson, 2005). There are likely more species that have not yet been discovered due to limited accessibility (Stalker et al., 2016). Species in most sections are diploid with a base chromosome number of 10 (x = 10), except *Rhizomatosae* which contains diploids (2n = 20) and tetraploids (2n = 40), *Erectoides* which has diploids (2n = 20) and some aneuploids, and *Arachis* which contains diploids (2n = 20) and two tetraploids (2n = 40) (Stalker et al., 2016). Based on intron sequences, the section *Arachis* has the highest diversification rate estimated so far for legume species at about .95 speciation events per million years; this rate attributed to its geocarpy and autogamy characteristics (Moretzsohn et al., 2013). Section *Arachis* includes cultivated peanut (*A. hypogaea*, 4x = 40) and 30 wild peanut species including the wild, non-domesticated allotetraploid *Arachis monticola* (4x = 40), three aneuploids or dysploids (2n = 2x

= 18), and 26 diploids (2n = 2x = 20) (Krapovickas and Gregory, 1994; Peñaloza and Valls, 1997; Lavia, 1998; Valls and Simpson, 2005). Species in the *Arachis* genus are widely distributed across South America from Northeast Brazil to southern Uruguay and from the western foothills of the Andes to the eastern Atlantic coast due to human cultivation (Valls et al., 1985; Stalker et al., 2016). Species can be found growing in diverse environments ranging from outcrops with almost no soil to areas of thick, gummy clay, among other harsh environments (Simpson et al., 2001; Stalker et al., 2016).

In the section *Arachis*, three genomic groups (A, B, and D) have been described based on cross-compatibility data and chromosome morphology (Smartt and Stalker, 1982; Stalker, 1991). The A genomic group is characterized by the presence of a small chromosome pair, termed the A-chromosome pair, that has a lower level of euchromatin condensation in comparison to other chromosomes (Husted, 1936). The B genomic group was first characterized by the lack of the A-chromosome pair and was fairly inclusive until it was later divided into B, F, and K genomic groups based on FISH mapping of rDNA loci and heterochromatin detection. F and K genomic groups both have centromeric bands on most chromosomes but differ in the amount and distribution of heterochromatin (Seijo et al., 2004; Robledo et al., 2009; Robledo and Seijo, 2010; Moretzsohn et al., 2013). In contrast, B genome species do not have centromeric heterochromatin (Robledo and Seijo, 2010). There is only one species, *A. glanduliferia*, in section *Arachis* that has a D genome characterized by the presence of six subtelocentric or submetacentric chromosome pairs. This species stands in stark contrast to the A, B, F, and K genome species that have mainly metacentric chromosomes (Fernandez and Krapovickas, 1994; Stalker, 1991).

Cultivated peanut, *A. hypogaea,* is a segmental allotetraploid thought to have originated in southern Bolivia or northern Argentina about 6,250 years before present or more recently based on DNA divergence between the Tifrunner reference genome and the *A. ipaensis* reference genome (Bertioli et al., 2016, 2020). The maternal progenitor *Arachis duranensis* donated the A genome and the paternal progenitor *Arachis ipaensis* donated the B genome (Grabiele et al., 2012). In *Arachis*, cpDNA is inherited maternally, and cpDNA alignment grouped six cultivated varieties, *A. monticola,* and *A. duranensis* together, indicating *A. duranensis* is the maternal progenitor (Kochert et al., 1996; Grabiele et al., 2012). The cross between the progenitors produced a sterile AB hybrid due to the A and B chromosomes not pairing during meiosis (Krapovickas and Gregory, 1994, 2007). Either through spontaneous doubling or fusion of unreduced gametes, a fertile tetraploid was made from the sterile hybrid. Both *A. hypogaea* and *A. monticola* share this tetraploid ancestor.

The origin of peanut was not a spontaneous event in the wild, rather *A. ipaensis* was brought to the eastern foothills of the Andes mountains and cultivated alongside *A. duranensis* by ancient inhabitants of South America (Bertioli et al., 2016). Only one population of *A. ipaensis* is currently known and it is within the range of *A. duranensis*. Given that *Arachis magna,* the closest relative of *A. ipaënsis,* is 500 km away from the only known population of *A. ipaensis* and this distance is too vast for a species with geocarpic fruit to travel, it is inferred that *A. ipaënsis* was brought to the Andes by man (Bertioli et al., 2016). In a garden in the Andes mountains, the two progenitors were likely crossed by insect pollination (Simpson et al., 2001). Allotetraploids generally have multiple origins given that species can hybridize multiple times; however, not only does peanut have a single origin, the progenitors were likely only one or a few individuals of each diploid progenitor species (Grabiele et al., 2012). This is supported by the six

botanical varieties having uniform 5S rDNA and cpDNA and significantly less molecular variation than wild diploid species. Cultivated peanut and *Arachis* species have 5S rDNA and cpDNA that are easily distinguishable (Grabiele et al., 2012). This is also supported by all varieties of *A. hypogaea* having identical size, number, and distribution of rDNA loci (Seijo et al., 2004). Lack of molecular variation indicates there has been little to no introgression from wild species into cultivated peanut and there is limited genetic variability among landraces and commercial cultivars.

Due to the narrow bottleneck, *A. hypogaea* and *A. monticola* are extremely similar in sequence and morphology. They are interfertile, have the same chromosome complements (Seijo et al., 2004), have high sequence similarity, and are indistinguishable by isozymes (Lu and Pickersgill, 1993) and RAPD markers (Cunha et al. 2008). However, they are considered separate taxa, since *A. monticola* has wild type (small, single-pod) fruits, *A. hypogaea* cannot persist in natural environments without maintenance by humans (Krapovickas and Gregory, 1994, 2007), and the two species are distinguishable by AFLP, SRAP, and SSR markers (Bravo et al., 2006; Milla et al., 2005). Morphological and genetic variance between the two are explained by genetic drift and the selection pressure. *A. hypogaea* underwent domestication selection pressures for shorter, stronger pegs, larger pods, pod internode suppression, upright plant growth, and shorter branches while *A. monticola* did not.

Introgression of wild *Arachis* alleles into cultivated peanut

The genetic bottle neck resulting from only one or a few tetraploid ancestors of *A. hypogaea* and *A. monticola* explains the lack of genetic diversity in cultivated peanut in comparison to other crops (Kochert et al, 1996.; Moretzsohn et al., 2005). There has been little natural introgression from wild diploid species; however, the high speciation rate in *Arachis* has

yielded a rich, wild *Arachis* germplasm with resistances to various pests and pathogens for potential introgression into cultivated peanut (Moretzsohn et al., 2013). For example, *Arachis cardenasii* displays a hypersensitive response to *Meloidogyne arenaria,* a root-knot nematode (Nelson et al., 1990), and has been used for cultivar improvement (Simpson et al., 1993). Another example is *Arachis stenosperma,* which shows high resistance to late leaf spot and rust (Leal-Bertioli et al., 2010). However, wild *Arachis* species have been underutilized for improvement of cultivated peanut. A major barrier to utilizing these wild species is due to sterility barriers caused by ploidy differences and genetic incompatibilities, since most wild *Arachis* species are diploid with only one genome type and cultivated peanut is a segmental allotetraploid with both A and B genomes (Stalker et al., 2016). Molecular markers during selection can reduce linkage drag by maintaining high resistance QTLs (quantitative trait loci) and decreasing undesirable linked traits such as poor seed shape, shell thickness, reticulation, catenate pods, and small pods and seeds.

There are various ways to introgress wild *Arachis* alleles into cultivated peanut. The hexaploid pathway involves crossing wild diploid *Arachis* ($2n = 20$) with cultivated peanut ($2n = 4x = 40$), doubling the resulting F_1 hybrid cuttings, or sometimes seeds, ($2n = 3x = 30$) with 0.2% colchicine, and backcrossing the synthetic hexaploid hybrid ($2n = 6x = 60$) to cultivated peanut several times to lose chromosomes. Backcrossing hexaploids with *A. hypogaea* results in semi-sterile pentaploids ($5x = 50$), which may produce a few seeds after self-pollination and can lead to stabilized tetraploids ($4x = 40$) after a few generations of self-pollination (Stalker et al., 2016). Once tetraploid, the plants can be used for cultivar development. The semi-sterility of hexaploids makes these materials difficult to use in a breeding program, since phenotypic selection on the small number of seeds produced is impossible. Despite hundreds of tetraploid lines being

produced from the hexaploidy pathway, the traits of interest were lost once the materials reached the tetraploid stage (Stalker et al., 2016). As an alternative to backcrossing the hexaploids to *A. hypogaea*, the hexaploids can be allowed to self-pollinate for several generations and some may spontaneously lose chromosomes and eventually stabilize at the tetraploid level (Stalker et al., 2016). While the spontaneous loss of chromosomes is infrequent, this method has been successfully used with hexaploids derived from *A. hypogaea* x *A. cardenasii*, which resulted in many tetraploid lines segregating for morphological traits like seed size and color as well as resistance such as leaf spot and nematode resistance (Company et al., 1982; Stalker et al., 2002ab). However successful, this method limits introgression to one wild *Arachis* species and requires more time while other methods allow two or more wild species to be used simultaneously.

Another introgression method is a diploid/tetraploid pathway, in which two wild species, one with an A genome and one with a B- or B-compatible genome, are both doubled with 0.2% colchicine. Then, these induced tetraploids are crossed, and the tetraploid progeny are subsequently backcrossed with an elite peanut line (Simpson, 2001). This method requires colchicine to double two different lines successfully; therefore, a more succinct method is to cross the two wild species at the diploid level, double the resulting F_1 hybrid (2n = 20), and then backcross this synthetic tetraploid to cultivated peanut (Simpson, 2001). This method has been used extensively in *Brassica* and is increasingly being used in peanut (Rahman 2013; Zhan et al. 2017; Leal-Bertioli 2018; Ballén-Taborda 2019). This method was used to create TXAg-6 and TXAg-7, lines with high nematode resistance but low yields and poor seed and pod quality due to linkage drag (Simpson et al., 1993). The introgressed, resistance-encoding segment comprised a third to half the chromosome in these lines (Nagy et al., 2010). Fortunately, RAPD markers

associated with the nematode resistance were identified and then used to produce high-yielding, nematode resistance cultivars, demonstrating the usefulness of this introgression pathway (Burow et al., 1996). This pathway has another benefit in addition to ease and time as compared to the hexaploidy pathway. In addition to making the variability of the diploid *Arachis* species available for introgression into peanut, allotetraploids made from wild *Arachis* species can have additional phenotypic variability in plant morphology, physiology, anatomy and biochemistry, resulting from their new combination of genomes as well as from their increased ploidy level (Leal-Bertioli et al. 2017). For example, heterozygosity, subgenome interactions, and the gigas effect in allotetraploids have been shown to result in beneficial traits such as increased plant vigor and chlorophyll content, among other traits that peanut breeders could exploit in their breeding programs (Ramsey and Ramsey 2014; Leal-Bertioli et al. 2017).

Desirable traits for introgression in *Arachis*

Resistance to fall armyworm in Arachis

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a moth that is a major defoliating pest in the Americas and has recently become an economically devastating, invasive pest in Sub-Saharan Africa (Sparks, 1986; Goergen et al., 2016), as well as in Asia. Fall armyworm feeds on more than 80 economically important crops, including peanut. A thirteen-billion-dollar loss to fall armyworm infestation was reported for maize, sorghum, rice, and sugarcane in Africa from 2016 to 2017 (Abrahams et al., 2017). In the state of Georgia, a fall armyworm infestation caused more than $17 million in peanut losses in 1977 (Todd and Suber, 1980). Without control, fall armyworm is a major defoliator that can cause high yield reductions, seriously affecting production in developing countries with limited access to pesticides and safety equipment to apply them properly (Cock et al., 2017; Day et al., 2017; Bateman et al.,

2018). Producing fall armyworm resistant cultivars will reduce reliance on pesticides, increase food security, and promote sustainable agriculture in these at-risk countries. In addition, resistant cultivars are needed to mitigate the insecticide resistance reported in FAW populations (Yu, 1991).

A limitation to producing cultivars highly resistant to fall armyworm is that only moderate levels of host resistance have been found in a few peanut cultivars such as 'Southeastern Runner 56-15' (Hammons 1970; Leuck and Skinner 1971), 'Florunner' and 'Tifton 8' (Todd et al., 1991). Fortunately, previous studies have identified wild peanut relatives such as *A. cardenasii* K 10017, *A. correntina* (PI 261870), *A. ipaensis* K 30076, and A. *villosa* (PI 261872) as potential donors of strong fall armyworm antibiosis and antixenosis (Lynch et al., 1981; Yang et al., 1993). The most extensive of these two studies was Lynch et al. (1981), which screened 14 *Arachis* species for fall armyworm resistance and found that *A. cardenasii, A. correntina, and A. villosa* displayed antibiosis through high fall armyworm mortality rates and inhibition of fall armyworm development and displayed antixenosis due to fall armyworm non-preference to feed on these materials (Lynch et al., 1981). Yang et al. (1993) found that *A. ipaensis* showed antibiosis through a high fall armyworm mortality rate of 79% just 8 days after the start of the experiment. Further work is necessary to introgress fall armyworm resistance from these wild *Arachis* species into a cultivated background useful for peanut breeders.

Resistance to rust and late leaf spot in Arachis

Late leaf spot, caused by *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash, Videira & Crous, and rust, caused by *Puccinia arachidis* Speg., are economically important foliar pathogens that often co-occur with *Passalora arachidicola* (Hori) U. Braun (early leaf spot), which have been reported to cause yield losses up to 80% without fungicide

control in India (Subrahmanyam et al., 1984). Late leaf spot and rust both degrade seed quality and haulm mass, quality, and nutritional value, affecting livestock feed in West Africa (Pande and Rao, 2001; Gajjar et al., 2014). There are many fungicides available that control both late leaf spot and rust, of which chlorothalonil has been the most widely used for over 30 years (Hagan et al., 2004). Many small-scale farmers in the semi-arid tropics lack the finances and technical expertise required to use fungicides effectively to control late leaf spot and rust; thus, breeding resistant cultivars is necessary to help these farmers as well as to decrease fungicide use and the cost of production (Pande and Rao, 2002). Despite fungicide accessibility in developed countries, there is still a need for resistant cultivars due to the monetary and time costs of the multiple fungicide applications and the environmental effects of these fungicides.

Late leaf spot is characterized by round, black lesions that occur on the abaxial side of peanut leaves within one week of fungal germination (Clevenger et al., 2018). Spores are deposited in the soil, so the fungus starts at the bottom of the canopy and moves upward. Due to sporulation occurring 20 to 30 days after infection, a secondary infection during the growing season is common. Highly susceptible lines have been reported to lose all their leaves one month prior to maturity. Loss of leaves and fungal growth on the leaves reduces photosynthesis and leads to lower peanut yields (Clevenger et al., 2018). Resistance is multigenetic and has several components, including percent defoliation, incubation period, latency period, lesion number and diameter, and sporulation (Aquino et al., 1995). Several sources of moderate leaf spot resistance have been reported in cultivated peanut germplasm. For example, 500 United States peanut plant introductions were tested, and 33 were found to have partial leaf spot resistance (Anderson et al., 1993). Using these resistance sources, many runner peanut cultivars such as ‘Georganic’ and ‘Tifrunner’ with partial resistance to late leaf spot have been produced in the United States

(Gorbet et al., 1987; Gorbet and Shokes, 2002; Holbrook and Culbreath, 2007; Holbrook et al., 2008). However, cultivars with higher late leaf spot resistance from genetically diverse backgrounds are desired, and resistant *Arachis* species are a promising source of resistance.

Fourteen wild *Arachis* species have been identified as potential sources of strong late leaf spot resistance (Stalker, 2017). Late leaf spot resistance has been associated with low yield due to either linkage or pleiotropic effects; therefore, utilizing molecular markers tightly linked to resistance to select against linkage drag must be a focus when producing resistant cultivars (Iroume and Knauft, 1987). The most effective and widely used source of late leaf spot resistance was derived from *A. cardenasii* (Company et al., 1982). Late leaf spot resistance QTL explaining up to 60% of phenotypic variation were identified in a population made from TG-24 and GPBD 4, in which the latter is derived from a cross with *A. cardenasii* (Khedikar et al., 2010; Sujay et al., 2012; Pandey et al., 2016). Using a F_2 population from cross between *A. duranensis* and *A. stenosperma,* Leal-Bertioli et al. (2009) identified 5 QTL for late leaf spot resistance, in which alleles from *A. stenosperma* increased resistance. These QTL showed dominant to additive effect ratios less than 0.55, indicating expression may be additive or partially dominant, and one QTL explained almost half the phenotypic variance by itself (Leal-Bertioli et al., 2009). The effectiveness of late leaf spot QTL can depend largely on the environment, so late leaf spot QTL should be pyramided to produce peanut cultivars that are highly resistant in a diverse range of environments (Leal-Bertioli et al., 2009; Khedikar et al., 2010; Sujay et al., 2012). Therefore, there is a need to identify more late leaf spot QTL derived from additional *Arachis* species to pyramid into cultivated peanut.

Rust is an air borne pathogen characterized by orange pustules on the abaxial surface of the leaves that rupture to release urediniospores (Mondal et al., 2007). Unlike late leaf spot, the

infected leaves dry, wither, but remain attached to the plant and its life cycle is much shorter as new infections can occur every 7 to 20 days. Multiple infections during the growing season can lead to rapid spread of rust. Like late leaf spot, chemical control is effective but costly, so resistant cultivars are critical in an integrated rust management strategy. However, a major limitation to breeding rust-resistant cultivars is that only moderate levels of rust resistance have been identified in *A. hypogaea* germplasm (Subrahmanyam et al., 1982).

Many wild *Arachis* accessions are immune or highly resistant to rust, but introgression has been limited due to linkage drag (Mondal et al., 2007; Subrahmanyam et al. 1982; Pande and Rao 2001; Fávero et al. 2009). Markers linked to rust resistance have begun to enable introgression of rust QTL, minimization of linkage drag, and pyramiding of multiple rust resistance QTL. Similar to late leaf spot resistance, rust resistance has many components that work additively, including longer incubation and latent periods, decreased sporulation, fewer pustules per leaf, decreased pustule diameter, and lesser leaf area damage, and lower disease score (Dwivedi et al., 2002). A few studies have identified rust resistance QTL derived from the A-genome species *A. cardenasii* GKP10017 and the B-genome species *A. magna* K30097 (Khedikar et al. 2010; Sujay et al. 2012; Leal-Bertioli et al., 2015). Sujay et al. (2012) identified 5 rust resistance QTL in the same linkage group that explained up to 63% to 83% rust phenotypic variation; these QTL likely originate from *A. cardenasii* GKP 10017. The populations used in Sujay et al. (2012) both have *A. hypogaea* 'GPBD$_4$' as a parent, which has *A. hypogaea* 'ICGV 86855' as a parent, which in turn originated from a cross between *A. hypogaea* and *A. cardenasii* GKP 10017 (Shirasawa et al., 2018). The QTL that explained the most rust phenotypic variation at 83% has been validated and introgressed into three cultivated varieties through marker-assisted backcrossing, improving yield by 56 to 96% in rust infected

environments (Varshney et al., 2014). Leal-Bertioli et al. (2015) identified 13 rust resistance QTL from *A. magna* K 30097. One of these QTLs showed rust resistance to four components of resistance, including IA, TLA, SLA, and incubation period, and explained up to 59% of rust phenotypic variation. These QTL identified by Leal-Bertioli et al. (2015) are distinct from those identified by Sujay et al. (2012) and can be pyramided into the same peanut cultivars to yield more effective and more robust resistance. Further identification of rust resistance QTL derived from different wild *Arachis* species could enable pyramiding of more diverse rust resistance genes in peanut cultivars thereby increasing rust resistance strength and durability.

Resistance to tomato spotted wilt orthotospovirus in Arachis

One of the most severe pathogens devastating peanut production in the United States is tomato spotted wilt orthotospovirus. It has an extensive host range of at least 800 plant species and has caused severe losses in tobacco, pepper, potato, and some ornamental crops (Goldbach and Peters, 1996; Srinivasan et al., 2014). It is transmitted to peanut by tobacco thrips (*Frankliniella fusca* Hinds.) and western flower thrips (*Frankliniella occidentalis* Pergande); however, *F. fusca* is the main vector as it reproduces more effectively on peanut (Todd et al., 1996). *Frankliniella fusca* preferably colonizes on seedlings, which are more susceptible than adult plants, and overwinters in weed bridge crops (Pappu et al., 1999; Shrestha et al., 2015). Tomato spotted wilt orthotospovirus causes chlorotic patterns, concentric ring spots, plant stunting, occasional contorting of peg and kernel shape, lower seed weight, and lower seed number per plant (Culbreath et al., 1992; Srinivasan et al., 2017). Although tomato spotted wilt orthotospovirus is not transmitted through seed, seeds from infected mother plants can have concentric ring spots on the seed coat. Plants affected earlier in the season more commonly display severe stunting, and yield is more dramatically decreased (Culbreath et al., 1992). These

plants infected early in the season can also act as an additional source of inoculum for thrips, enabling further spread of the virus (Lyerly et al., 2002). It is extremely difficult to control tomato spotted wilt orthotospovirus due to the difficulties in suppressing thrip populations (Lyerly et al., 2002). Ullman et al. (1997) found that small numbers of thrips can result in high rates of tomato spotted wilt orthotospovirus spread. In addition, insecticides may increase shallow probing behavior leading to increases in pathogen spread (German et al., 1992). Lastly, insecticides are costly, so the deployment of resistant peanut cultivars is desired.

Tomato spotted wilt orthotospovirus was first identified in 1971 in Texas, and since 1985, epidemics have become more prevalent and destructive in peanut (Culbreath et al., 1997; Srinivasan et al., 2017). In the 1980s, popular peanut cultivars including Florunner and 'GK-9' were completely susceptible to tomato spotted wilt orthotospovirus, and yield losses reached tens of millions of dollars annually (Culbreath et al., 1993; Srinivasan et al., 2017). In the 1990s, cultivars with moderate resistance were used along with chemical management of the insect vector to reduce losses. Current cultivars such as 'GA-10T' and 'Florun-107' possess greater resistance but are not completely effective under high thrip and tomato spotted wilt orthotospovirus pressures and must be used along with chemical and cultural controls (Branch and Culbreath, 2011; Srinivasan et al., 2017; Tillman and Gorbet, 2015). The insecticide phorate reduces thrips feeding damage, stunting and yield loss by upregulating plant defense genes that down regulate virus replication (Culbreath et al., 2008; Todd et al., 1996; Jain et al., 2015). Other narrow-spectrum insecticides applied as in-furrow treatments such as imidacloprid are starting to be used more regularly (Srinivasan et al., 2017). Cultural practices include altering planting date, practicing conservation tillage, and twin row planting (Baldwin et al., 2001).

Peanut cultivars have quantitative resistance and, in some cases, tolerance to tomato spotted wilt orthotospovirus (Shrestha et al., 2013). Major genes resulting in a hypersensitive response have been found in tomato and pepper, but a hypersensitive response has not been observed in peanut (Moury et al., 1998; Stevens et al., 1991). Cultivars may be resistant to either the vector by repressing thrip feeding and development and decreasing the accumulation of tomato spotted wilt orthotospovirus viral copies or the tomato spotted orthotospovirus virus itself; however, resistance mechanisms are not yet well characterized (Srinivasan et al., 2017). There has been a recent increase in tomato spotted wilt orthotospovirus occurrences, and most of the southeast is planted with just a few resistant cultivars (Srinivasan et al., 2017). For example, in Georgia in 2016, about 80% of planted cultivated peanut was 'GA-06G' (Srinivasan et al., 2017). Therefore, there is great utility in mapping and integrating new tomato spotted wilt orthotospovirus resistance into cultivated peanut and determining its mode of resistance.

Since high levels of resistance have not been found in cultivated peanut germplasm, wild *Arachis* species are a promising source of resistance. Lyerly et al. (2002) tested 46 *Arachis* accessions for resistance to tomato spotted wilt orthotospovirus in the greenhouse by artificial inoculation and found nine to have no disease symptoms. After testing with more virulent tomato spotted wilt orthotospovirus isolates, *A diogoi* GKP 10602 and *A. correntina* GKP 9530 were identified as highly resistant. Milla (2003) identified five AFLP markers on the same chromosome linked to tomato spotted wilt orthotospovirus resistance in an F_2 population made from a cross between two A-genome wild species, the highly susceptible *A. kuhlmannii* (VRGeSv 7639) and the highly resistant *A diogoi* GKP 10602. In Brazil, wild *Arachis* species *A. gregoryi* VS 14957, *A. stenosperma* V 13832, *A. kuhlmannii* V 8979, *A. kuhlmannii* V 9912, *A. kuhlmannii* V 7639, and *A. villosa* VMiIrLbGv 14309 were found to have promising resistance

to thrips, *E. flavens*, for introgression (Janini et al., 2010). Further breeding efforts are needed to introgress these resistances from wild *Arachis* species into cultivated peanut.

Molecular tools for introgression of *Arachis* alleles into cultivated peanut

Utilizing markers associated with resistances can improve the speed of introgression and selection of resistance and can help track the size of introgressed segments to reduce linkage drag. Due to higher variation within and among wild *Arachis* species as compared to cultivated peanut, most molecular research with *Arachis* species has been focused on describing relationships between *Arachis* species rather than being used for crop improvement (Stalker and Mozingo, 2001). Molecular maps of wild *Arachis* species aid in marker-assisted selection by grouping markers and associated resistance QTL into linkage groups (Stalker et al., 2016).

The first map for any *Arachis* species was constructed by Halward et al. (1993) in a cross between the A-genome species, *A. stenosperma* and *A. cardenasii*; it had 117 RFLP markers grouped in 11 linkage groups. Milla (2003) used AFLP markers to make a map used to identify markers related to tomato spotted wilt orthotospovirus in an F_2 population made from crossing two A-genome wild species, *A. kuhlmannii* (VRGeSv 7639) and *A diogoi* GKP 10602. The availability of SSR markers and then later SNPs allowed more saturated maps of A-genome species to be made (Leal-Bertioli et al., 2009; Moretzsohn et al., 2005). A map with almost 2,000 SNP, SSR and SSCP markers using progeny from a cross between two *A. duranensis* accessions was made by Nagy et al. (2012). Maps of B- and K-genome species such as *A. ipaensis, A. magna*, and *A. batizcoi* have also been made and compared to A-genome maps (Guo et al., 2012; Moretzsohn et al., 2005). A lot of synteny was found between the A- and B-genome species as well as several inversions and translocations (Guo et al., 2012). In 2001, the first tetraploid map was made using RFLP markers using materials made from crossing the cultivar

'Florunner' and the synthetic tetraploid TxAG-6 [*A. batizocoi* × (*A. cardenasii* × *A. diogoi*)], which had high resistance to nematode (Burow et al., 2001). Foncéka et al. (2009) later developed a map with SSR markers with 21 linkage groups using progeny from crossing the cultivar 'Fleur 11' with the synthetic tetraploid *A. ipaënsis* × *A. duranensis*. These molecular maps are important for identifying the location of important genes.

QTL associated with desirable genes are essential for pyramiding them into peanut cultivars. The agronomically-useful first trait to be linked to molecular markers was resistance to root-knot nematode originating from *A. cardenasii* (Burow et al., 1996; Garcia et al., 1996). This resistance was thought to be contributed mostly by a single, dominant gene, meaning it may not be very durable under high selection pressures. Therefore, further studies mapped nematode resistance QTL from another diverse source, *A. stenosperma,* to be pyramided into peanut cultivars (Ballén-Taborda et al., 2019). Other QTL derived from wild *Arachis* species have been identified for resistance to early leaf spot (Stalker and Mozingo, 2001), late leaf spot (Leal-Bertioli et al., 2009; Sujay et al., 2011), rust (Khedikar et al., 2010), and tomato spotted wilt orthotospovirus (Milla, 2003). In addition, markers association with positive impact on agronomic traits such as number of flowers, seed and pod number per plant, and length, size, pod maturity, and oil content have been identified (Fonceka et al., 2012; Huang et al., 2012).

The most recent advances in molecular tools in peanut have been the release of the sequences of the diploid progenitors of peanut, *A. ipaensis* and *A. duranensis,* and cultivated peanut as well as the Axiom_*Arachis*2 SNP chip (Bertioli et al., 2016, 2019; Clevenger et al., 2018; Korani et al., 2019). These sequenced genomes have opened the door to further study the evolution of their genome structures and the dynamics of their molecular evolutionary processes as well as to identify desirable genes and other functional elements more accurately. Most

importantly, these genome sequences have allowed SNP genotyping resources to be made, greatly improving upon SSR markers. Over 15,000 SSR markers have been used successfully to study the genetic diversity in *Arachis* and to map desirable QTL (Pandey et al., 2012; Tang et al., 2007), but SSR markers are limited in number, time consuming, and expensive. SNPs offer the advantage of abundance; 66,099 high quality SNPs have been identified from *Arachis* and 58,233 of those SNPs have been tiled on the Axiom_*Arachis* SNP array (Clevenger et al., 2017; Pandey et al. 2017). This 58k SNP array represents the A and B genomes fairly at 51.5% and 48.5%, respectively, and includes SNPs from *A. hypogaea* (76.7%), *A. duranensis* (6.2%), *A. batizocoi* (4.7%), *A. magna* (4.5%) *A. stenosperma* (4.1%) and *A. cardenasii* (3.8%) (Clevenger et al., 2017; Pandey et al. 2017). This powerful tool has enabled high resolution trait mapping of many resistances, speeding up breeding efforts.

Utilization of allotetraploids for improvement of cultivated peanut

Wild *Arachis* species have a wide range of genetic and phenotypic variability, including strong resistance to many economically devastating insects and pathogens (Stalker, 2017). This variability presents an opportunity to both widen genetic diversity in peanut and to introgress phenotypic traits such as increased plant biomass, flower count, and pathogen and insect resistances into cultivated peanut. As previously discussed, some species such as *A. cardenasii* have already been used successfully to introgress leaf spot and nematode resistance into cultivated peanut, demonstrating the value of these species for introgression of desirable traits (Simpson et al. 2003; Tallury et al. 2014). The easiest pathway for introgression of desirable traits is through synthesized allotetraploids. Efficient employment of novel phenotypic variation from these allotetraploids in peanut cultivar enhancement is dependent on the knowledge of the genetic and phenotypic diversity present in the materials. This study sought to fill the knowledge

gap in characterization of *Arachis*-derived allotetraploids by examining many allotetraploids comprising of various interspecific hybrid combinations for desirable morphological and reproductive characteristics as well as for resistance to pathogens and insects such as fall armyworm, rust, and tomato spotted wilt orthotospovirus. This study also sought to provide additional knowledge and tools to ease the use of these materials in peanut breeding programs. For example, the negative consequence of weak peg strength in *Arachis* species being introgressed along with desirable alleles into allotetraploids and thus peanut breeding materials was examined and discounted. Additionally, KASP markers distinguishing A- and B/K-genomes were made to further ease use of these materials in breeding programs. The goal of this study was to provide peanut breeders with the resources to create high-yielding peanut cultivars with strong, durable resistance derived from wild *Arachis* species to promote global food security.

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CHAPTER 2

MORPHOLOGICAL AND REPRODUCTIVE CHARACTERIZATION OF NASCENT ALLOTETRAPLOIDS CROSS-COMPATIBLE WITH CULTIVATED PEANUT (*ARACHIS HYPOGAEA* L.)

[1] C.M. Levinson, Y. Chu, X. Luo, H.T. Stalker, D. Gao, C.C. Holbrook, and P. Ozias-Akins. 2021. Genetic Resources and Crop Evolution. Reprinted here with permission of publisher. The final publication is available at https://link.springer.com/article/10.1007/s10722-021-01161-0.

Abstract

Peanut improvement is limited by a narrow genetic base. However, this obstacle can be circumvented by incorporating phenotypic variability from wild, diploid *Arachis* species through interspecific hybridizations. In this study, four allotetraploid interspecific hybrids *IpaCor*^{4x} (*A. ipaensis* x *A. correntina*), *IpaDur*^{4x} (*A. ipaensis* x *A. duranensis*), *IpaSten*^{4x} (*A. ipaensis* x *A. stenosperma*), and *ValSten*^{4x} (*A. valida* x *A. stenosperma*) were created and morphologically characterized through the following parameters: flower count, flower size, flower banner pigmentation, leaf area and weight, leaf hairiness, main stem height, internode length, percent of reproductive nodes, biomass, 100 pod weight, and 100 seed weight. For every trait, except for flower banner absorption at 380 nm, at least one or more allotetraploids differed from the cultivated peanut control. In general, these allotetraploids had a greater production of flowers during the growing season, larger flowers, larger and hairier leaves, taller main stems, longer primary laterals, longer internodes, lower percentage of reproductive nodes, heavier plant body masses, and smaller seeds and pods. This phenotypic diversity can be utilized directly in ornamental and forage breeding, while for oil and food crop breeding, this diversity will likely need to be selected against while desirable traits such as disease and insect resistance and abiotic stress tolerances derived from the wild diploid species are maintained.

Introduction

Peanut (*Arachis hypogaea* L.) is an allotetraploid species (AABB; 2*n*=4*x*=40), and evidence suggests that it arose from a natural polyploidization of the diploid hybrid between *A.*

ipaensis (BB; 2*n*=2*x*=20) and *A. duranensis* (AA; 2*n*=2*x*=20) (Bertioli et al. 2016). The difference in ploidy levels between cultivated peanut and its wild relatives resulted in a crossing barrier (Simpson 2001) and constricted genetic diversity in peanut. Subsequently, peanut cultivar improvement is limited by the narrow genetic base of this species (Holbrook et al. 2014). However, there are more than 80 wild *Arachis* species with a wide range of genotypic and phenotypic variability that can be used to expand peanut's genetic base (Stalker et al. 2016). The predominant use of *Arachis* species in peanut breeding programs has been for introgression of insect and disease resistances into cultivated peanut (Stalker et al. 2016); however, these materials could offer desirable phenotypic traits in addition to biotic and abiotic stress resistances and tolerances.

Due to peanut being an allotetraploid and most wild *Arachis* species being diploid, an efficient way to introgress genetic variability from wild *Arachis* species into cultivated peanut is to produce allotetraploid interspecific hybrids that are cross-compatible to peanut. This method has been used extensively in *Brassica* and is increasingly being used in peanut (Rahman 2013; Zhan et al. 2017; Leal-Bertioli 2018; Ballén-Taborda 2019). In addition to making the variability of the diploid *Arachis* species available for introgression into peanut, allotetraploids made from wild *Arachis* species can have additional phenotypic variability in plant morphology, physiology, anatomy and biochemistry, resulting from their new combination of genomes as well as from their increased ploidy level (Leal-Bertioli et al. 2017). For example, heterozygosity, subgenome interactions, and the gigas effect in allotetraploids have been shown to result in beneficial traits such as increased plant vigor and chlorophyll content, among other traits that peanut breeders could exploit in their breeding programs (Ramsey and Ramsey 2014; Leal-Bertioli et al. 2017).

Our peanut pre-breeding program seeks to produce neotetraploids from a selected set of diploid species as a genetic resource to enlarge the genetic variability of cultivated peanut. The selection of diploids was mainly based on their strong, diverse resistances to many insect and disease pests. Specifically, we chose *A. correntina* (Burkart) Krapov. and W.C. Gregory (PI 262808, GKP 9530), *A. duranensis* Krapov. and W.C. Gregory (PI 468197, GKBSPSc 30060), *A. ipaensis* Krapov. and W.C. Gregory (PI 468322, GKBSPSc 30076), *A. stenosperma* Krapov. and W.C. Gregory (PI 666100, V10309), and *A. valida* Krapov. and W.C. Gregory (PI 468154, GK 30011), which have been reported to be resistant to aflatoxin, early leaf spot, late leaf spot, peanut rust, cylindrocladium black rot, groundnut rosette virus, tomato spotted wilt virus, peanut stunt virus, peanut mottle virus, nematodes, and numerous insect pests (Stalker 2017).

Efficient use of novel germplasm is dependent on the knowledge of the genetic and phenotypic diversity present in the materials. Germplasm characterization of agronomic traits is especially useful for germplasm intended to be used for crop improvement. However, there is a gap between the realized agronomic germplasm characterization and the needed level of characterization to identify and employ novel phenotypic variation in germplasm enhancement (Ferreira 2006). Therefore, when depositing the neotetraploids into a germplasm bank, it is important to provide a description of their morphological and reproductive characteristics and to inform breeders of the range of diversity presented in these materials. The goal of this study was to characterize morphological and reproductive traits of these nascent allotetraploids to promote efficient utilization of these materials in breeding programs after their release. This was done by building a catalog of descriptors, including flower count, flower size, flower banner pigmentation, leaf area and weight, leaf hairiness, main stem height, internode length, percentage of reproductive nodes, biomass, 100 pod weight, and 100 seed weight, of these materials.

Materials and Methods

Plant Materials

Three A genome diploid species, *A. correntina* (Burkart) Krapov. and W.C. Gregory (PI 262808, GKP 9530), *A. duranensis* Krapov. and W.C. Gregory (PI 468197, GKBSPSc 30060), and *A. stenosperma* Krapov. and W.C. Gregory (PI 666100, V10309), and one B genome species, *A. ipaensis* Krapov. and W.C. Gregory (PI 468322, GKBSPSc 300076) were crossed following the BB x AA crossing regime to create the diploid hybrids at North Carolina State University (Raleigh, NC). The A genome diploid species *A. stenosperma* Krapov. and W.C. Gregory (PI 666100, V10309) and the B genome diploid species *A. valida* Krapov. and W.C. Gregory (PI 468154, GK 30011) were crossed to create the diploid hybrids at the University of Georgia (Athens, GA). Four allotetraploids, *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x}, were created from the diploid hybrids by colchicine treatment at the University of Georgia Tifton Campus. The cultivated peanut check for these experiments was *A. hypogaea* '13-2113' [a selected runner-type breeding line from (C1805-617-2 x 'Florida-07') x 'Georgia-06G']. C1805-617-2 is a selection from 'Tifguard' x 'Florida-07'.

S_2 seeds from the four allotetraploids and seeds from 13-2113 were coated in Vitavax PC (Vitavax, Crompton, Middlebury, CT) and treated overnight in 0.5% Florel Growth Regulator (Lawn and Garden Products Inc., Fresno, CA) to break dormancy on April 5th for the allotetraploids and April 26th, 2018, for 13-2113, respectively. The day after dormancy treatment, the seeds were planted in Jiffy pots filled with Promix growth medium (Premier Tech Horticulture, Quakertown, PA). On May 14th, the seedlings were transplanted to the center of the

1.82 m wide beds with 1 m distance between two neighboring plants and 4 m distance between plants in neighboring rows. The field was planted in a randomized complete block design with seven blocks, each containing one 13-2113, three *IpaCor*^{4x}, three *IpaDur*^{4x}, one *IpaSten*^{4x}, and two *ValSten*^{4x} plants. Different numbers of plants per genotype were sown due to variation in seed availability. The field was treated with Provost fungicide spray four times each two weeks apart starting on June 12th. The allotetraploids grew large and started to intertwine against each other one month after transplanting. Since flower counts from individual plants would be inaccurate if the neighboring plants grew into each other, 30.8 cm tall Choppers garden edging (Emsco Group, Girard, PA) were installed to create a circle with a diameter of 1 m of edging around each plant to separate them. The garden hedges were installed in a circle with a radius of 0.5 m around each plant.

Characterization

Flower Count

Numbers of flowers were counted from 50 to 108 days after transplanting between 8:30 to 11:00 am. Data collection was performed twice a week.

Flower Size

Between 35 and 39 days after transplanting, four flowers were collected from each plant in 15 ml falcon tubes (Corning CoStar, Corning, NY) containing a moist Kimwipe (Kimerly-Clark, Neenah, WI) to keep flowers from wilting during collection. Two flowers were taken from the primary lateral (n+1) branch(es) and two from the secondary lateral (n+2) branch(es). Flowers were dissected and scanned; measurements on hypanthium area (cm^2); banner area (cm^2); banner height (cm), banner width (cm), and left wing area (cm^2) were taken with ASSESS 2.0 software (APS Press).

Flower Banner Pigmentation

The flower banners dissected from the four flowers per plant previously used for flower size measurements were used to measure banner pigmentation. The banners were first weighed before they were put into 2 ml tubes (Phenix Research, Swedesboro, NJ) with 4, 3.2 mm diameter, beads (BioSpec, Bartlesville, OK). The tubes were then submerged in liquid nitrogen and vortexed until the banner was a fine powder. The volume of 100% ethanol added to the pulverized banner was 10 times banner weight. The tubes were vortexed for about 3 s and then spun down at 3000 rpm for 5 min. 200 ul of the supernatant was loaded into a spectrometry plate for reading, with 100% ethanol blanks as controls. Readings were normalized to the 100% ethanol, and only readings at wavelengths of 380, 415, 450, 473 and 495 nm were above the blank ethanol.

Leaf Area and Weight

At 101 and 102 days after transplanting, the first four newly emerged and fully expanded leaves on the main stem were collected from each plant and scanned for leaf area measurement using the ASSESS 2.0 software (APS Press). Leaf area was expressed as cm^2. Fresh weights of the collected leaves were taken immediately after collection. The leaves were dried for 48 h at 30 °C and then dry weights were taken.

Leaf Hairs

Between 114 and 119 days after transplanting, the most distal fully expanded leaflet was collected from two n+1 branches from each plant. The number of leaf hairs at the edge of the mature peanut leaflet within 1,000 µm was counted under the microscope.

Main Stem Height

Main stem height of plants was measured with a meter stick 91 days after transplanting when flowering in all genotypes plateaued.

Internode Length and Differentiation of Nodes

The length of n+1 branches was measured with meter sticks upon harvest, 120 days after transplanting. The total number of nodes on each branch was counted. The internode length was derived by dividing the branch length by the number of nodes.

The differentiation of reproductive and vegetative nodes was documented on the main stem and primary laterals. The presence or absence of flower buds and/or pegs was used to determine whether the node was reproductive or vegetative, respectively.

Plant biomass

After removing the pods, the above ground tissue of each plant was dried in the greenhouse (25 to 32 °C) for 7 days. Weight of dried tissue was taken.

Pod and Seed Traits

After harvest, pods were examined for the presence or absence of a beak, and 100 pod weight was measured. The pods were shelled, and 100 seed weight was taken. For lines that yielded less than 100 pods and/or seeds, 100 pod and seed weight were extrapolated by dividing the measured weight by the number of pods/seeds and multiplying by 100. For 13-2113, the seeds were bulked from the plants before 100 seed and 100 pod weight were taken; therefore, this genotype was excluded from the statistical analysis for these traits. However, the bulked 100 pod weight and 100 seed weight were plotted to give a reference point for the allotetraploids.

Statistical Analysis

The flower count was analyzed using a standard two-way analysis of variance (ANOVA) with repeated measures. Genotype and days after transplanting were fixed effects, and block was

a random effect. GLIMMIX procedure in SAS 9.4 (SAS Institute) was used to test the fixed effects. The residual-normality assumption was evaluated based on residual Q-Q plot, and the plot implied that the flower number was approximately normally distributed. Compound symmetry (CS) covariance structure was chosen to describe the correlation of flower counts between repeated measurements. One-way ANOVA was performed to determine the genotype effect on plant reproductive and morphological characteristics assessed using the following parameters: flower size, flower banner pigmentation, leaf area and weight, leaf hairiness, main stem height, internode length, percentage of reproductive nodes, biomass, 100 pod weight, and 100 seed weight. The *IpaCor*^{4x}, *IpaDur*^{4x}, and *ValSten*^{4x} replications in each block were averaged so that each allotetraploid had one data point per block and were equally represented in the statistical analysis. Means of each parameter among the treatments were separated based on the Tukey's Test ($\alpha = 0.05$) results.

Results

Flower Count, Size, and Banner Pigmentation

Genotype, days after transplanting, and genotype by days after transplanting interactions were all significant indicators of flower count (Table 2.1). *IpaCor*^{4x} had the greatest flower counts from 50 to 108 days after transplanting (Fig. 2.1), with an average count of 47.45 flowers per day (Table 2.2). While *IpaSten*^{4x} and *ValSten*^{4x} had fewer flowers than *IpaCor*^{4x}; their flower counts were stable throughout the data collection period with average flower counts of 14.54 and 35.57 per day, respectively (Table 2.2). *IpaDur*^{4x} had a flowering trend similar to 13-2113, in which both had obvious decreasing counts beginning 78 days after transplanting, although this trend was even more prominent in 13-2113. *IpaDur*^{4x} had an average flower count of 23.86 per

day (Table 2.2). Overall, 13-2113 had the lowest average flower count at 11.50 flowers per day (Table 2.2).

Significant genotypic effects on hypanthium area, banner area, banner width, banner height, and left-wing area were found (Table 2.3). All the allotetraploids, except for *IpaDur*^{4x}, had significantly greater hypanthium area as compared to the cultivated control (Fig. 2.2A). *IpaCor*^{4x} had the largest hypanthium area, in which the average area was more than three times that of the cultivated line. The trend in banner area and wing area were similar (Fig. 2.2B, C). *IpaCor*^{4x} and *ValSten*^{4x} had significantly larger banner and wing area than the cultivated control, while *IpaSten*^{4x} was not statistically different from the cultivated control and *IpaDur*^{4x} was even smaller than the cultivated control. All the genotypes had yellow wings, but only *IpaSten*^{4x} and *ValSten*^{4x} had completely yellow banners, with thin red stripes originating from where the hypanthium and banner connect and spread outwards (Fig. 2.2D). 13-2113, *IpaCor*^{4x}, and *IpaDur*^{4x} had banners with a yellow center and orange edge, though the intensity of the orange differed with *IpaDur*^{4x} having a soft orange banner edge color, *IpaCor*^{4x} a dark orange banner edge, and 13-2113 falling in between the soft orange of *IpaDur*^{4x} and the dark orange of *IpaCor*^{4x} (Fig. 2.2D).

Significant genotypic effects on banner pigment at the following wavelengths were found: 380, 415, 450, 473, and 495 nm (Table 2.3). Although the banner pigment at 380 (ultraviolet-A) nm was above the 100% ethanol background, a significant genotypic effect on its absorbance was not detected. *IpaSten*^{4x} and *ValSten*^{4x}, the two genotypes with yellow flowers (Fig. 2.2D), had the same level of absorbance at all the wavelengths (Fig. 2.3). At 415 nm (violet), the absorbance of all the genotypes was the same, except for *IpaCor*^{4x}, which had a lower absorbance than 13-2113, *IpaDur*^{4x}, and *IpaSten*^{4x}. The absorbance at both 450 nm (blue)

and 473 nm (cyan) followed the same trend, in which only *IpaCor*^{4x} and *ValSten*^{4x} had significantly lower absorbance than 13-2113. Absorbance at 495 nm (cyan/green) was much lower for all the genotypes as compared to the other wavelengths, and only *IpaSten*^{4x} and *ValSten*^{4x} had significantly lower levels of absorbance than 13-2113.

Leaf Area, Weight, and Hair

Significant genotypic effects on leaf area, fresh leaf weight, dry leaf weight, and leaf hairs were found (Table 2.3). All the allotetraploids had significantly larger and heavier leaves than the cultivated control as measured by leaf area and leaf weight (fresh and dry) (Fig. 2.4A, B, C). In addition, the range of leaf weight was much greater for all the allotetraploids as compared to the cultivated control. All the allotetraploids had significantly greater numbers of hairs than 13-2113 (Fig. 2.4D, E).

Plant Size

Significant genotypic effects on main stem height, main stem percentage of reproductive nodes, primary lateral length, internode length, primary lateral percentage of reproductive nodes, and plant biomass were found (Table 2.3). 13-2113 and *IpaSten*^{4x} had the shortest main stem with an average height of 13.8 and 15.2 cm, respectively (Fig. 2.5A). *ValSten*^{4x} and *IpaCor*^{4x} had longer main stems with an average of 22.0 and 27.1 cm, respectively. However, *IpaDur*^{4x} had the longest main stem height by far with an average of 65.6 cm, which was 4.75 times greater than 13-2113. Only *IpaCor*^{4x} and *IpaDur*^{4x} had reproductive nodes on their main stems, and *IpaCor*^{4x} had the highest percentage of reproductive nodes at 4.82% while *IpaDur*^{4x} had a percentage of 0.87% (Fig. 2.5B). Primary lateral length did not follow the same trend as main stem height, except that 13-2113 had the shortest primary lateral length at 41 cm (Fig. 2.5C). All the allotetraploids had much longer primary laterals than 13-2113. *IpaSten*^{4x}, with the shortest

primary lateral length of the allotetraploids, still had a length 3 times longer than 13-2113. Meanwhile, *ValSten*^{4x}, with the longest primary lateral length of the allotetraploids, was 4.5 times longer than 13-2113. For internode length, 13-2113 was again classified as having the shortest of the five genotypes with an average internode length of 1.9 cm (Fig. 2.5D). All the allotetraploids had internode lengths at least 2 times greater than 13-2113. *IpaDur*^{4x} and *IpaSten*^{4x} had greater internode lengths than *IpaCor*^{4x}, while *ValSten*^{4x} had the longest internode length overall. For primary lateral percentage of reproductive nodes, 13-2113 had the highest average at 43.2% (Fig. 2.5E). This percentage was about 3 times greater than the lowest percentage, which was *IpaSten*^{4x} at 15.6%, and about 1.5 times greater than the next highest percentage, which was *ValSten*^{4x} at 29.2%. Lastly, 13-2113 and *IpaSten*^{4x} had the lowest plant body mass at 90.3 and 144.7 g, respectively (Fig. 2.5F). *IpaDur*^{4x} and *IpaCor*^{4x} had plant biomass 2 times greater than 13-2113, while *ValSten*^{4x} was 3 times greater than 13-2113.

100 Pod and Seed Weight

Significant genotypic effects on allotetraploid 100 pod and seed weights were not found (Table 2.3). All the allotetraploids had low 100 pod weights ranging from the lowest at 21.2 g for *IpaCor*^{4x} to the highest at 25.4 g for *ValSten*^{4x} (Fig. 2.6A, C). These weights were only 24% to 29% of the weight (86.9g) for 13-2113. Similarly, all the allotetraploids had low 100 seed weight ranging from the lowest at 14.4 g for *IpaSten*^{4x} to the highest at 16.5 g for *ValSten*^{4x} (Fig. 2.6B, C). These weights were only 19% to 22% of the weight (76.6g) for 13-2113.

Discussion

Wild *Arachis* species have a wide range of genetic and phenotypic variability, including strong resistances to many economically devastating insect and disease pests (Stalker 2017). This variability presents an opportunity to both widen genetic diversity in peanut and to introgress

phenotypic traits such as increased plant biomass, flower count, and disease and insect resistances into cultivated peanut. Some species such as *A. cardenasii* have already been used successfully to introgress leaf spot and nematode resistance into cultivated peanut, demonstrating the value of these species for introgression of desirable traits (Simpson et al. 2003; Tallury et al. 2014). The four allotetraploids reported in the study are cross compatible to cultivated peanut and therefore, they can be readily used for peanut cultivar improvement. The documentation of the morphological and reproductive characterization of these materials allows phenotypic traits such as plant vigor (demonstrated by increased plant biomass, plant height, flower production, among others) to be introgressed into peanut breeding lines. In the case of undesirable phenotypic traits characterized for these materials, plans for selection against these traits should be considered.

To fill the gap in characterization of these neotetraploids, they were examined in-depth for both morphological and reproductive characteristics. For most of the traits, at least one or more allotetraploid differed from the cultivated peanut control; therefore, these materials offer a wide range of phenotypic diversity that can be utilized for peanut breeding. Overall, the allotetraploids tended to have more flowers over the growing season, larger flowers, larger and hairier leaves, taller main stems, longer primary laterals, longer internodes, lower percentages of reproductive nodes, heavier plant body masses, and smaller seeds and pods. Greater leaf hair density may be a trait that breeders choose to select for in order to increase resistance to some insect pests, since hair density has been demonstrated as one defense mechanism to some insect pests (Mohammad et al. 2019). In addition, the allotetraploids with larger flowers and more flowers over the growing season such as *IpaCor*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} could be used for ornamental breeding. This absence of decline in flower production demonstrated by these three

allotetraploids is likely influenced by the perenniality of *A. correntina* and *A. stenosperma* (Stalker and Simpson 1995). Likewise, allotetraploids with heavy plant biomass such as *IpaCor*^{4x}, *IpaDur*^{4x}, and *ValSten*^{4x} could be used for forage breeding. On the contrary, breeders aiming to introgress biotic and abiotic stress resistances and tolerances into cultivars used as oil or food crop will need to backcross these materials to their desired peanut cultivars. Linkage drag for small pod and seed size, low percentage of reproductive nodes, long primary lateral length, and long internode length should be selected against in each breeding cycle.

The findings of this study complement a previous report Leal-Bertioli et al. (2017), which compared 26 anatomical, morphological, and physiological traits of six *Arachis* neotetraploids, their parental diploids, and *A. hypogaea* grown in a greenhouse environment. Despite different growing environments (greenhouse versus the field) and testing different allotetraploids, both Leal-Bertioli et al. (2017) and this study found that allotetraploids had high levels of plant vigor and biomass. Leal-Bertioli et al. (2017) reported this greater vigor by increased stem height, aerial dry biomass, leaf size, leaf canopy, root biomass, and leaf cell size as compared to the parental diploids, while this study identified increased vigor by increased flower production through the growing season, flower size, leaf area and weight, main stem height, internode length, and plant biomass as compared to a peanut breeding line. In addition, both Leal-Bertioli et al. (2017) and this study found that seed size and yield of these allotetraploids were small. The increased plant vigor of allotetraploids does not seem to be correlated to reproductive vigor; therefore, plant breeders will have to backcross these materials to peanut breeding lines while selecting against small seed size, low yield, and fragile pegs.

More recent studies have begun to characterize biotic and abiotic stress resistance and tolerance levels of *Arachis*-derived allotetraploids to inform breeders of the value of these

materials for traits such as late leaf spot and rust resistance, nematode resistance, and drought stress tolerance (Dutra et al. 2018; Leal-Bertioli et al. 2018). Leal-Bertioli et al. (2018) and Ballén-Taborda et al. (2019) built upon the previously discussed report Leal-Bertioli et al. (2017) and found that the *IpaDur*^{4x} V14167 allotetraploid had wild *Arachis* species traits such as long branches and small seeds but higher levels of late leaf spot and rust resistance as compared to Runner-886, a susceptible control. The *IpaDur*^{4x} 30060 in this study was produced from a cross between *A. ipaensis* and a different accession of *A. duranensis* than the accession Leal-Bertioli et al. (2018) used. This is an important distinction, since resistance to pests is variable between accessions within an *Arachis* species (Stalker and Campbell 1983). One example of variation for resistance within a species is *A. correntina.* Stalker and Campbell (1983) identified *A. correntina* 9530 (PI 262808), the same accession used in this study to make the *IpaCor*^{4x} allotetraploid, as well as *A. correntina* (Manfredi #5 and #36) as sources for potato leafhopper resistance, but only *A. correntina* 9530 and Manfredi #36 as sources for corn earworm resistance. Furthermore, another study identified *A. correntina* 9548 (PI 262881) as a source of resistance to two spotted spider mites but not Manfredi #36 (Johnson et al. 1977). Therefore, producing allotetraploids with the same species but different accessions creates an even greater resource pool for breeders and is not redundant.

The genetic resources produced in this study widen the available pool of diversity for peanut breeders for a wide range of phenotypic traits. The future direction of this research is to continue the momentum of using this allotetraploid method to bridge wild diploid species with tetraploid peanut to reach the success that has been achieved in *Brassica*. Therefore, these materials and data are being provided to a germplasm bank to allow breeders the opportunity to use and mine these materials for their phenotypic diversity. In addition, we will study the disease

and insect resistance levels in these materials so that breeders can use these materials for resistance introgression in addition to introgression of morphological and reproductive traits. Populations of these allotetraploids crossed to peanut breeding lines could be produced so that QTL of these morphological and reproductive traits can be mapped and shared with breeders to make introgression more efficient.

Conclusions

Comprehensive morphological and reproductive characterization of four unique allotetraploids was performed from field grown materials in this study. Most of these allotetraploids produced more flowers over the growing season, larger flowers, larger and hairier leaves, taller main stems, longer primary laterals, longer internodes, lower percentages of reproductive nodes, heavier plant body masses, and smaller seeds and pods than cultivated peanut, although there were exceptions for certain allotetraploids. These germplasm resources will be released so that further characterization and utility in peanut breeding will become possible to improve peanut production.

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Table 2.1. Type III tests of fixed effects of genotype, days after transplanting and genotype by days after transplanting interaction on flower count.

Effect	F or X^2 value	Df(n), df(d)	P-value
Genotype	21.17	4, 23	< 0.0001***[a]
Days after transplanting	29.80	17, 493	< 0.0001***
Genotype x days after transplanting	7.98	68, 493	< 0.0001***

[a]*P < .05. **P < .01. ***P < .001

Table 2.2. Tukey-Kramer grouping for genotype least squares means (alpha = 0.05) on flower count mean separation across all days after transplanting.

Genotype	Estimated Mean	Tukey-Kramer Grouping
13-2113	11.50	C
IpaCor^{4x}	47.45	A
IpaDur^{4x}	23.87	BC
IpaSten^{4x}	14.54	C
ValSten^{4x}	30.59	A

Table 2.3. ANOVA output testing the genotype effects on plant reproductive and morphological characteristics assessed using the following parameters: flower size, flower banner pigmentation, leaf area and weight, leaf hairiness, main stem height, internode length, percentage of reproductive nodes, biomass, 100 pod weight, and 100 seed weight.

Parameter	F or X^2 value	Df(n), df(d)	P-value
Hypanthium area	57.11	4, 28	< 0.0001***
Banner area	47.15	4, 28	< 0.0001***
Banner width	55.53	4, 28	< 0.0001***
Banner height	43.77	4, 28	< 0.0001***
Left wing area	49.85	4, 28	< 0.0001***
Banner pigmentation: 380 nm	2.40	4, 29	0.073
Banner pigmentation: 415 nm	5.15	4, 29	0.0029**
Banner pigmentation: 450 nm	4.19	4, 29	0.0035**
Banner pigmentation: 473 nm	4.19	4, 29	0.0085**
Banner pigmentation: 495 nm	16.11	4, 29	< 0.0001***
Leaf area	10.12	4, 25	< 0.0001***
Leaf weight (fresh)	12.73	4, 25	< 0.0001***
Leaf weight (dry)	8.34	4, 25	0.0003***
Leaf hair	9.47	4, 29	< 0.0001***
Main stem height	211.33	4, 25	< 0.0001***
Primary lateral length	26.71	4, 29	< 0.0001***
Internode length	35.25	4, 29	< 0.0001***
Percent reproductive nodes (n)	4.16	4, 29	0.0088**
Percent reproductive nodes (n+1)	21.39	4, 29	< 0.0001***
Plant biomass	9.27	4, 29	< 0.0001***
100 pod weight	1.45	3, 20[b]	0.26 [b]
100 seed weight	0.55	3, 21[b]	0.65 [b]

[a]*P < .05. **P < .01. ***P < .001

[b] 13-2113 was not included in 100 seed and 100 pod analysis

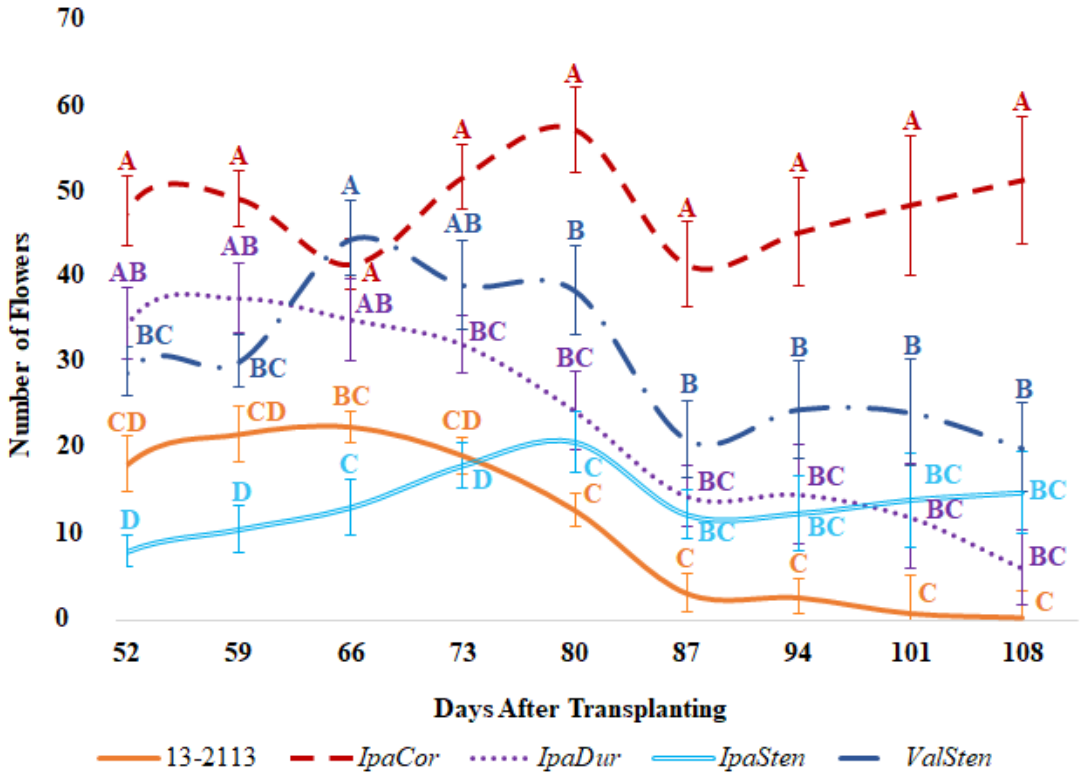

Figure 2.1. Graph of number of flowers for each genotype over the course of 8 weeks, from 52 to 108 days after transplanting.

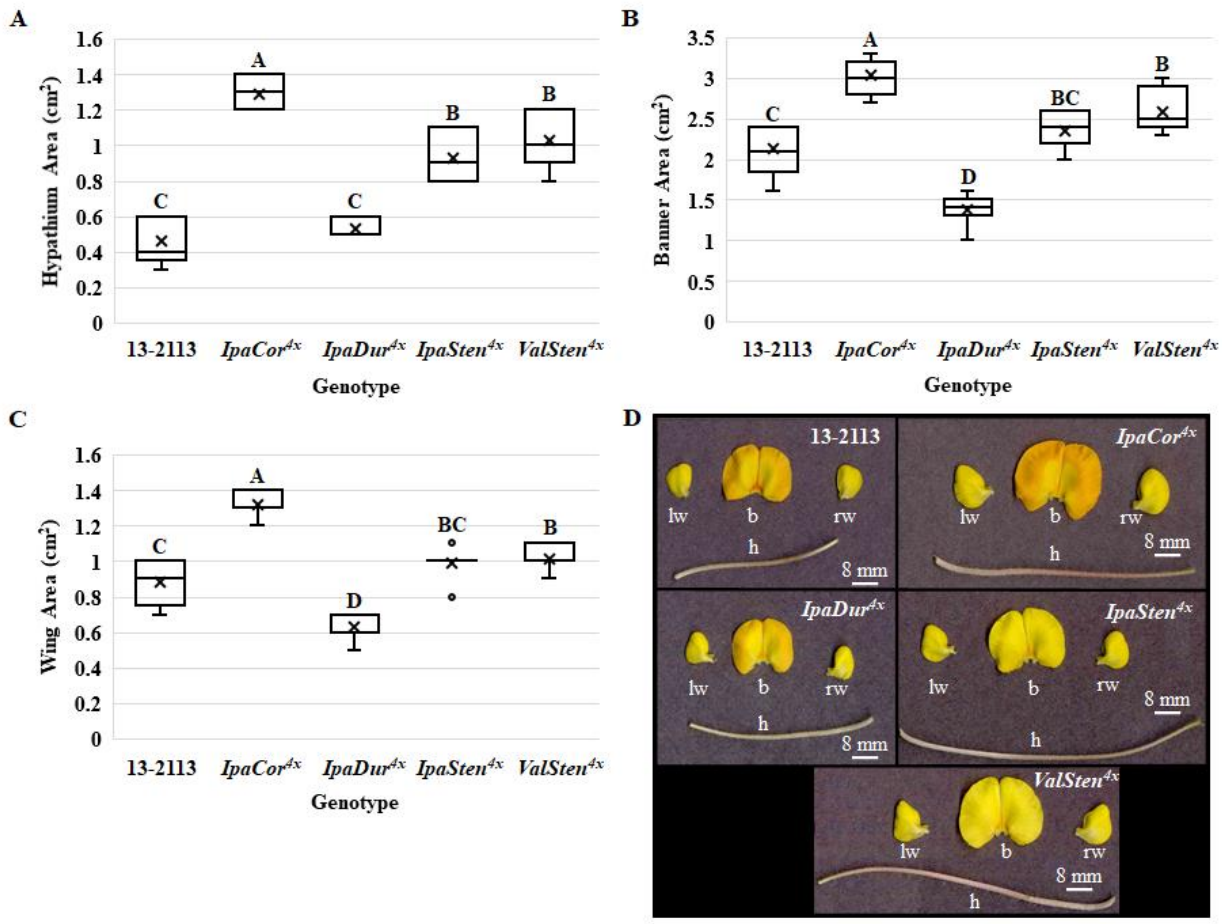

Figure 2.2 Box plots of (A) hypanthium area, (B) banner area, and (C) wing area (D) Scans of dissected flowers from secondary lateral branches, in which banner is labeled b, hypanthium h, left wing-lw, and right-wing rw.

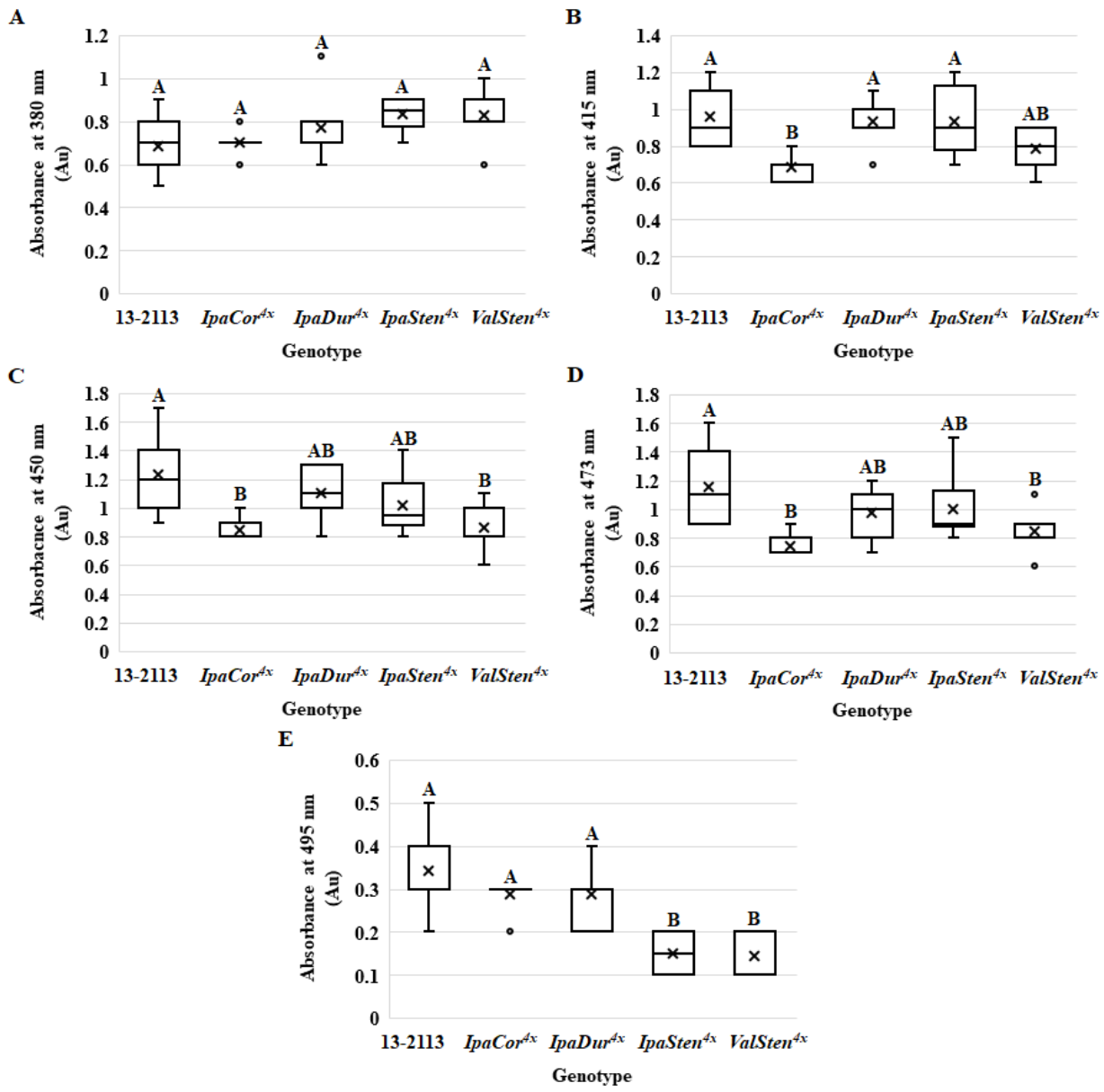

Figure 2.3. Box plots of absorbance at (A) 380, (B) 415, (C) 450, (D) 473, and (E) 495 nm.

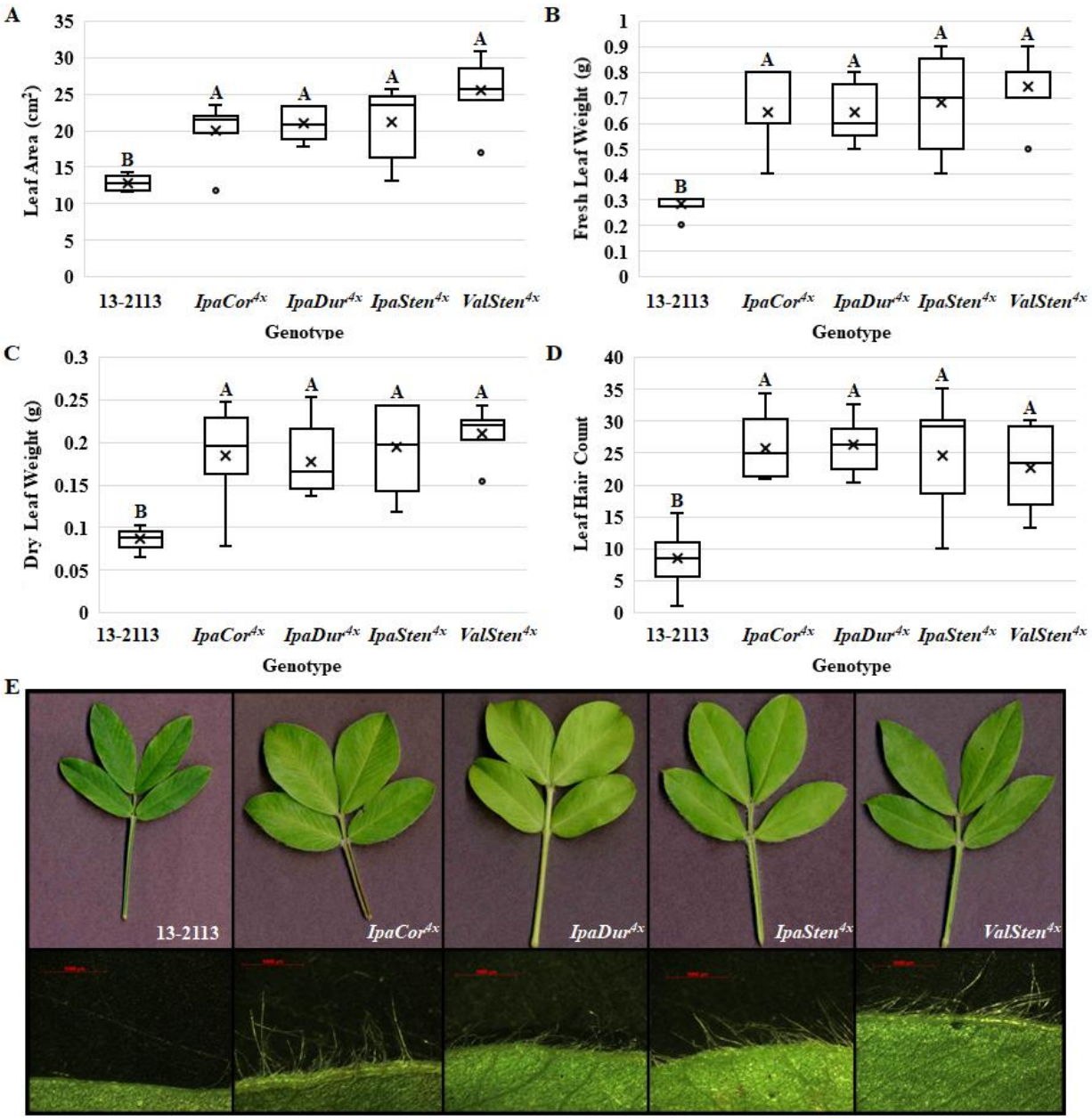

Figure 2.4. Box plots of (A) leaf area, (B) fresh leaf weight, (C) dry leaf weight, and (D) leaf hair count (E) Scans of the most distal, fully expanded leaf excised from the main stem (top) and pictures of the edge of the bottom, right leaflet with a 1,000 um scale bar in red.

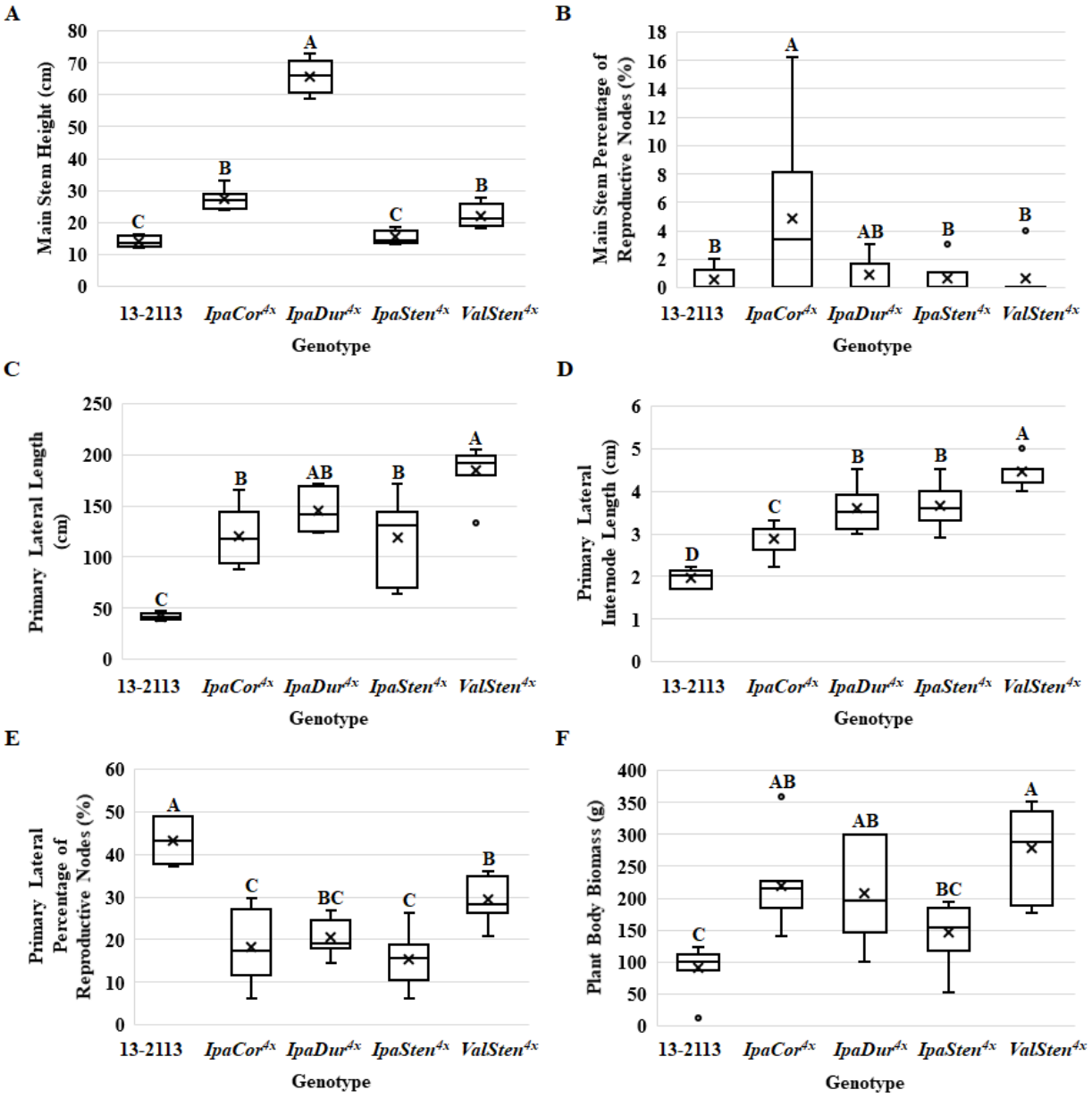

Figure 2.5. Box plots of (A) main stem height, (B) main stem percentage of reproductive node, (C) primary lateral length, (D) primary lateral internode length, (E) primary lateral percentage of reproductive nodes, and (F) plant body biomass.

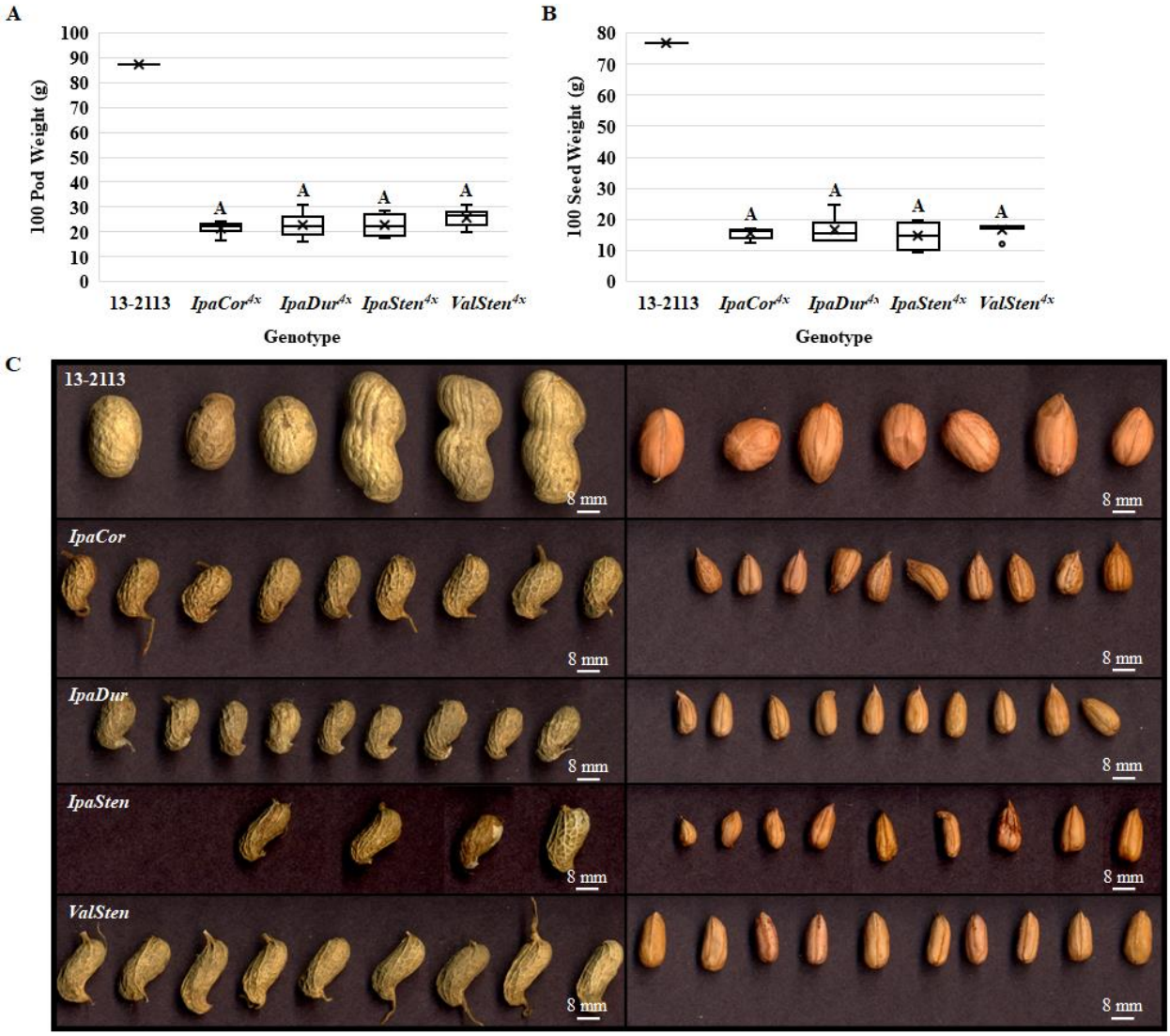

Figure 2.6. Box plots of (A) 100 pod weight and (B) 100 seed weight (C) Scans of pods (left) and seeds (right).

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CHAPTER 3

ANATOMICAL CHARACTERISTICS CORRELATED TO PEG STRENGTH IN *ARACHIS*

[1] C.M. Levinson, Y. Chu, M. Levinson, K.M. Marasigan, H.T. Stalker, C.C. Holbrook, and P. Ozias-Akins. *Accepted*. Peanut Science. Reprinted here with permission of publisher.

Abstract

Wild, diploid *Arachis* species are a great source of biotic and abiotic stress resistances and tolerances for peanut breeding programs; however, these species also have undesirable characteristics such as small seed size, low yield, and weak peg strength. Peg strength has been shown to have a positive, linear relationship with yield in cultivated peanut. Therefore, the weak peg strength of wild *Arachis* species could be detrimental to yield and needs to be selected against when introgressing useful alleles from wild species into elite germplasm. To enable breeders to effectively utilize these wild species, we sought to characterize peg strength and anatomical characteristics correlated with peg strength in seven diploid *Arachis* species, as well as four allotetraploids, six (cultivated peanut lines x allotetraploid) F_1 hybrids, and two cultivated peanut breeding lines. For each genotype, five mature pegs were tested for peg strength and cross-sections for three of the five pegs were subsequently taken and analyzed for peg anatomical characteristics including total peg cross-section area, mean bundle cap area, total bundle cap area, bundle cap as a percentage of peg area, bundle cap number, mean distance between bundle caps, total distance between bundle caps, and tannin cell count. Genotype was a significant indicator for peg strength and all the anatomical characterization parameters ($P < 0.05$). Peg strength was positively and highly correlated with peg area, mean bundle cap area, total bundle cap area, and bundle cap number. Peg strength comparable to that of peanut breeding lines was recovered in the F_1 hybrids. Because weak peg strength in the wild species appears to be recessive, strong pegs can likely be selected during the process of introgression.

Introduction

Peanut (*Arachis hypogaea* L.) is grown worldwide as an oil, cash, food, and feed crop, and is a key source for protein, calories, vitamins, and minerals (Suchoszek-Lukaniuk et al., 2011; Guimón and Guimón, 2012). About 46 million tons of peanut are produced globally each year, but average peanut yields in developing countries are only a fraction of the average yield achieved in the United States (FAOSTAT, 2018). Developing high yielding peanut cultivars with strong resistance/tolerance to biotic and abiotic stress, strong peg strength, and plant vigor is critical to improve peanut productivity (Bantilan et al., 2003; Holbrook et al., 2015). However, the narrow genetic base of cultivated peanut limits crop improvement (Bertioli et al., 2016; Stalker et al., 2016). Yet, there are more than 80 wild peanut relatives and those analyzed to date have much higher levels of genetic polymorphism than cultivated peanut. Introgression of wild segments into cultivated peanut has been found to not only improve disease resistance but also to strengthen a number of agronomic traits such as flowering precocity, seed and pod number or length and size, and pod maturity (Fonceka et al., 2012; Moretzsohn et al., 2013; Stalker et al., 2016). However, wild *Arachis* species also have undesirable traits such as small seed size, a trailing growth habit, and weak peg strength that have to be selected against when used in peanut breeding programs (Fonceka et al., 2012; Stalker et al., 2013; Stalker, 2017).

Peanut is a unique crop that flowers above ground but whose fruit develop underground. In the process of pod formation, the peg, a needle-like structure, extends geotropically from the base of a fertilized flower after fertilization. Ovules are located at the tip of the peg and after penetrating the soil the peg tip and ovules swell to develop into a pod and seed, respectively. Most harvesting of peanut in the United States is mechanized. At the time of seed maturity, the peanut plant is inverted from the soil by an inverter while retaining the peg and pods that are

attached to the plant. A peanut combine then collects the pods, but if the peg is broken in the process of inverting, the pod is lost in the field. Therefore, peg strength is an important agronomic trait for peanut and variability of peg strength in cultivated peanut results in differential levels of yield loss (Sorensen et al., 2015; Sorensen et al., 2017). Peg strength is affected by genotype, fungal and viral infection, maturity, peg age, and field conditions (Bauman and Norden, 1971; Troeger et al., 1976; Thomas et al., 1983; Chapin and Thomas, 2005; Sorensen et al., 2015; Sorensen et al., 2017). Significant differences in peg strength among peanut cultivars can be detected, and there is enough phenotypic variability to produce cultivars with improved peg strength (Thomas et al., 1983). Decreased peg strength was correlated with infection by southern stem rot (*Sclerotium rolfsii*) and increased peg strength was correlated with infection by tomato spotted wilt orthtospovirus (Thomas et al., 1976; Chapin and Thomas, 2005). The increased peg strength of plants infected with tomato spotted wilt orthtospovirus is irrelevant to yield due to the severe stunting caused by this virus and the consequent major yield losses (Srinivasan et al., 2017). Therefore, selecting for resistance alleles to both these pathogens as well as for peg strength alleles will likely increase peanut yields.

Anatomical characteristics associated with peg strength have been studied in cultivated peanut. All pegs have vascular bundles and associated fiber bundle caps arranged in a ring surrounding stelar pith cells that gradually disintegrate with maturity, ultimately forming a hollow stele. Additionally, most pegs have epidermal hairs (Thomas et al., 1983). However, pegs of different genotypes have been found to vary in size and shape of their fiber bundle caps, degree of lignification in fiber bundle caps, number of cambial and parenchymatous cells between vascular bundles, number of xylem vessels per vascular bundle, and size of tannin cells (Thomas et al., 1983; Tiwari et al., 1988). Cultivated peanut genotypes with strong pegs have

bigger, crescent-shaped fiber bundle caps, fewer rows of interfascicular cambium and parenchyma cells between vascular bundles, and more xylem vessels per vascular bundle as compared to genotypes with weak pegs (Thomas et al., 1983). Peg diameter and number of vascular bundles per peg are not related to peg strength (Thomas et al., 1983; Tiwari et al., 1988).

Weak peg strength of *Arachis* species has not been documented, but other undesirable traits such as small seed size, long peg length, low yield, and trailing branch habit have been recorded (Fonceka et al., 2012; Stalker, 2017). Some of these undesirable traits have been found in early generations of introgressed materials such as TxAG-6, which had strong nematode resistance but low yield and poor pod and seed quality but can be selected against using molecular markers (Church et al., 2000; Stalker, 2017). Therefore, as the first objective of this study, peg strength in several *Arachis* species was measured. Since direct crossing between wild diploid species and cultivated peanut (allotetraploid) would result in sterile diploids, allotetraploids were made from diploid interspecific hybrids and chromosomes were doubled to bridge the crossing barrier. Allotetraploids have additional phenotypic variation due to novel interactions between the newly combined genomes, increased chromosome content leading to the gigas effect, and new allele dosages (Tal, 1980; Li et al., 1996; Ramsey and Schemske, 1998; Ni et al., 2009; Leal-Bertioli et al., 2017). Therefore, it is important to study peg strength in these allotetraploids. It is possible that ploidy level could affect peg strength since peg strength is correlated to bundle cap area and increased ploidy level was reported to be associated with larger plant tissues in *Arachis* (Leal-Bertioli et al., 2017). In this study, the peg strengths of seven *Arachis* species, four allotetraploids, six (peanut breeding line x allotetraploid) F_1 hybrids, and

two cultivated breeding lines were measured. To elucidate differences in peg strength among these materials, their peg anatomy was also documented.

Materials and Methods

Plant Materials

Wild *Arachis* species, *A. cardenasii* Krapov. and W.C. Gregory (PI 262141, 10017; abbrev.: *Card*), *A. correntina* (Burkart) Krapov. and W.C. Gregory (PI 262808, GKP 9530; abbrev.: *Cor*), *A. duranensis* Krapov. and W.C. Gregory (PI 468197, GKBSPSc 30060; abbrev.: *Dur*), *A. ipaensis* Krapov. and W.C. Gregory (PI 468322, GKBSPSc 300076; abbrev.: *Ipa*), *A. stenosperma* Krapov. and W.C. Gregory (PI 338280, 410; abbrev.: *Sten*410), *A. stenosperma* (PI 666100, V10309; abbrev.: *Sten*V10309), and *A. valida* Krapov. and W.C. Gregory (PI 468154, GK 30011; abbrev.: *Val*) were obtained from Dr. H.T. Stalker at North Carolina State University (NCSU). Two accessions of *A. stenosperma* were included because both have been used to produce allotetraploids in the UGA Tifton and Athens peanut breeding programs, and resistances to pests have been found to vary between accessions within *Arachis* species (Stalker and Campbell, 1983). *IpaDur*^{4x}, *IpaCor*^{4x}, *IpaSten*V10309^{4x}, and *ValSten*V10309^{4x} allotetraploids were created from the diploid hybrids by colchicine treatment at the University of Georgia (UGA) Tifton Campus (Table 3.1). Cultivated peanut controls included *A. hypogaea* '13-2113' and *A. hypogaea* '13-1014,' selected breeding lines from [(C1805-617-2 x 'Florida-07') x 'Georgia-06G'] in which C1805-617-2 was a selection from 'Tifguard' x 'Florida-07'. Line 13-2113 was selected with the ADSNP124 (A09 6720287) marker (Chu et al., 2016) to have an A09 *A. cardenasii* introgression that confers nematode resistance. Hybrids were made by crossing the allotetraploids to the cultivated peanut controls. These hybrids were confirmed using the Axiom_Arachis2 SNP array (ThermoFisher Scientific, Waltham, MA) (Clevenger et al., 2018;

Korani et al., 2019). DNA was extracted from newly formed leaves of the germinated plants with the Qiagen DNeasy Plant mini kit (Qiagen, Germantown, MD), and SNP calling was performed with Axiom Analysis Suite (Version 1.2). Genetic markers were grouped into six categories by the software depending on the quality and separation of markers 1) Monomorphic, 2) PolyHighResolution, 3) NoMinorHom, 4) OfftargetVariant, 5) CallRateBelowThreshold, and 6) Other. The markers in the PolyHighResolution class were used for analysis since the grouping of samples was unambiguous and all of the samples passed quality control.

In April, 2018, *A. hypogaea* x allotetraploid seeds and in May, 2018, diploid *Arachis*, allotetraploid, and cultivated control seeds were coated in Vitavax PC (Vitavax, Crompton, Middlebury, CT) and treated overnight in 0.5% Florel Growth Regulator solution (Lawn and Garden Products Inc., Fresno, CA) to break dormancy. Forty-eight hours later, seeds were planted in #123 7.62 cm round x 11.43 cm deep Jiffy pots (Harris Seeds, Rochester, NY) filled with Promix growth medium (Premier Tech Horticulture, Quakertown, PA). On 18 April, the (*A. hypogaea* x allotetraploid) plants were transplanted from the greenhouse to a 20 m by 4 m plot in the Gibbs Farm in Tifton, GA in a completely randomized design. On 14 May, the allotetraploids and cultivated peanut controls were transplanted from the greenhouse to a 10 m by 40 m plot in the UGA NESPAL field in Tifton, GA, in a randomized complete block design with seven replications. In the same field in an adjacent plot 50 m long and 8 m wide, each diploid *Arachis* species was planted as a block spaced approximately 3.7 m apart from neighboring species blocks, on 14 May. While planted in a test area adjacent to the allotetraploids and cultivated controls in a randomized complete block design, the diploid *Arachis* species were randomized separately. Standard field management was applied.

Peg Strength

Upon harvest, five pegs from plants free of tomato spotted wilt orthtospovirus were evaluated for each genotype. To minimize the effect of uneven maturity on peg strength, pods at the orange stage of maturity were selected (Anco and Thomas, 2020). For cultivated peanut and hybrids from *A. hypogaea* x allotetraploid crosses, the saddle area of each pod was scraped with a single-edge razor blade to expose the mesocarp layer and pods with orange-colored mesocarp were selected. For the allotetraploids and wild diploid species, most of the double-pods had a long isthmus connecting the two pods. Therefore, the dorsal side of the pod connected directly to the peg was scraped to determine its maturity. Stems with selected pods were excised from each plant. Pods and pegs with any physical damage were excluded. The selected pod was detached carefully from the peg by using a digital force gauge (IMADA, model DS2-11, Northbrook, IL) that was modified with special grips to hold the pod. Detachment force of these pegs was measured at the break point of detachment. Peg ends attached to both the stem and the pods were excised and preserved in FAA fixative (10% formaldehyde, 50% ethanol, 5% acetic acid, and 35% distilled water) for 48 hrs then stored in 80% ethanol prior to anatomical characterization.

Anatomical characterization

Of the five pegs per genotype, the three most intact pegs, as determined by clean excision sites and clean break point from the pods, were selected for anatomical characterization. Cross-sections were taken from both the proximal (the part of the peg connecting to the plant stem) and distal (the part of the peg connecting to the pod) positions for each peg and were then stored separately. Each cross-section was made by wedging the peg into a small incision in a block of Styrofoam to keep the peg secure and cutting freehand using a double-edged razor blade (Personna, Knoxville, TN). The cross sections were kept separately in 2 mL tubes (Phenix Research, Swedesboro, NJ) suspended in 80% ethanol until microscopic examination and

imaging. Slides were prepared by first placing two coverslips approximately 5 mm apart on the slide and then transferring the cross-sections to the gap using a 2 mL disposable transfer pipette (ThermoFisher Scientific, Waltham, MA) so that the cross-sections were suspended in 80% ethanol between the two coverslips. A third coverslip was placed on top of the two coverslips with the suspended cross-section between them. Images were taken using a Zeiss AxioCam camera on a Zeiss Axioskop 2 plus microscope (Carl Zeiss Microscopy, Thornwood, NY, USA) with transmitted light and ultraviolet light at 5x and 10x magnification. Fifty µm scale bars were added to each photo using the Zeiss AxioVision Program.

Fiber bundle cap number and tannin cell number were counted directly from the microscope images. Tannin cell count was included even though it was not expected to correlate with peg strength. Tannin cells may play an important role in defense response (Beckman, 2000), and the documentation of variation may be considered in relevant future studies. Peg area, bundle cap area, and distance between bundle caps were measured from the images with Assess 2.0 (APS Press) (Fig. 3.1). However, the images were first analyzed with Materials Image Processing and Automated Reconstruction (MIPAR™) software (Sosa et al., 2014) to find the ratio of pixels to the 50 µm scale bar in the photos. The ratio was 41 px to 50 µm for 5x magnification images and 75 px to 50 µm for 10x magnified photos; all images were 1,296 px by 1,018 px. In Assess 2.0, 5x images were calibrated by imputing the dimensions 1.58 mm x 1.24 mm, while 10x images were calibrated by imputing 0.864 mm x 0.679 mm.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using JMP software (SAS Institute, Cary, NC) to determine the genotype effect on peg strength as well as peg anatomy according to the following parameters: peg area, bundle cap area, total bundle cap area, ratio of

bundle cap area to peg area, bundle cap number, distance between bundle caps, total distance between bundle caps, and tannin cell count. ANOVA to identify genotype effect on peg anatomy was performed on proximal and distal data separately due to proximal and distal cross-sections originating from the same peg. This meant the cross-sections were not independent observations, since peg, not cross-section, was the experimental unit. For bundle cap area and distance between bundle caps, each data point was the mean for that cross-section so that the data would be balanced, because different genotypes had different numbers of bundle caps. Total bundle cap area and total distance between bundles caps were only measured for proximal peg cross sections, since distal cross sections were more degraded making it more difficult to see all the bundle caps clearly. Means of each parameter among the treatments were separated based on the Tukey's Test ($\alpha = 0.05$) results. Correlation analysis was performed with JMP software (SAS Institute) through pairwise comparisons to find Pearson product-moment correlations between peg strength and the peg anatomy parameters.

Results

Peg Strength

Genotype had a significant effect on peg strength (Table 2). The general trend in peg strength was that, numerically, cultivated breeding lines and F_1 hybrids had higher mean peg strength than the allotetraploids, or diploid *Arachis* species. A noticeable exception from this trend was *Val*, which had a mean detachment force of 3.91 N as compared to 5.30 N of 13-1014 and 4.24 N of 13-2113 (Fig. 3.2). However, *Val* had a much tighter distribution of peg strength than the cultivated controls. Unlike *Val*, four of the seven diploid *Arachis* species had significantly lower peg strength than the cultivated controls. In addition, the allotetraploids did not have peg strength significantly different than the diploids, from which they were derived.

Furthermore, two of the seven F_1 hybrids did not have significantly stronger pegs than the weakest species *Sten*V10309, and four of the seven F_1 hybrids did not have significantly stronger pegs than the next three weakest *Arachis* species, *Card*, *Dur*, and *Sten*410.

Peg and Bundle Cap Area

Genotype had a significant effect on proximal and distal peg area, proximal and distal mean bundle cap area, proximal total bundle cap area, and proximal total bundle cap area as a percentage of peg area (Table 3.2). Although both proximal and distal cross-sections had a similar trend, the proximal peg area data had more significant groups than the distal data (Fig. 3.3a, 3.3b). All the diploid *Arachis* species had significantly smaller proximal peg area than the cultivated breeding line 13-2113, except for *Val* (Fig. 3.1, 3.3). Only three of the seven *Arachis* species had significantly smaller proximal peg area compared to both cultivated breeding line controls. *Card*, the second weakest in peg strength, had the numerically smallest proximal peg area at 0.28 mm^2, which is about half the mean peg area of *Val* at 0.63 mm^2 and a third the mean peg area of 13-2113, the genotype with the largest peg area of 0.97 mm^2. None of the F_1 hybrids and allotetraploids differed significantly from the cultivated breeding lines for proximal peg area, except for *IpaDur* which was significantly smaller than 13-2113. On the contrary, except for (13-1014 x *ValSten*)_F_1 and (13-2113 x *ValSten*)_F_1, none of the F_1 hybrids and allotetraploids differed significantly from *Card*, the genotype with the smallest proximal peg area.

Bundle cap area for proximal and distal cross-sections was similar, with the cultivated controls having the largest bundle caps and some of the *Arachis* species having the smallest bundle caps, but in general, proximal cross-sections had greater mean bundle cap area than distal cross-sections (Fig. 3.4a, 3.4b). The cultivated breeding lines had the greatest proximal bundle cap areas at 3,483 and 4,203 μm^2 for 13-1014 and 13-2113, respectively (Fig. 3.4a). The range in

bundle cap area between the diploid *Arachis* species was large. *Card* had a mean bundle cap area of 1,149 μm^2, a third of the mean bundle cap area of 13-1014, while *Val* had a mean bundle cap area of 2,290 μm^2, two thirds the mean bundle cap area of 13-1014.

Trends in proximal total bundle cap area and proximal bundle cap area were the same. Numerically, the cultivated breeding lines had the largest total bundle cap area at 75,213 μm^2 for 13-2113 and 57,857 μm^2 for 13-1014 (Fig. 3.5). Only *IpaDur* and *ValSten* as well as five of the seven diploid *Arachis* species had significantly lower total bundle cap areas than the cultivated controls. *Val* and *Ipa* had the greatest total bundle cap area of the *Arachis* species at 29,102 and 24,550 μm^2, respectively. *Card*, *Cor*, and *Sten*V10309 had the smallest total bundle cap areas at 13,562, 14,186, and 14,906 μm^2, respectively, which are each about a fifth of the total bundle cap area of the cultivated control 13-2113.

Despite a significant genotype effect on the total bundle cap area as a percentage of peg area, Tukey's test did not parse out the genotypes into many distinct groupings. Only the bundle cap area as a percentage of total peg area for 13-2113 at 7.72 % and for *ValSten*, *Cor*, and *Ipa* at 4.49, 3.75, and 4.12 %, respectively, were found to be statistically different. All other genotypes were not significantly different (Fig. 3.6).

Bundle Cap Number

Genotype had a significant effect on proximal and distal bundle cap number (Table 3.2). Numerically, the general trend of bundle cap number was similar for proximal and distal cross-sections, in which the cultivated breeding lines had the greatest number of bundle caps and Cor had the smallest number of bundle caps (Fig. 3.7a, 3.7b). In general, proximal bundle cap counts were greater than distal counts. No genotypes had significantly different proximal and distal bundle cap counts except for five of the *Arachis* species (Fig. 3.7a). 13-1014 and 13-2113 had a

mean of 18.0 and 17.3 proximal bundle caps, while *Cor*, the genotype with the fewest bundle caps at 9.3, had about half of their bundle cap counts. The *Arachis* species with the most bundle caps were *Dur* and *Ipa* with a mean of 13.3 and 14.0 proximal bundle caps and 10.3 and 10.3 distal bundle caps, respectively.

Distance between bundle caps

Genotype had a significant effect on distance between bundle caps in proximal and distal sections and on total distance between bundle caps in proximal sections (Table 3.2). In general, the mean distance between bundle caps was greater for distal cross-sections than proximal cross-sections. Despite genotype being a significant indicator of proximal distance between bundle caps, Tukey's test differentiated the genotypes into just two significance groups (Fig. 3.8). *Cor* had a significantly higher mean distance between bundle caps in proximal peg sections than 13-2113, (13-2113 x *IpaCor*)_F_1, (13-2113 x *IpaDur*)_F_1, (13-1014 x *IpaSten*)_F_1, and *IpaSten*. All genotypes were the same for distance between bundle caps in distal cross-sections and total distance between bundle caps in proximal cross-sections.

Tannin Cell Count

Genotype had a significant effect on tannin cell count per cross-section for both distal and proximal cell counts; however, the trends in tannin cell count differed between distal and proximal tannin cell counts (Table 3.2). In general, distal peg cross-sections had two to three times as many tannin cells than proximal peg cross-sections (Fig. 3.9a and 3.9b). For proximal cross-sections, the cultivated breeding lines had the numerically highest tannin cell counts at 26.0 and 25.7 for 13-2113 and 13-1014, respectively; however, (13-1014 x *ValSten*)_F_1, *IpaSten*, *ValSten*, *Card*, and *Val*, were not significantly different from the cultivated controls. The four genotypes with the numerically lowest number of tannin cells were *IpaDur*, *Ipa*, *Dur*, and (13-

2113 x *IpaDur*)_F_1 with 0.5, 1.0, 2.0, and 2.0 tannin cells, respectively. For distal cross-sections, 13-2113 had the highest mean tannin cell count of 70.3. 13-1014 had a mean of 45.3 tannin cells and was only significantly greater than (13-2113 x *ValSten*) and *Sten*V10309.

Correlation of peg strength and anatomy parameters

Peg strength was correlated with approximately half of the peg anatomy parameters (Table 3.3). The highest correlations with peg strength were, proximal peg area (R = 0.80), proximal bundle cap area (R = 0.78), total proximal bundle cap area (R = 0.75), and proximal bundle cap number (R = 0.74). Peg strength was not correlated to total bundle cap area as a percentage of peg area, distal bundle cap number, proximal or distal distance between bundles, proximal total distance between bundles, or proximal or distal tannin cell count (Table 3.3). Both proximal and distal peg area were correlated with one another and proximal and distal bundle cap area, total proximal bundle cap area, and proximal and distal bundle cap count, yet neither were correlated with total proximal bundle cap area as a percentage of peg area.

Discussion

Peg strength has a direct impact on harvest efficiency since weak pegs tend to break off when the peanuts are inverted in the field. The weakness in peg strength among the wild species could compromise yield, which should be selected against while introgressing other beneficial alleles from wild species. The most important findings in this study were that genotype had a significant effect on peg strength and that while most of the allotetraploids and diploid *Arachis* species were significantly weaker than breeding line 13-1014, peg strength of F_1 hybrids were not significantly different from the cultivated breeding lines. Therefore, peg strength was recovered in the F_1 hybrids upon crossing. This suggested that weak peg strength in the wild

Arachis species may be recessive, and it will likely segregate among the progenies of interspecific breeding populations.

In *Arachis* materials, peg strength was strongly correlated with peg diameter, bundle cap area, and proximal bundle cap number. The finding that bundle cap area and bundle cap number were correlated with peg strength agreed with previous reports (Thomas et al., 1983; Tiwari et al., 1988). However, Tiwari et al. (1988) also found that peg strength was not correlated with peg diameter, but peg area–a direct function of peg diameter–was the anatomical characteristic with the greatest correlation to peg strength found in this study. This difference might be due to the inclusion of wild, *Arachis* species, which had peg areas about half that of the cultivated breeding lines. The increased variation introduced by *Arachis* species may have enabled a difference in peg area to be detected. Another explanation could be that the current study was able to capture peg area more precisely by taking a photo with the microscope and then analyzing the area with Assess 2.0 (APS Press) instead of measuring peg area through an eyepiece micrometer (Tiwari et al., 1988). In the current study, proximal peg area had the highest correlation with peg strength ($R = 0.80$), and the other peg anatomical characteristics that were correlated with peg strength were also highly correlated with proximal peg area. Therefore, only one of these peg anatomical characteristics needs to be measured to predict peg strength. Bundle cap area was the best predictor of peg strength ($R = 0.78$) in this study and was also correlated to peg strength by Tiwari et al. (1988) and Thomas et al. (1983).

The results of this study also differ from those reported by Thomas et al. (1983), who found that 2 to 4 rows of interfascicular cambium and parenchyma cells separated vascular bundles in weak pegs as compared to 3 to 5 in strong pegs. In this study, the distance between bundle caps was measured instead of counting rows of cells because it was easier and thus more

accurate to distinguish the ends of bundle caps than to distinguish the rows of cells. However, the current study did not find the distance between bundle caps to be correlated with peg strength. The difference between this study and Thomas et al. (1983) may be due to capturing this anatomical character by different methods–counting rows of cells versus measuring a distance–since the cells between bundle caps may be different sizes in different genotypes. In addition, Thomas et al. (1983) observed that strong pegs had crescent-shaped bundle caps. While this study did not seek to quantify bundle cap shape, this trend of strong pegs having crescent-shaped bundle caps was not anecdotally observed. In Fig. 3.1, the genotype with the weakest pegs, *Sten*V10309 had bundle caps that were more crescent-shaped than those of 13-2113, the cultivated breeding line with the second strongest pegs.

An unexpected but important result to emphasize is that *Val* had a peg strength of 3.9 N that was comparable to the cultivated breeding lines' peg strengths of 5.3 and 4.2 N. While in general, peg strength of the *Arachis* species was low, the relatively high peg strength of *Val* suggests that some *Arachis* species may have beneficial alleles for peg strength that could be used for cultivar improvement. A peg strength of 3.9 N is low as compared to peanut cultivars (Thomas et al., 1983), but there may be wild *Arachis* alleles that increase peg strength in cultivars when in the right genetic background. This claim is encouraged by the finding of Fonceka et al. (2012) that identified wild *Arachis* alleles that increased seed size in a BC_2F_1 population, despite wild *Arachis* species having small seeds.

The mean peg strengths of the allotetraploids were similar to those of the wild *Arachis* species, and three of the four allotetraploids were significantly weaker than the breeding line 13-1014. In addition, all of the allotetraploids had statistically similar peg strengths to the weakest *Arachis* species, *Sten*V10309. It was hypothesized that the peg strength of the allotetraploids

would be greater than the wild, *Arachis* species if peg strength was partly due to increased peg area. It was also thought that the allotetraploids would have larger bundle caps than the diploid *Arachis* species because of the gigas effect, in which increased chromosome content leads to larger cell size and larger plant tissues. Leal-Bertioli et al. (2017) compared the morphological characteristics of multiple allotetraploids with wild *Arachis* species and cultivated peanut and found that allotetraploids had increased plant size and higher photosynthetic capacity but not increased seed size or mass. Peg strength may be an additional characteristic to seed size and mass that allotetraploids do not gain due to increased ploidy level. Improved peg strength in peanut cultivars is likely due to selection for alleles that increase peg strength. Also, while the allotetraploids had numerically larger proximal peg areas than the *Arachis* species (except for *Val*) this difference was not found to be significant ($P>0.05$). This suggested that increased ploidy level may not increase peg area or peg strength.

A limitation of this study was small sample size, which was caused by few pods remaining attached to plants of the *Arachis* species. Although there were more than 10 plants per genotype, pegs tend to break during harvest leaving mature pods and seeds under the soil resulting in few pods attached to the plant. Coupled with the naturally indeterminate maturity of peanut, the weak peg strength resulted in a great difficulty in finding pods at the right maturity. A larger sample size would have likely further distinguished the peg strength and peg anatomical characteristics of the different breeding materials. Despite this limitation, significant differences in peg strength and anatomy were detected with sufficient clarity to correlate peg strength and peg anatomy parameters and to show that peg strength comparable to cultivated peanut is recovered in the F_1 hybrids. This finding should encourage further use of *Arachis* species as donors of disease resistance in peanut breeding programs.

Conclusions

Cultivated breeding lines had higher peg strength than allotetraploids and *Arachis* species in general. Peg strength was positively and highly correlated with proximal and distal peg area, proximal and distal mean bundle cap area, proximal total bundle cap area, and proximal bundle cap number. Since peg strength comparable to the peanut breeding lines was recovered in the F_1 hybrids, this suggests that few backcrosses may be needed to select against weak peg strength. Furthermore, peanut breeders will indirectly select against weak peg strength as they select for elite yield potential in their breeding programs, so special attention to weak peg strength is unnecessary. In addition, there may be positive alleles for peg strength in some wild, *Arachis* species such as *Val*, that could be introgressed into cultivated peanut and could result in new yield improvements. Peanut breeders should be encouraged that the weak peg strength of *Arachis* species should not hinder the introgression of beneficial alleles from wild, *Arachis* species into peanut cultivars.

Acknowledgments

This work was supported by the National Science Foundation (grant # MCB-1543922) and by the AFRI NIFA Fellowships Grant Program: Predoctoral Fellowships project accession no. 1019105 from the USDA National Institute of Food and Agriculture. The authors would like to express their appreciation to Dr. Dongying Gao of the Center for Applied Genetic Technologies, University of Georgia, Athens, Georgia, for producing and sharing the *ValSten* diploid hybrid cutting and to Dr. Jason Wallace of the Department of Crop and Soil Sciences, University of Georgia, Georgia, for assisting in the approach to data analysis.

Table 3.1. Genetic materials tested and their abbreviations and ploidy level.

Genotype	Abbreviation	Ploidy
A. hypogaea 13-2113	13-2113	Tetraploid
A. hypogaea 13-1014	13-1014	Tetraploid
(13-2113 x *IpaCor*^{4x}_S_0)_F_1	(13-2113 x *IpaCor*)_F_1	Tetraploid
(13-2113 x *IpaDur*^{4x}_S_0)_F_1	(13-2113 x *IpaDur*)_F_1	Tetraploid
(13-1014 x *IpaDur*^{4x}_S_0)_F_1	(13-1014 x *IpaDur*)_F_1	Tetraploid
(13-1014 x *IpaSten*V10309^{4x}_S_0)_F_1	(13-1014 x *IpaSten*)_F_1	Tetraploid
(13-2113 x *ValSten*V10309^{4x}_S_0)_F_1	(13-2113 x *ValSten*)_F_1	Tetraploid
(13-1014 x *ValSten*V10309^{4x}_S_0)_F_1	(13-1014 x *ValSten*)_F_1	Tetraploid
IpaCor^{4x}_$S_{0:2}$	*IpaCor*	Tetraploid
IpaDur^{4x}_S_0_S_1	*IpaDur*	Tetraploid
*IpaSten*V10309^{4x}_S_0_S_1	*IpaSten*	Tetraploid
*ValSten*V10309^{4x}_S_0_S_1	*ValSten*	Tetraploid
A. cardenasii 10017	*Card*	Diploid
A. correntina 9530	*Cor*	Diploid
A. duranensis 30060	*Dur*	Diploid
A. ipaensis 30076	*Ipa*	Diploid
A. stenosperma 410	*Sten*410	Diploid
A. stenosperma V10309	*Sten*V10309	Diploid
A. valida (PI 468154)	*Val*	Diploid

Table 3.2. ANOVA output testing the genotype effect on *Arachis* peg strength as well as peg anatomical characteristics assessed using the following parameters: peg area, bundle cap area, total bundle cap area, ratio of bundle cap area to peg area, bundle cap number, distance between bundle caps, and tannin cell count.

Parameter	*F* value	Df(n), df(d)	P-value
Peg strength	6.06	18, 76	< 0.0001***[a]
Peg area: Proximal	4.91	18, 38	< 0.0001***
Peg area: Distal	3.58	18, 38	0.0005***
Bundle cap area: Proximal	4.03	18, 36	0.0002***
Bundle cap area: Distal	24.14	18, 29	< 0.0001***
Total bundle cap area: Proximal	7.81	18, 34	< 0.0001***
Bundle cap area as a percentage of peg area: Proximal	3.12	18, 34	0.0021**
Bundle cap number: Proximal	8.93	18, 34	< 0.0001***
Bundle cap number: Distal	5.38	18, 37	< 0.0001***
Distance between bundle caps: Proximal	3.14	18, 36	0.0017**
Distance between bundle caps: Distal	2.18	18, 27	0.033*
Total distance between bundle caps: Proximal	2.11	18, 28	0.037*
Tannin cell count: Proximal	2.68	18, 33	0.0069**
Tannin cell count: Distal	6.89	18, 31	< 0.0001***

[a]*P < .05. **P < .01. ***P < .001

Table 3.3. Pairwise comparisons of peg strength and peg anatomy parameters. Only significant correlations are shown.

	Peg Strength	PA[b]: Proximal	PA: Distal	BCA: Proximal	BCA: Distal	TBCA: Proximal	BCA%: Proximal	BC: Proximal	BC: Distal	DBC: Proximal	DBC: Distal	TDBC: Proximal	TC: Proximal
PA: Proximal	0.80***	-											
PA: Distal	0.70***	0.78***	-										
BCA: Proximal	0.78***	0.89***	0.69**	-									
BCA: Distal	0.66**	0.7***	0.60**	0.79***	-								
TBCA: Proximal	0.75***	0.88***	0.75***	0.97***	0.76***	-							
BCA%: Proximal				0.64**	0.51*	0.68**	-						
BC: Proximal	0.74***	0.67**	0.81***	0.62**	0.64**	0.71***	0.5*	-					
BC: Distal		0.47*	0.65**	0.51*	0.50*	0.65**	0.62**	0.74***	-				
DBC: Proximal							-0.67**	-0.53*	-0.74***	-			
DBC: Distal											-		
TDBC: Proximal										0.49*	0.49*	-	
TC: Proximal		0.53*		0.68**	0.72***	0.68**	0.57*						-
TC: Distal				0.68**	0.58**	0.75***	0.67**	0.48*	0.56*	-0.48*			0.57*

[a]*P < .05. **P < .01. ***P < .001

[b]Peg area abbreviated as PA, bundle cap area as BCA, total bundle cap area as TBCA, total bundle cap as a percentage of peg area BCA%, bundle cap number as BC, distance between bundle caps as DBC, total distance between bundle caps TDBC, and tannin cell count as TC.

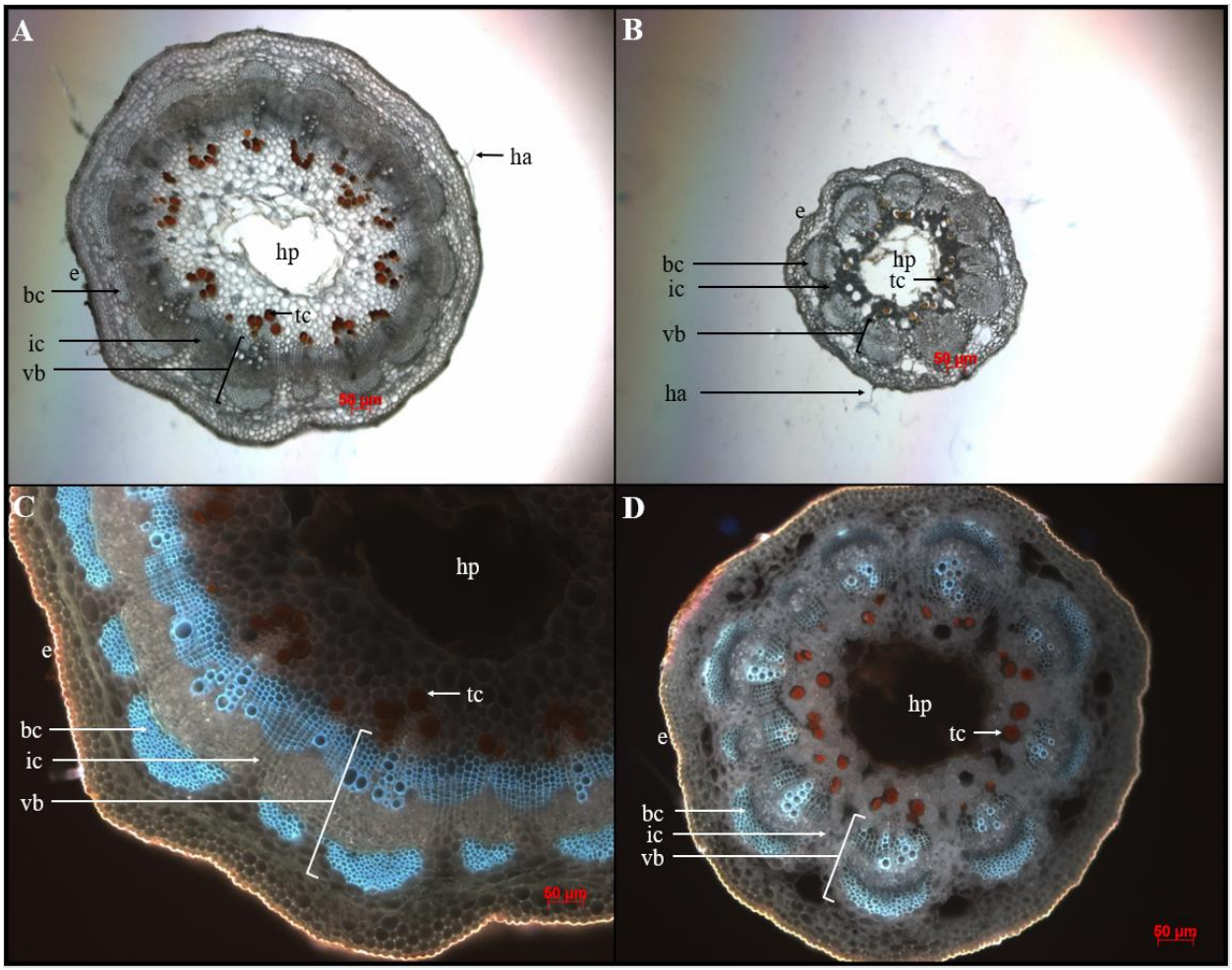


Figure 3.1. *Arachis* peg cross sections illustrating the anatomical features of a strong (left) and weak (right) peg. A) 13-2113 proximal peg cross section at 5x magnification; B) *Sten*V10309 proximal peg cross section at 5x magnification; C) 13-2113 proximal peg cross section at 10x under UV light; D) *Sten*V10309 proximal peg cross section at 10x under UV light, bc = bundle cap; e = epidermis; ha = epidermal hair; hp = hollow pith; ic = interfascicular cambium; tc = tannin cell; vb = vascular bundle. All scale bars are 50 µm.

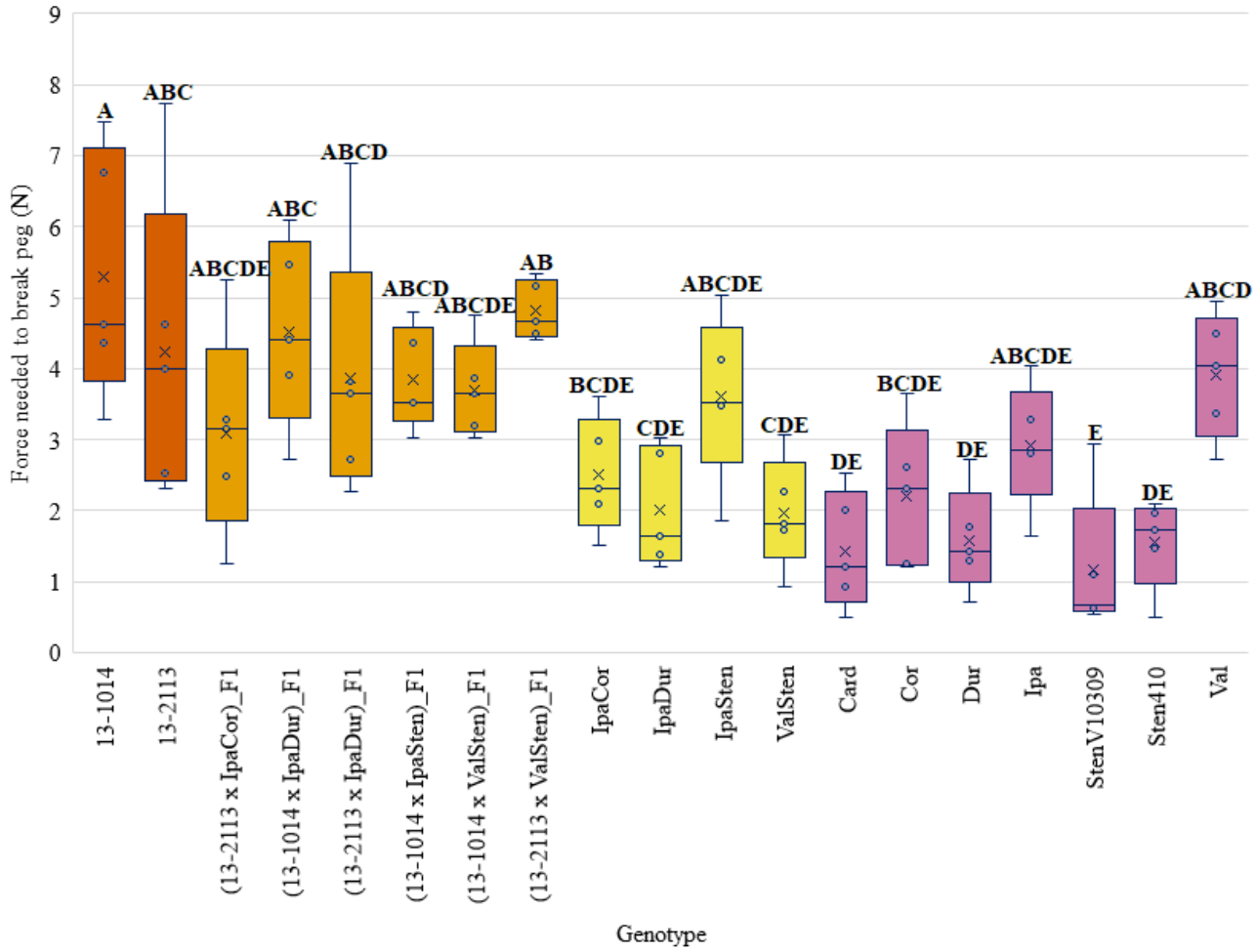
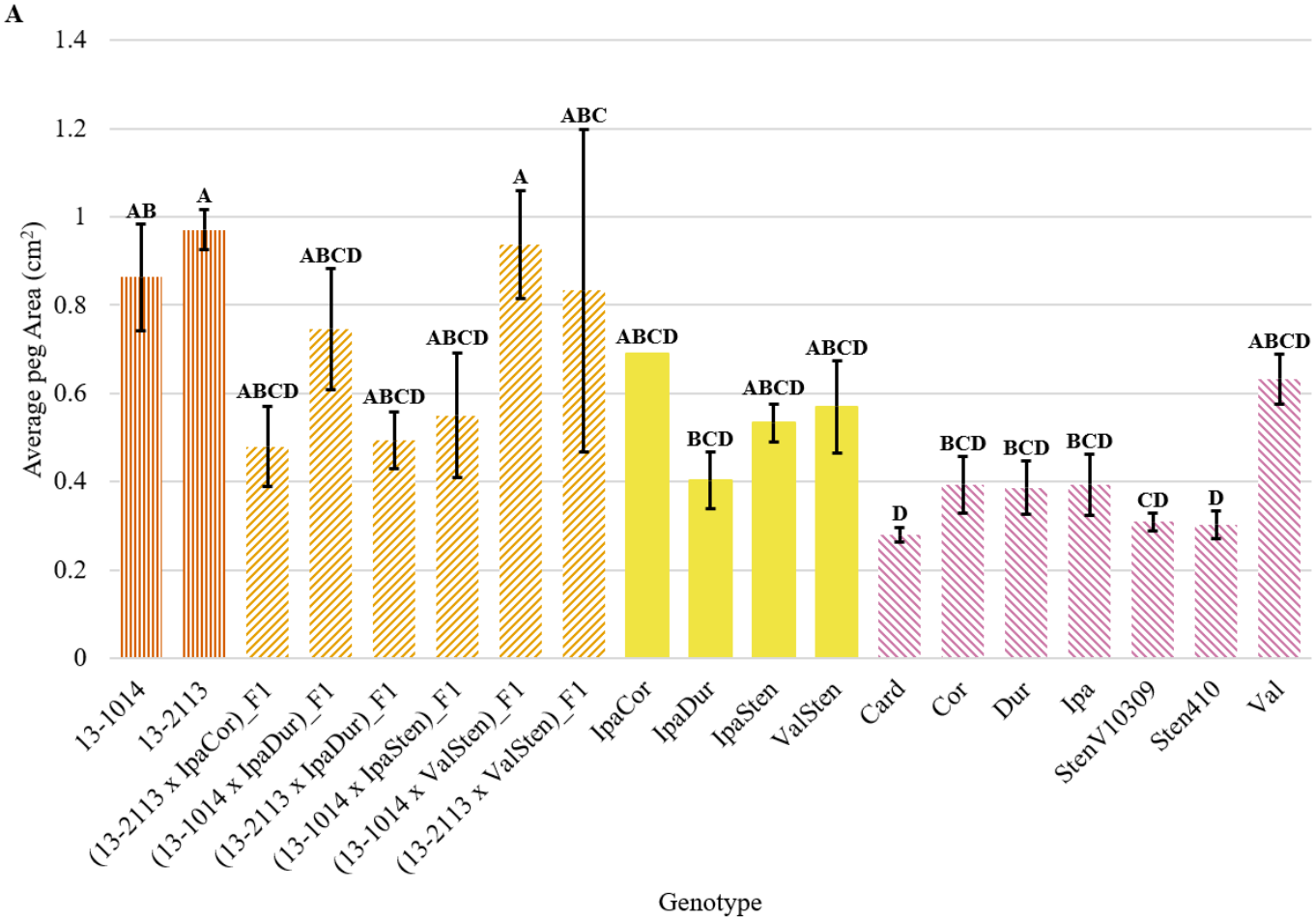


Figure 3.2. Peg strength of cultivated breeding lines (dark orange), (breeding line x allotetraploid) hybrids (light orange), allotetraploids (yellow), and wild *Arachis* diploids (pink).

A


1.4
1.2
1
0.8
0.6
0.4
0.2
0
Average peg Area (cm²)
AB
A
ABCD
ABCD
ABCD
ABCD
A
ABC
ABCD
BCD
ABCD
ABCD
D
BCD
BCD
BCD
CD
D
ABCD
13-1014
13-2113
(13-2113 x IpaCor)_F1
(13-1014 x IpaDur)_F1
(13-2113 x IpaDur)_F1
(13-1014 x IpaSten)_F1
(13-1014 x ValSten)_F1
(13-2113 x ValSten)_F1
IpaCor
IpaDur
IpaSten
ValSten
Card
Cor
Dur
Ipa
StenV10309
Sten410
Val
Genotype

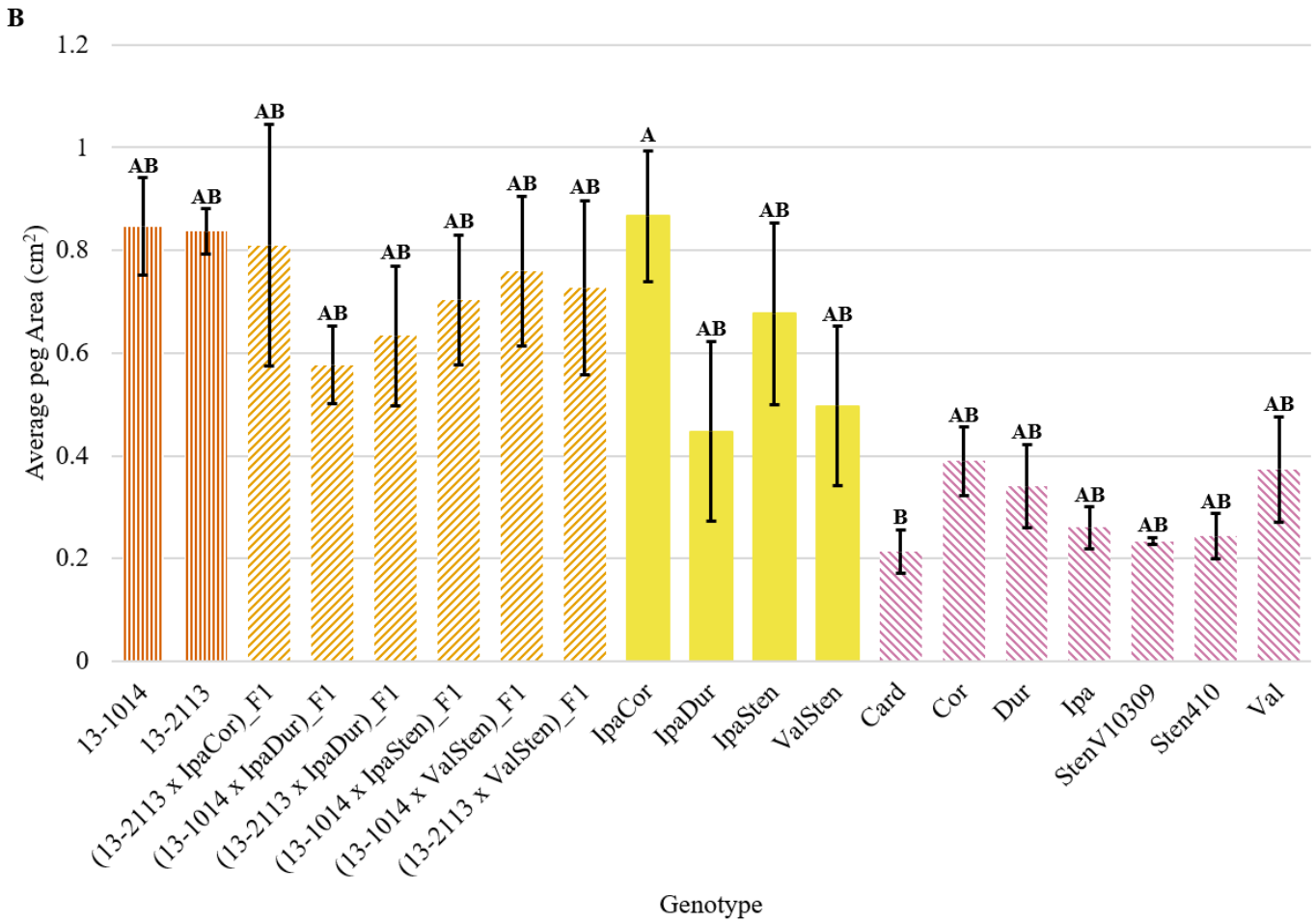
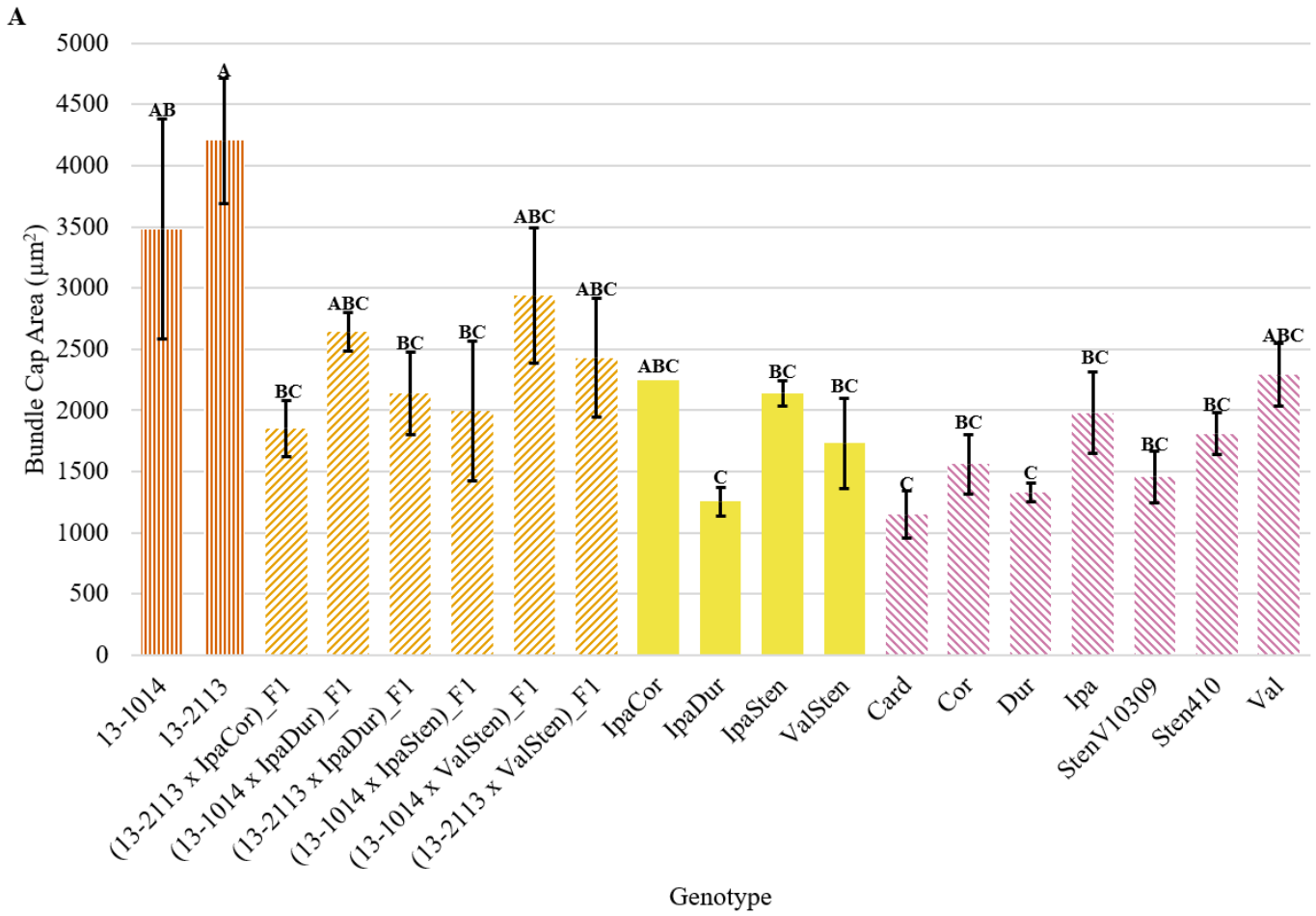


Figure 3.3. Peg area of cultivated breeding lines (dark orange, vertical stripes), (breeding line x allotetraploid) hybrids (light orange, right diagonal stripes), allotetraploids (solid yellow), and wild *Arachis* diploids (pink, left diagonal stipes). Error bars represent standard error. (A) Proximal. (B) Distal.


A
Bundle Cap Area (µm²)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
AB
A
BC
ABC
BC
BC
ABC
ABC
ABC
C
BC
BC
C
BC
C
BC
BC
ABC
13-1014
13-2113
(13-2113 x IpaCor)_F1
(13-1014 x IpaDur)_F1
(13-2113 x IpaDur)_F1
(13-1014 x IpaSten)_F1
(13-1014 x ValSten)_F1
(13-2113 x ValSten)_F1
IpaCor
IpaDur
IpaSten
ValSten
Card
Cor
Dur
Ipa
StenV10309
Sten410
Val
Genotype

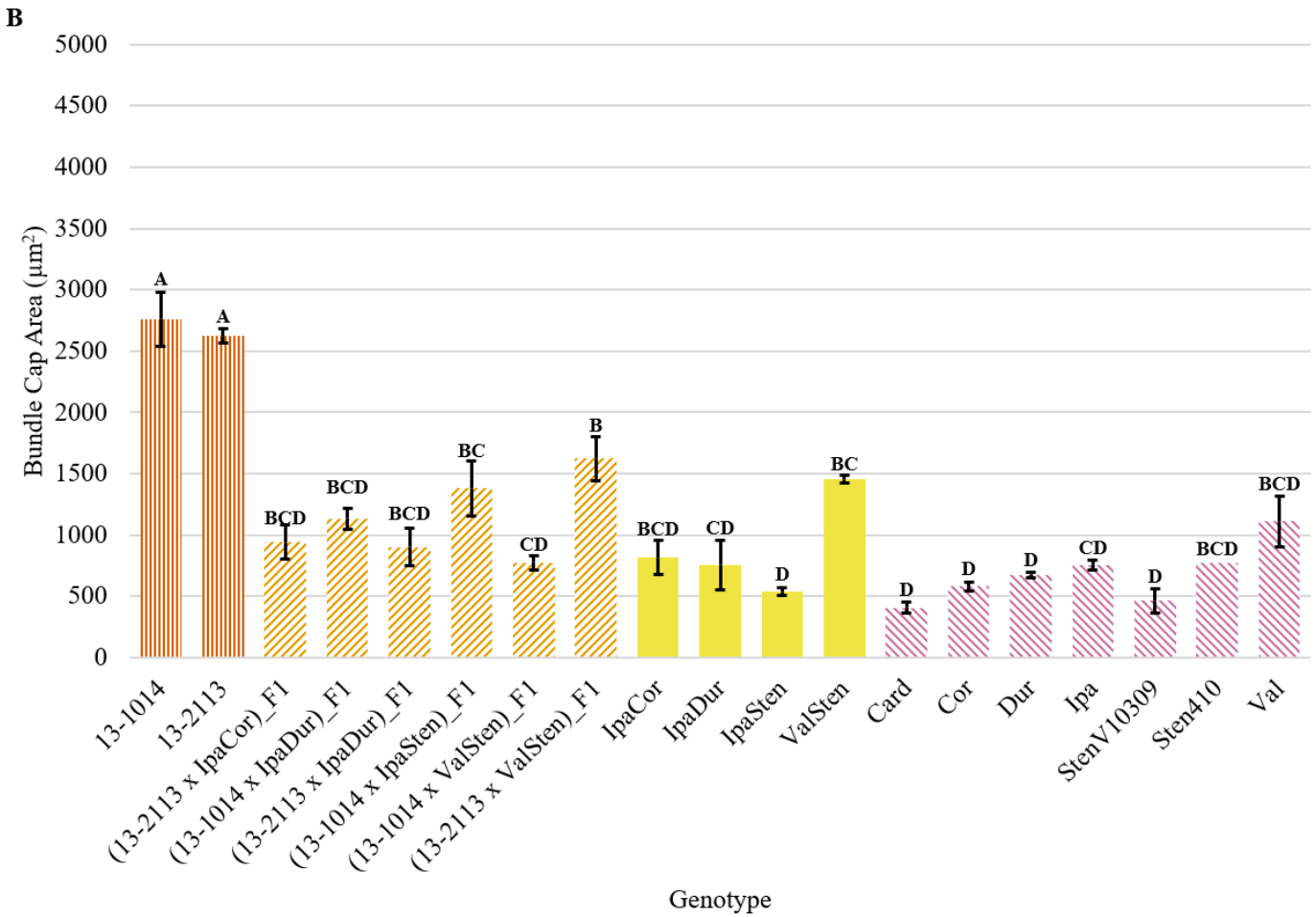


Figure 3.4. Bundle cap area of cultivated breeding lines (dark orange, vertical stripes), (breeding line x allotetraploid) hybrids (light orange, right diagonal stripes), allotetraploids (solid yellow), and wild *Arachis* diploids (pink, left diagonal stipes). Error bars represent standard error. (A) Proximal. (B) Distal.

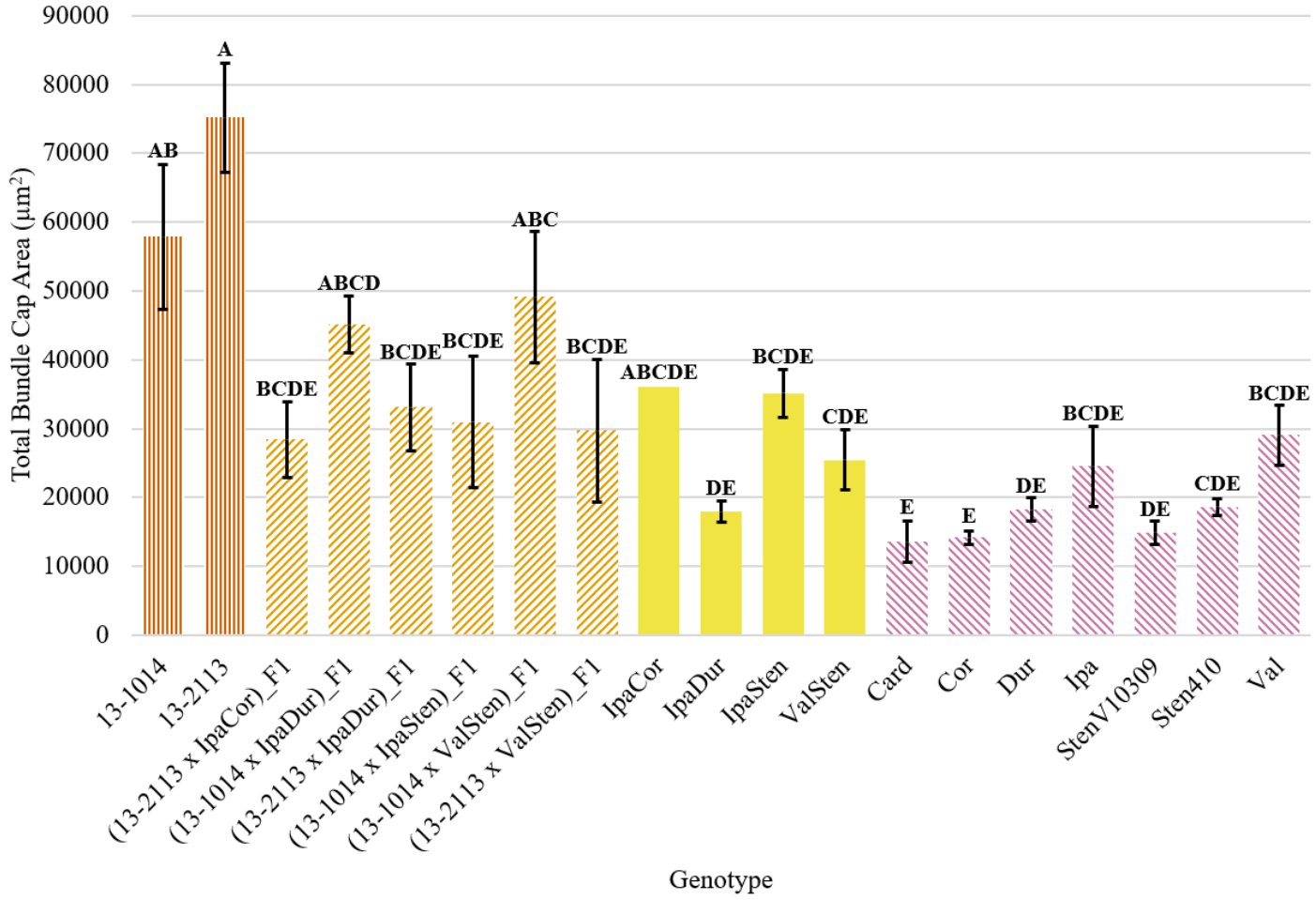


Figure 3.5. Total bundle cap area of proximal peg cross-sections of cultivated breeding lines (dark orange, vertical stripes), (breeding line x allotetraploid) hybrids (light orange, right diagonal stripes), allotetraploids (solid yellow), and wild *Arachis* diploids (pink, left diagonal stipes). Error bars represent standard error.

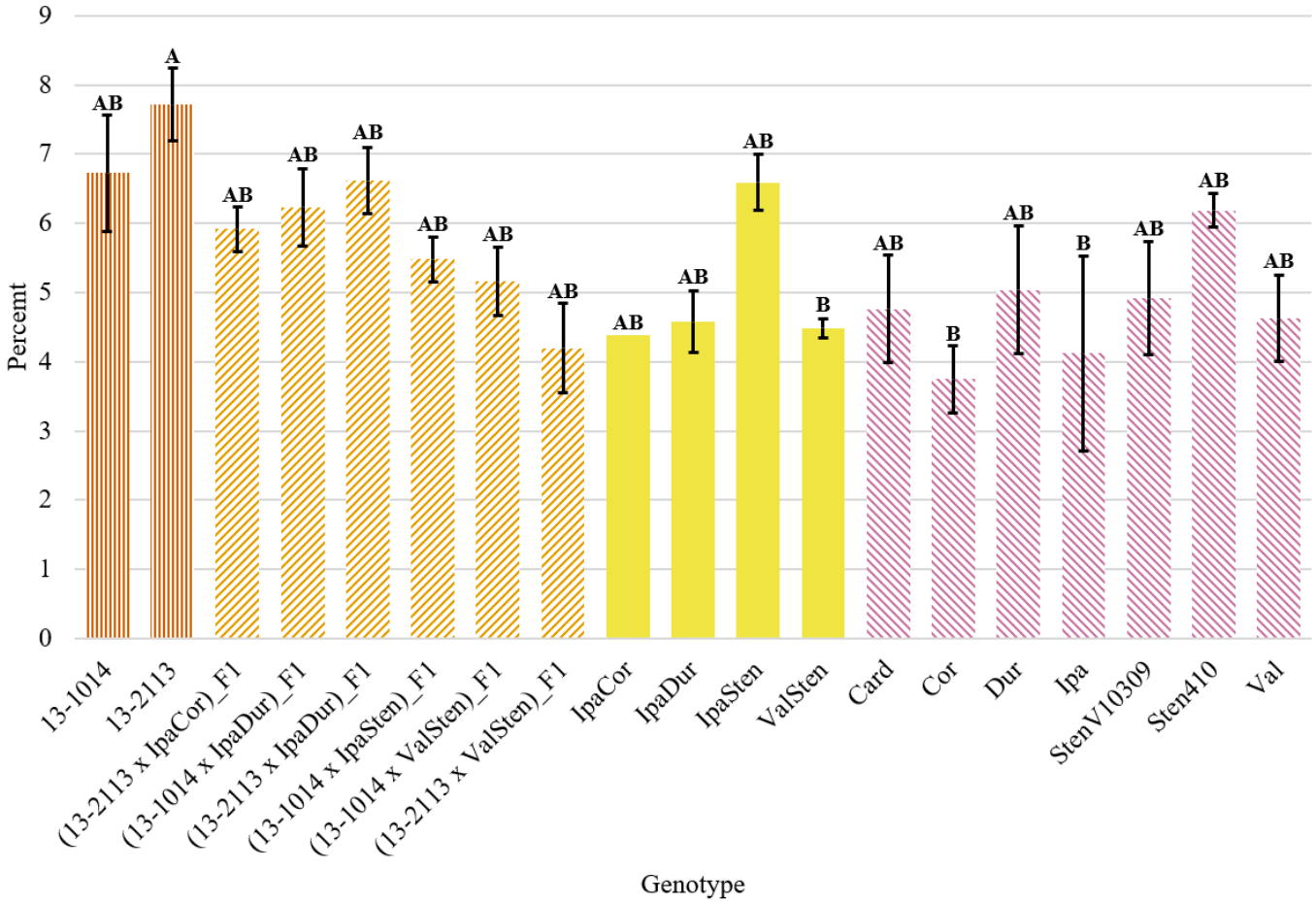
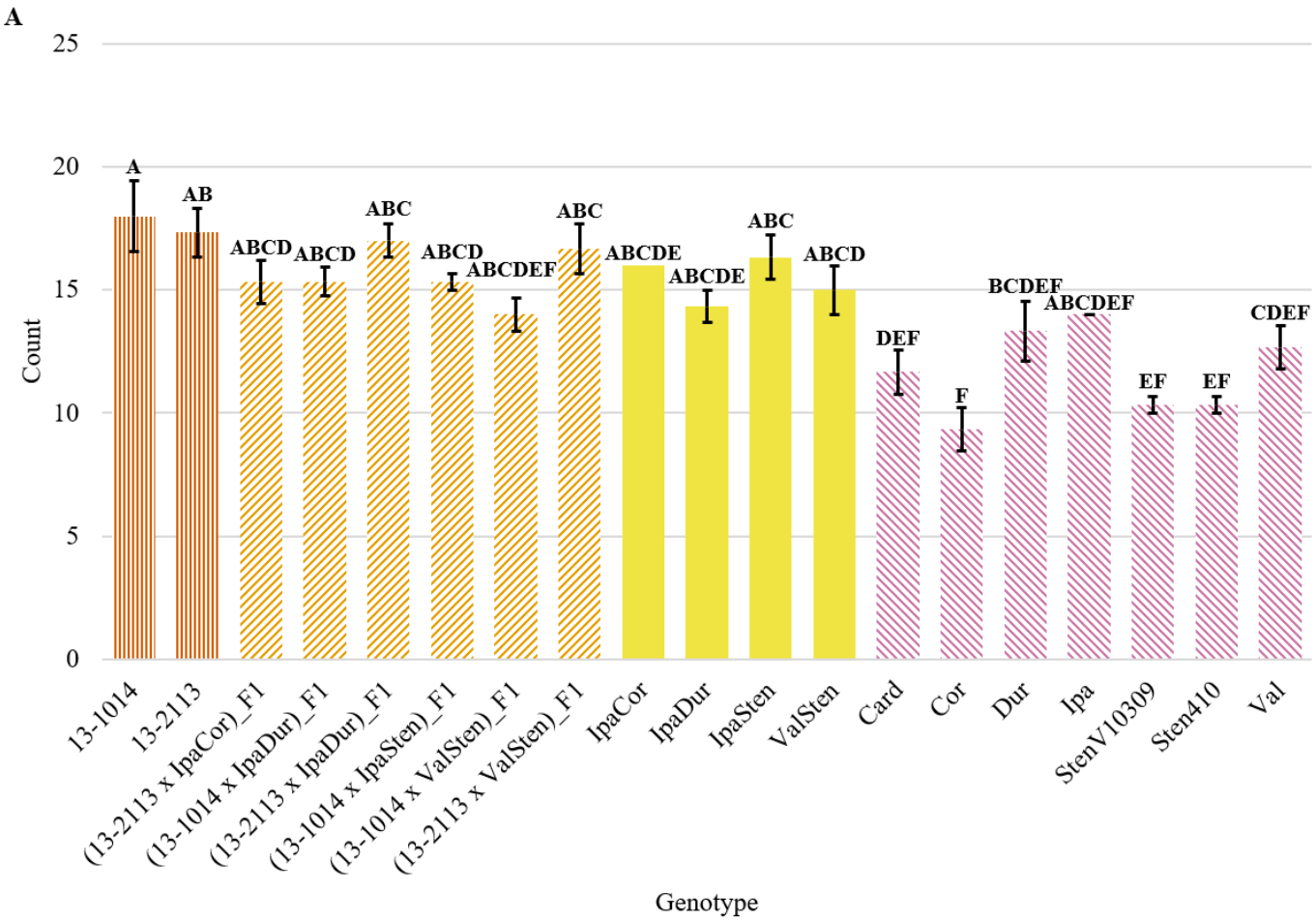


Figure 3.6. Total bundle cap area as a percentage of peg area for proximal peg cross-sections of cultivated breeding lines (dark orange, vertical stripes), (breeding line x allotetraploid) hybrids (light orange, right diagonal stripes), allotetraploids (solid yellow), and wild *Arachis* diploids (pink, left diagonal stipes). Error bars represent standard error.

A


25
20
15
10
5
0
Count
A
AB
ABCD
ABCD
ABC
ABCD
ABCDEF
ABC
ABCDE
ABCDE
ABC
ABCD
DEF
F
BCDEF
ABCDEF
EF
EF
CDEF
13-1014
13-2113
(13-2113 x IpaCor)_F1
(13-1014 x IpaDur)_F1
(13-2113 x IpaDur)_F1
(13-1014 x IpaSten)_F1
(13-1014 x ValSten)_F1
(13-2113 x ValSten)_F1
IpaCor
IpaDur
IpaSten
ValSten
Card
Cor
Dur
Ipa
StenV10309
Sten410
Val
Genotype

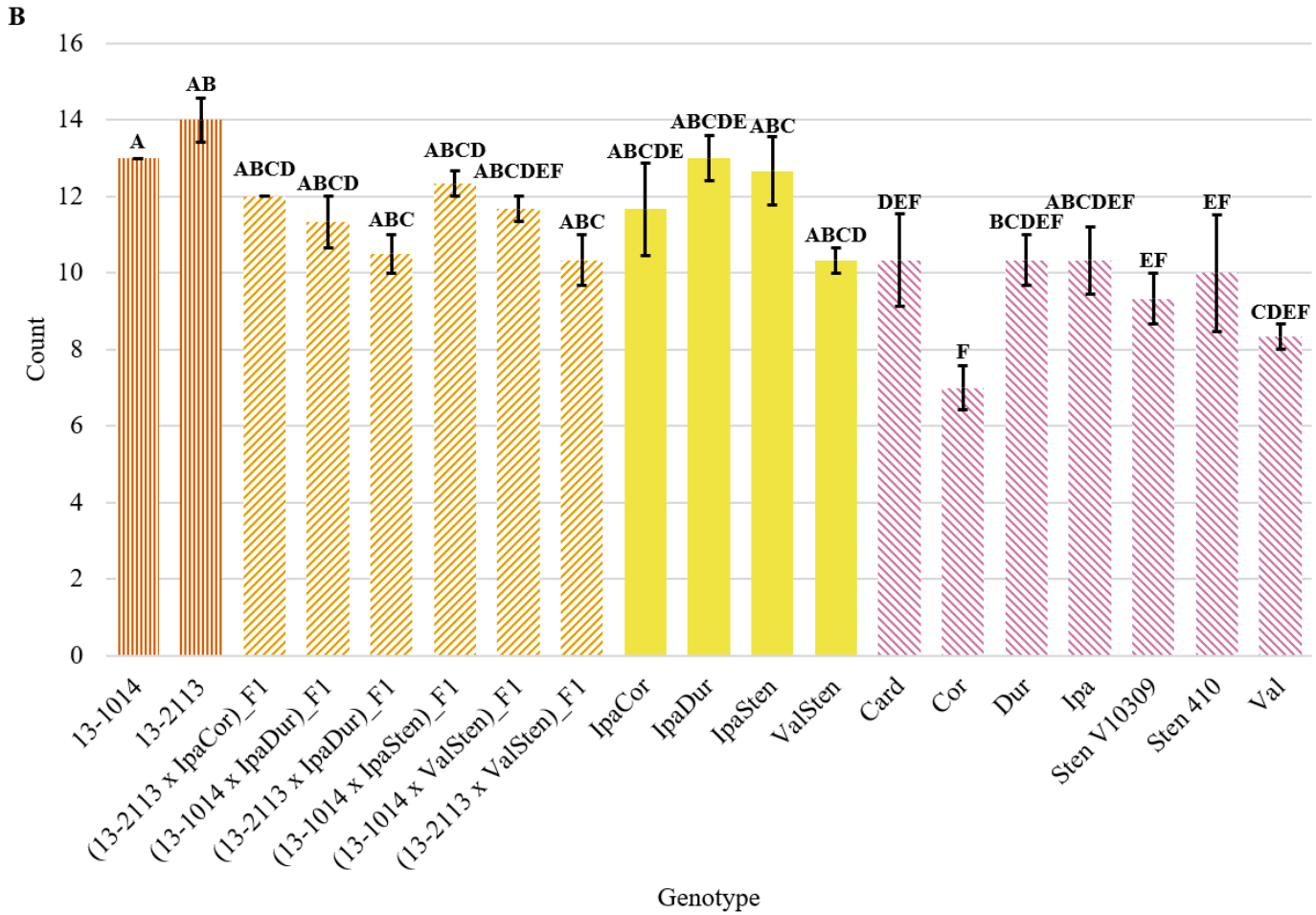


Figure 3.7. Bundle cap count of cultivated breeding lines (dark orange, vertical stripes), (breeding line x allotetraploid) hybrids (light orange, right diagonal stripes), allotetraploids (solid yellow), and wild *Arachis* diploids (pink, left diagonal stipes). Error bars represent standard error. (A) Proximal. (B) Distal.

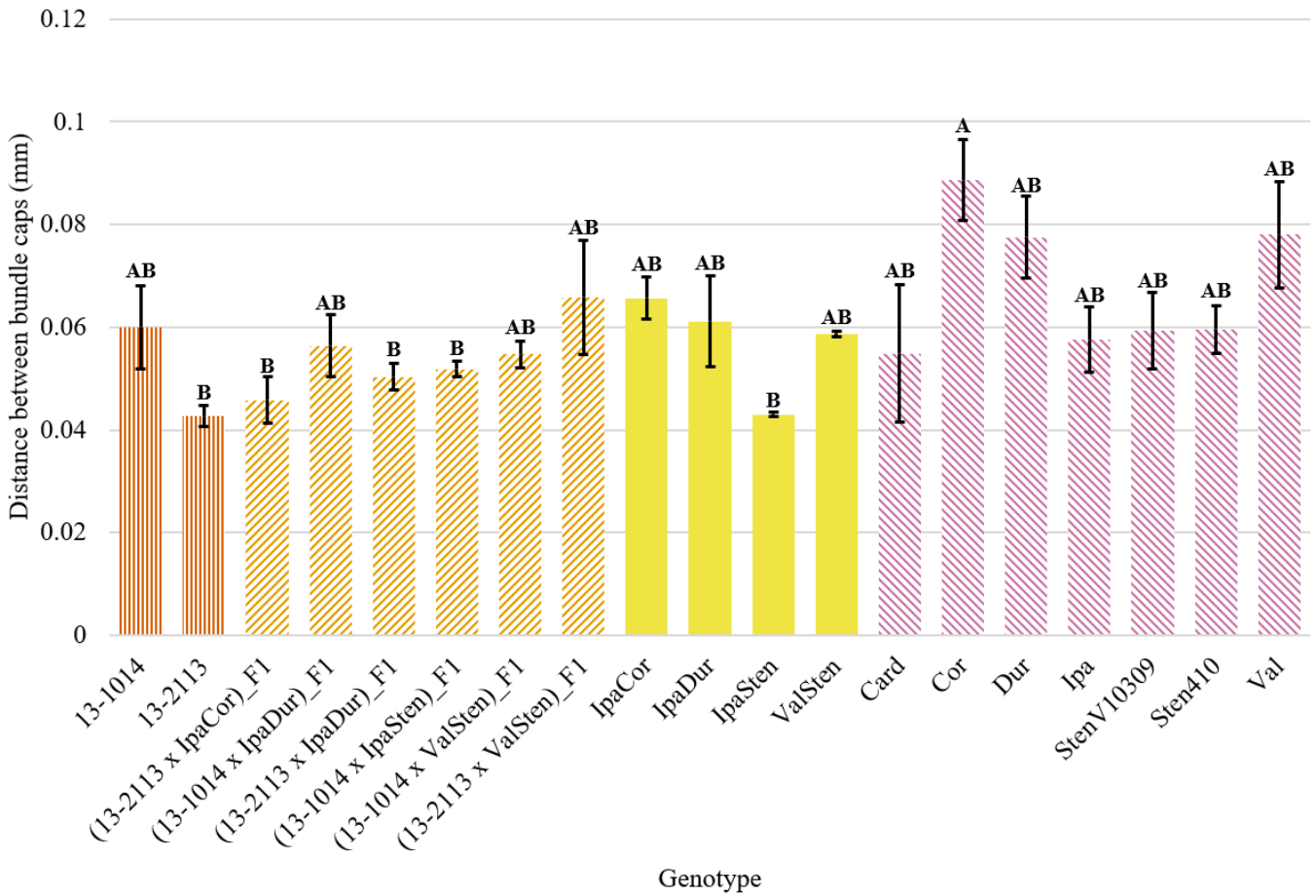
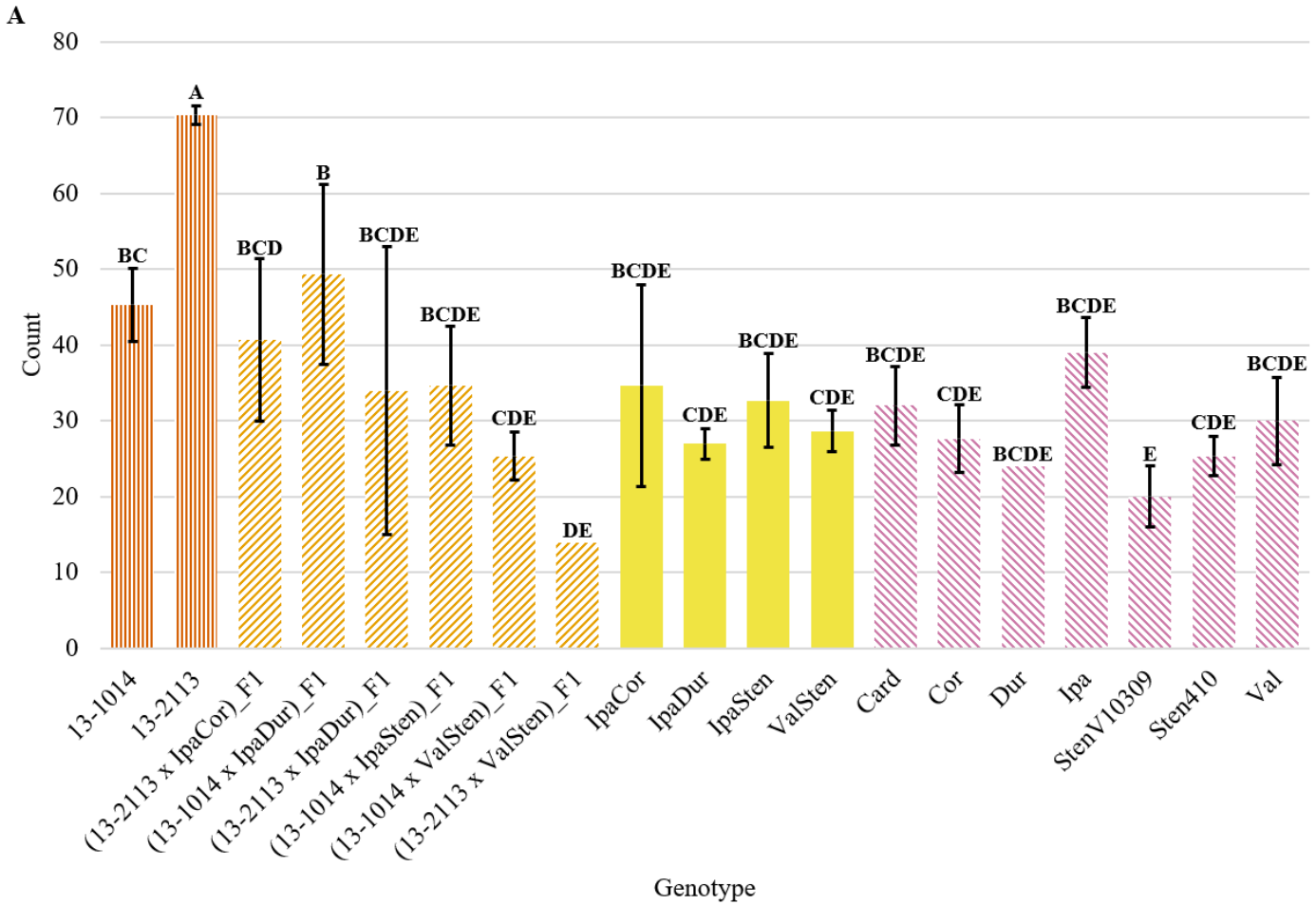


Figure 3.8. Distance between bundle caps of proximal peg cross-sections of cultivated breeding lines (dark orange, vertical stripes), (breeding line x allotetraploid) hybrids (light orange, right diagonal stripes), allotetraploids (solid yellow), and wild *Arachis* diploids (pink, left diagonal stipes). Error bars represent standard error.


A
80
70
60
50
40
30
20
10
0
Count
A
B
BC
BCD
BCDE
BCDE
CDE
DE
BCDE
CDE
BCDE
CDE
BCDE
CDE
BCDE
E
CDE
BCDE
13-1014
13-2113
(13-2113 x IpaCor)_F1
(13-1014 x IpaDur)_F1
(13-2113 x IpaDur)_F1
(13-1014 x IpaSten)_F1
(13-1014 x ValSten)_F1
(13-2113 x ValSten)_F1
IpaCor
IpaDur
IpaSten
ValSten
Card
Cor
Dur
Ipa
StenV10309
Sten410
Val
Genotype

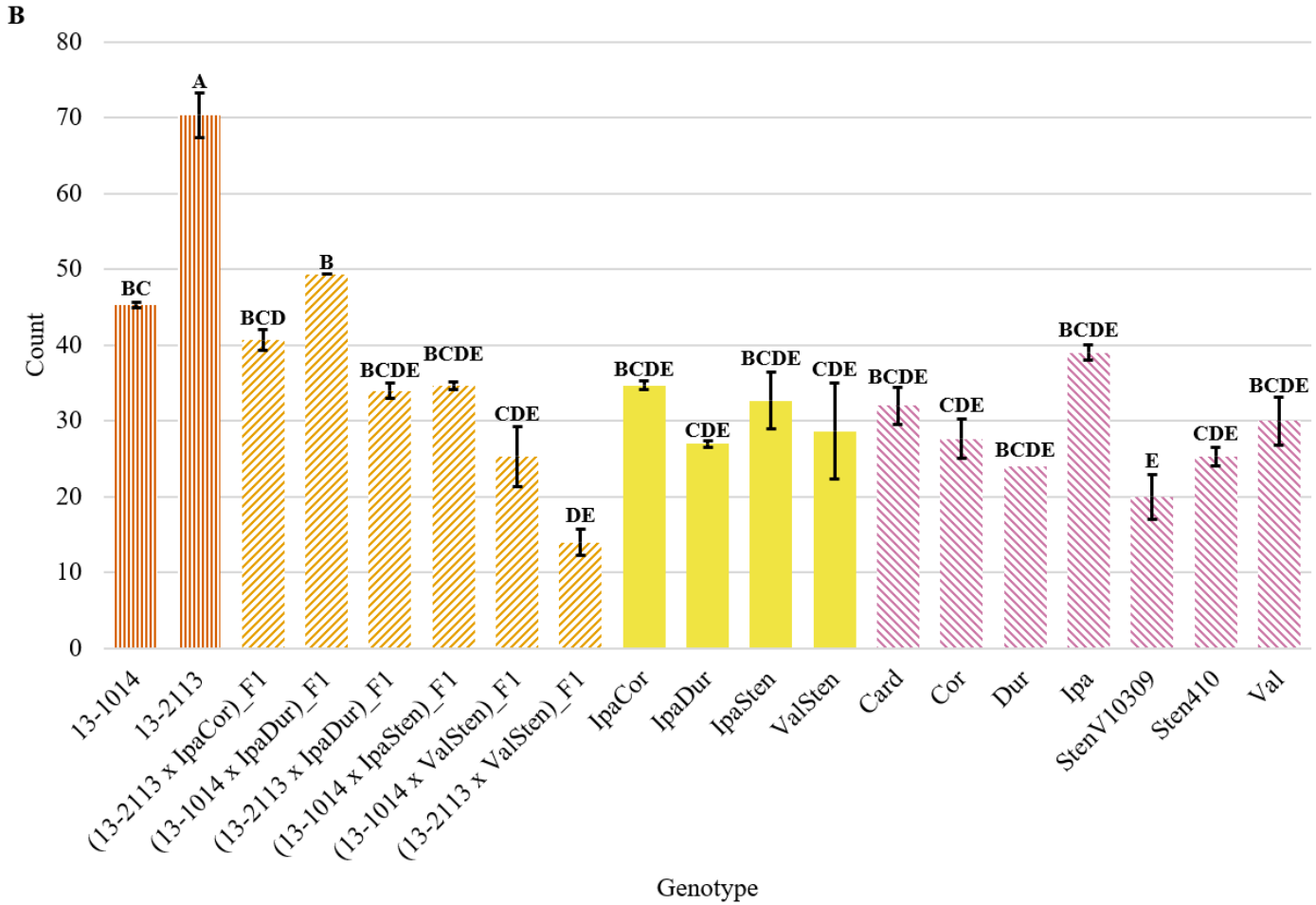


Figure 3.9. Tannin cell count per cross-section of cultivated breeding lines (dark orange, vertical stripes), (breeding line x allotetraploid) hybrids (light orange, right diagonal stripes), allotetraploids (solid yellow), and wild *Arachis* diploids (pink, left diagonal stipes). Error bars represent standard error. (A) Proximal. (B) Distal.

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CHAPTER 4

DIVERSITY OF ULTRAVIOLET REFLECTION PATTERN IN *ARACHIS* FLOWERS

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Abstract

A large portion of global agriculture is dependent on insect pollination, especially by bees. Yields of insect-pollinated crops are often managed by the addition of honeybees (*Apis mellifera*) to increase pollination, yet wild bee species (*Bombus* spp.) also increase pollination and fruit set for many globally important crops. Plant biodiversity within and surrounding crop fields can attract wild bee species and increase pollination in those fields. Peanut is an important global food crop known to attract honeybees and wild bee pollinators, and current breeding efforts include introgression of desirable alleles from wild *Arachis* species into cultivated peanut. Some wild *Arachis* species derived breeding materials have been documented to have early flowering, high flowering throughout the growing season, and large flowers as compared to cultivated peanut, which can increase benefits for bees. However, the presence and diversity of UV nectar guides to aid bees in detection of flowers and orientation after landing have not been studied in *Arachis*. This study sought to document the presence and diversity of nectar guides in two peanut cultivars, two peanut breeding lines, 8 wild *Arachis* species, 19 unique allotetraploids, and four BC_1F_3 lines. All *Arachis* genotypes studied had UV nectar guides and genotype was a significant indicator of flower size and nectar guide size according to the following parameters: banner area, area of UV absorption on the banner (abaxial and adaxial), area of UV absorption on the banner as a percentage of total banner area (abaxial and adaxial), left wing area, area of UV absorption on the left wing (abaxial and adaxial), and area of UV absorption on the left wing as a percentage of total left wing area (abaxial and adaxial). Further studies are needed to evaluate the use of ornamental *Arachis* species and floriferous peanut

cultivars for promoting wild bee abundance and diversity in urban landscapes and for use as cost-effective border crops for pollinator recruitment.

Introduction

Successful reproduction and fruit set of most flowering plants, including valuable food crops, are dependent on pollination, especially bee pollination, and pollinating insects can increase fruit or seed quality or quantity of most major crops (Klein et al., 2007). Yields of insect-pollinated crops are often managed by bringing beehives of honeybees (*Apis mellifera*) to the fields to increase pollination. However, wild bee species (*Bombus* spp.) are also vital to our cropping systems, and for over 40 globally important crops, wild pollinators improved pollination efficiency and increased fruit set compared to honeybees alone (Garibaldi et al., 2013). Plant biodiversity within and surrounding crop fields provide habitats for wild bee species (*Bombus* spp.) and can increase pollination in agroecosystems (Garibaldi et al., 2013; Nicholls and Altieri, 2013; Blaauw and Isaacs, 2014; Tonietto et al., 2016). Better understanding pollination cues of major crops could help inform future decisions regarding increasing wild pollinator abundance and diversity.

Flowers have adapted diverse advertising strategies to attract bees, including flower size, shape, scent, color, and pattern, which often correspond to morphological and sensory characteristics of pollinators. Bees can associate visual cues such as flower color and shape with nectar and pollen rewards and will use these cues to optimize foraging times (Spaethe et al., 2001). Bees are sensitive to ultraviolet (UV), blue, and green wavelengths, and their detection of large flowers (about 15 mm in diameter or larger) is dependent on high contrast between flower and green foliage background color (Spaethe et al., 2001). For detection of small flowers (around 8 mm in diameter or less), bees (*Bombus* spp.) fly closer to the ground and use a different

neuronal channel focused on green contrast only. For example, Spaethe et al. (2001) found that bees took 22% longer to detect large, UV-absorbing white flowers than large, yellow flowers but 72% longer to identify small, yellow flowers than small, UV-absorbing white flowers due to using color contrast to identify large flowers and green contrast to identify small flowers. While flower detection of small and large flowers both involve color contrast, honeybees and wild bee species appear to ignore flower brightness during foraging (Vorobyev and Brandt, 1997; Spaethe et al., 2001).

While flower color is vital in attracting pollinators from a distance, flower color and UV patterns are important to orientation and landing (Papiorek et al., 2015). Many plants have adapted to have UV absorbance and reflectance flower patterns that act as nectar guides for bees that direct pollinators to the nectar and pollen to increase pollination success (Orbán and Plowright, 2013). In radially symmetric flowers with nectar guides, generally apical parts of flower petals have UV reflecting carotenoids and the central flower parts have UV absorbing flavonoids creating a "bullseye" pattern (Thompson et al., 1972; Harborne and Smith, 1978). In addition to UV absorbing flavonoids, another type of UV absorbing pigment, dearomatized isoprenylated phloroglucinols, have been identified in some species on the anthers and ovarian wall that have been found to have both a visual and defensive function, such as being a deterrent to insect feeding (Gronquist et al., 2001). While most radially symmetric flowers have radially symmetric nectar guides, most bilaterally symmetric flowers have bilaterally symmetric nectar guides (Dafni and Kevan, 1996). Whether radially or bilaterally symmetric, these nectar guides orientate pollinators toward the center of the flower where nectar and pollen are produced.

Peanut (*Arachis hypogaea* L.) is an annual, self-pollinated crop that is grown worldwide on about 25.9 million ha as an oil, cash, food, and feed crop (FAOSTAT, 2019). Like most

crops, the predominant breeding goal for peanut is increased yield either directly through selection of genes that increase yield or indirectly such as through improved yield retention via increased biotic and abiotic stress tolerance and/or resistance with wild *Arachis* species as donors of high, durable resistances (Stalker, 2017). While previous reports (Girardeau and Leuck, 1967; Rashad et al., 1979) found that large bee species that trip flowers increased peanut yields in some varieties, a more recent report found bees did not increase yield in newer peanut varieties and suggested that selection for other desirable traits may have resulted in development of varieties not attractive to flower-tripping bees (Blanche et al., 2005). Most *Arachis* species, including peanut, are self-pollinating species and are not dependent on bee-pollination; however, peanut has been documented as attractive to honeybees, many wild bee species, and other insect pollinators despite lacking a nectary, meaning insect visitation is likely for pollen foraging (Vogel, 1997; Hammons and Leuck, 1966; Leuck and Hammons, 1969; Blanche et al., 2005). Therefore, peanut, as well as *Arachis* species such as the high-density flowering species *A. glabrata* and *A. pintoi* used as cover crops and as alternatives to turf grass in lawns, could be beneficial for increasing plant biodiversity to provide habitats for wild bees in agricultural and urban landscapes (Anderson et al., 2015; Kröning et al., 2019). While peanut farmers may or may not have increased yield due to bee-pollination, peanut may increase abundance and diversity of wild bees, which can impact nearby farms growing crops dependent on bee-pollination.

Despite the documentation of bee-pollination in *Arachis* and the extensive morphological characterization of *Arachis* that has been performed, UV reflectance pattern diversity in peanut as well as the *Arachis* genus has not been documented. It is unknown if *Arachis* species have flowers with UV reflectance patterns. Since *Arachis* species generally have yellow and orange

flowers and yellow flowers have been found more likely than other colored flowers to contain nectar guides (Horovitz and Cohen, 1972; Guldberg and Atsatt, 1975; Primack, 1982), it is likely that peanut and species in the genus *Arachis* have UV reflectance patterns. Furthermore, given the wide pool of genetic and phenotypic variation in the *Arachis* genus (Kochert et al., 1996; Moretzsohn et al., 2004), different species may have distinct flower UV reflectance patterns. This study documented flower UV absorbance and UV reflectance levels in cultivated peanut, diploid *Arachis* species, wild *Arachis* species-derived allotetraploid interspecific hybrids cross-compatible to peanut, as well as BC_1F_3 hybrids derived from crosses between cultivated peanut and allotetraploids to determine if *Arachis* flowers have nectar guides, and if so, to document the diversity of ultraviolet reflection pattern in *Arachis* flowers.

Materials and Methods

Plant Materials

Diploid, wild *Arachis* species, *A. correntina* (Burkart) Krapov. and W.C. Gregory (PI 262808, GKP 9530; abbrev.: *Cor*9530), *A. duranensis* Krapov. and W.C. Gregory (PI 468197, GKBSPSc 30060; abbrev.: *Dur*30600), and *A. ipaensis* Krapov. and W.C. Gregory (PI 468322, GKBSPSc 30076; abbrev.: *Ipa*30076) were used to generate the diploid hybrids, *Ipa*30076*Cor*9530 and *Ipa*30076*Dur*30060 in 2016 at North Carolina State University (NCSU). Wild *Arachis* species, *A. batizocoi* Krapov. and W.C. Gregory (PI 298639, K 9484; abbrev.: *Bat*9484), *A. cardenasii* Krapov. and W.C. Gregory (PI 261874, GKP 10017; abbrev.: *Card*), *A. correntina* (PI 262881, GKP 9548; abbrev.: *Cor*9548), *A. duranensis* Krapov. and W.C. Gregory (V14167; abbrev.: *Dur*14167, SeSn 2848; abbrev.: *Dur*2848, and K 7988; abbrev.: *Dur*7988), *A. gregoryi* A. Gripp, C.E. Simpson, and J.F.M. Valls (PI 476116; VSGr 6389; abbrev.: *Greg*6389), *A. magna* Krapov., W.C. Gregory, and C.E. Simpson (PI 468340; K30097; abbrev.: *Mag*30097),

A. stenosperma Krapov. and W.C. Gregory (V 10309; abbrev.: *Sten*10309, PI 338280; HLK 410; abbrev.: *Sten*410), *A. valida* Krapov. and W.C. Gregory (PI 468154; GK 30011; abbrev.: *Val*30011) and *A. villosa* Benth. (V 12812; abbrev.: *Villo*12812) were used to create diploid hybrids *Bat*9484*Dur*2848, *Bat*9494*Sten*10309, *Greg*6368*Sten*V10309, *Ipa*30076*Cor*9548, *Ipa*30076*Dur*14167, *Ipa*30076*Sten*10309, *Ipa*30076*Villo*12812, *Mag*30097*Dur*7988, *Mag*30097*Sten*7382, *Mag*30097*Sten*10309, and *Val*30011*Sten*V10309 at the University of Georgia (UGA) Athens Campus. All allotetraploids were derived from the diploid hybrids by colchicine treatment of F_1 hybrid cuttings at the UGA Athens Campus, except for *Ipa*30076*Dur*30060 and *Ipa*30076*Cor*9530, which were generated at the UGA Tifton Campus. *Ipa*30076*Cor*9530, *Ipa*30076*Dur*30060, *Ipa*30076*Sten*10309, and *Val*30011*Sten*10309 allotetraploids, used as pollen donors, were crossed to cultivated peanut breeding lines, *A. hypogaea* '13-2113' or '13-1014' and subsequently backcrossed to these breeding lines (Table 4.1). Breeding lines 13-2113 and 13-1014 were selected from [(C1805-617-2 x 'Florida-07' (Gorbet and Tillman, 2009)) x 'Georgia-06G' (Branch, 2007)], in which C1805-617-2 was a selection from 'Tifguard' (Holbrook et al., 2008) x 'Florida-07'. Line 13-2113 was selected with the ADSNP124 (A09 6720287) marker (Chu et al., 2016) to have an A09 *A. cardenasii* introgression that confers nematode resistance. Nomenclature for plant materials tested in this study is summarized in Table 4.1

Due to limited field space, UV reflectance evaluation was performed on plant materials grown in two different environments, field and greenhouse, in which two peanut cultivars and three allotetraploids were grown in both environments. The greenhouse materials included two peanut cultivars 'NC3033' and 'Tifrunner', three allotetraploids, and eight wild *Arachis* species. The field grown materials included the two peanut cultivars, four BC_1F_3 lines derived from four

unique allotetraploids, and 13 allotetraploids. The *Ipa*30076*Sten*410 and *Ipa*30076*Sten*10309 seeds were accidently mixed, so *IpaSten* data may come from plants from either or both allotetraploids (Table 4.1).

On 5 May 2020, the 13 allotetraploid lines and on 18 May 2020, NC3033 and Tifrunner seeds were coated in Vitavax PC (Vitavax, Crompton, Middlebury, CT) and treated overnight in 0.5% Florel Growth Regulator (Lawn and Garden products Inc., Fresno, CA) to break dormancy. The allotetraploid seeds were germinated sooner to account for slower growth of the allotetraploids as compared to the peanut cultivars. Twenty-four hours later, seeds were planted in #123 7.62 cm round x 11.43 cm deep Jiffy pots (Harris Seeds, Rochester, NY) filled with Promix growth medium (Premier Tech Horticulture, Quakertown, PA). On 2 June, the 13 allotetraploid lines and peanut cultivars were transplanted from the greenhouse to the BlackShank farm in Tifton, GA, in a randomized design. On 1 June 2020, *IpaCor*2_BC_1F_3, *IpaDur*4_BC_1F_3, *IpaSten*_ BC_1F_3, and *ValSten*1_ BC_1F_3 seeds were coated in Vitavax PC and directly seeded into plots at the Rigdon farm, located one mile from the BlackShank farm, in Tifton, GA. Standard field management was applied except fungicides were withheld due to testing the materials for resistance to white mold and late leaf spot.

On 20 July, seeds for greenhouse cultivation were coated in Vitavax PC and treated overnight in 0.5% Florel Growth Regulator to break dormancy. Seeds were then planted in #123 7.62 cm round x 11.43 cm deep Jiffy Pots and transplanted approximately one month later into 121.92 cm round x 27.94 cm deep pots filled with Promix growth medium. Normal plant management was applied in the greenhouse except that fungicide treatments were withheld due to also testing the materials for rust resistance.

Ultraviolet photography

A Nikon D 5000 digital camera was used to take photos in a glasshouse in natural light. The UV/IR cut color correcting hot mirror filter was removed from the camera by Kolari Vision (New Jersey, USA) to make it sensitive to UV, visible, and IR light. The camera was equipped with a Baader U-Venus-Filter 2" (350nm) and an adapter Baader Hyperion DT Ring HDT54/52 (M54 to M52) # 2958052 (Mammendorf, Germany), which allowed transmission of UV light from 320 to 380 nm and blocked the rest of the spectral range from 200 to 320 nm and from 380 to 1,120 nm.

Flower Characterization

Between 78 and 92 days after transplanting, five flowers from three plants of each genotype (15 flowers per genotype total) for the allotetraploids and peanut cultivars at the BlackShank farm were collected in 15 ml falcon tubes (Corning CoStar, Corning, NY) containing a moist Kimwipe (Kimerly-Clark, Neenah, WI) to keep flowers from wilting during collection. On 109 days after planting, five flowers from three plants of each BC_1F_3 line were collected. Between 112 and 221 days after planting, five flowers from three plants of each genotype grown in the greenhouse were collected. The greenhouse collection period was long mostly due to some wild *Arachis* species taking a long time to produce flowers. NC3033, Tifrunner, and *Ipa* only had flowers collected from two plants, while *Val* only had flowers collected from one plant. Flowers were dissected and photographed; measurements of banner area (cm^2), area of UV absorbance on banner (abaxial and adaxial) (cm^2), left wing area (cm^2), and area of UV absorbance on left wing (abaxial and adaxial) (cm^2) were measured with Assess 2.0 (APS Press) (Fig. S4.1). Identical analysis was performed on the right wing but was not reported due to high similarity to left wing results. Each image was calibrated by using rulers

photographed in each image. Measurements of flowers collected from the same plant were not independent observations since, plant, not flower, was the experimental unit. Therefore, the measurements for the five flowers per plant were averaged to make one data point, meaning each genotype had three data points per tested parameter. Lastly, flowers collected from the greenhouse were scanned to obtain regular, non-UV, photos of these materials.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using RStudio (RStudio, Inc.) to determine the effect of the two different environments (field environment collected between 18 August to 18 September 2020 and greenhouse environment collected from 9 November 2020 to 26 February 2021) on UV reflection in *Arachis* flowers according to the following parameters: area of UV absorption on the banner (abaxial and adaxial), area of UV absorption on the banner as a percentage of total banner area (abaxial and adaxial), area of UV absorption on the left wing (abaxial and adaxial), area of UV absorption on the left wing as a percentage of total left wing area (abaxial and adaxial). ANOVA was also performed to determine the effect of the two different environments on banner area and left wing area. Means of each parameter among the genotypes were separated based on the Tukey's Test ($\alpha = 0.05$) results with RStudio. Only genotypes grown in both the field and greenhouse were included in ANOVA and Tukey's analysis, including NC3033, Tifrunner, *IpaCor*2, *IpaDur*4, and *ValSten*1.

One-way analysis of variance (ANOVA) was performed using RStudio (RStudio, Inc.) to determine the effect of genotype on flower size in *Arachis* and on UV reflection in *Arachis* flowers according to the following parameters: banner area, area of UV absorption on the banner (abaxial and adaxial), area of UV absorption on the banner as a percentage of total banner area (abaxial and adaxial), area of UV absorption on the adaxial as well as the abaxial side of the left

wing (abaxial and adaxial), area of UV absorption on the adaxial as well as abaxial left wing as a percentage of total left wing area (abaxial and adaxial). Means of each parameter among the genotypes were separated based on the Tukey's Test ($\alpha = 0.05$) results with RStudio. ANOVA and subsequent Tukey's analysis were performed separately on materials from the greenhouse and materials from the field.

Results

Environment (field versus greenhouse) had a significant effect on area of UV absorption on the banner (adaxial), area of UV absorption on the banner as a percentage of banner area (adaxial), area of UV absorption on the left wing (adaxial), and area of UV absorption on the left wing as a percentage of left wing area (adaxial) (Table 4.2). No environment effect was observed on banner area, left wing area, area of UV absorption on the left wing (abaxial), nor area of UV absorption on the left wing as a percent of left wing area. In addition, no flowers collected from the field nor the greenhouse had UV absorption on the abaxial side of the banner. Flowers from the field had greater total area of UV absorption on the banner (adaxial) and area of UV absorption as a percent of banner area (adaxial) than flowers from the greenhouse (Supplementary Figure 2). Similarly, flowers from the field had greater total area of UV absorption on the left wing (adaxial) and area of UV absorption as a percent of left wing area (adaxial) than flowers from the greenhouse (Supplementary Figure 3). The UV absorption pattern on the adaxial side of the banner remained consistent within each genotype (Fig. S4.4). The peanut cultivars, NC3033 and Tifrunner, had UV absorption areas on the banner that were shaped similarly to a vertical cross section of a mushroom while the allotetraploids had heart-shaped cross-sections whether grown in the field or greenhouse (Fig. S4.4).

Genotype had a significant effect on banner area, left wing area, and all parameters of UV reflection on *Arachis* flowers collected from the greenhouse (Table 4.2). *IpaCor*2 and *Cor* had the largest banner area at 2.11 and 2.23 cm^2, respectively, which were numerically larger than the cultivated peanut genotypes but statistically larger than *Dur*, *Mag*, and *Sten*10309, which had banner areas of 0.68, 0.96, and 0.97 cm^2, respectively (Fig. S4.5A). The only genotype with significantly different banner area from a peanut cultivar was *Dur*. *IpaCor*2 and *Cor* the largest left wing area at 0.51 and 0.56 cm^2, respectively, and the latter was significantly greater than all four cultivated peanut genotypes (Fig. S4.5B). Only *IpaCor*2 and *Cor* were significantly different from at least one cultivated peanut genotype for left wing area. All the genotypes had yellow wings, but only *ValSten*1, *Sten*410, and *Sten*10309 had completely yellow banners (Fig. S4.6). The remaining genotypes had banners with a yellow center and orange edge, though the intensity of the orange varied. All genotypes except *Ipa*, *Mag*, and two of the three *Cor* plants had thin red stripes originating from where the hypanthium and banner connect and spread outwards on their banners.

Numerically, the four cultivated peanut genotypes had the largest total area of UV absorption on the adaxial side of the banner as well largest UV absorption area as a percent of banner area (Fig. 4.1) 13-2113 had the largest total area of UV absorption at 0.56 cm^2 and Tifrunner had the largest UV absorption as a percent of banner area at 32.5%. The total area of UV absorption of the four cultivated peanut genotypes was not significantly larger than the allotetraploids with the exception of Tifrunner and 13-2113 being significantly larger than *IpaDur*4; however, they were significantly larger than all the *Arachis* species except *Val*. All the wild *Arachis* species had total UV absorption areas smaller than half of the total UV absorption areas of the cultivated peanut genotypes. The area of UV absorption as a percentage of banner

area of the four cultivated peanut genotypes was not significantly larger than the allotetraploids *IpaCor*2 and *ValSten*1, but most were significantly larger than *IpaDur*4. Similar to total UV absorption, the area of UV absorption as a percent of total banner area of the cultivated peanut genotypes was significantly larger than most wild *Arachis* species. As previously mentioned, NC3033 and Tifrunner have mushroom-shaped UV absorption areas with wings on the widest section of the UV absorption area that extended downwards towards the hypanthium (Fig. 4.2). These characteristic wings were absent in 13-1014 and 13-2113, which had a more intermediate UV pattern between the mushroom shape of the two peanut cultivars and the heart shape of the allotetraploids and some wilds (Fig. 4.2).

While the cultivated peanut genotypes had larger total UV absorption on the abaxial side of the left wing than all the wild *Arachis* species, *IpaCor*2 had the largest UV absorption area of 0.14 cm^2 (Fig. 4.3A). When accounting for left wing area, 13-2113 and Tifrunner had the largest percent of UV absorption at 32.37 and 31.44%, respectively, and then *IpaCor*2 had the next largest at 27.84% (Fig. 4.3B). The percent UV absorption of the four peanut cultivated genotypes was larger than that of *IpaDur*4, *ValSten*1, and all the wild *Arachis* species, although only 13-2113 and Tifrunner were significantly larger than the majority of the wild *Arachis* species. NC3033 had very narrow wings, distinctive from the rest of the genotypes, with UV absorption on the distal tips of the wings; the distinctive wing shape of NC3033 makes it difficult to compare the UV absorption shape with the other genotypes (Fig. 4.2). Tifrunner and 13-1014 had UV absorption areas that ran vertically along the entire distal-most edge of the left and right wing, while the rest of the genotypes had UV absorption just on the upper tip of the distal-most edge (Fig. 4.2).

All genotypes had areas of UV absorption on the adaxial side of the left wing less than half the area of UV absorption on the abaxial side of the wing, except for the breeding lines 13-1014 and 13-2113, in which the former had a mean adaxial UV absorption area of about three-fourths that of the abaxial UV absorption area (Fig. 4.4). 13-1014, 13-2113, and *IpaCor*2 had significantly larger total area of UV absorption than all the other allotetraploids and wild *Arachis* species, but when accounting for wing area, only 13-1014 and 13-2113 had significantly larger percent of UV as compared to *IpaDur*4, *ValSten*1, and all the wild *Arachis* species. While smaller, UV absorption area on the adaxial side of the wings was located in the same position as on the abaxial side of the wings, in the upper tip of the wings (Fig. S4.7). For 13-1014 and 13-2113, adaxial UV absorption did not run the entire distal-most edge like on the abaxial side but was present only in the upper region of the wing. Once dissected, the wings laid flat for the UV photos, except the wings of NC3033, which curve inwards towards the keel and reproductive organs (Fig. S4.7).

Genotype had a significant effect on banner area, left wing area, and all parameters of UV reflection on *Arachis* flowers collected from the field (Table 4.2). As with the greenhouse collected flowers, *IpaCor*2 had the largest banner of 2.28 cm^2 but was only numerically larger than the two peanut cultivars, NC3033 and Tifrunner, which had banner areas of 1.86 and 1.62 cm^2, respectively (Table 4.3). The next three genotypes, *MagSten*2, *ValSten*3, and *MagSten*1 with the largest banners all were made with *Sten*10309 or *Sten*7382. Despite *IpaCor*2 having the largest banner area, *IpaCor*1 had the smallest at 1.05 cm^2. The BC_1F_3 lines had similar average banner sizes ranging from 1.29 to 1.12 cm^2 for *IpaCor*2_BC_1F_3 and *IpaDur*4_$BC1F_3$, respectively. *IpaCor*2 had a significantly larger left wing area of 0.52 cm^2 as compared to the cultivated controls, NC3033 and Tifrunner, which had areas of 0.31 and 0.34 cm^2, respectively.

Similar to banner area, *MagSten*2, *MagSten*1, and *ValSten*1 had the next largest left wing area after *IpaCor*2, and *IpaCor*1 had the smallest banner area.

The peanut cultivars had significantly larger total UV absorption areas on the adaxial side of their banners of 0.99 cm^2 for NC3033 and 0.63 cm^2 for Tifrunner as compared to the BC_1F_3 genotypes and the majority of the allotetraploids (Table 4.3). When banner area was accounted for, the percent of UV absorption of 53.41 % for NC3033 and 38.75% for Tifrunner was significantly greater than for all the BC_1F_3 genotypes and allotetraploids, except Tifrunner was similar to *BatDur*2 and *BatSten*1. *GregSten*1 had the smallest total UV absorption on the adaxial side of the banner at 0.10 cm^2 and the smallest percent of UV absorption at 16.88%. BC_1F_3 individuals derived from the four allotetraploids and 13-2113 and 13-1014 had UV absorption areas on the adaxial side of the banner shaped similarly to flowers of 13-2113 and 13-1014 collected from the greenhouse (Fig. 4.2, 4.5). While variations in size were found, the allotetraploids all had centralized, heart-shaped UV absorption areas that were narrowest proximal to the hypanthium (Fig. 4.5).

*IpaCor*2 and NC3033 had total UV absorption areas of 0.120 and 0.116 cm^2, respectively, on the abaxial side of the left wing that where significantly larger than most of the allotetraploids, but not significantly larger than three of the four BC_1F_3 genotypes (Table 4.3). *IpaDur*4 and *IpaCor*1 had the smallest areas of UV absorption on their left wings at 0.035 and 0.039 cm^2, respectively. When accounting for left wing area, NC3033 and Tifrunner had the highest percent of UV absorption at 38.05 and 26.90%, respectively, and the percent of NC3033 was significantly larger than *IpaCor*2. Only *BatSten*1 and *BatDur*2 had similar percentages of UV absorption on the abaxial side of the left wing as compared to NC3033. As was found with

flowers collected from the allotetraploids in the greenhouse, the UV absorption of the BC_1F_3 flowers and allotetraploid flowers was confined to the distal tip of the wings.

All genotypes had area of areas of UV absorption on the adaxial side of the left wing less than that on the abaxial side (Table 4.3). *BatDur*2 had the great area of UV absorption on the adaxial side of the left wing at 0.082 cm^2, significantly greater than all the BC_1F_3 genotypes and most of the allotetraploids, but not significantly greater than the peanut cultivars, NC3033 and Tifrunner, which had areas of 0.073 and 0.043 cm^2, respectively. When accounting for left wing area, NC3033 had the highest percent of UV absorption on the adaxial side of the left banner at 24.40%, not significantly greater than *BatDur*2, which had an UV absorption at 20.15%. The three genotypes with the smallest total and percent of UV absorption on the adaxial side of the wings were *IpaDur*4_BC_1F_3, *IpaSten*_BC_1F_3, and *ValSten*1_BC_1F_3. Furthermore, *ValSten*1_BC_1F_3 had almost no UV absorption on the abaxial side of the left wing, with a mean UV absorption area of 0.001 cm^2. While smaller, UV absorption area on the adaxial side of the wings was located in the same place as on the abaxial side of the wings, in the upper tip of the wings (Fig. S4.8).

Discussion

Arachis species, including peanut, can provide pollen for wild bee species and may promote abundance and diversity of important wild pollinators; however, visual cues that bees can associate with pollen rewards such as contrasting color and UV absorption and reflectance on flowers have not been characterized in *Arachis*. Since yellow flowers have been found to be more likely than other colored flowers to contain nectar guides (Horovitz and Cohen 1972; Guldberg and Atsatt 1975; Primack, 1982) and *Arachis* species generally have yellow or orange and yellow flowers, it was thought that peanut and *Arachis* species would have UV reflectance

patterns. This study found that all genotypes, including two peanut cultivars, two peanut breeding lines, 8 wild *Arachis* species, 19 unique allotetraploids, and four BC_1F_3 lines, had nectar guides. These nectar guides were bilaterally symmetrical consistent with previous findings that bilaterally symmetric flowers have bilaterally symmetric nectar guides (Dafni and Kevan, 1996). All genotypes had UV absorption located centrally on the banner with UV absorption extending towards the junction of banner and hypanthium and had UV absorption on the distal tips of the abaxial and adaxial sides of the wings.

While all studied genotypes had nectar guides, the size and shape of these nectar guides varied. In general, cultivated peanut genotypes had greater total UV absorption and UV absorption as a percent of banner area on the adaxial side of the banner than the wild *Arachis* species. This same trend occurred for UV absorption on the abaxial side of the wings. This complements the finding of Horth et al. (2014), which found that mean nectar guide size was smaller in wild populations as compared to cultivated populations of *Rudbeckia hirta*. It is unknown if larger UV absorption on the adaxial side of the banner and abaxial side of the wings increases or decreases *Arachis* flower conspicuousness for detection by bees or if it affects bee landing and orientation after detection. In other species when UV nectar guides are completely obscured in both radially and bilaterally symmetric flowers, bee visitation has been found to decrease (Peter and Johnson, 2008; Klomberg et al., 2019). However, the effect of altering the size of UV absorption and reflectance without eliminating the UV pattern on bee visitation frequency has not been extensively studied, especially in bilateral symmetrical flowers. In radially symmetrical flowers, increasing the area of UV absorbance of *Hypoxis camerooniana* flowers did not affect bee visitation but increased bee visitation of *R. hirta, R. flugida*, and *Argentina anserina* flowers (Horth et al., 2014; Rae et al., 2012; Klomberg et al., 2019).

All *Arachis* genotypes had “large” flowers, greater than 8 mm in diameter as defined by Spaethe et al. (2001), meaning bees likely use the color-contrast neuronal channel visualizing blue, green, and UV wavelengths to detect *Arachis* flowers. Therefore, flowers with a greater contrast to a foliage background color should be most conspicuous to bees. A small area of UV absorption surrounded by a large area of UV reflectance characteristic of wild *Arachis* species may stand out more in UV-absorbing foliage as compared to flowers with a large area of UV absorption surrounded by a small area of UV reflectance more characteristic of the cultivated peanut genotypes tested in this study (Fig. S4.9). Further studies elucidating the relationship between UV absorption and UV reflection area with bee flower detection and visitation on bilaterally symmetric flowers are needed. A study to determine the effect of UV absorption area on bee visitation in *Arachis* could use UV-absorbing chemicals such as butyl methoxydibenzoylmethane and ethylhexyl methoxycinnamate to manipulate the size of UV absorbance to various degrees in a few genotypes similar to the study performed by Klomerg (et al., 2019). Using unscented, UV-absorbing chemicals would keep other flower cues consistent, such as flower shape and size, while only altering UV reflectance area.

Furthermore, for large flowers (> 8 mm), UV is not the only wavelength bees utilize to identify flowers. Blue and green color contrast with foliage in *Arachis* would increase flower conspicuousness as well. Levinson et al. (2021) found that the banners of peanut breeding line 13-2113 and four allotetraploids, *IpaCor*2, *IpaDur*4, *IpaSten*10309, and *ValSten*1 had absorption at wavelengths of 380, 415, 450, 473 and 495 nm significantly higher than the blank ethanol control but not for wavelengths in the green interval. Not only is this finding of UV detection at 380 consistent with UV detection in this study, but it suggests that flowers from this peanut breeding line and these four allotetraploids derived from five unique *Arachis* species absorb blue

wavelengths (450, 473 and 495 nm) and reflect green wavelengths, which would contrast with *Arachis* foliage that absorbs primarily green wavelengths. Furthermore, it would be interesting to test two additional camera filters, one that only allows blue and one that only allows green wavelengths to pass through, and to stack UV, blue, and green wavelength photos of *Arachis* flowers still attached to the plant to better understand the contrast of all wavelengths between *Arachis* flowers and foliage.

For flower detection by bees, it is also critical to consider flower visibility beyond just flower color (Chittka et al., 2001). First, flower size affects flower detection time, in which flowers under 8 mm in diameter take wild bees longer to find (Spaethe et al., 2001). In this study, cultivated peanut genotypes, BC_1F_3 lines, allotetraploids, and wild *Arachis* species had flowers with diameters greater than 8 mm and their banner areas were generally similar. Therefore, banner size differences may not cause differences in bee detection of flowers of different *Arachis* species. Another aspect of flower visibility is the growth habit of the plant coupled with flower morphology. Wild *Arachis* species and allotetraploids derived from wild *Arachis* species have prostrate growth, in which branches grow flat along the ground (Stalker et al., 2016). This growth habit coupled with the long hypanthium area characteristic of wild *Arachis* species and allotetraploids allow flowers to rise above the foliage of the plant likely being easier to detect by bees (Stalker et al., 2016; Levinson et al., 2021). In contrast, domesticated peanut has been selected for more compact growth with shorter branches that curve upwards and cultivated peanut is also known to have a shorter hypanthium than wild *Arachis* species, meaning flowers are more likely to be at least partially hidden from view by plant foliage (Stalker et al., 2016; Levinson et al., 2021).

Total flower production of different genotypes must also be considered when determining its attractiveness to bees. Positive alleles for flowering precocity have been identified in some allotetraploids (Fonceka et al., 2012) and allotetraploids with significantly higher flower counts over the growing season as compared to cultivated peanut have been identified (Levinson et al., 2021). Furthermore, Levinson et al., 2021 found that allotetraploids made with perennial species like *A. correntina* and *A. stenosperma* do not have a decline in flower production unlike cultivated peanut, in which the peanut breeding line had a decline from its maximum production of about 20 flowers/ day from 52 to 66 days after transplanting to close to 5 flowers / day 87 days after transplanting. This is a large difference compared to the flower production of *IpaCor* allotetraploids (the same genotype as *IpaCor*2 in this study) that produced over 40 flowers/ day from 52 to 108 days after transplanting (Levinson et al., 2021). In general, cultivated peanuts may have fewer flowers that may be harder to detect than some allotetraploid and *Arachis* species; however, studies including pollinator counts are needed to determine differences in bee visitation between wild *Arachis* species, allotetraploids, and cultivated peanut. If differences are found, alleles contributing to high, durable flower count in allotetraploids such as in *IpaCor*2 could be introgressed into cultivated peanut along with efforts currently being done to introgress pathogen and insect resistance to produce cultivars more attractive to bees. These cultivars could be important for providing habitats for wild bees as farmers may be more easily incentivized to use high-flowering peanut cultivars that are profitable as compared to non-agronomic flowering plants as border rows to increase pollination and provide habitats for bees.

A secondary finding of the study was a significant effect of the two different environments (field environment from 18 August to 18 September 2020 and greenhouse environment from 9 November 2020 to 26 February 2021) on the area of UV absorption on the

banner (adaxial), area of UV absorption on the banner as a percentage of banner area (adaxial), area of UV absorption on the left wing (adaxial), and area of UV absorption on the left wing as a percentage of left wing area (adaxial). However, no environment effect was observed on banner area, left wing area, area of UV absorption on the left wing (abaxial), nor area of UV absorption on the left wing as a percent of left wing area. Therefore, while flower size remained similar between genotypes in the two different environments, variation of UV absorption on the adaxial side of the banner and on the adaxial side of the wings can be displayed. Due to differences in both collection timing and environment, further studies are needed to identify the causes of UV variation in different environments. Future studies could focus on light quality and quantity, temperature, plant development stage, among others, as potential causes in nectar guide variation.

Conclusions

This study is the first to look at UV nectar guides in *Arachis* and found that nectar guides of various sizes are present in both wild *Arachis* species, allotetraploids, and cultivated peanut. However, a wide knowledge gap about nectar guides in crops, particularly those with bilaterally symmetric flowers, still exists, and this study is only one step towards narrowing this gap. Learning more about what attributes to flower attractiveness of various crops, including attractiveness caused by area and pattern of UV absorption, can lead to better decision-making regarding wild pollinator recruitment for crop fields (Frankie et al., 2009; Cameron et al., 2011; Bommarco et al., 2012). Increasing plant biodiversity in urban and agricultural landscapes is one way to increase wild bee abundance and diversity. Ornamental *Arachis* species should be evaluated for promoting wild bee abundance and diversity in urban landscapes while high-

flowering peanut cultivars should be evaluated for use as cost-effective border crops for pollinator recruitment for crop fields.

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Table 4.1. Genetic materials tested and their abbreviations and ploidy level.

Greenhouse: Plant Materials	Abbreviation	Ploidy Level
A. hypogaea cv. NC3033	**NC3033**	Tetraploid
A. hypogaea cv. Tifrunner	**Tifrunner**	Tetraploid
A. hypogaea 13-1014	13-1014	Tetraploid
A. hypogaea 13-2113	13-2113	Tetraploid
(*Ipa*30076 x *Cor*9530)^{4x}	***IpaCor*2**	Tetraploid
(*Ipa*30076 x *Dur*30600)^{4x}	***IpaDur*4**	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**	Tetraploid
A. cardenasii GKP 10017	*Card*	Diploid
A. correntina HLK 9530	*Cor*	Diploid
A. duranensis GKBSPSc 30600	*Dur*	Diploid
A. ipaensis K 30076	*Ipa*	Diploid
A. magna K 30092	*Mag*	Diploid
A. stenosperma HLK 410	*Sten*410	Diploid
A. stenosperma V 10309	*Sten*10309	Diploid
A. valida GK 30011	*Val*	Diploid
Field: Plant Materials	**Abbreviation**	**Ploidy Level**
A. hypogaea cv. NC3033	**NC3033**	Tetraploid
A. hypogaea cv. Tifrunner	**Tifrunner**	Tetraploid
[13-1014 x (13-2113 x IpaCor2)]_BC1F2:1_F3	*IpaCor*2_BC_1F_3	Tetraploid
13-1014 x (13-1014 x IpaDur4)]_BC1F2:1_F3	*IpaDur*4_$BC1F_3$	Tetraploid
[13-2113 x (13-2113 x IpaSten10309)]_BC1F2:1_F3	*IpaSten*_$BC1F_3$	Tetraploid
[13-1014 x (13-1014 x ValSten1)]_BC1F2:1_F3	*ValSten*1_$BC1F_3$	Tetraploid
(*Bat*9484 x *Dur*2848)^{4x}	*BatDur*2	Tetraploid
(*Bat*9494 x *Sten*10309)^{4x}	*BatSten*1	Tetraploid
(*Greg*6389 x *Sten*10309)^{4x}	*GregSten*1	Tetraploid
(*Ipa*30076 x *Cor*9548)^{4x}	*IpaCor*1	Tetraploid
(*Ipa*30076 x *Cor*9530)^{4x}	***IpaCor*2**	Tetraploid
(*Ipa*30076 x *Dur*14167)^{4x}	*IpaDur*1	Tetraploid

(*Ipa*30076 x *Dur*30600)^{4x}	***IpaDur*4**	Tetraploid
(*Ipa*30076 x *Sten*410)^{4x} and/or (*Ipa*30076 x *Sten*10309)^{4x}	*IpaSten*	Tetraploid
(*Ipa*30076 x *A. villosa*12812)^{4x}	*IpaVillo*1	Tetraploid
(*Mag*30097 x *Dur*7988)^{4x}	*MagDur*1	Tetraploid
(Mag30097 x Sten7382)^{4x}	*MagSten*1	Tetraploid
(*Mag*30097 x *Sten*10309)^{4x}	*MagSten*2	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**	Tetraploid

Table 4.2. ANOVA output testing the effect of the two different environments, on flower size in *Arachis* and UV reflection in *Arachis* flowers according to the following parameters: banner area, left wing area, area of UV absorption on the banner (adaxial), area of UV absorption on the banner as a percentage of total banner area (adaxial), area of UV absorption on the left wing (abaxial and adaxial), and area of UV absorption on the left wing as a percentage of total left wing area (abaxial and adaxial). The second and third section of the table show ANOVA output testing the genotype effect of greenhouse plants and field plants, respectively according to the aforementioned parameters.

Field versus Greenhouse: Parameter	*F* value	Df(n)[a], df(d)[b]	P-value
Banner area	0.25	1,26	0.62
Left wing area	0.2	1,26	0.66
BUV[c] adaxial	7.3	1,26	0.012*[d]
BUVP[e] adaxial	7	1,26	0.014*
LWUV[f] abaxial	0.23	1,26	0.63
LWUVP[g] abaxial	0.19	1,26	0.89
LWUV adaxial	8.43	1,26	0.0074**
LWUVP adaxial	9.82	1,26	0.0042**
Greenhouse Genotype: Parameter	*F* value	Df(n)[a], df(d)[b]	P-value
Banner area	4.21	14, 25	<0.001***
Left wing area	9.79	14, 25	<0.001***
BUV adaxial	10.89	14, 25	<0.001***
BUVP adaxial	8.81	14, 25	<0.001***
LWUV abaxial	7.12	14, 25	<0.001***
LWUVP abaxial	8.14	14, 25	<0.001***
LWUV adaxial	11.99	14, 25	<0.001***
LWUVP adaxial	10.70	14, 25	<0.001***
Field Genotype: Parameter	*F* value	Df(n)[a], df(d)[b]	P-value
Banner area	5.87	18, 38	<0.001***

Left wing area	4.07	18, 38	<0.001***
BUV adaxial	27.56	18, 38	<0.001***
BUVP adaxial	23	18, 38	<0.001***
LWUV abaxial	5.73	18, 38	<0.001***
LWUVP abaxial	5.84	18, 38	<0.001***
LWUV adaxial	4.19	18, 38	<0.001***
LWUVP adaxial	5.87	18, 38	<0.001***

[a]The df(n), degrees of freedom of the numerator, is based on the number of environment types or genotypes
[b]The df(d), degrees of freedom of the denominator, is based on the total number of data points
[c]BUV stands for area of UV absorption on the banner
[d]*P < .05. **P < .01. ***P < .001
[e]BUVP stands for area of UV absorption on the banner as a percentage of total banner area
[f]LWUV stands for area of UV absorption on the left wing
[g]LWUVP stands for area of UV absorption on the left wing as a percentage of total left wing area

Table 4.3. Genotypes grown in the field and their means for banner area (BA), left wing area (LWA), area of UV absorption on the adaxial side of the banner (BUV Ad.) and on the abaxial and adaxial side of the left wing (LWUV Ab. and LWUV Ad.), and area of UV absorption on the adaxial side of the banner as a percentage of total banner area (BUVP Ad.) and on the abaxial and adaxial side of the left wing as a percent of total left wing area (LWUVB Ab.) and adaxial (LWUVB Ad.) and their Tukey's HSD level ($\alpha = 0.05$). Bolded genotypes were also grown in the greenhouse. Conditional formatting was applied within each column in Excel adding a color gradient to the values, in which red values are larger and blue values are smaller, to visualize the range of values more easily.

Genotype	BA	LWA	BUV Ad.	BUVP Ad.	LWUV Ab.	LWUVP Ab.	LWUV Ad.	LWUVB Ad.
Tifrunner	1.62 abcdef	0.34 bc	0.63 b	38.75 b	0.094 ab	26.90 abc	0.043 abc	12.86 abc
*IpaCor*2_BC$_1$F$_3$	1.29 bcdef	0.35 abc	0.30 g	23.47 efg	0.077 abc	21.48 bcd	0.024 bc	6.46 cd
*IpaDur*4_BC1F$_3$	1.12 def	0.32 bc	0.32 fg	28.10 cde	0.068 abc	21.50 bcd	0.014 c	4.44 cd
*IpaSten*_BC1F$_3$	1.25 cdef	0.32 bc	0.27 g	21.63 efg	0.081 abc	26.04 abcd	0.013 c	4.64 cd
*ValSten*1_BC1F$_3$	1.23 def	0.31 bc	0.35 defg	28.48 cde	0.057 bc	17.69 bcd	0.001 c	00.20 d
*BatDur*2	1.50 bcdef	0.35 abc	0.50 bcde	33.39 bcd	0.096 ab	26.60 abcd	0.082 a	20.15 ab
*BatSten*1	1.59 abcdef	0.31 bc	0.56 bc	36.03 bc	0.086 abc	27.38 ab	0.046 abc	14.60 abc
*GregSten*1	1.86 abcde	0.38 abc	0.10 fg	16.88 g	0.049 bc	12.88 d	0.026 bc	6.75 cd
*IpaCor*1	1.05 f	0.24 c	0.23 g	22.04 efg	0.039 c	17.10 bcd	0.014 c	6.56 cd
IpaCor2	2.28 a	0.52 a	0.52 bcd	23.31 efg	0.120 a	22.67 bcd	0.055 abc	10.46 bcd
*IpaDur*1	1.36 bcdef	0.31 bc	0.40 cdefg	29.11 cde	0.054 bc	17.53 bcd	0.027 bc	08.87 bcd
IpaDur4	1.11 ef	0.26 bc	0.30 g	26.99 cdef	0.035 c	13.37 cd	0.019 bc	6.99 cd
IpaSten	1.28 cdef	0.38 abc	0.31 fg	24.57 defg	0.059 bc	19.48 bcd	0.023 bc	6.88 cd
*IpaVillo*1	1.65 abcdef	0.38 abc	0.39 cdefg	24.13 defg	0.076 abc	19.03 bcd	0.028 abc	8.02 bcd
*MagDur*1	1.58 abcdef	0.33 bc	0.33 efg	21.53 efg	0.047 bc	13.55 bcd	0.026 bc	7.57 cd
*MagSten*1	1.99 abc	0.41 ab	0.39 cdefg	20.45 efg	0.073 abc	18.48 bcd	0.031 abc	7.79 bcd

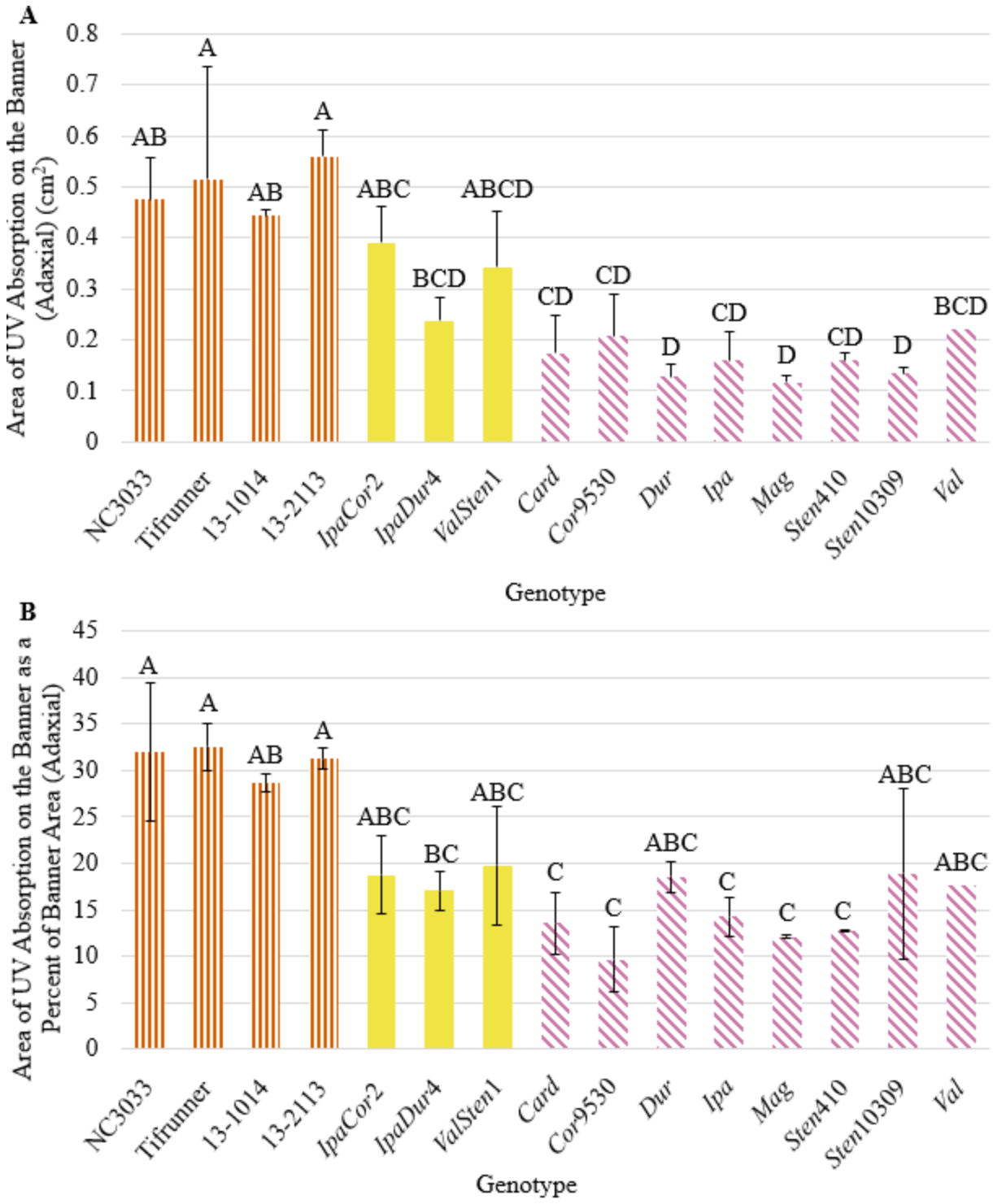

Figure 4.1. A) Area of UV absorption on the banner (adaxial) and B) area of UV absorption as a percentage of banner area (adaxial) of cultivated peanut genotypes (orange, vertical stripes),

allotetraploids (solid, yellow), and wild *Arachis* species (horizontal, pink stripes) collected from the greenhouse.

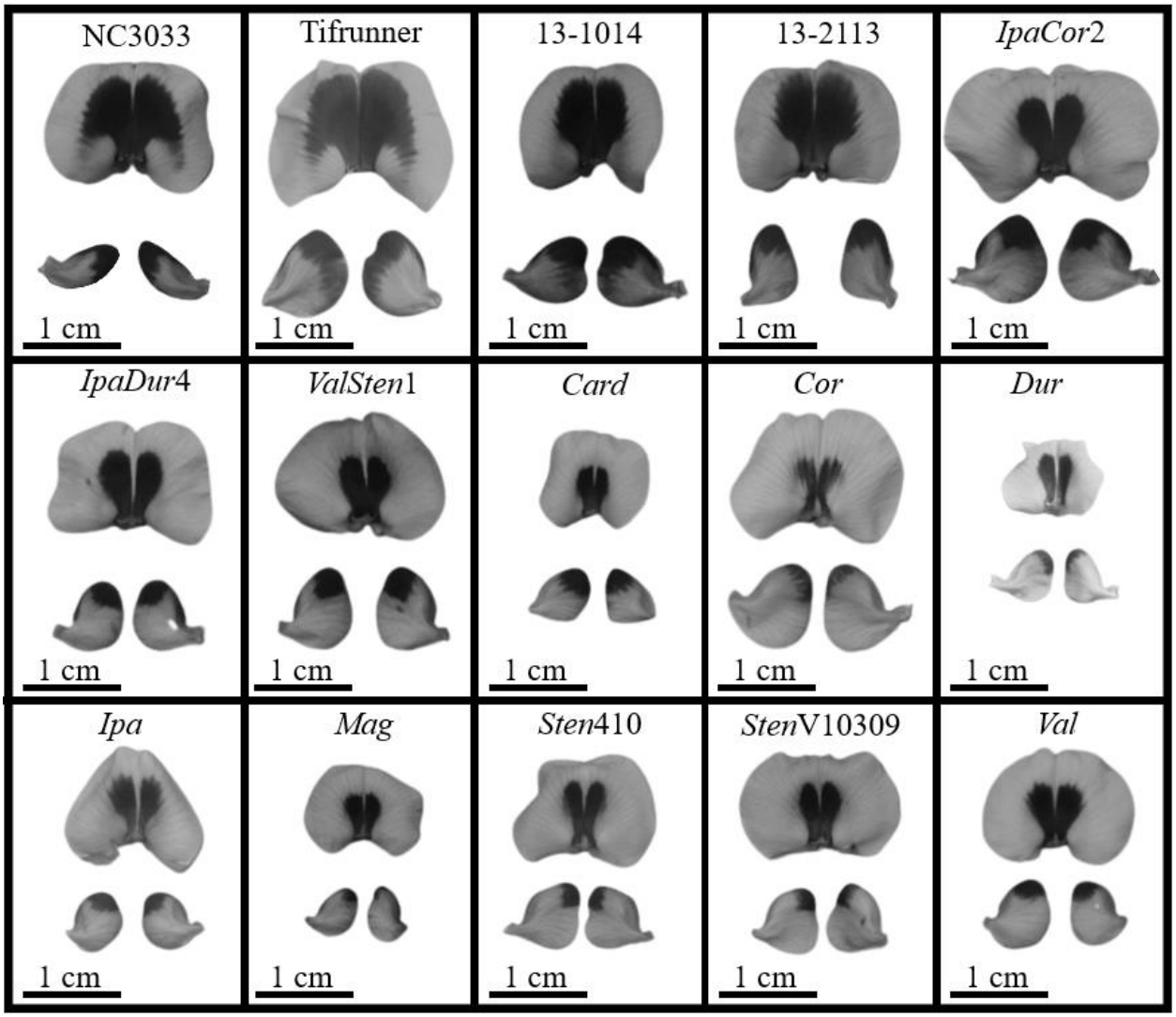

Figure 4.2. UV photos of dissected flowers from cultivated peanut genotypes, allotetraploids, and wild *Arachis* species collected from the greenhouse showing the adaxial side of the banners and abaxial side of the wings.

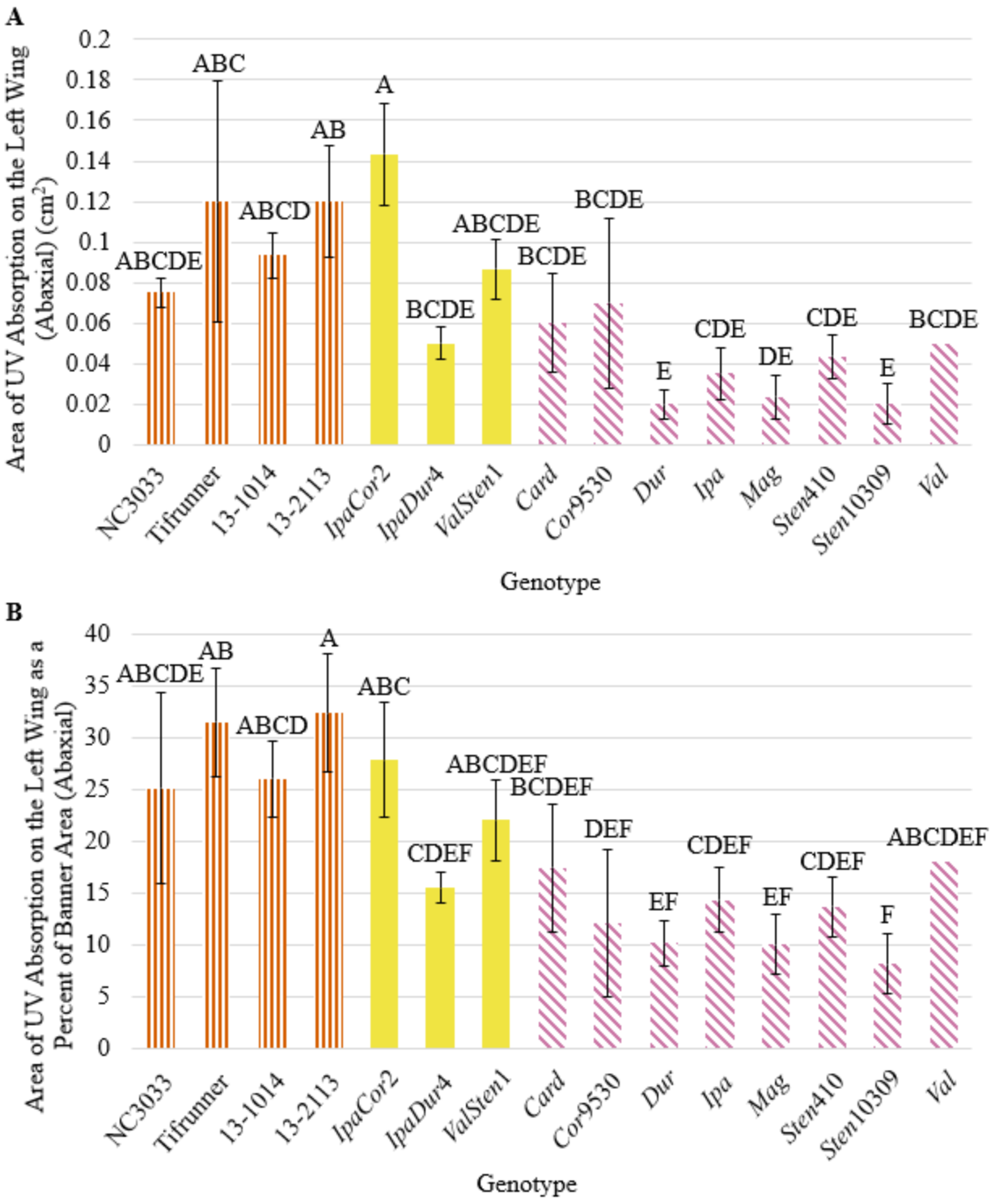

Figure 4.3. A) Area of UV absorption on the left wing (abaxial) and B) area of UV absorption as a percentage of left wing area (abaxial) of cultivated peanut genotypes (orange, vertical stripes),

allotetraploids (solid, yellow), and wild *Arachis* species (horizontal, pink stripes) collected from the greenhouse.

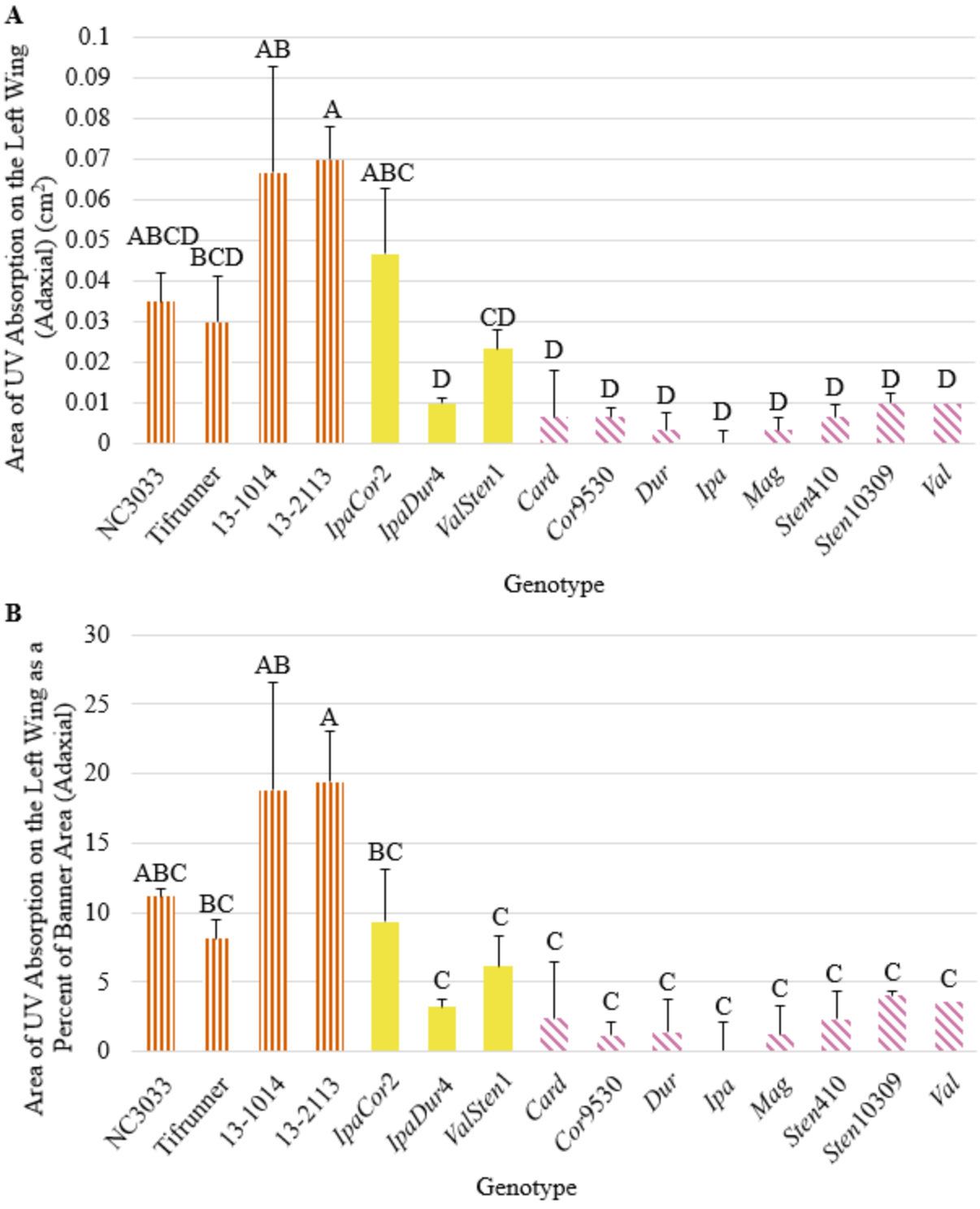

Figure 4.4. A) Area of UV absorption on the left wing (adaxial) and B) area of UV absorption as a percentage of left wing area (adaxial) of cultivated peanut genotypes (orange, vertical stripes),

allotetraploids (solid, yellow), and wild *Arachis* species (horizontal, pink stripes) collected from the greenhouse.

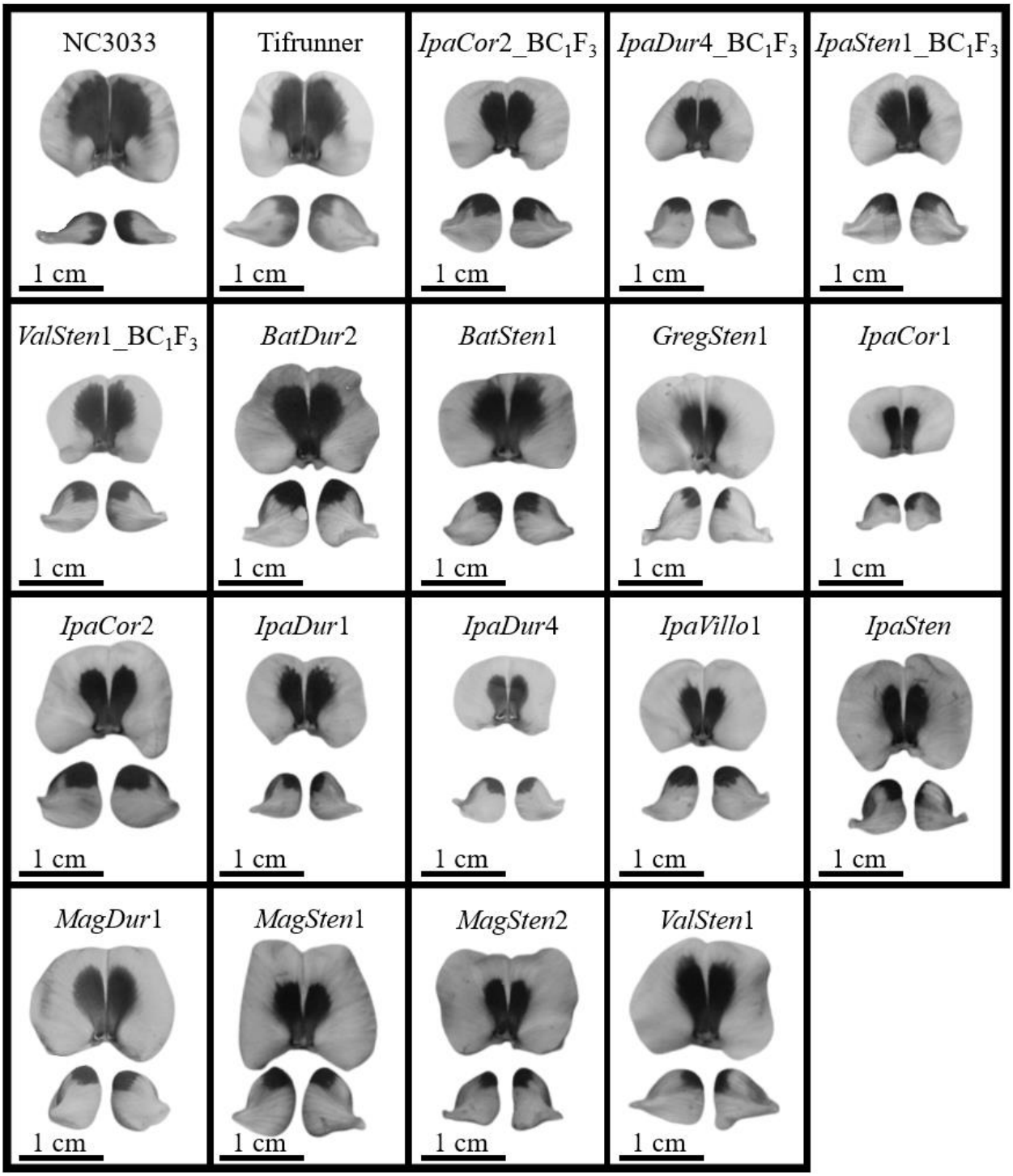

Figure 4.5. UV photos of dissected flowers from peanut cultivars and allotetraploids collected from the field showing the adaxial side of the banners and abaxial side of the wings.

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Appendix 4

Supplemental Figures

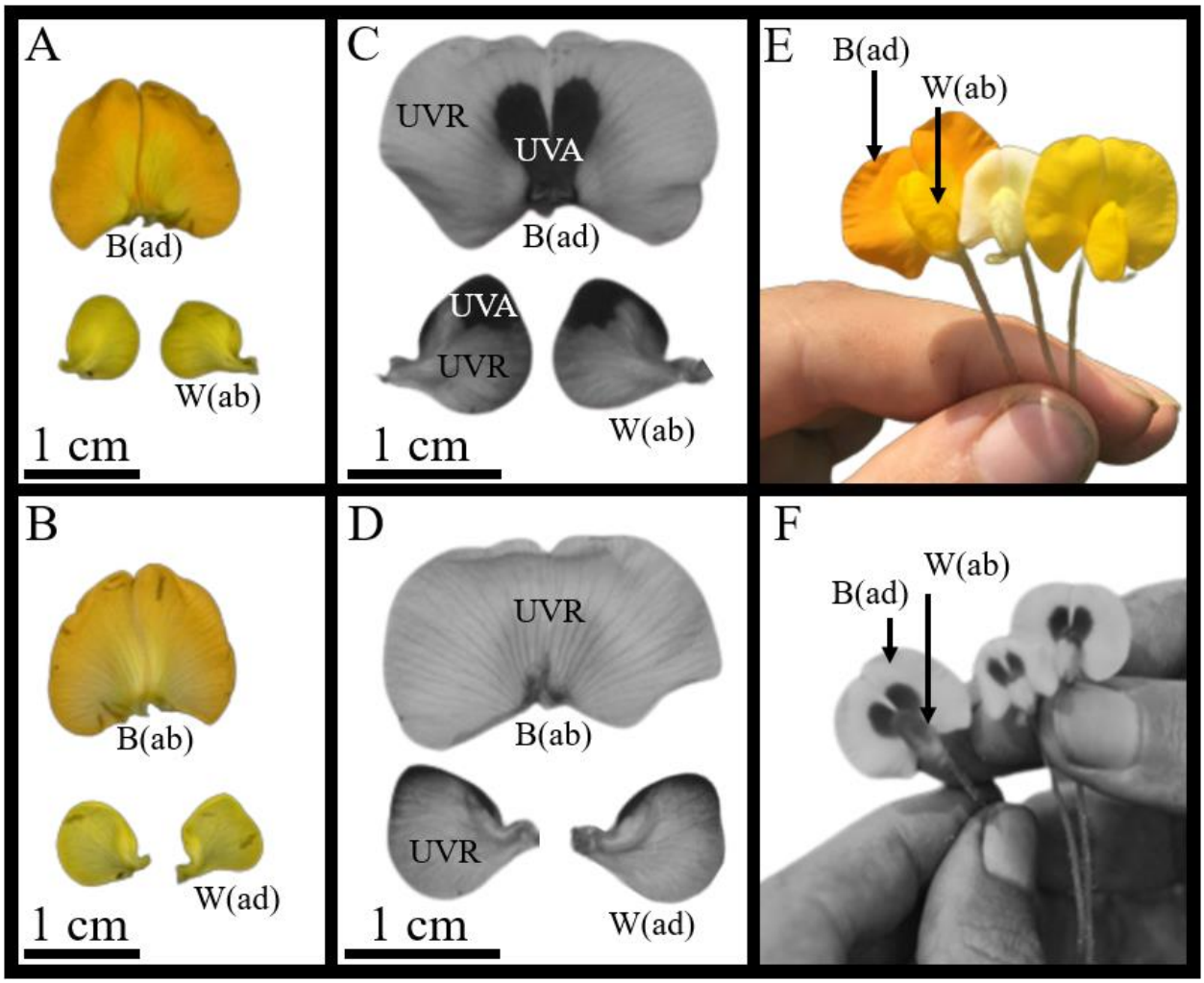

Figure S4.1. A and B) Scans of dissected *IpaCor*2 flowers, in which A) shows the adaxial side of the banner labeled B(ad) and abaxial side of the wings labeled W(ab) and B) shows the abaxial banner labeled B(ab) and the adaxial side wings labeled W(ad). C) and D) UV photos of *IpaCor*2 flowers, in which C) shows UV reflectance (grey) labeled UVR and UV absorbance (black) labeled UVA on the adaxial side of the banner and abaxial sides of the wings and D) shows UV reflectance on the abaxial side of the banner and adaxial side of the wings and small sliver of UV absorbance on the tips on the edges of the upper wings. E) Regular photo of the flowers from three different allotetraploids with the abaxial side of the banner labeled B(ad) and abaxial side of the wings labeled W(ab). F) UV photograph of the same flowers shown in E).

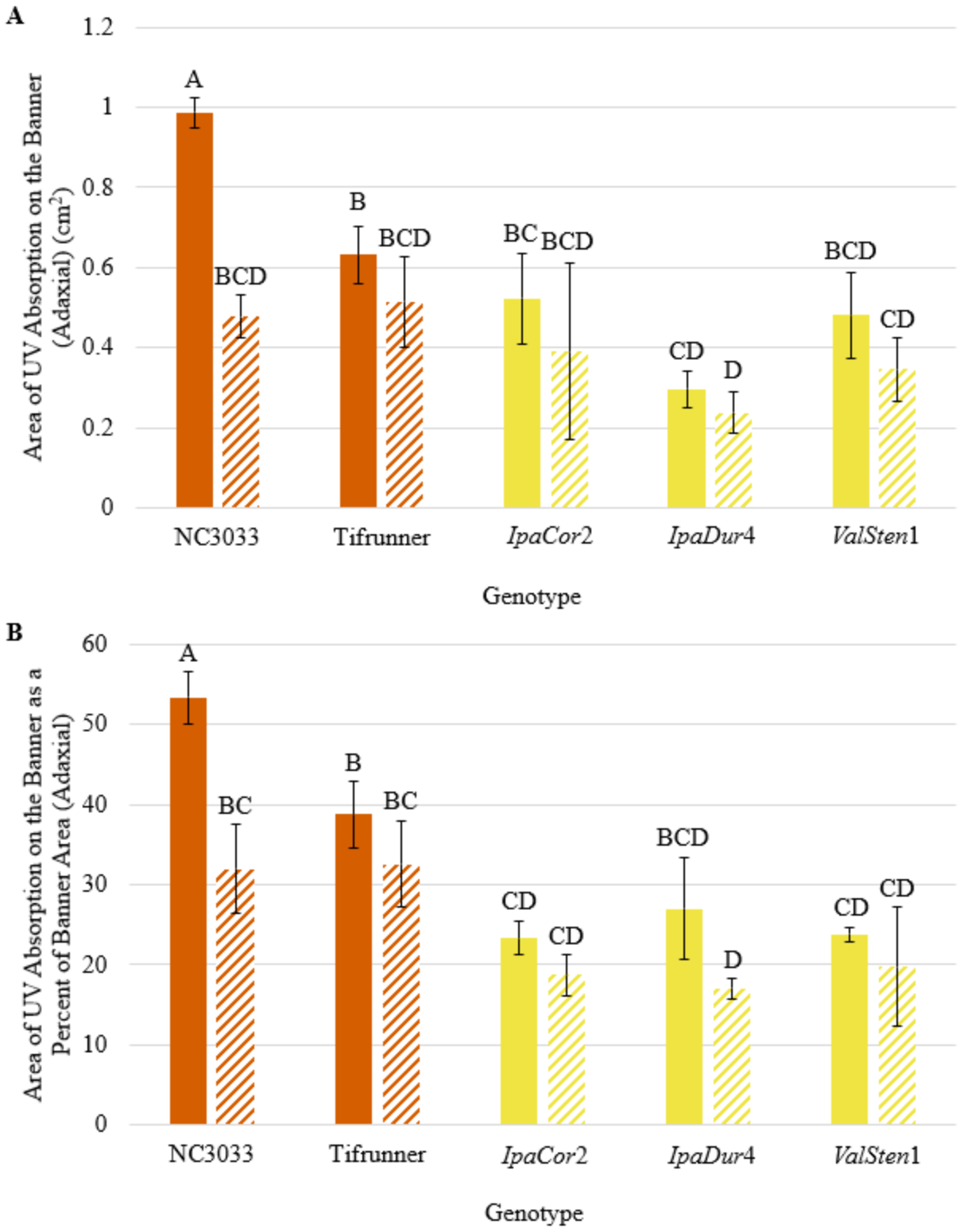

Figure S4.2. A) Area of UV absorption on the banner (adaxial) and B) area of UV absorption as

a percentage of banner area (adaxial) of cultivars (orange) and allotetraploids (yellow) in the field (solid) and in the greenhouse (horizontal stripes).

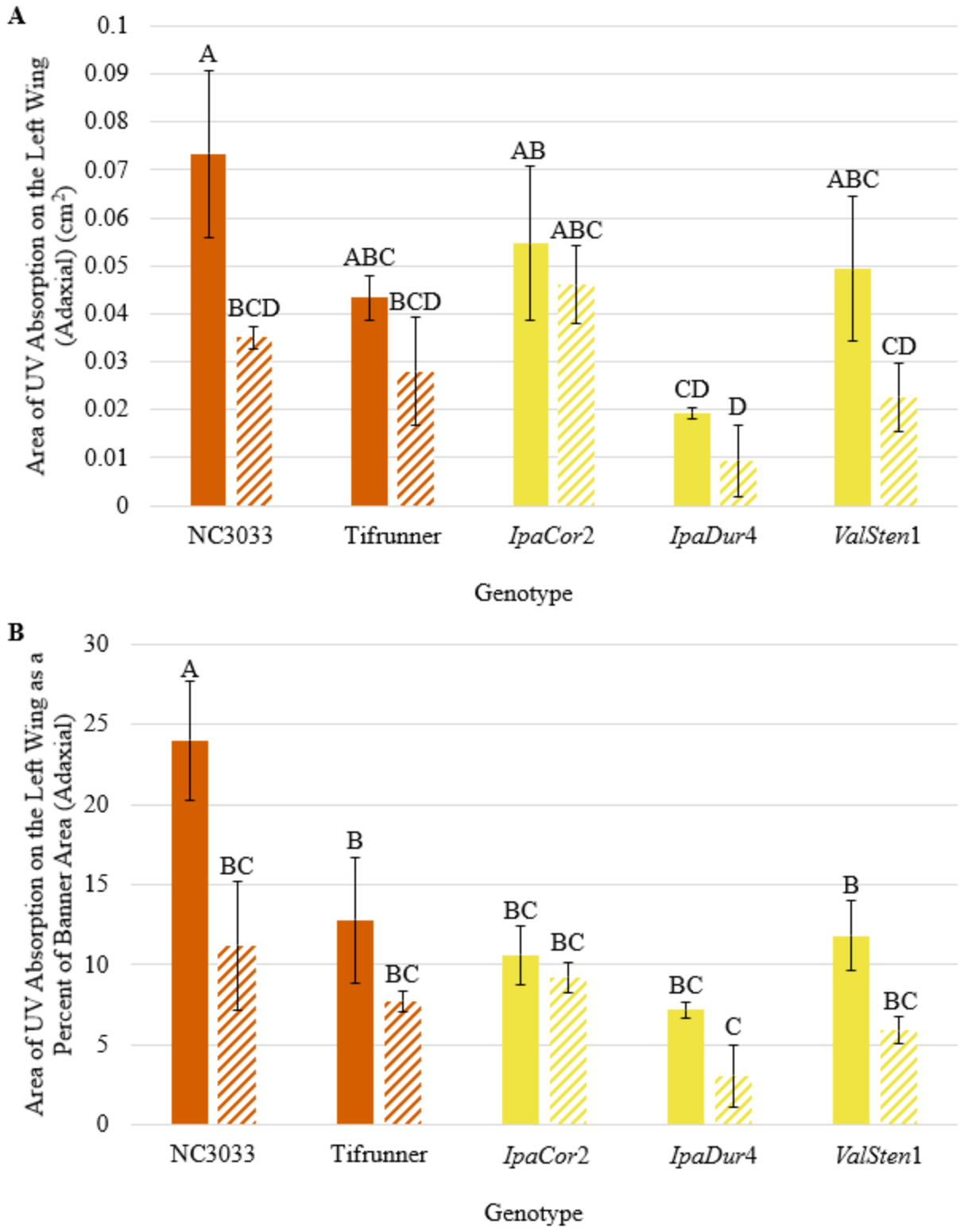

Figure S4.3. A) Area of UV absorption on the left wing (adaxial) and B) area of UV absorption

as a percentage of left wing area (adaxial) of cultivars (orange) and allotetraploids (yellow) in the field (solid) and in the greenhouse (horizontal stripes).

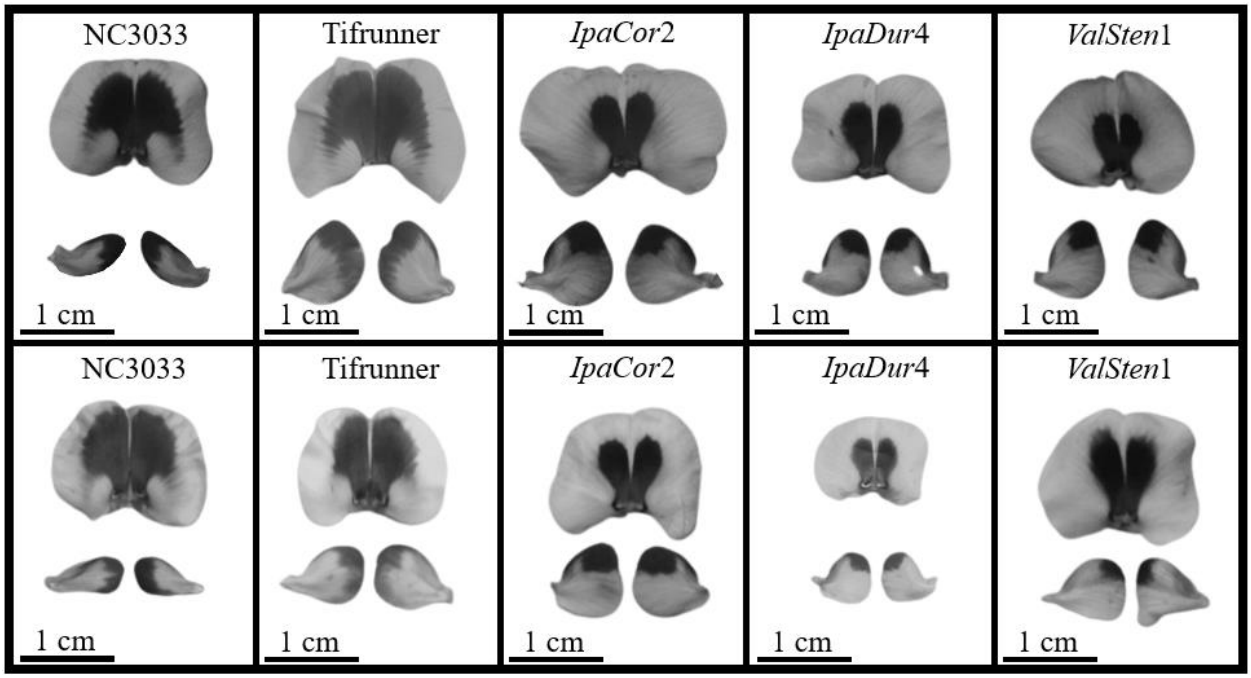

Figure S4.4. UV photos of dissected flowers in which the top row was collected from Black Shank farm and the bottom row was collected from the greenhouse.

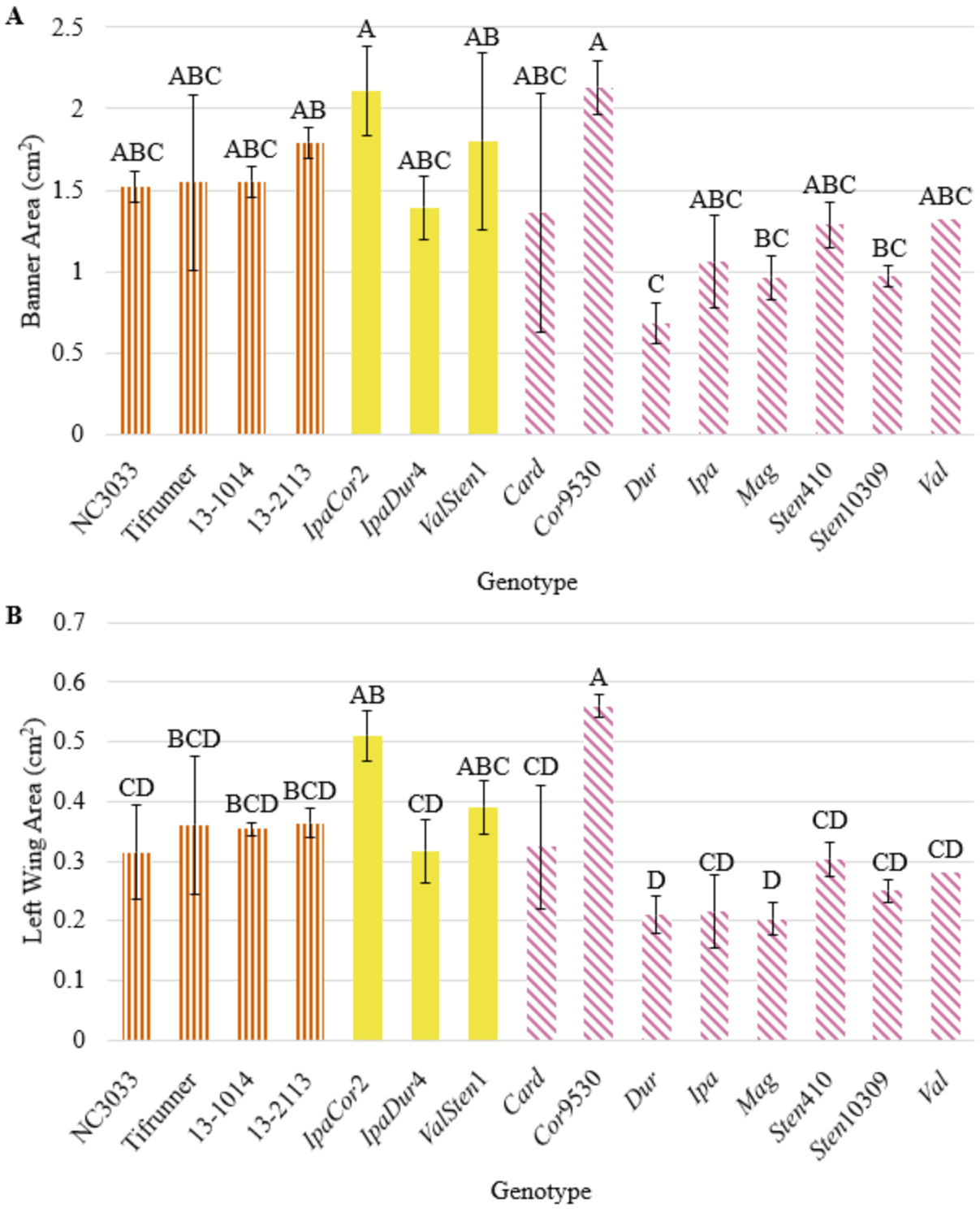

Figure S4.5. A) Banner area and B) left wing area of cultivars (orange vertical stripes),

allotetraploids (solid, yellow), and wild peanut species (pink horizontal stripes) collected from the greenhouse.

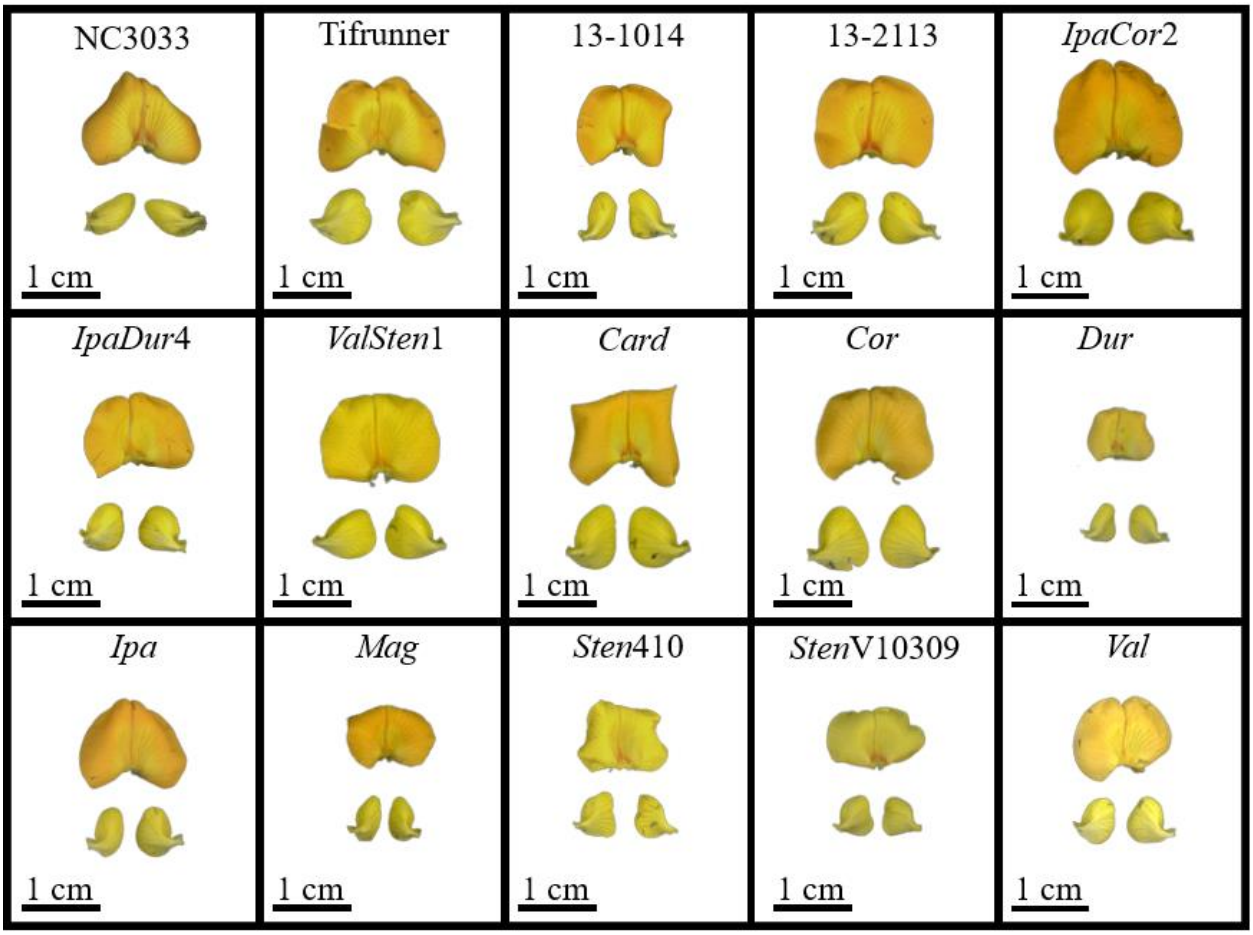

Figure S4.6. Scans of dissected flowers from cultivars, allotetraploids, and wild *Arachis* species collected from the greenhouse.

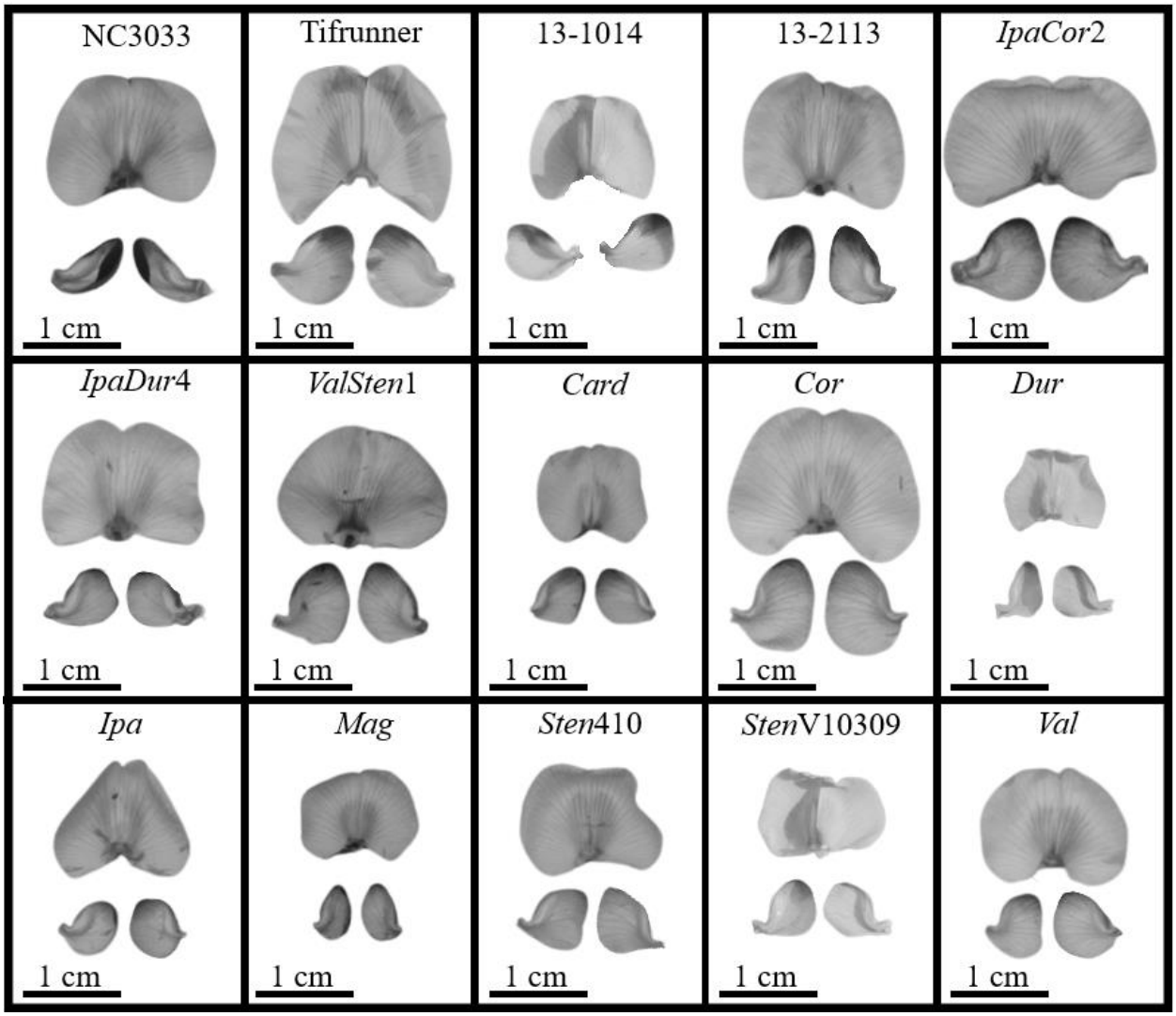

Figure S.7. UV photos of dissected flowers from cultivated peanut genotypes, allotetraploids, and wild *Arachis* species collected from the greenhouse showing the abaxial side of the banners and adaxial side of the wings.

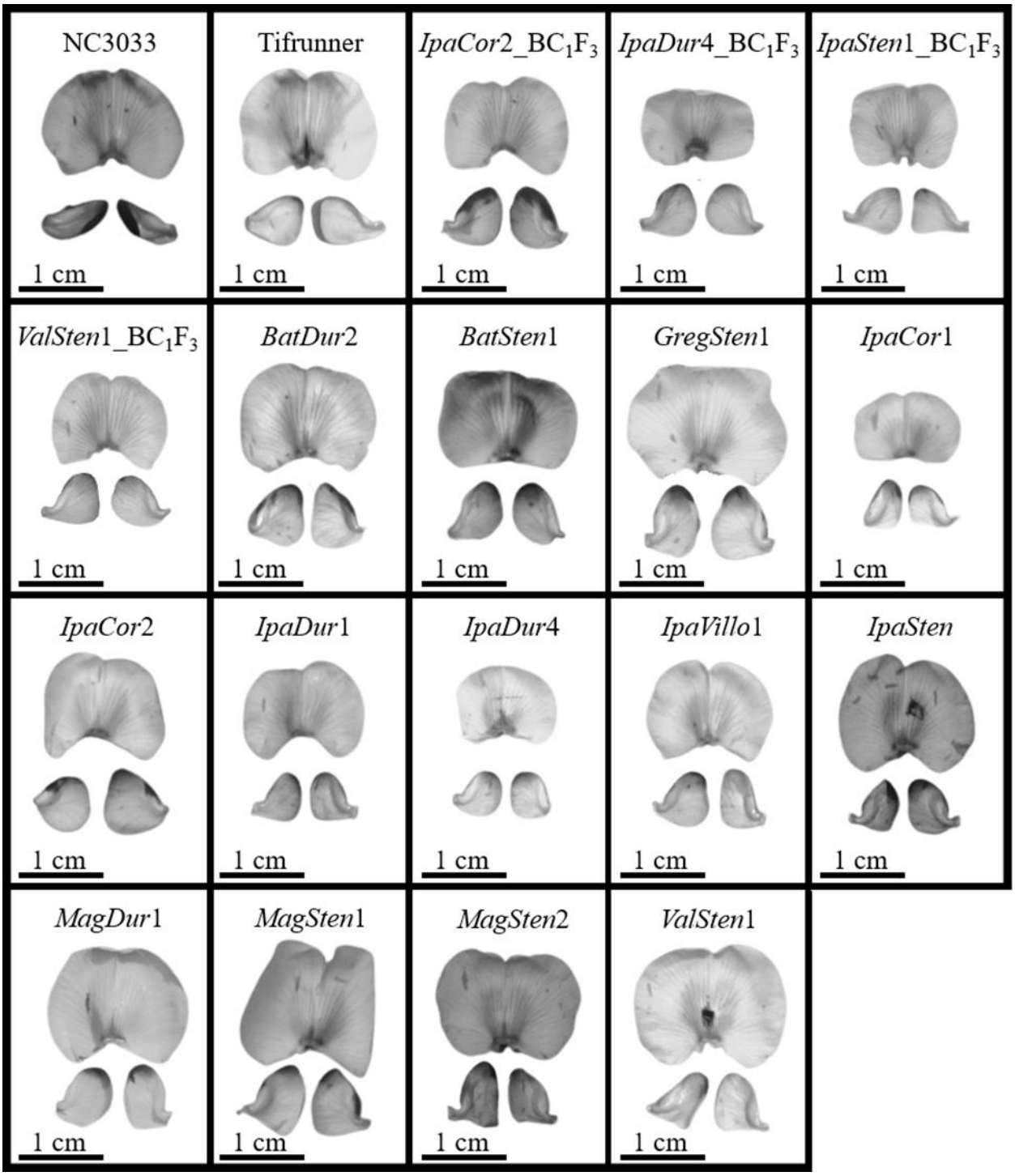
NC3033
Tifrunner
IpaCor2_BC1F3
IpaDur4_BC1F3
IpaSten1_BC1F3
ValSten1_BC1F3
BatDur2
BatSten1
GregSten1
IpaCor1
IpaCor2
IpaDur1
IpaDur4
IpaVillo1
IpaSten
MagDur1
MagSten1
MagSten2
ValSten1
1 cm

Figure S4.8. UV photos of dissected flowers from peanut cultivars, BC_1F_3 lines, and allotetraploids collected from the greenhouse showing the abaxial side of the banners and adaxial side of the wings.

Figure S4.9. UV photograph of a flower of a cultivated peanut breeding line surrounded by UV-absorbing peanut foliage.

CHAPTER 5

RESISTANCE TO FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) FEEDING IDENTIFIED IN NASCENT ALLOTETRAPLOIDS CROSS-COMPATIBLE TO CULTIVATED PEANUT (*ARACHIS HYPOGAEA L.*)

[1] C.M. Levinson, K.M. Marasigan, Y. Chu, H.T. Stalker, C.C. Holbrook, X. Ni, W.P. Williams, and P. Ozias-Akins. 2020. Peanut Science 47(3):123-134. Reprinted here with permission of publisher.

Abstract

Fall armyworm (FAW) is an economically devastating, invasive pest in Sub-Saharan Africa and can be a major pest in the Americas. This pest feeds on more than 80 plant species, including peanut, and threatens the food security of millions of people who rely on these crops in Sub-Saharan Africa. An integrated pest management strategy, including resistant crop cultivars, is needed to control FAW, since populations have been reported to develop insecticide resistance. Genetic sources of host resistance to FAW are limited in cultivated peanut; however, strong resistance to FAW was reported previously in peanut wild relatives. In this *in vitro* study, we tested diploid peanut relatives including *A. ipaensis* KG37006 (*Ipa*), *A. duranensis* 30060 (*Dur*), *A. correntina* 9530 (*Cor*9530), and *A. correntina* 9548 (*Cor*9548); allotetraploids including *IpaCor*9530^{4x}, *IpaDur*^{4x}; F_2 hybrids [*A. hypogaea* 13-1014 x *IpaCor*9530^{4x}]; and cultivated peanut lines *A. hypogaea* '13-1014' and 'Georgia Green' for FAW resistance to identify valuable materials in our breeding program. FAW development was measured by survival, larval weight, larval stage duration, pupation, pupal stage duration, moth emergence relative to pupation, and moth sex. All allotetraploids showed promise as donors for FAW resistance. This FAW resistance was derived primarily from *A. ipaensis*, but *A. duranensis* was also identified as a source of resistance, though more moderate. A high level of heterogeneity was found in *A. correntina* 9530, which likely contributed to the variable performance of this species in the bioassay. Producing hybrids and allotetraploids with wild *Arachis* species does not guarantee that each derived line from these crosses will be resistant, and since these lines are segregating, selection for resistance is still needed.

Introduction

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a major defoliating pest in the Americas and has recently become an economically devastating, invasive pest in Sub-Saharan Africa (Sparks, 1986; Goergen *et al*., 2016), as well as in Asia. FAW feeds on more than 80 economically important crops, including peanut. A thirteen-billion-dollar loss to FAW infestation was reported for maize, sorghum, rice, and sugarcane in Africa from 2016 to 2017 (Abrahams et al., 2017). Without control, FAW is a major defoliator that can cause high yield reductions, seriously affecting production in developing countries with limited access to pesticides and safety equipment to apply them properly (Cock et al., 2017; Day et al., 2017; Bateman et al., 2018; CABI, 2020). Producing FAW resistant cultivars will reduce reliance on pesticides, increase food security, and promote sustainable agriculture in these at-risk countries. In addition, resistant cultivars are needed to mitigate the insecticide resistance reported in FAW populations (Yu, 1991).

Genetic sources with strong FAW resistance are limited in cultivated peanut due to its narrow genetic base (Stalker et al., 2016). Peanut is an allotetraploid species (AABB; $2n=4x=40$), and evidence suggests that it arose from a natural polyploidization event involving hybridization of the diploid species *A. ipaensis* (BB; $2n=2x=20$) and *A. duranensis* (AA; $2n=2x=20$) (Bertioli et al., 2016). The difference in ploidy levels between cultivated peanut and its wild relatives results in crossing barriers and constricts genetic diversity in peanut. Moderate levels of host resistance to FAW have been found in a few peanut cultivars such as ‘Southeastern Runner 56-15’ (Hammons 1970, Leuck and Skinner 1971), ‘Florunner’ and ‘Tifton 8’ (Todd et al., 1991). On the other hand, more than 80 wild peanut relatives have diverse levels of resistance against a wide range of peanut insect pests and diseases (Stalker et al., 2016; Stalker, 2017).

Wild peanut relatives, such as *Arachis cardenasii*, already have proved to be useful sources of resistance to multiple pests including late leaf spot, early leaf spot, root-knot nematode, Cylindrocladium black rot, and Sclerotinia blight (Simpson and Starr, 2001; Stalker and Mozingo, 2001; Gowda et al., 2002; Simpson et al., 2003; Tallury et al., 2014). Previous studies have identified wild peanut relatives such as *A. cardenasii*, *A. correntina*, and *A. villosa* as potential donors of strong FAW antibiosis and antixenosis (Painter, 1951; Kogan and Ortman, 1978; Lynch et al., 1981; Ortega et al., 2016). These *Arachis* species displayed antibiosis through high FAW mortality rates and inhibition of FAW development and displayed antixenosis due to FAW non-preference to feed on these materials (Lynch et al., 1981). In addition, *A. ipaensis* showed antibiosis through a high FAW mortality rate (Yang et al., 1993). However, further work has not resulted in FAW resistance being introgressed from these wild relative species into a cultivated background useful for peanut breeders. This study identified FAW resistance in newly created allotetraploids that are cross compatible to cultivated, allotetraploid peanut. Resistant allotetraploid lines identified in this study can be used in breeding programs in the United States and shared with breeding programs in East and West Africa to introgress FAW resistance into elite cultivars. The long-term goal of this study is to create FAW resistant peanut cultivars that can protect yields in the United States and increase yields in regions with limited access to pesticides in Africa.

Materials and Methods

Plant Materials

Wild *Arachis* species, *A. ipaensis* Krapov. and W.C. Gregory (PI 468322, GKBSPSc 30076; abbrev.: *Ipa*), *A. duranensis* Krapov. and W.C. Gregory (PI 468197, GKBSPSc 30060; abbrev.: *Dur*), *A. correntina* (Burkart) Krapov. and W.C. Gregory (PI 262808, GKP 9530;

abbrev.: *Cor*9530) and (PI 262881, GKP 9548; abbrev.: *Cor*9548) were grown and the diploid hybrids generated in 2016 at North Carolina State University (NCSU). *IpaDur*^{4x} and *IpaCor*9530^{4x} allotetraploids were created from the diploid hybrids by colchicine treatment of F_1 hybrid cuttings at the University of Georgia (UGA) Tifton Campus. The susceptible *A. hypogaea* controls included 'Georgia Green' (Branch, 1996) and '13-1014,' a breeding line selected from [(C1805-617-2 x 'Florida-07' (Gorbet and Tillman, 2009)) x 'Georgia-06G' (Branch, 2007)]. C1805-617-2 is a selection from 'Tifguard' (Holbrook et al., 2008) x 'Florida-07'.

Due to limitations of labor and pesticide-free plants required for bioassay, the resistance evaluation was performed in three experiments with *Ipa* and allotetraploids with *Ipa* as one parent being tested in all of the experiments (Table 5.1). The first experiment, aimed to test the level of resistance in *IpaDur*^{4x}, was conducted in July 2018 and included seven genotypes as treatments: *Ipa*, *Dur*, Georgia Green, and four S_2 generation *IpaDur*^{4x} plants. The allotetraploids were each designated an arbitrary number to make distinguishing them easier (Table 5.1). *IpaDur*_1 and *IpaDur*_2 originated from the same *IpaDur*_$S_{0:1}$ plant, while *IpaDur*_3 and *IpaDur*_ 4 originated from the same *IpaDur*_$S_{0:5}$ plant. The second experiment was designed to test the level of resistance in *IpaCor9530*^{4x}; it was carried out in May 2019 and included 10 genotypes as treatments: *Ipa*, *Cor*9530, *Cor*9548, *A. hypogaea* 13-1014, three S_1 generation *IpaCor9530*^{4x} plants, and three F_1 plants made from a cross between *A. hypogaea* 13-1014 and three different S_0 *IpaCor9530*^{4x} plants (Table 5.1). *Cor* (PI 261870) was previously reported to have FAW resistance (Lynch et al., 1981), but was not included in this study due to lack of seed supply. The third experiment was conducted in November 2019 to directly compare the level of resistance detected in materials from the first two experiments. The third experiment included eight genotypes as treatments: *Ipa*, *Dur*, *Cor*9530, Georgia Green, *A. hypogaea* 13-1014, one

IpaDur^{4x} S_2 plant, and two *IpaCor9530*^{4x} S_1 plants (Table 5.1). *IpaDur*_5 was an S_2 plant that originated from one of the *IpaDur*^{4x} lines tested in the July 2018 experiment. *IpaCor*_4 originated from *IpaCor*_1 tested in the May 2019 experiment, while *IpaCor*_5 originated from *IpaCor*_2. In this last experiment, four genotypes were terminated early because the plants grew slowly in the short-day length season and failed to produce enough leaves to complete the test. The earliest date of termination was 17 DAI (days after infestation) for genotype *Dur*.

Fall Armyworm Resistance Evaluation

For each of the three experiments, seeds were coated in Vitavax PC (Vitavax, Crompton, Middlebury, CT) and treated overnight in 0.5% Florel Growth Regulator (Lawn and Garden Products Inc., Fresno, CA) to break dormancy. Seeds were then planted in #123 7.62 cm round x 11.43 cm deep Jiffy Pots (Harris Seeds, Rochester, NY) and transplanted approximately one month later into 121.92 cm round x 27.94 cm deep pots filled with Promix growth medium **(**Premier Tech Horticulture, Quakertown, PA). Normal plant management was applied in the greenhouse except that insecticide treatments were withheld.

When plants were three months old, FAW eggs (corn strain) were obtained from USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS. Thirty replications were tested for each genotype, in which one replication comprised one FAW larva fed on the excised leaflets from a genotype. Within 24 h of egg hatching, the neonate larvae were gently transferred with a soft tip paintbrush from the bag of FAW egg masses onto a fresh, newly emerged leaflet placed in a 100 mm x 15 mm Petri dish (ThermoFisher Scientific). Each Petri dish contained a sheet of 9 cm diameter Whatman No. 1 filter paper (ThermoFisher Scientific) supported by a cotton round (Equate Beauty) that was saturated with approximately 4 ml of deionized water. The day that the neonate larvae were transferred onto the leaflets was considered day 0 after

infestation (0 DAI). Petri dishes were sealed with Parafilm® M laboratory film (Bemis Company, Inc.) for the first week to prevent the FAW from escaping. For the next three days, Petri dishes with dead FAW larva were replenished with another neonate larva. On the day a Petri dish was restarted with a new FAW larva, that day was considered 0 DAI for that particular plate. The Petri dishes were examined daily to relocate FAW larvae that may have moved off of the leaflets and onto or underneath the filter paper. Daily inspection ensured that FAW larvae would have the opportunity to feed on plant material, and that the filter paper and cotton pad could be moistened if they had become dry. Fresh leaflets were offered every other day for one week, then fresh leaves were offered daily to meet the need of increased larval feeding. The moistened cotton pad and filter paper were changed as the frass from FAW larva accumulated and contaminated the filter paper.

The following parameters were evaluated to study the effect of plant genotype on FAW growth and development: survival, larval weight, larval stage duration, pupation, pupal stage duration, moth emergence relative to pupation, and moth sex. Low survival, larval weight, pupation, and emergence as well as high larval stage duration and pupal stage duration indicated deceleration of normal FAW growth and development. Sex ratio was an indicator of genotype effect on FAW fitness in which a deviation from a 1:1 (male to female) indicated a genotypic effect of the host plants on FAW sexual dimorphism. Sex ratio was included, since diet and other environmental effects have been found to negatively affect adult insect, including FAW, sex ratios (Bull, 1981; Murúa and Virla, 2004). Biased sex ratio could be utilized as one of the valuable tactics for sustained pest management in multiple cropping systems. FAW survival was documented daily. Larval weight was recorded at 14 DAI. Larval stage duration documented the number of days from first-instar larva stage to pupal formation. Pupation and relative moth

emergence described completed pupation and moth emergence relative to the number of pupa formed, respectively. Pupal stage duration denoted the number of days recorded from pupal development to moth emergence. Pupal weight was recorded on the day of complete pupal formation. Moth sex (male or female) was determined after emergence.

Genotyping the diploid species

Cor demonstrated morphological diversity; therefore, we genotyped the diploid species using the Affymetrix Axiom_*Arachis*2 SNP array (Clevenger et al., 2018; Korani et al. 2019) consisting of 47,000 features (ThermoFisher Scientific). DNA was extracted by the Qiagen DNeasy Plant mini kit (Qiagen, Germantown, MD). SNP calling was performed with Axiom Analysis Suite (Version 1.2). Genetic markers were grouped into six categories by the software depending on the quality and separation of markers 1) Monomorphic 2) PolyHighResolution 3) NoMinorHom 4) OfftargetVariant 5) CallRateBelowThreshold 6) Other. Only the 5,342 markers in the PolyHighResolution class were used for analysis since the grouping of samples was unambiguous and all the samples passed quality control.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using JMP software (SAS Institute) to determine the genotype effect on FAW growth and development according to the following parameters: larval weight, larval stage duration, pupal stage duration, and pupal weight. Chi-squared tests were performed with JMP software to determine the genotype effect on the following categorical parameters used to assess FAW development: survival at 8 DAI and 14 DAI (dead or alive), pupation (pupated or did not pupate), relative moth emergence (emerged or did not emerge), and moth sex (male or female). For relative moth emergence, data (emerged or did not emerge) was only included for FAW that pupated. Means of each parameter among the

treatments were separated based on the Tukey's Test ($\alpha = 0.05$) results with JMP software. Tukey's Test for binomial data was not supported with JMP software, so RStudio (RStudio, Inc.) was used by running genotype effect on survival at 8 DAI and 14 DAI, pupation and relative moth emergence as a generalized linear model and then Tukey's Test ($\alpha = 0.05$) was performed on the model results. Genotypes with less than four replications for a measurement were excluded from statistical analysis. Therefore, larval stage duration, pupation, pupal weight, pupal stage duration, relative moth emergence, and moth analysis only included eight genotypes for the May 2019 experiment with *Ipa* and *IpaCor*_3 excluded and only four genotypes for the November 2019 experiments with *Cor*, *Dur*, *Ipa*, and *IpaDur*_5 excluded due to being terminated before pupal formation. The data for both the May and November 2019 experiments were still valid, since they were maintained according to experimental design and the controls were continued.

Results

Survival

Significant genotypic effect on FAW survival at 8 DAI and 14 DAI was found for the May 2019 experiment and the November 2019 experiment, but not for the July 2018 experiment (Table 5.2). However, Tukey-Kramer significant differences between genotypes were not found for neither 8 DAI nor 14 DAI for all three experiments (Table S5.1). In all three experiments, the cultivated control genotypes had the highest FAW survival (Fig. 5.1). For the July 2018 experiment (Fig. 5.1A), the allotetraploid *IpaDur*_2 had the greatest FAW mortality. Only 30% (9 of the 30) of FAW fed exclusively on *IpaDur*_2 survived to the end of the experiment and completed their life cycle. The larval survival curves on *Ipa* and *Dur* were below that of the susceptible cultivated peanut control.

Unlike the other two experiments, the May 2019 experiment (Fig. 5.1B) had a high mortality of FAW larvae just four days after the onset of the experiment. This is likely due to a trained entomologist performing the July 2018 and November 2019 experiments. However, all allotetraploids had survival curves below the susceptible control. Both accessions of *A. correntina* were similar to the susceptible control, although *Cor*9530 lost five more FAW larvae (17% more) than *Cor*9548.

In the November 2019 experiment (Fig. 5.1C), *Ipa*, *Dur*, *Cor*, and *IpaDur*_5 had to be terminated early due to insufficient plant material to sustain the FAW feeding. However, FAW larvae fed on susceptible controls showed almost identical survival curves as the previous two experiments. The FAW larva survival curve on *Cor*9530 closely followed survival curves of the susceptible controls. *IpaCor*_4, a progeny line from *IpaCor*_1 that showed high FAW mortality in the May 2019 experiment (Fig. 5.1B), also had a survival curve similar to the controls. *Ipa* and *Dur* had similar survival curves to the July 2018 experiment (Fig. 5.1C) until the treatments were terminated. *IpaCor*_5 (Fig. 5.1C), a progeny line from *IpaCor*_2 (Fig. 5.1B) that had high FAW mortality in the May 2019 experiment, had the greatest FAW mortality in the November 2019 experiment.

Larval Weight

Genotype had a significant effect on larval weight in all of the experiments (Table 5.2). All five *IpaDur*^{4x} allotetraploid plants significantly reduced FAW larval weight compared to the cultivated peanut genotypes included as experimental controls (Fig. 5.2). For the July 2018 experiment, the allotetraploid *IpaDur*_2 that had the greatest FAW mortality also had the numerically lowest larval weight (Figs. 1A, 2A). As for *IpaCor9530*^{4x}, three out of five tested allotetraploid plants (*IpaCor*_1, *IpaCor*_4, and *IpaCor*_5) significantly suppressed larval weight

(Figs. 2B, C). The other two *IpaCor9530*^{4x} plants (*IpaCor*_2 and *IpaCor*_3) also reduced larval weight, yet the level of reduction did not show statistical significance when compared to the cultivated peanut controls (Fig. 5.2B). This is partly due to high mortality of FAW larvae in the May 2019 experiment (Fig. 5.1B), which led to small sample size, and affected statistical power for larval weight data (Fig. 5.2B). For example, while the larval weight on *IpaCor*_1 was 35% of the control and found to be significantly different, the *Ipa* larval weight was only 13.5% of the control but not significantly different due to the small sample size caused by mortality of FAW larvae on *Ipa* leaves. When the two allotetraploid lines were tested side-by-side in the November 2019 experiment (Fig. 5.2C), *IpaDur*_5 demonstrated a stronger level of FAW resistance by reducing FAW larval weight to a greater extent than *IpaCor9530*_4 and *IpaCo*r9530_5.

Diploid parents of both allotetraploid lines were included in this study to determine the source of FAW resistance. Compared to the cultivated peanut control, *Ipa* significantly suppressed FAW weight gain in the July 2018 and November 2019 experiments (Figs. 2A, C). *Dur* also significantly reduced the weight of FAW larvae but to a numerically lesser extent than that of *Ipa* in the November 2019 experiment. Conflicting results were found between the May 2019 (Fig. 5.2B) and November 2019 (Fig. 5.2C) experiments for *Cor*9530. A significant, positive effect on larval weight was observed in the May 2019 experiment (Fig. 5.2B) and a significant suppressive effect was found in the November 2019 experiment (Fig. 5.2C). *Cor*9548 was tested only in one experiment (Fig. 5.2B) and it was found to increase larval weight gain. A high level of genetic heterogeneity was found in *Cor*9530. Among the three genotyped *Cor*9530 plants, 1,259 out of 5,342 total markers (23.5%) were found to be polymorphic. On the contrary, only 23 (0.4%) and 27 (0.5%) polymorphic markers were found with *Ipa* and *Dur*, respectively. Therefore, the conflicting feeding response on *Cor*9530 could be due to the genetic diversity

within this accession. In the May 2019 experiment (Fig. 5.2B), three 13-1014 x *IpaCor* F_2 plants were found to be segregating for larval weight. The (13-1014 x *IpaCor*_1)_F_2 plant demonstrated a significantly greater increase in larval weight compared to the cultivated control. The (13-1014 x *IpaCor*_2)_F_2 plant also showed a similar effect on larval weight compared to the cultivated control. The (13-1014 x *IpaCor*_3)_F_2 plant demonstrated high suppression of FAW growth similar to the *IpaCor9530*^{4x}.

Larval Stage Duration

Genotype had a significant effect on larval stage duration in all of the experiments (Table 5.2). In all three experiments, the cultivated controls had the shortest larval stage duration (Fig. 5.3). Compared to the cultivated control, *Ipa* significantly reduced larval growth and development by extending larval stage duration in the July 2018 experiment (Fig. 5.3A). Likewise, all allotetraploids significantly extended larval stage duration, except for *IpaDur*_1 in the July 2018 experiment (Fig. 5.3A). *IpaCor*_5, the allotetraploid with the greatest FAW mortality in the November 2019 experiment (Fig. 5.1B), significantly outperformed *IpaCor*_4 by greatly extending larval stage duration (Fig. 5.4B). While *Ipa* significantly extended larval stage duration, *Dur*, *Cor*9530, and *Cor*9548 did not differ significantly from the cultivated controls.

Pupation

Genotype had a significant effect on pupation in all of the experiments (Table 5.2). For the July 2018 and May 2019 experiments, Tukey-Kramer significant differences between genotypes were not found; however, 13-1014 had significantly more pupated FAW than *IpaCor*_5 (Table S5.1). Across all the experiments, the cultivated controls had the highest number of FAW to pupate (Fig. 5.4). For the July 2018 experiment, *IpaDur*_2, which had the highest FAW mortality (Fig. 5.1A), also had the fewest FAW to pupate (Fig. 5.4A). For the May

2019 experiment (Fig. 5.4B), pupation was heavily influenced by FAW mortality early in the experiment. Despite this artifact, all allotetraploids had a lower number of FAW to pupate than the cultivated control. Both *Cor*9530 and *Cor*9548 had a similar number of FAW to pupate as compared to the cultivated control. Line (13-1014 x *IpaCor*_1)_F_2 also had a similar number of FAW to pupate as compared to the cultivated controls, while (13-1014 x *IpaCor*_2)_F_2 and (13-1014 x *IpaCor*_3)_F_2 had much lower numbers of FAW to pupate as compared to the cultivated controls. For the November 2019 experiment, *IpaCor*_4 had a similar number of FAW to pupate as the cultivated controls (Fig. 5.4C), while *IpaCor*_5 had significantly less pupa form as compared to 13-1014 (Table S5.1).

Pupal Weight

Genotype had a significant effect on average pupal weight across all experiments (Table 5.2). All allotetraploids, except *IpaCor*_1 in the May 2019 experiment, had significantly lower pupal weight than the cultivated controls (Fig. 5.5). *IpaDur*_3 and *IpaDur*_4 had significantly lower pupal weight than *IpaDur*_1 (Figs. 5A, C). *IpaCor*_2 had the lowest pupal weight of all the allotetraploids in the May 2019 experiment (Fig. 4.5B). *IpaCor*_5, the progeny line of *IpaCor*_2, had the lowest pupal weight in the November 2019 experiment (Fig. 5.5C). *Ipa*, *Dur*, and *Cor*9548 all had significantly lower pupal weight than the respective cultivated control. However, *Cor*9530 was not statistically different from the cultivated control in the May 2019 experiment (Fig. 5.5B).

Pupal Stage Duration

Genotype had a significant effect on pupal stage duration in the July 2018 experiment and the May 2019 experiment, but not in the November 2019 experiment (Table 5.2). Across all three experiments, *Ipa*, *Dur*, *Cor*9530, and *Cor*9548 were not significantly different from the

cultivated controls (Fig. 5.6). However, *IpaCor*_1 and (13-1014 x *IpaCor*_2)_F_2 had significantly longer pupal stage duration than the cultivated control, indicating these materials impeded pupal stage development (Fig. 5.6B).

Relative Moth Emergence

Genotype had a significant effect on relative moth emergence in the November 2019 experiment but not the July 2018 or May 2019 experiments (Table 5.2), and Tukey-Kramer significant differences between genotypes for relative moth emergence were not found for any of the three experiments (Table S5.1). All of the allotetraploids, except for *IpaCor*_2 and *IpaCor*_4 (Fig. 5.7B, C), supported less relative moth emergence than the cultivated controls (Fig. 5.7). Allotetraploids had a high number of aborted pupae with the highest being 38.5% (5 out of 13 pupa) for *IpaCor*_5, then 25% (5 out of 20 pupa) for *IpaDur*_4, and 18.9% (4 out of 22) for *IpaDur*_3 (Fig. 5.7A, C). In comparison, Georgia Green had 4.2% (1 out of 24) and 7.1% (2 out of 28) aborted pupa in the July 2018 and November 2019 experiments, respectively (Fig. 5.7A, C). *Arachis hypogea* 13-1014 had 7.1% (2 out of 28) and 6.9% (2 out of 29) aborted pupa in the July 2018 and November 2019 experiments, respectively (Fig. 5.7A, C). The overall low number of 8 moths formed on *IpaCor*_5, a progeny line of *IpaCor*_2, was due to early FAW mortality and high pupa abortion (Figs. 5.1B, 5.7B). All larvae fed on *Ipa*, *Dur*, *Cor*9530, and *Cor*9548 showed similar relative moth emergence as compared to the cultivated controls. Also, larvae fed on *Cor*9530 showed similar relative moth emergence as compared to the larvae fed on *Cor*9548 (Fig. 5.7B). Lastly, the (13-1014 x *IpaCor*)_F_2 plants all had similar relative moth emergence when compared to the control (Fig. 5.7B).

Moth Sex

Genotype did not have a significant effect on moth sex in the July 2018, May 2019, nor November 2019 experiment (Table 5.2).

Discussion

The wild peanut-derived allotetraploid genotypes showed promise as sources for FAW resistance for peanut breeding programs, since all *IpaDur*^{4x} and *IpaCor*9530^{4x} allotetraploids significantly reduced larval weight (except *IpaCor*_2 and *IpaCor*_3), increased pupal stage duration (except *IpaDur*_1), and decreased pupal weight (except *IpaCor*_1) as compared to the controls. All allotetraploid genotypes showed lower survival and pupation than the cultivated genotypes evaluated as well as lower relative moth emergence (except *IpaCor*_2 and *IpaCor*_4). The most promising allotetraploid line, *IpaDur*_2, had the lowest larval weight and the greatest FAW mortality in the July 2018 experiment. This was further supported by *IpaDur*_5, which significantly reduced larval and pupal weight in the November 2019 experiment. *IpaCor*_2 and its progeny *IpaCor*_5 were shown as a promising line as they both suppressed FAW growth in the May and November 2019 experiments. Since segregation for FAW resistance in the allotetraploids was found, further selections for FAW resistance in individual lines will be necessary for effective utilization of these materials in a breeding program.

Primarily *Ipa*, but also *Dur*, were shown to be donor sources for FAW resistance, by reducing larval and pupal weight, survival and pupation. The conclusion that *Ipa* is valuable as a source for FAW resistance confirms a previous report (Yang et al., 1993). However, a 79% mortality rate at 8 DAI was reported for *A. ipaensis* in a similar bioassay with five wild *Arachis* species and *A. hypogaea* (Yang et al., 1993). The bioassay did differ in the use of terminal buds from field-grown plants rather than expanded leaves from greenhouse-grown plants. Two of our three experiments showed generally higher survival for *Ipa* with 8 DAI mortality rates of 16.7%,

80%, and 20% for the July 2018, May 2019, and November 2019 experiments, respectively. The high mortality of FAW fed on *Ipa* leaves in the May 2019 experiment may be an artifact of this experiment having overall higher FAW mortality than the other two experiments (Fig. 5.1), influenced by a trained entomologist performing the July 2018 and November 2019 experiments. Overall, the mean *Ipa* mortality rate was 38.9% across the three experiments. However, the low larval weight of *Ipa* supports the previous conclusions (Yang et al., 1993). Another FAW bioassay study (Lynch et al., 1981) tested 14 wild *Arachis* species, not including *Ipa* and *Dur*, and recommended *Cor* (PI 261870) as a source for FAW resistance. *Cor* (PI 261870) impeded FAW survival and development and also showed antixenosis in a free-choice preference test. We tested *Cor*9530 and *Cor*9548 instead of *Cor* (PI 261870), because *Cor* (PI 261870) seeds were not available. Unlike the previously reported results, conflicting results were found regarding the FAW resistance in *Cor*9530 plants tested in these experiments. Genotyping detected a high level of SNP variation within *Cor9530,* suggesting this diploid species accession is highly heterogeneous, which may explain the contradictory bioassay results of FAW resistance within this species. In addition, *IpaCor*_1, *IpaCor*_2, and *IpaCor*_3 were derived from crosses between *Ipa* and different *Cor9530* individuals, so genetic difference between these lines can be ascribed to parental heterogeneity or genetic segregation in the allotetraploids.

This study builds upon previous reports by focusing on allotetraploids that are cross compatible to *A. hypogaea* and are therefore valuable to breeding programs, instead of focusing solely on diploid, wild *Arachis* species. In the May 2019 experiment, varied levels of FAW resistance were detected among the three tested F_2 lines from 13-1014 x *IpaCor9530*^{4x} cross. The (13-1014 x *IpaCor*_1)_F_2 plant had an even greater larval weight than *A. hypogaea* 13-1014, while the (13-1014 x *IpaCor*_3)_F_2 plant was similar to the allotetraploids, suggesting the FAW

resistance from *IpaCor9530*^{4x} segregated in the F_2 populations. An expanded study of a population derived from the tested (13-1014 x *IpaCor*)_F_2 materials is needed to determine the inheritance pattern and conduct genetic mapping of FAW resistance QTL.

The major limitation of this study is that all three experiments were confined to *in vitro* bioassays using excised peanut leaflets. Due to the required labor for these bioassays that included daily examination of each plate for 44 days per experiment, only a limited number of genotypes could be tested. A progression to this study would be to confirm these results in a field trial and then to map FAW resistance QTL in a mapping population. However, it is difficult to maintain a peanut field with FAW infestation in the southeastern U.S. because of the abundance of natural insect enemies in the peanut fields throughout the growing season and the sporadic nature of FAW as a pest on peanut plants. However, a field study could be performed in collaboration with partners in Africa, where FAW is a newly invasive pest and FAW pressures are high.

Conclusions

This study built upon previous reports by testing FAW resistance in the unique allotetraploids instead of just wild, diploid *Arachis* species alone. The allotetraploids examined in this study showed strong FAW resistance, making them more useful than peanut cultivars previously found to have only moderate levels of FAW resistance. Furthermore, these allotetraploids are cross-compatible with peanut cultivars, making this resistance accessible for peanut breeders. These FAW resistant allotetraploids will be shared with breeding programs in the United States and Africa so that FAW resistance can be introgressed into elite peanut cultivars to effectively reduce the impact of the FAW (as an invasive pest) on peanut production in countries where growers have limited access to pesticides. At the same time, FAW resistant

peanut cultivars can protect yields in the United States, increase yields in regions with limited use of pesticides, decrease reliance on pesticides, and promote organic peanut production and sustainable agriculture in general.

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Table 5.1. Genetic materials tested in each fall armyworm feeding bioassay and their abbreviations and ploidy level.

July 2018 Bioassay: Plant Materials	Abbreviation	Ploidy Level
Georgia Green	**Georgia Green**[a]	Tetraploid
A. ipaensis 30076	***Ipa***	Diploid
A. duranensis 30060	***Dur***	Diploid
IpaDur30060^{4x}_$S_{0:1}$_$S_{1:2}$	*IpaDur*_1	Tetraploid
IpaDur30060^{4x}_$S_{0:1}$_$S_{1:4}$	*IpaDur*_2	Tetraploid
IpaDur30060^{4x}_$S_{0:5}$_$S_{1:2}$	*IpaDur*_3	Tetraploid
IpaDur30060^{4x}_$S_{0:5}$_$S_{1:4}$	*IpaDur*_4	Tetraploid
May 2019 Bioassay: Plant Materials	**Abbreviation**	**Ploidy Level**
A. hypogaea 13-1014	**13-1014**	Tetraploid
A. ipaensis 30076	***Ipa***	Diploid
A. correntina 9530	***Cor*9530**	Diploid
A. correntina 9548	*Cor*9548	Diploid
IpaCor9530^{4x}_$S_{0:2}$_S_1	*IpaCor*_1	Tetraploid
IpaCor9530^{4x}_$S_{0:5}$_S_1	*IpaCor*_2	Tetraploid
IpaCor9530^{4x}_$S_{0:6}$_S_1	*IpaCor*_3	Tetraploid
(13-1014 x *IpaCor*9530^{4x}_$S_{0:2}$)_$F_{1:4}$_F_2	(13-1014 x *IpaCor*_1)_F_2	Tetraploid
(13-1014 x *IpaCor*9530^{4x}_$S_{0:5}$)_$F_{1:4}$_F_2	(13-1014 x *IpaCor*_2)_F_2	Tetraploid
(13-2113 x *IpaCor*9530^{4x}_$S_{0:6}$)_$F_{1:4}$_F_2	(13-1014 x *IpaCor*_3)_F_2	Tetraploid
November 2019 Bioassay: Plant Materials	**Abbreviation**	**Ploidy Level**
Georgia Green	**Georgia Green**	Tetraploid
A. hypogaea 13-1014	**13-1014**	Tetraploid
A. ipaensis 30076	***Ipa***	Diploid
A. duranensis 30060	***Dur***	Diploid
A. correntina 9530	***Cor9530***	Diploid
IpaDur30060^{4x}_$S_{2:1}$	*IpaDur*_5[b]	Tetraploid
IpaCor9530^{4x}_$S_{0:2}$_$S_{1:9}$	*IpaCor*_4[c]	Tetraploid
IpaCor9530^{4x}_$S_{0:5}$_$S_{1:7}$	*IpaCor*_5[c]	Tetraploid

[a]Bolded genotypes were tested in more than one experiment
[b] *IpaDur*_5 originated from one of the *IpaDur* lines tested in the July 2018 experiment
[c] *IpaCor*_4 originated from *IpaCor*_1 and *IpaCor*_5 originated from *IpaCor*_2

Table 5.2. ANOVA and chi-squared test output testing the genotype effect on FAW growth and development according to the following parameters: survival at 8 DAI and 14 DAI, larval weight, larval stage duration, pupation, pupal stage duration, pupal weight, relative moth emergence, and moth sex.

Parameter	F or X^2 value	df(n) [a], df(d) [b]	P-value
Survival at 8DAI (July 2018)	8.66	6	0.19
Survival at 8DAI (May 2019)	92.62	9	< 0.0001***[c]
Survival at 8DAI (Nov. 2019)	26.44	7	0.0004***
Survival at 14DAI (July 2018)	11.81	6	0.07
Survival at 14DAI (May 2019)	117.61	9	< 0.0001***
Survival at 14DAI (Nov. 2019)	35.41	7	< 0.0001***
Larval weight (July 2018)	11.14	6, 156	< 0.0001***
Larval weight (May 2019)	23.60	9, 151	< 0.0001***
Larval weight (Nov. 2019)	29.92	7, 205	< 0.0001***
Larval stage duration (July 2018)	12.13	6, 131	< 0.0001***
Larval stage duration (May 2019)	29.60	7, 124	< 0.0001***
Larval stage duration (Nov. 2019)	38.01	3, 92	< 0.0001***
Pupation (July 2018)	20.94	6	0.0019**
Pupation (May 2019)	82.15	7	< 0.0001***
Pupation (Nov. 2019)	38.24	3	< 0.0001***
Pupal Weight (July 2018)	22.68	6, 131	< 0.0001***
Pupal Weight (May 2019)	14.91	7, 124	< 0.0001***
Pupal Weight (Nov. 2019)	84.12	3, 92	< 0.0001***
Pupal stage duration (July 2018)	2.66	6, 117	.019*
Pupal stage duration (May 2019)	8.92	7, 113	< 0.0001***
Pupal stage duration (November 2019)	1.32	3, 79	0.27
Relative moth emergence (July 2018)	11.45	6	0.077
Relative moth emergence (May 2019)	6.14	7	0.052
Relative moth emergence (Nov. 2019)	10.66	3	< 0.014*

Moth sex (July 2018)	7.32	6	0.29
Moth sex (May 2019)	11.92	7	0.10
Moth sex (Nov. 2019)	4.29	3	0.23

[a]The df(n), degrees of freedom of the numerator, is based on the number of plant genotypes tested

[b]The df(d), degrees of freedom of the denominator, is based on the total number of replicates, in which a replicate is one plate with one FAW, for all plant genotypes tested

[c]*P < .05. **P < .01. ***P < .001

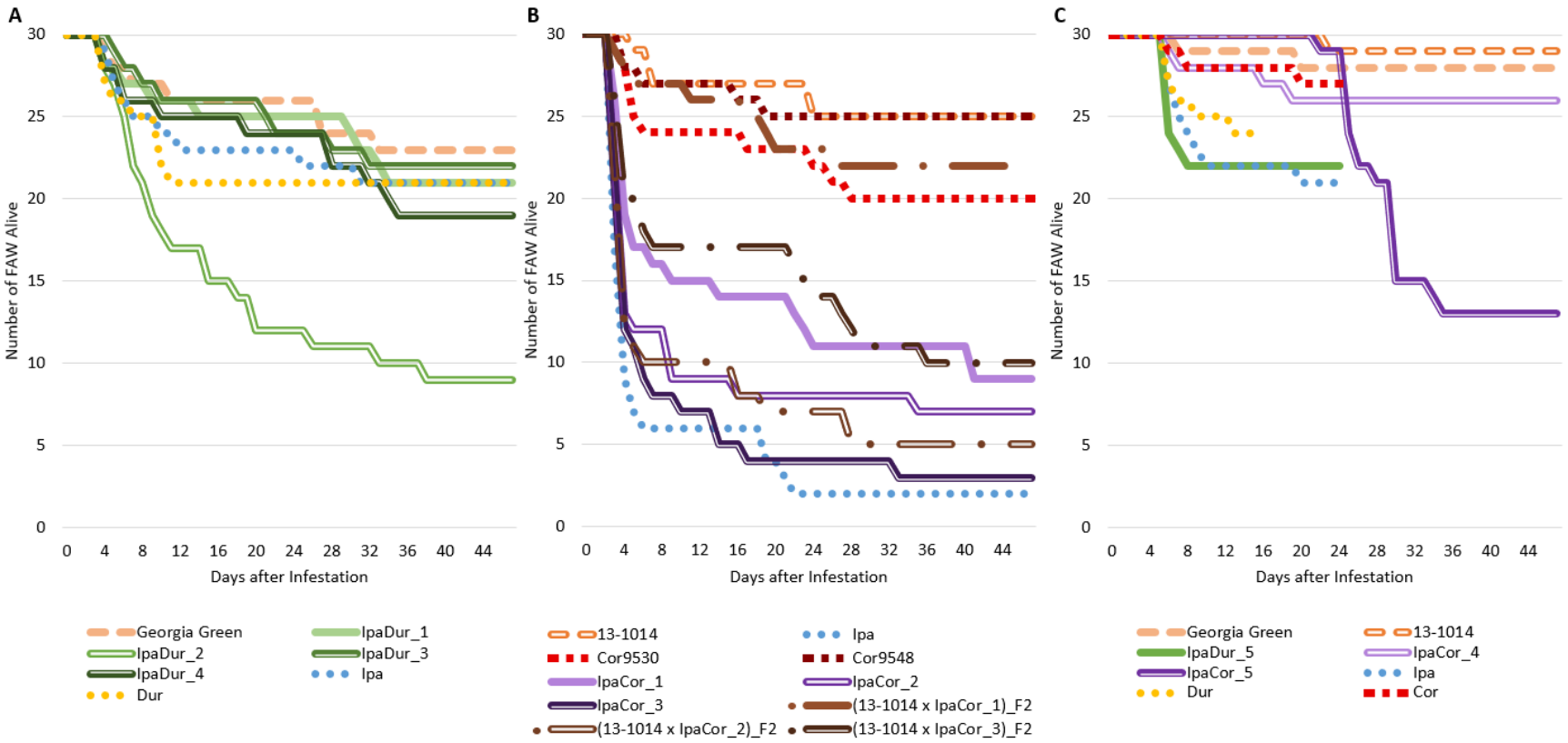

Figure 5.1. Survival of FAW throughout the life cycle during the experimental period. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment.

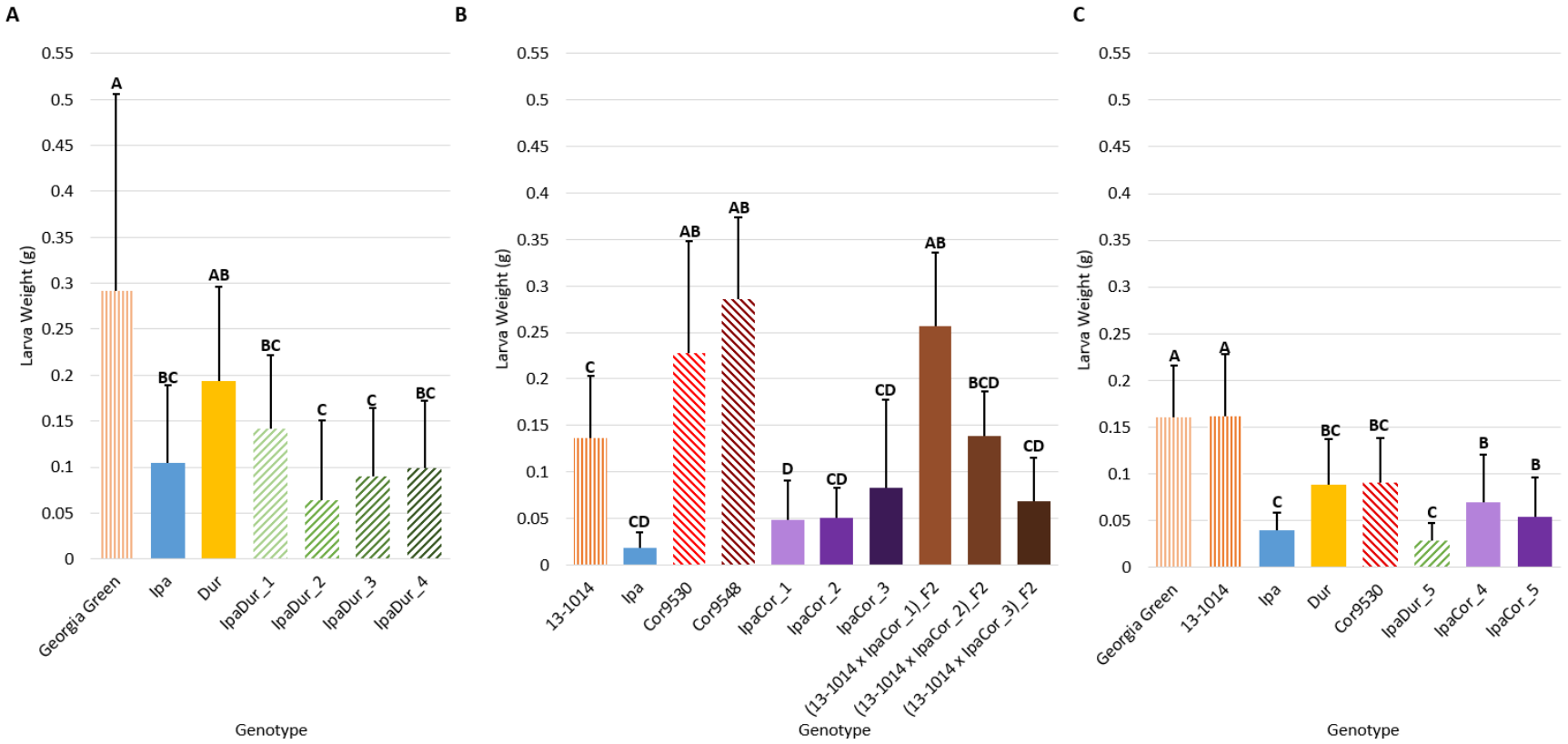

Figure 5.2. Larva weight on day 14 after feeding on different peanut leaflets. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment. Error bars represent standard error. Tukey's HSD significance levels were calculated within each experiment, so these significance groupings cannot be compared between the three experiments. Genotypes within an experiment with the same Tukey's HSD letter are not significantly different ($\alpha = 0.05$).

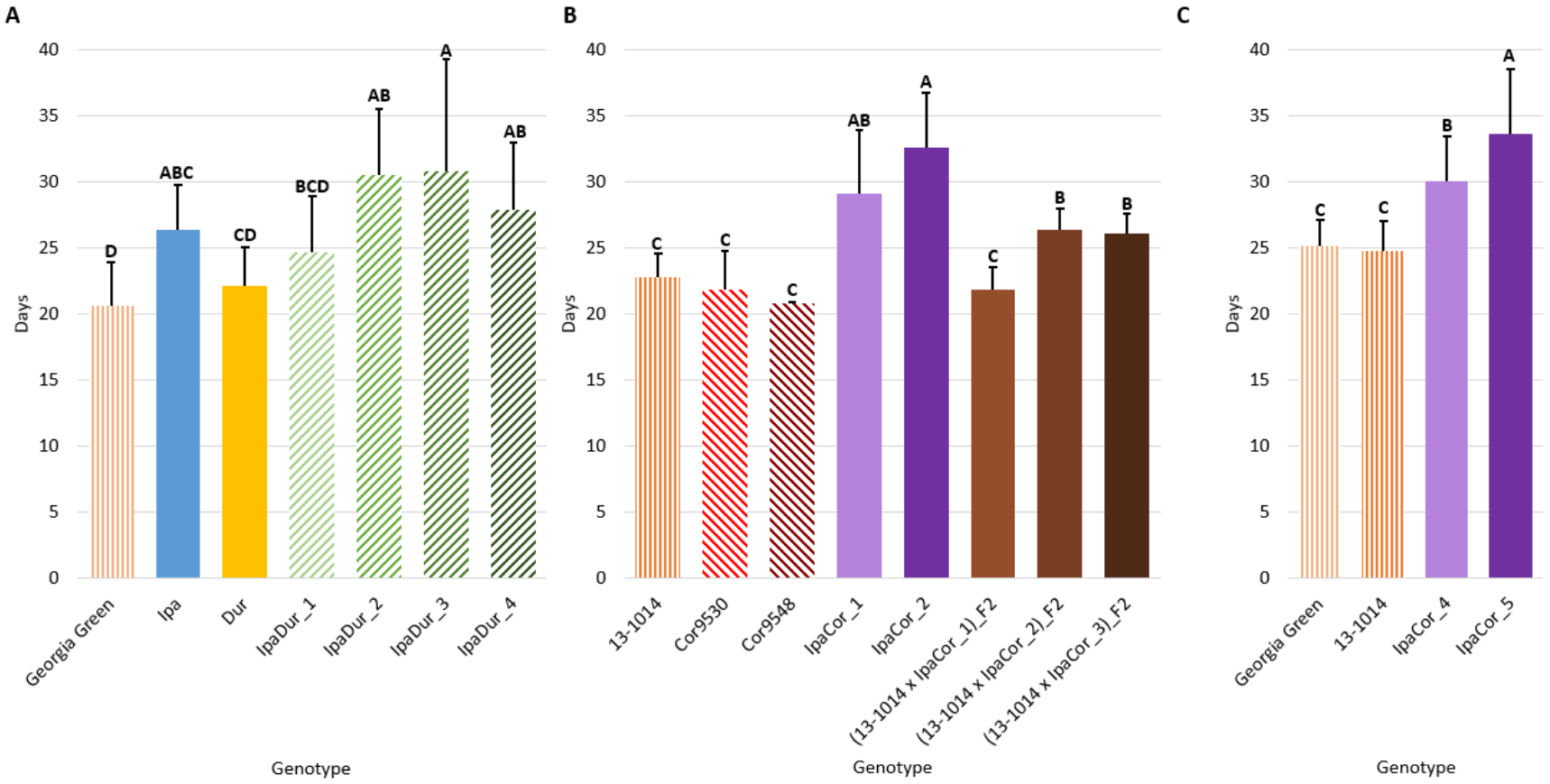

Figure 5.3. Larval stage duration. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment. Error bars represent standard error. Tukey's HSD significance levels were calculated within each experiment, so these significance groupings cannot be compared between the three experiments. Genotypes within an experiment with the same Tukey's HSD letter are not significantly different ($\alpha = 0.05$).

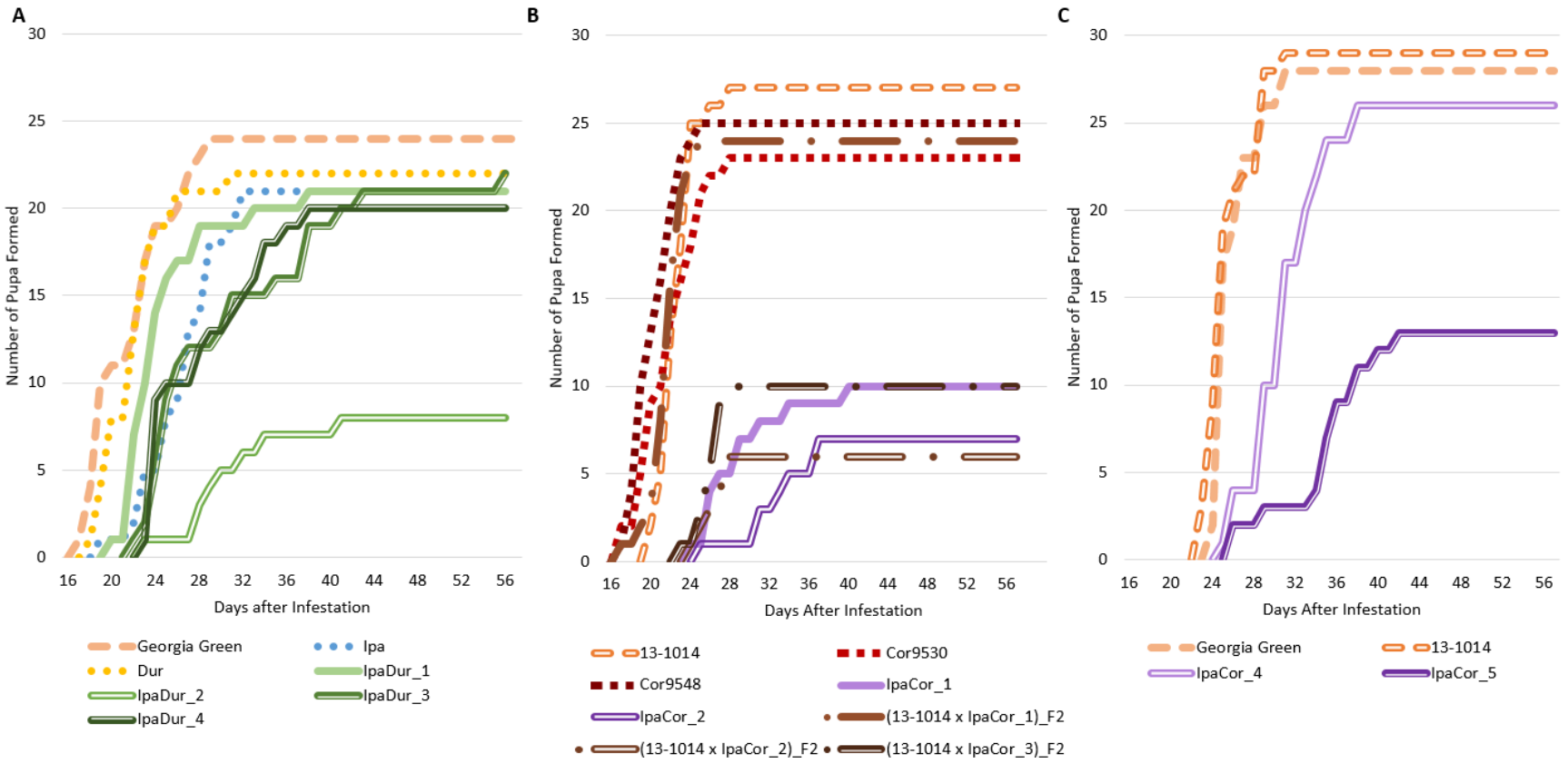

Figure 5.4. Pupation. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment.

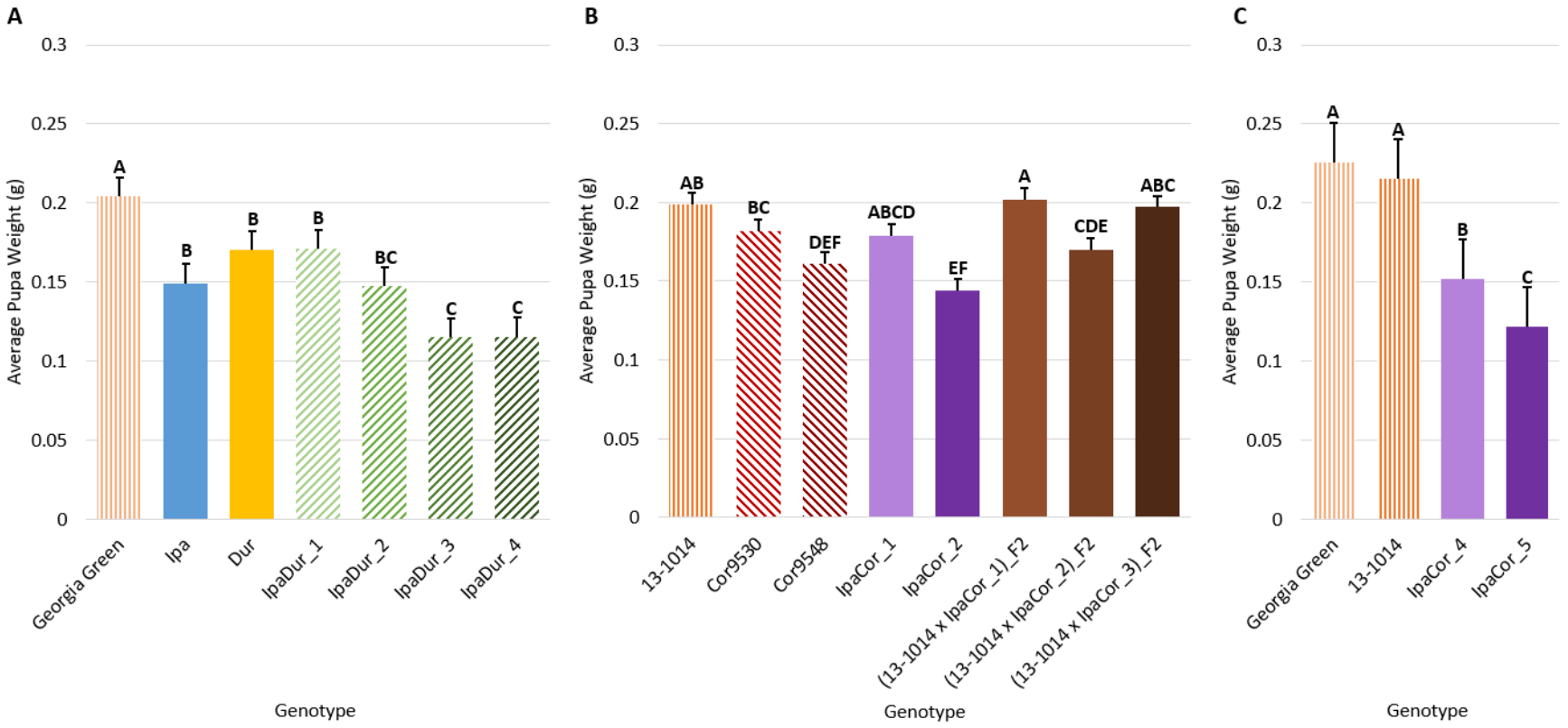

Figure 5.5. Pupal weight. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment. Error bars represent standard error. Tukey's HSD significance levels were calculated within each experiment, so these significance groupings cannot be compared between the three experiments. Genotypes within an experiment with the same Tukey's HSD letter are not significantly different ($\alpha = 0.05$).

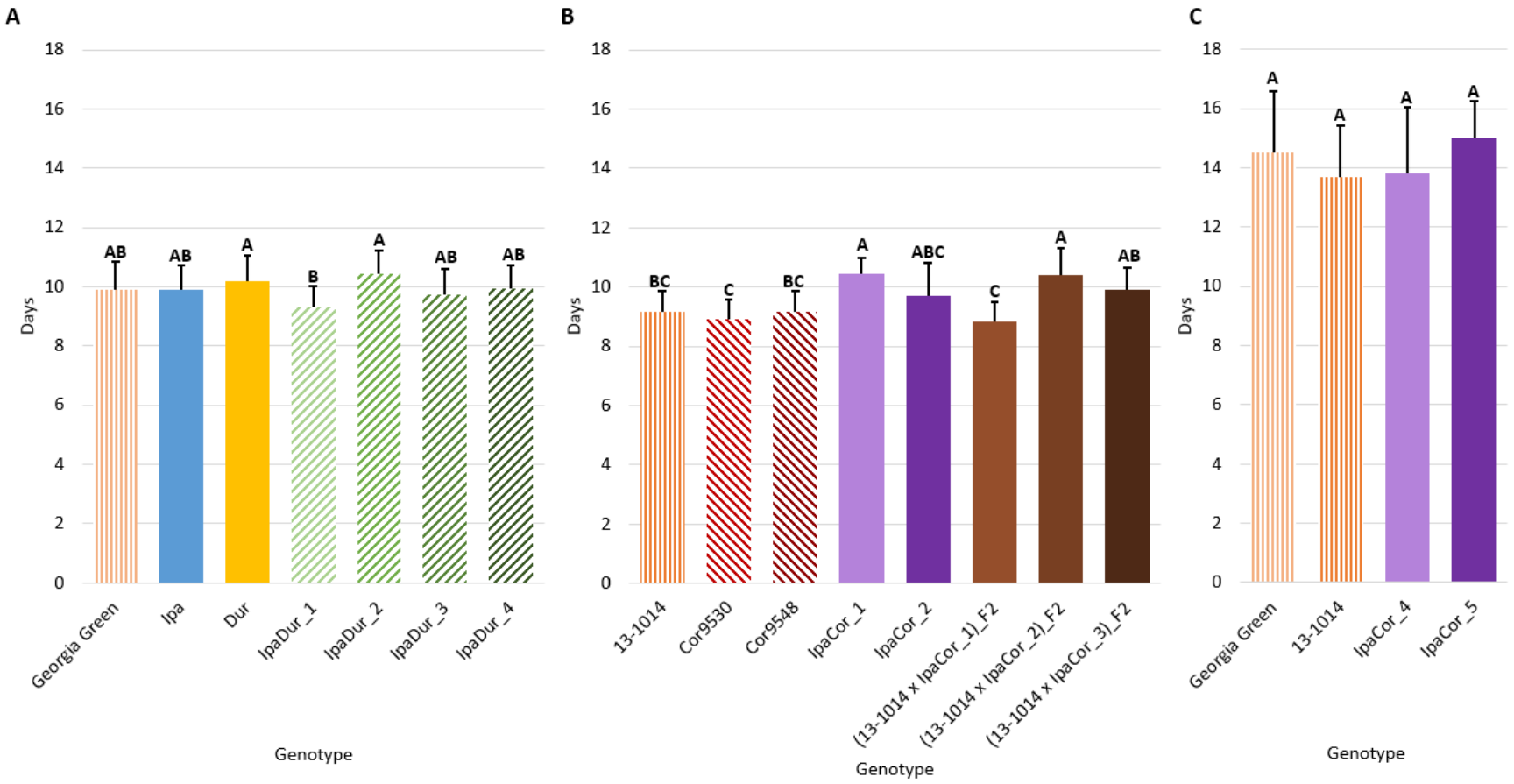

Figure 5.6. Pupal stage duration. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment. Error bars represent standard error. Tukey's HSD significance levels were calculated within each experiment, so these significance groupings cannot be compared between the three experiments. Genotypes within an experiment with the same Tukey's HSD letter are not significantly different ($\alpha = 0.05$).

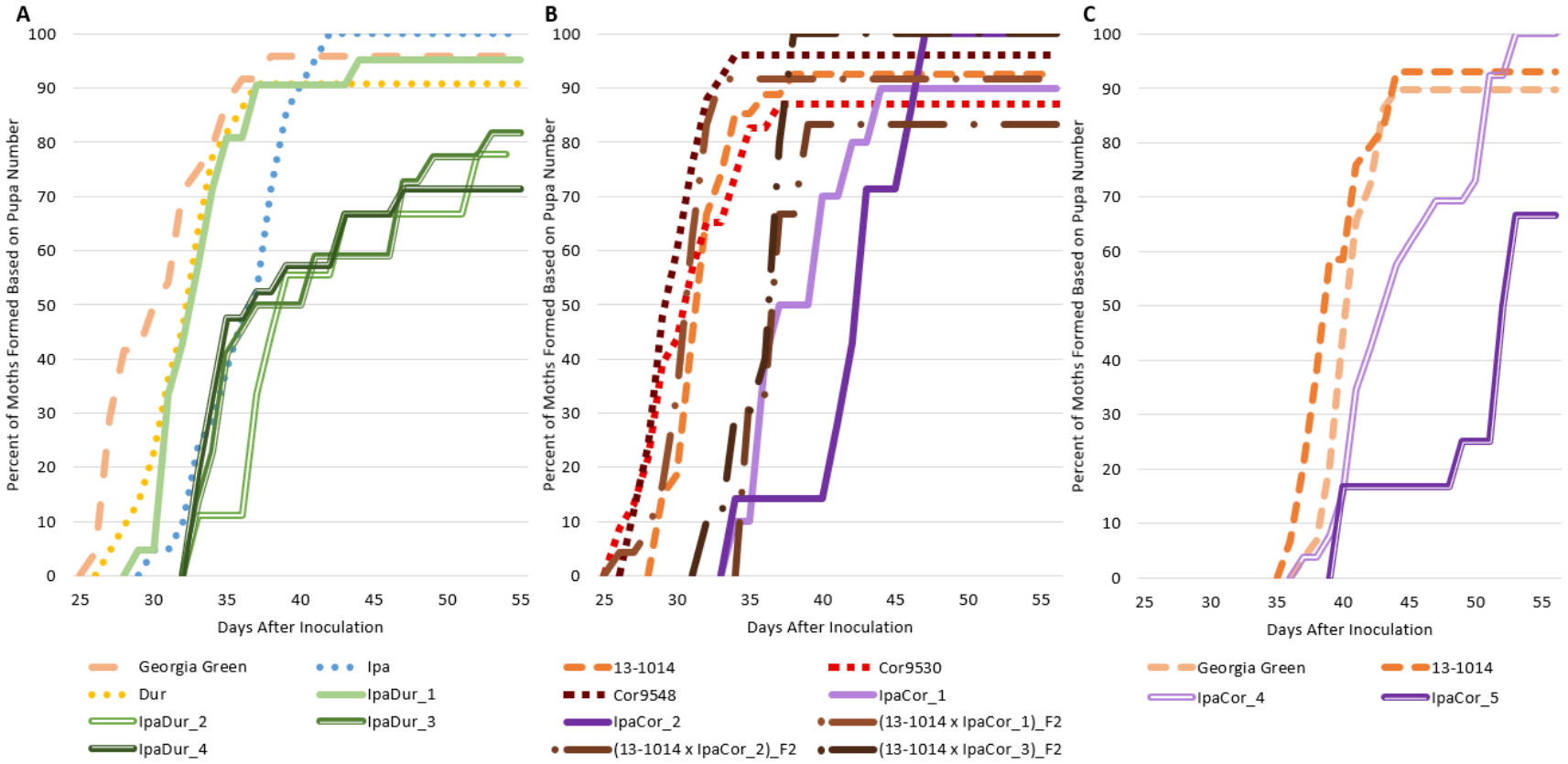

Figure 5.7. Percent of moths formed based on pupa number. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment

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Appendix 5

Supplemental Tables

Table S5.1. Tukey-Kramer grouping ($\alpha = 0.05$) for genotype for all binomial data, including survival at A) 8 DAI and B) 14 DAI, C) pupation and D) moth emergence, for each bioassay. Each replication was coded as either 0 for dead, did not pupate or did not emerge, while 1 was coded for alive, pupated or emerged.

A) 8 DAI.

July 2018 Bioassay: Survival at 8 DAI (0-Dead, 1-Alive)		
Genotype	Estimate	Tukey-Kramer Grouping
Georgia Green	0.90	a
Ipa	0.80	a
Dur	0.83	a
IpaDur_1	0.90	a
IpaDur_2	0.67	a
IpaDur_3	0.90	a
IpaDur_4	0.87	a
May 2019 Bioassay: Survival at 8 DAI (0-Dead, 1-Alive)		
Genotype	Estimate	Tukey-Kramer Grouping
13-1014	0.90	a
Ipa	0.13	a
Cor9530	0.80	a
Cor9548	0.90	a
IpaCor_1	0.55	a
IpaCor_2	0.40	a
IpaCor_3	0.27	a
(13-1014 x IpaCor_1)_F2	0.9	a
(13-1014 x IpaCor_2)_F2	0.33	a
(13-1014 x IpaCor_3)_F2	0.57	a
November 2019 Bioassay: Survival at 8 DAI (0-Dead, 1-Alive)		
Genotype	Estimate	Tukey-Kramer Grouping
Georgia Green	1.00	a

13-1014	1.00	a
A. ipaensis	0.73	a
A. duranensis	0.80	a
A. correntina 9530	0.93	a
IpaDur_5	0.73	a
IpaCor_4	0.93	a
IpaCor_5	1.00	a

B) 14 DAI.

July 2018 Bioassay: Survival at 14 DAI (0-Dead, 1-Alive)		
Genotype	Estimate	Tukey-Kramer Grouping
Georgia Green	0.87	a
Ipa	0.70	a
Dur	0.77	a
IpaDur_1	0.83	a
IpaDur_2	0.57	a
IpaDur_3	0.87	a
IpaDur_4	0.83	a
May 2019 Bioassay: Survival at 14 DAI (0-Dead, 1-Alive)		
Genotype	Estimate	Tukey-Kramer Grouping
13-1014	0.90	a
Ipa	0.13	a
Cor9530	0.80	a
Cor9548	0.90	a
IpaCor_1	0.43	a
IpaCor_2	0.30	a
IpaCor_3	0.13	a
(13-1014 x IpaCor_1)_F2	0.87	a
(13-1014 x IpaCor_2)_F2	0.33	a
(13-1014 x IpaCor_3)_F2	0.57	a
November 2019 Bioassay: Survival at 14 DAI (0-Dead, 1-Alive)		
Genotype	Estimate	Tukey-Kramer Grouping
Georgia Green	1.00	a
13-1014	1.00	a
A. ipaensis	0.73	a
A. duranensis	0.80	a
A. correntina 9530	0.93	a
IpaDur_5	0.73	a
IpaCor_4	0.93	a

IpaCor_5	1.00	a

C) Pupation.

July 2018 Bioassay: Pupation (0-Did not pupate, 1-Pupated)		
Genotype	Estimate	Tukey-Kramer Grouping
Georgia Green	0.80	a
Ipa	0.70	a
Dur	0.73	a
IpaDur_1	0.70	a
IpaDur_2	0.30	a
IpaDur_3	0.73	a
IpaDur_4	0.67	a
May 2019 Bioassay: Pupation (0-Did not pupate, 1-Pupated)		
Genotype	Estimate	Tukey-Kramer Grouping
13-1014	0.90	a
Cor9530	0.77	a
Cor9548	0.83	a
IpaCor_1	0.33	a
IpaCor_2	0.23	a
(13-1014 x IpaCor_1)_F2	0.8	a
(13-1014 x IpaCor_2)_F2	0.20	a
(13-1014 x IpaCor_3)_F2	0.33	a
November 2019 Bioassay: Pupation (0-Did not pupate, 1-Pupated)		
Genotype	Estimate	Tukey-Kramer Grouping
Georgia Green	0.97	ab
13-1014	0.97	a
IpaCor_4	0.87	ab
IpaCor_5	0.40	b

D) Moth emergence.

July 2018 Bioassay: Moth Emergence (0-Did not emerge, 1-Emerged)		
Genotype	Estimate	Tukey-Kramer Grouping
Georgia Green	0.96	a
Ipa	1.00	a
Dur	0.91	a
IpaDur_1	0.90	a
IpaDur_2	0.78	a
IpaDur_3	0.82	a
IpaDur_4	0.75	a
May 2019 Bioassay: Moth Emergence (0-Did not emerge, 1-Emerged)		
Genotype	Estimate	Tukey-Kramer Grouping
13-1014	0.93	a
Cor9530	0.83	a
Cor9548	0.96	a
IpaCor_1	0.90	a
IpaCor_2	1.00	a
(13-1014 x IpaCor_1)_F2	0.92	a
(13-1014 x IpaCor_2)_F2	0.83	a
(13-1014 x IpaCor_3)_F2	1.00	a
November 2019 Bioassay: Moth Emergence (0-Did not emerge, 1-Emerged)		
Genotype	Estimate	Tukey-Kramer Grouping
Georgia Green	0.93	a
13-1014	0.93	a
IpaCor_4	1.00	a
IpaCor_5	0.67	a

CHAPTER 6

RESISTANCE TO RUST (*PUCCINIA ARACHIDIS* SPEG.) IDENTIFIED IN NASCENT ALLOTETRAPLOIDS CROSS-COMPATIBLE WITH CULTIVATED PEANUT (*ARACHIS HYPOGAEA* L.)

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Abstract

Peanut rust, caused by *Puccinia arachidis* Speg., is a foliar disease that plagues peanut production along with early and late leaf spots, *Passalora arachidicola* (Hori) U. Braun and *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash, Videira & Crous, respectively. Rust can cause up to 80% yield losses without control and is widespread in tropical countries but is also a sporadic problem in the United States. An integrative plant management strategy with rust resistant peanut cultivars is needed to decrease dependence on costly fungicides and increase yields for farmers who cannot afford or do not have access to fungicides. Only moderate levels of rust resistance have been found in cultivated peanut germplasm, but fortunately, high resistance to rust has been identified in wild *Arachis* species that can be introgressed into peanut cultivars. In this study, 16 diploid, wild *Arachis* species, five diploid, interspecific hybrids, 11 unique, allotetraploid interspecific hybrids, and two cultivated peanut controls were tested for resistance to rust. Resistance was evaluated *in vitro* by incubation time, susceptibility index (calculated based on the number of lesions of different diameters)/ leaf area, total number of lesions/ leaf area, and total number of sporulating lesions/ leaf area. All wild *Arachis* species tested were very highly resistant to rust, except for *A. ipaensis*, the B-genome progenitor of cultivated peanut. Additionally, all allotetraploids not produced with *A. ipaensis* as a parent did not show symptoms for rust. Any of these nine synthetic allotetraploids, *BatCor*, *BatDur*1, *BatDur*2, *BatSten*1, *GregSten*, *MagCard*, *MagDio*, *MagDur*, and *ValSten*1 are recommended for progression to QTL mapping of rust resistance. These resistance QTLs can be pyramided into peanut cultivars to protect yields in the United States and to increase yields in

tropical, developing countries for farmers that cannot afford, or do not have access to, costly fungicides.

Introduction

Peanut rust, a foliar disease caused by *Puccinia arachidis* Speg., is a widespread problem in countries with warm, tropical climates. The disease reduces peanut yield and quality and indirectly increases management costs (Subrahmanyam et al., 2015). Rust often co-occurs with early leaf spot (caused by *Passalora arachidicola* (Hori) U. Braun [syn. *Cercospora arachidicola* (S. Hori)]), and late leaf spot (caused by *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash, Videira & Crous [syn. *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton]), which have been reported to cause up to 80% yield losses in India in the absence of fungicide control (Subrahmanyam et al., 1984). Although rust is primarily a pathogen that afflicts tropical countries and has only sporadic outbreaks in the USA, global climate change may continue to increase its impact in the USA by increasing the frequency of tropical storms that carry rust inoculum into the USA and by expanding the range in which rust can overwinter (Power, 2014). Yield loss estimates due to rust alone are unavailable for the USA due to it being a localized issue in warm regions and its co-occurrence with leaf spots; however, losses due to damage caused by rust and early and late leaf spot and increased fungicide costs were about $32 million in Georgia in 2011 (Williams-Woodward, 2013). Crop rotations, eradicating volunteer peanut plants to reduce inoculum source, and allowing one-month fallow periods are cultural practices applied by some farmers who do not have access to or cannot afford fungicides in countries such as India, Haiti, and Guyana, (Subrahmanyam, 1997). Even for farmers that can use fungicides, there is still a likelihood that rust populations will develop resistance when exposed to frequent fungicide applications (Smith and Littrell, 1980). Therefore, rust resistant,

high-yielding peanut cultivars are an important part of an integrated pest management strategy to control rust in tropical countries as well as the USA.

A major limitation to breeding rust-resistant cultivars is that only moderate levels of resistance have been identified in *A. hypogaea* germplasm (Power et al., 2019). Fortunately, high resistance to rust has been identified in numerous wild *Arachis* species, including the readily usable A- and B-genome *Arachis* species, and resistance can be introgressed into cultivated peanut (Subrahmanyam et al., 1982, 1983; Pande and Rao, 2001; Fávero et al., 2009). Cultivated peanut is an allotetraploid (AABB; $2n=4x=40$) species and the majority of wild *Arachis* species are diploid ($2n=2x=20$); the most efficient way to introgress genes from wild *Arachis* species into cultivated peanut is to cross A- and B-genome species to produce allotetraploid interspecific hybrids that are cross-compatible to peanut. Then, the wild *Arachis* derived allotetraploids are backcrossed to peanut cultivars. Markers linked to rust resistance allow quick introgression as well as pyramiding of multiple QTLs. Rust resistant QTLs have been identified in the A-genome species *A. cardenasii* GKP 10017 and the B-genome species *A. magna* K 30097. One major QTL from each species is being used in peanut breeding programs to introgress rust resistance into peanut cultivars (Khedikar et al., 2010; Sujay et al., 2012; Leal-Bertioli et al., 2015). For example, Gowda et al. (2002) released a Spanish bunch, rust resistant cultivated genotype 'GPBD 4,' in which resistance was derived from *A. cardenasii*. GPBD 4 had a mean rust score of three on a scale of one to nine, in which one was equivalent to no disease and nine was equivalent to 80 to 100% disease (Gowda et al., 2002). While GPBD 4 still develops rust, it does so at far lower levels than most cultivated germplasm, and one rust resistance QTL derived from *A. cardenasii* but identified in a population derived from GPBD 4, has been shown to improve yields by 56 to 96% in rust infected environments (Gowda et al., 2002; Varshney et al., 2014).

While progress towards resistant rust cultivars is being made, more major rust resistance QTLs need to be identified for further pyramiding in peanut cultivars to strengthen resistance and more importantly, to increase resistance durability to rust population pressures. This study identified rust resistance in newly synthesized allotetraploids that are cross compatible with cultivated peanut. The long-term goal of this study is to create rust resistant peanut cultivars that can protect yields and decrease the need for fungicides in the USA and to increase yields in tropical, developing countries for farmers who cannot afford or access costly fungicides.

Materials and Methods

Plant Materials

Diploid, wild *Arachis* species, *A. correntina* (Burkart) Krapov. and W.C. Gregory (PI 262808, GKP 9530; abbrev.: *Cor*9530), *A. duranensis* Krapov. and W.C. Gregory (PI 468197, GKBSPSc 30060; abbrev.: *Dur*), and *A. ipaensis* Krapov. and W.C. Gregory (PI 468322, GKBSPSc 30076; abbrev.: *Ipa*) were used to generate the diploid hybrids, *IpaCor*2 and *IpaDur*4 in 2016 at North Carolina State University (NCSU). Wild *Arachis* species, *A. batizocoi* Krapov. and W.C. Gregory (PI 298639, K 9484; abbrev.: *Bat*), *A. cardenasii* Krapov. and W.C. Gregory (PI 261874, GKP 10017; abbrev.: *Card*), *A. correntina* (*Cor*9530) and (PI 262881, GKP 9548; abbrev.: *Cor*9548), *A. diogoi* (PI 331200; GK 10602; abbrev.: *Dio*), *A. duranensis* Krapov. and W.C. Gregory (V 14167; abbrev.: *Dur*1, SeSn 2848; abbrev.: *Dur*2, and K 7988; abbrev.: *Dur*3), *A. gregoryi* A. Gripp, C.E. Simpson, and J.F.M. Valls (PI 476116; VSGr 6389; abbrev.: *Greg*), *A. magna* Krapov., W.C. Gregory, and C.E. Simpson (PI 468337; K 30092; abbrev.: *Mag*1 and PI 468340; K 30097; abbrev.: *Mag*2), *A. stenosperma* Krapov. and W.C. Gregory (V 10309; abbrev.: *Sten*1 and PI 338280; HLK 410; abbrev.: *Sten*2), *A. valida* Krapov. and W.C. Gregory (PI 468154; GK 30011; abbrev.: *Val*) and *A. villosa* Benth. (V 12812; abbrev.: *Villo*) were used

to create diploid hybrids *BatCor*, *BatDur*1, *BatDur*2, *BatSten*1, *GregSten*, *IpaCor*1, *IpaDur*1, *IpaSten, IpaVillo*1, *MagCard, MagDio, MagDur,* and *ValSten*1 at the University of Georgia (UGA) Athens Campus. All allotetraploids were derived from the diploid hybrids by colchicine treatment of F_1 hybrid cuttings at the UGA Athens Campus, except for *IpaDur*4 and *IpaCor*2, which were generated at the UGA Tifton Campus.

The resistance evaluation was performed in two separate experiments, in which four wild *Arachis* species and two interspecific hybrid combinations, *IpaCor*2 and *ValSten*1, were tested in both experiments (Table 6.1). Allotetraploids that had more than one plant tested were each designated an arbitrary number to make distinguishing them easier (Table 6.1). The first experiment performed in 2017 included *A. hypogaea* 'Georgia Green' (Branch, 1996) as a susceptible control, while the 2020 experiment included *A. hypogaea* 'Runner 886' as a susceptible control due to seed availability.

Rust Resistance Evaluation

For both experiments, seeds were coated in Vitavax PC (Vitavax, Crompton, Middlebury, CT) and treated overnight in 0.5% Florel Growth Regulator (Lawn and Garden Products Inc., Fresno, CA) to break dormancy. Seeds were then planted in #123 7.62 cm round x 11.43 cm deep Jiffy Pots (Harris Seeds, Rochester, NY) and transplanted approximately one month later into 121.92 cm round x 27.94 cm deep pots filled with Promix growth medium (Premier Tech Horticulture, Quakertown, PA). Normal plant management was applied in the greenhouse except that fungicide treatments were withheld. One week before each experiment, leaves infected with rust were collected from untreated border rows in Tifton, GA and rust spores were collected in sterile vials using a vacuum pump. Great care was taken to avoid collecting late leaf spot spores. Spores were kept at 4 °C until the day of inoculation.

For the 2017 experiment, seven newly and fully expanded leaves were collected from primary laterals from one plant per genotype (Table 6.1). Each leaf was washed, and then its petiole was cut diagonally underwater. The petiole was then wrapped in sterilized, water-soaked cotton and the leaf was placed in a 100 mm x 15 mm petri dish (ThermoFisher Scientific) with the abaxial side upwards. Each sterilized petri dish contained a 76 mm x 25 mm x 1 mm microscope slide (ThermoFisher Scientific) on top of a sheet of 9 cm diameter Whatman No. 1 filter paper (ThermoFisher Scientific) supported by cotton wool that was saturated with approximately 4 ml of deionized water. The cotton-wrapped petiole was in contact with the wet filter paper, while the leaflets were positioned on top of the microscope slide to avoid their contact with the wet filter paper following the method of Guimaraes et al. (2017). Mounted leaves were inoculated with a spore suspension of 0.005% Tween 20 at 1.5 x 10^5 urediniospores/mL of *P. arachidis* using a soft paint brush. Inoculated leaves were kept in the dark for 48 h at approximately 26 °C, after which they were incubated with a photoperiod of 16-hr light and 8-hr dark. The leaves were checked daily for newly emerged rust pustules to document incubation period. Susceptibility was evaluated 25 days after inoculation using the following parameters: total number of lesions/ leaf area (cm^2) (TLA), number of sporulated lesions/leaf area (cm^2) (SLA), and susceptibility index /leaf area (cm^2) (IA) as described by (Leal-Bertioli et al., 2015). IA was calculated with the scale of Savary et al. (1989), with the following modifications made by Leal-Bertioli et al. (2015): index was the number of lesions times a number that reflected lesion size/reaction. $I = \Sigma(s * n)/LA$, where s = lesion size (1 = necrotic aborted lesion, 2−6 = ruptured, sporulating pustules, varying between 0.5 and 3 mm in diameter), n = number of lesions of a particular size, LA = leaf area (cm^2). Spore count and classification was performed with a stereoscope microscope, and leaf area was measured by

scanning the leaves and then using Assess 2.0 (APS Press) for image analysis. The 2020 experiment was performed the same as the 2017 experiment, except 30 replications per genotype were tested, leaves were excised from five plants per genotype, mounted leaves were inoculated with a spore suspension of 0.025% Tween 20 at 1.7 x 10^6 urediniospores/mL, and the experiment was ended 28 days after inoculation.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using RStudio (RStudio, Inc.) to determine the genotype effect on rust resistance according to the following parameters: incubation period, IA, TLA, and SLA. Means of each parameter among the genotypes were separated based on the Tukey's Test ($\alpha = 0.05$) results with RStudio. *Greg* and *IpaDur*1 were excluded from incubation period analysis in the 2017 experiment because each only had one replication to develop rust pustules. Genotypes that presented no rust symptoms, and therefore had no incubation period, were artificially tabulated as 100 days after inoculation for statistical analysis.

Results

Significant genotypic effect on all rust resistance parameters was found for both the 2017 and 2020 experiments (Table 6.2). Between both rust resistance experiments, 30 out of the 39 unique, materials did not show rust symptoms had had no lesions at the end of each experiment (Table 6.3). In the 2017 experiment, the susceptible control, two wild *Arachis* species, *Greg* and *Ipa*, and three allotetraploids, *IpaCor*1, *IpaDur*1, and *IpaVillo*1, developed rust pustules. *Greg* and *IpaDur*1 only had one replication that developed rust pustules, so they were excluded from ANOVA analysis evaluating genotype effect on incubation time; however, these replications developed sporulating pustules, meaning they did not show a hypersensitive response. The

susceptible cultivated control, Georgia Green, had the shortest incubation period of 14.33 days, while *Ipa, IpaCor*1, and *IpaVillo*1 had incubation periods within 15 to 16 days (Table 6.3). In the 2020 experiment, the susceptible control, one wild *Arachis* species, *Ipa*, and two allotetraploids, *IpaCor*2_2 and *IpaDur*4, developed rust pustules. The susceptible cultivated control 886 also had the shortest incubation period of 13.95 days, while *Ipa*, *IpaCor*2_2, and *IpaDur*4 had incubation periods between 14 and 15 days (Table 6.3).

In 2017 rust experiment, the susceptible cultivated control Georgia Green had the highest IA score, but *Ipa*, with an IA score of 1.42, was not significantly different from Georgia Green (Table 6.3). *IpaCor*1, *IpaDur*1, and *IpaVillo*1 had IA scores of 0.92 or less, which were not significantly different from the highly resistant genotypes that did not develop rust pustules (Table 6.3). In the 2020 experiment, *Ipa* had the highest the IA score of 1.94 and showed greater susceptibility than the susceptible control, 886, which had an IA score of 0.76. *Ipa* had a significantly higher IA score than *IpaCor*2_2.

As expected, the susceptible control had the highest IA, TLA, SLA in the 2017 experiment. However, *Ipa* and *IpaCor*1 had similar TLA and SLA scores as compared to the susceptible control. In the 2020 experiment, *Ipa* demonstrated greater susceptibility to rust with a TLA and SLA score twice as much as the susceptible control. *IpaCor*2_2 showed a TLA and SLA comparable to the susceptible control. While *IpaCor*2_2 demonstrated susceptibility in the 2020 experiment, *IpaCor*2^{2x} had no rust incidence in the 2017 experiment and *IpaCor*2_1 had no rust incidence in the 2017 experiment.

Discussion

All the wild peanut-derived allotetraploid genotypes that were not produced with *A. ipaensis* as a parent showed promise as sources for rust resistance for peanut breeding programs,

since they were highly resistant to rust, with no evidence of disease development in any assay. Therefore, these nine interspecific hybrids, *BatCor*, *BatDur*1, *BatDur*2, *BatSten*1, *GregSten*, *MagCard*, *MagDio*, *MagDur*, and *ValSten*1 made from 13 unique *Arachis* accessions are recommended to peanut breeding programs for rust resistance introgression.

*IpaCor*2 performed variably in the two experiments. *IpaCor*2^{2x} and *IpaCor*2_1 had no rust incidence in the 2017 and 2020 experiment, respectively, while *IpaCor*2_2 demonstrated susceptibility similar to the cultivated peanut control in the 2020 experiment. This variability in resistance of *IpaCor2* may be due to a high level of heterogeneity in *A. correntina* 9530, complementing results found by Levinson et al. (2020). This study tested different *A. correntina* 9530 plants as well as allotetraploid lines derived from this wild *Arachis* species for resistance to fall armyworm in detached leaf assays, and these plants had variable performance (Levinson et al., 2020). Three *A. correntina* 9530 plants were genotyped with the Affymetrix Axiom_Arachis2 SNP array (Clevenger et al., 2018; Korani et al., 2019) and 1,259 out of 5,342 total markers (23.5%) were found to be polymorphic (Levinson et al., 2020). This was a high level of heterogeneity when compared to *A. ipaensis* and *A. duranensis*, which had 23 (0.4%) and 27 (0.5%) polymorphic markers, respectively (Levinson et al., 2020). The *IpaCor*2 plants tested in this study were made from crosses between *A. ipaensis* and different *A. correntina* 9530 plants, so genetic difference between these plants may be due to accession heterogeneity or genetic segregation.

Of all the wild *Arachis* species tested, only one species, *Ipa*, the B-genome progenitor of peanut, was susceptible to rust. In the 2020 experiment, *Ipa* showed higher susceptibility than the susceptible control, 886, with greater IA, TLA, and SLA scores than 886. This affirms previous rust bioassays that found most *Arachis* species, especially those in section *Arachis*, to be highly

resistant to rust (Subrahmanyam et al., 1983; Fávero et al., 2009). Like this study, Pande and Rao (2001), Fávero et al. (2009), and Leal-Bertioli et al. (2015) found *A. ipaensis* to be as susceptible or even more susceptible than cultivated peanut. Fávero et al. (2009) also found sporulation on six accessions of *A. stenosperma*, two accessions of *A. valida*, and one accession of *A. magna*, which were not tested in this study. So far, high resistance to rust as not been identified in *A. hypogaea* germplasm (Power et al., 2019; Subrahmanyam et al., 1982; Pande and Rao, 2001; Fávero et al., 2009). The lack of high resistance to rust in cultivated germplasm was contributed in part by having a highly susceptible progenitor as well as the ploidy barrier between allotetraploid peanut and its diploid, highly resistant *Arachis* relatives.

This study builds upon previous reports by identifying rust resistant allotetraploids that are cross compatible to *A. hypogaea* and are therefore valuable to breeding programs, instead of focusing solely on diploid, wild *Arachis* species. A few studies have identified rust resistance QTLs derived from the A-genome species *A. cardenasii* GKP10017 and the B-genome species *A. magna* K 30097 (Khedikar et al., 2010; Sujay et al., 2012; Leal-Bertioli et al., 2015). Sujay et al. (2012) identified five rust resistance QTLs in the same linkage group that explained up to 63% to 83% phenotypic variation; these QTLs likely originated from *A. cardenasii* GKP 10017. The populations used in Sujay et al. (2012) both have *A. hypogaea* GPBD 4 as a parent, which has *A. hypogaea* 'ICGV 86855' as a parent, which in turn originated from a cross between *A. hypogaea* and *A. cardenasii* GKP 10017 (Shirasawa et al., 2018). The QTL that explained the most rust phenotypic variation at 83% has been validated and introgressed into three cultivated varieties through marker-assisted backcrossing, improving yield by 56 to 96% in rust infected environments (Varshney et al., 2014). Leal-Bertioli et al. (2015) identified 13 rust resistance QTLs from *A. magna* K 30097. One of these QTLs contributed to four components of rust

resistance, including IA, TLA, SLA, and incubation period, and explained up to 59% of phenotypic variation. Another resistance QTL explaining up to 35% of phenotypic variation was located in the same linkage group, just 25.4 to 33.1 cM away from the major QTL. These QTL identified by Leal-Bertioli et al. (2015) are distinct from those identified by Sujay et al. (2012) and can be pyramided into the same peanut cultivars to yield more effective and more robust resistance. All nine rust resistant allotetraploids identified in this study have unique *Arachis* parents as compared to these two previous studies; therefore, any of these allotetraploids could be used to map new rust resistance QTL for further stacking of resistance QTLs in cultivated peanut. However, the greatest chance of identifying a major QTL would come from a mapping population using an allotetraploid that has a unique A- and B-genome species. For example, *BatCor*, *BatDur*1, *BatDur*2, *BatSten*1, *GregSten*, *MagDio*, and *ValSten*1 would be good candidates for future rust resistance QTL mapping.

One limitation of this study was that both experiments were confined to *in vitro* bioassays using excised peanut leaves rather than having these *in vitro* experiments in addition to field evaluations. However, past rust field evaluations have been complicated by other fungal pathogens such as *Colletotrichum* spp., *Leptosphaerulina crassiasca* Sechet., early leaf spot, *Myrothecium roridum* Tode ex. Fr., and late leaf spot (Subrahmanyam et al., 1983; Sujay et al., 2012). Furthermore, while peanut rust is common in countries with warm, tropical climates, it only threatens USA peanut production every few years when brought by tropical storms (Bromfield, 1971; Subrahmanyam et al., 1985). When achieved, rust pressure is very inconsistent and fragmented within the same field, and results identifying resistant germplasm could easily result from avoidance of the pathogen instead of actual resistance. Lastly, the morphological differences, i.e., canopy structure, between wild *Arachis* species, allotetraploids,

and cultivated peanut can add variations to pathogen pressure in the field. Despite these complications, Subrahmanyam et al. (1983) tested wild *Arachis* species and cultivated peanut germplasm in the field and *in vitro* and while complications in the field study from three other fungal pathogens were encountered, the reactions to rust were the same in the field and the *in vitro* experiments. Six of the wild *Arachis* accessions tested by Subrahmanyam et al. (1983) were also tested in this study and all were found to be highly resistant presenting no rust symptoms in both studies. Therefore, *in vitro* bioassays like this study have produced consistent results with previous studies, have similar results to field rust experiments, and have been used to map rust resistance QTL, indicating *in vitro* bioassays are sufficient for identifying rust resistant germplasm (Subrahmanyam et al., 1983; Khedikar et al., 2010; Sujay et al., 2012; Leal-Bertioli et al., 2015).

Conclusions

This study built upon previous reports by testing rust resistance in numerous allotetraploids instead of wild *Arachis* species alone. Nine allotetraploids demonstrated high resistance to rust, making them a better source of rust resistance than cultivated peanut germplasm, which has only been found to have moderate levels of resistance. These allotetraploids are cross-compatible with peanut cultivars and thus, available as a usable genetic resource for peanut breeders. A few of these unique allotetraploids will be used to map rust resistance QTLs so that they can be introgressed into peanut cultivars along with the previously identified rust resistance QTLs from *A. cardenasii* GKP 10017 and *A. magna* K30097. The long-term goal of this study is to create rust resistant peanut cultivars that can protect yields in the USA and to increase yields in tropical, developing countries for farmers that cannot afford, or do not have access, to costly fungicides.

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Table 6.1. Genetic materials tested in each rust bioassay and their abbreviations and ploidy level. Bolded genotypes were tested in both bioassays.

2017 Bioassay: Plant Materials	Abbreviation	Ploidy Level
A. hypogaea cv. Georgia Green	Georgia Green	Tetraploid
A. batizocoi K 9484	*Bat*	Diploid
A. cardenasii GKP 10017	***Card***	Diploid
A. correntina HLK 9530	***Cor*2**	Diploid
A. diogoi GK 10602	*Dio*	Diploid
A. duranensis V 14167	*Dur*1	Diploid
A. duranensis SeSn 2848	*Dur*2	Diploid
A. duranensis K 7988	*Dur*3	Diploid
A. gregoryi V 6389	*Greg*	Diploid
A. ipaensis K 30076	***Ipa***	Diploid
A. magna K 30092	***Mag*1**	Diploid
A. magna K 30097	*Mag*2	Diploid
A. stenosperma V 10309	***Sten*1**	Diploid
A. valida GK 30011	***Val***	Diploid
A. villosa V 12812	*Villo*	Diploid
(*Bat*9484 x *Cor*9530) ^{2x}	*BatCor*^{2x}	Diploid
(*Ipa*30076 x *Cor*9530)^{2x}	*IpaCor*2^{2x}	Diploid
(*Ipa*30076 x *Sten*10309)^{2x}	*IpaSten*^{2x}	Diploid
(*Mag*30092 x *Card*10017)^{2x}	*MagCard*^{2x}	Diploid
(*Mag*30092 x *Dio*10602)^{2x}	*MagDio*^{2x}	Diploid
(*Bat*9484 x *Dur*14167)^{4x}	*BatDur*1	Tetraploid
(*Bat*9484 x *Dur*2848)^{4x}	*BatDur*2	Tetraploid
(*Bat*9494 x *Sten*10309)^{4x}	*BatSten*1	Tetraploid
(*Greg*6368 x *Sten*10309)^{4x}	*GregSten*	Tetraploid
(*Ipa*30076 x *Cor*9530)^{4x}	***IpaCor*2**_1[a]	Tetraploid
(*Ipa*30076 x *Cor*9548)^{4x}	*IpaCor*1	Tetraploid
(*Ipa*30076 x *Dur*14167)^{4x}	*IpaDur*1	Tetraploid

(*Ipa*30076 x *Villo*12812)^{4x}	*IpaVillo*1	Tetraploid
(*Mag*30097 x *Dur*7988)^{4x}	*MagDur*_1[b]	Tetraploid
(*Mag*30097 x *Dur*7988)^{4x}	*MagDur*_2[b]	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**_1[c]	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**_2[c]	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**_3[c]	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**_4[c]	Tetraploid
2020 Bioassay: Plant Materials	Abbreviation	Ploidy Level
A. hypogaea Runner 886	886	Tetraploid
A. correntina GKP 9548	*Cor*1	Diploid
A. correntina HLK 9530	***Cor*2**	Diploid
A. ipaensis K 30076	***Ipa***	Diploid
A. duranensis K 30060	*Dur*4	Diploid
A. magna K 30092	***Mag*1**	Diploid
A. valida GK 30011	***Val***	Diploid
(*Ipa*30076 x *Cor*9530)^{4x}	***IpaCor*2**_2[a]	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**_5[b]	Tetraploid
(*Ipa*30076 x *Dur*30060)^{4x}	*IpaDur*4	Tetraploid

[a]These allotetraploids are the same genotype but different plants, and they are distinguished by the arbitrary numbers "_1" and "_2"

[b]*MagDur*_2 is progeny of *MagDur*_1 and are distinguished by the arbitrary numbers "_1" and "_2"

[c]These *ValSten*1 plants are sister lines and are distinguished by arbitrary numbers

Table 2. ANOVA output testing the genotype effect on rust resistance using the following parameters, incubation period, IA, TLA, and SLA for the 2017 and 2020 experiments.

2017 Experiment Parameters	*F* value	Df(n)[a], df(d)[b]	P-value
Incubation period	7970.4	34, 170	< 0.0001***[c]
IA	3.49	36, 186	< 0.0001***
TLA	3.00	36, 186	< 0.0001***
SLA	3.22	36, 186	< 0.0001***
2020 Experiment Parameters	*F* value	Df(n), df(d)	P-value
Incubation period	10890	9, 133	< 0.0001***
IA	4.46	10, 154	< 0.0001***
TLA	4.65	10, 154	< 0.0001***
SLA	4.50	10, 154	< 0.0001***

[a]The df(n), degrees of freedom of the numerator, is based on the number of plant genotypes tested

[b]The df(d), degrees of freedom of the denominator, is based on the total number of replicates for all genotypes tested

[c]*P < .05. **P < .01. ***P < .001

Table 3. Genetic materials tested in each rust bioassay, their mean for the rust resistance parameters, IA, TLA, and SLA, and their Tukey's HSD level. Tukey's HSD significance levels were calculated within each experiment, so these significance groupings cannot be compared between the two experiments. Genotypes within an experiment with the same Tukey's HSD letter are not significantly different ($\alpha = 0.05$). Bolded genotypes were tested in both bioassays.

2017: Genotype	Incubation Period	IA	TLA	SLA
Georgia Green	14.33 b	3.15 a	0.84 a	0.82 a
Bat	∞ a	0.00 b	0.00 b	0.00 b
Card	∞ a	0.00 b	0.00 b	0.00 b
***Cor*2**	∞ a	0.00 b	0.00 b	0.00 b
Dio	∞ a	0.00 b	0.00 b	0.00 b
*Dur*1	∞ a	0.00 b	0.00 b	0.00 b
*Dur*2	∞ a	0.00 b	0.00 b	0.00 b
*Dur*3	∞ a	0.00 b	0.00 b	0.00 b
Greg	-	0.08 b	0.11 b	0.04 b
Ipa	15.00 b	1.42 ab	0.52 ab	0.47 ab
***Mag*1**	∞ a	0.00 b	0.00 b	0.00 b
*Mag*2	∞ a	0.00 b	0.00 b	0.00 b
***Sten*1**	∞ a	0.00 b	0.00 b	0.00 b
Val	∞ a	0.00 b	0.00 b	0.00 b
Villo	∞ a	0.00 b	0.00 b	0.00 b
BatCor^{2x}	∞ a	0.00 b	0.00 b	0.00 b
*IpaCor*2^{2x}	∞ a	0.00 b	0.00 b	0.00 b
IpaSten^{2x}	∞ a	0.00 b	0.00 b	0.00 b
MagCard^{2x}	∞ a	0.00 b	0.00 b	0.00 b
MagDio^{2x}	∞ a	0.00 b	0.00 b	0.00 b
*BatDur*1	∞ a	0.00 b	0.00 b	0.00 b
*BatDur*2	∞ a	0.00 b	0.00 b	0.00 b
*BatSten*1	∞ a	0.00 b	0.00 b	0.00 b

GregSten	∞ a	0.00 b	0.00 b	0.00 b
IpaCor2_1	∞ a	0.00 b	0.00 b	0.00 b
*IpaCor*1	15.80 b	0.92 b	0.34 ab	0.34 ab
*IpaDur*1	-	0.71 b	0.22 b	0.22 b
*IpaVillo*1	16.00 b	0.52 b	0.17 b	0.17 b
*MagDur*_1	∞ a	0.00 b	0.00 b	0.00 b
*MagDur*_2	∞ a	0.00 b	0.00 b	0.00 b
ValSten1_1	∞ a	0.00 b	0.00 b	0.00 b
ValSten1_2	∞ a	0.00 b	0.00 b	0.00 b
ValSten1_3	∞ a	0.00 b	0.00 b	0.00 b
ValSten1_4	∞ a	0.00 b	0.00 b	0.00 b

2020: Genotype	Incubation Period	IA	TLA	SLA
886	13.95 b	0.76 ab	0.30 ab	0.30 ab
*Cor*1	∞ a	0.00 b	0.00 b	0.00 b
Cor2	∞ a	0.00 b	0.00 b	0.00 b
Ipa	14.64 b	1.94 a	0.69 a	0.65 a
*Dur*4	∞ a	0.00 b	0.00 b	0.00 b
Mag1	∞ a	0.00 b	0.00 b	0.00 b
Val	∞ a	0.00 b	0.00 b	0.00 b
IpaCor2_2	14.69 b	0.68 b	0.28 ab	0.27 ab
ValSten1_5	∞ a	0.00 b	0.00 b	0.00 b
*IpaDur*4	14.67 b	0.24 b	0.12 b	0.12 b

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CHAPTER 7

MORPHOLOGICAL, REPRODUCTIVE, AND TOMATO SPOTTED WILT ORTHOTOSPOVIRUS RESISTANCE CHARACTERIZATION AND QTL IDENTIFICATION IN WILD *ARACHIS* DERIVED BC_1F_1 INDIVIDUALS AND AN (*A. IPAENSIS* X *A. CORRENTINA*) DERIVED BC_1F_2 POPULATION

Introduction

Peanut (*Arachis hypogaea* L.) is grown worldwide as an oil, cash, food, and feed crop, and is a key source for protein, calories, vitamins, and minerals (Suchoszek-Lukaniuk et al., 2011). Peanut is widely cultivated in tropical and subtropical regions, and about 49 million tons of peanut are produced globally each year (FAOSTAT, 2019). However, peanut is affected by numerous insects and pathogens that decrease peanut yields and increase production costs. There are over 80 wild *Arachis* species with a wide range of genotypic and phenotypic variability that can be used to expand peanut's genetic base (Stalker et al., 2016). Some *Arachis* species such as *A. cardenasii* have already been used successfully to introgress leaf spot and nematode resistance into cultivated peanut, demonstrating the value of these species for introgression of desirable traits (Simpson et al., 2003; Tallury et al. 2014). Aside from *A. cardenasii*, wild *Arachis* species have been underutilized as sources of pathogen and insect resistance and as sources for desirable agronomic traits.

Arachis species resistance has especially been underutilized for tomato spotted wilt orthotospovirus. This virus is one of the most severe diseases devastating peanut production in the southeastern United States. It has an extensive host range of at least 800 plant species and is

transmitted primarily by tobacco thrips (*Frankliniella fusca* Hinds.) and western flower thrips (*Frankliniella occidentalis* Pergande) (Goldbach and Peters, 1996; Srinivasan et al., 2014). Tomato spotted wilt orthotospovirus causes chlorotic patterns, concentric ring spots, plant stunting, occasional contorting of peg and kernel shape, lower seed weight, and lower seed number per plant (Culbreath et al., 1992; Srinivasan et al., 2017). Plants affected earlier in the season more commonly display severe stunting, which dramatically decreases yield, and these plants also serve as an additional source of inoculum for thrips (Culbreath et al., 1992). It is extremely difficult to control tomato spotted wilt orthotospovirus due to the difficulties in suppressing thrips populations; small numbers of thrips can result in high rates of tomato spotted wilt orthotospovirus spread (Lyerly et al., 2002; Ullman et al., 1997). In addition, insecticides may increase shallow probing behavior leading to increases in pathogen spread (German et al., 1992). Lastly, insecticides are costly, so the deployment of resistance peanut cultivars is desired.

Tomato spotted wilt orthotospovirus was first identified in 1971 in Texas, and since 1985, epidemics have become more prevalent and destructive in peanut (Culbreath et al., 1997; Srinivasan et al., 2017). In the 1980s, popular peanut cultivars including ‘Florunner’ and ‘GK-9’ were completely susceptible to tomato spotted wilt orthotospovirus, and yield losses reached tens of millions of dollars annually (Culbreath et al., 1993; Srinivasan et al., 2017). Current cultivars such as ‘GA-10T’ and ‘Florun-107’ possess greater resistance but are not completely effective under high thrips and tomato spotted wilt orthotospovirus pressure and must be used along with chemical and cultural controls (Branch and Culbreath, 2011; Srinivasan et al., 2017; Tillman and Gorbet, 2015). There has been a recent increase in tomato spotted wilt orthotospovirus occurrence, and most of the southeast is planted with just a few moderately resistant cultivars (Srinivasan et al., 2017). For example, in Georgia in 2016, about 80% of planted cultivated

peanut was ‘GA-06G’ (Srinivasan et al., 2017). Therefore, there is great utility in mapping and integrating new, strong tomato spotted wilt orthotospovirus resistance from wild *Arachis* species into cultivated peanut.

Since high levels of resistance have not been found in cultivated peanut germplasm, wild *Arachis* species are a promising source of resistance. Lyerly et al. (2002) tested 46 *Arachis* accessions for resistance to tomato spotted wilt orthotospovirus in the greenhouse by artificial inoculation and found nine to have no disease symptoms. After testing with more virulent tomato spotted wilt orthotospovirus isolates, *A diogoi* GKP 10602 and *A. correntina* GKP 9530 were identified as highly resistant. Milla et al. (2003) identified five AFLP markers on the same chromosome linked to tomato spotted wilt orthotospovirus resistance in an F_2 population made from a cross between two A-genome wild species, the highly susceptible *A. kuhlmannii* (VRGeSv 7639) and the highly resistant *A diogoi* GKP 10602. This is the current extent of QTL mapping for tomato spotted wilt orthotospovirus resistance from wild *Arachis* species. This study sought to identify tomato spotted wilt orthotospovirus resistance QTL from *A. correntina* GKP 9530 and four other wild *Arachis* species for deployment in peanut breeding programs to be pyramided into elite peanut cultivars to protect peanut yields in the southeastern United States.

While the primary goal of this study was to identify tomato spotted wilt orthotospovirus resistance QTL in BC_1F_1 individuals derived from four unique allotetraploids and in an *IpaCor*^{4x} BC_1F_2 population, these materials have other important agronomic traits that can be used in peanut breeding programs for cultivar improvement. Therefore, the secondary goal of this study was to characterize these materials and identify putative QTL for the following traits: flower

production, flower size, leaf hair density, growth habit, flowering on the main stem, peg length, 100-pod weight, 100-seed weight, shell-out ratio, seed coat color, and leaf spot severity.

Materials and Methods

Plant Materials

Three A genome diploid species, *A. correntina* (Burkart) Krapov. and W.C. Gregory (PI 262808, GKP 9530), *A. duranensis* Krapov. and W.C. Gregory (PI 468197, GKBSPSc 30060), and *A. stenosperma* Krapov. and W.C. Gregory (PI 666100, V10309), and one B genome species, *A. ipaensis* Krapov. and W.C. Gregory (PI 468322, GKBSPSc 300076) were crossed following the BB x AA crossing regime to create the diploid hybrids at North Carolina State University (Raleigh, NC). *Arachis stenosperma* V10309 and the B genome diploid species *A. valida* Krapov. and W.C. Gregory (PI 468154, GK 30011) were crossed to create the diploid hybrids at the University of Georgia Athens Campus. Four allotetraploids, *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x}, were created from the diploid hybrids by colchicine treatment at the University of Georgia Tifton Campus.

IpaCor^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} allotetraploids, used as pollen donors, were crossed to cultivated peanut breeding lines, *A. hypogaea* '13-2113' and '13-1014' (Supplementary Table 7.1). These breeding lines were selected from [(C1805-617-2 x 'Florida-07' (Gorbet and Tillman, 2009)) x 'Georgia-06G' (Branch, 2007)], in which C1805-617-2 was a selection from 'Tifguard' (Holbrook et al., 2008) x 'Florida-07'. Line 13-2113 was selected with the ADSNP124 (A09 6720287) marker (Chu et al., 2016) to incorporate an A09 *A. cardenasii* introgression that confers nematode resistance. Additionally, *IpaCor*^{4x} was crossed to the cultivated peanut breeding line '5-646-10,' which was derived from (Florida-07 x Tifguard) and has susceptibility to root knot nematode but good yield and grade and high O/L content. Twenty-

five, 80, 60, and 37 *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} derived BC_1F_1 individuals were produced, respectively. The *IpaCor*^{4x} derived BC_1F_1 individuals were made from three different *IpaCor*^{4x} sister allotetraploid lines (*IpaCor*^{4x}_$S_{0:2}$, *IpaCor*^{4x}_$S_{0:5}$, and *IpaCor*^{4x}_$S_{0:6}$), *IpaDur*^{4x} derived BC_1F_1 individuals from three sister lines (*IpaDur*^{4x}_$S_{0:1}$, *IpaDur*^{4x}_$S_{0:5}$, and *IpaDur*^{4x}_$S_{0:6}$), *IpaSten*^{4x} derived BC_1F_1 individuals from one allotetraploid (*IpaSten*^{4x}_$S_{0:1}$), and *ValSten*^{4x} derived BC_1F_1 individuals from three sister lines (*ValSten*^{4x}_$S_{0:2}$, *ValSten*^{4x}_$S_{0:4}$, and *ValSten*^{4x}_$S_{0:5}$) (Supplementary Table 7.1). All BC_1F_1 seeds were coated in Vitavax PC (Vitavax, Crompton, Middlebury, CT) and treated overnight in 0.5% Florel Growth Regulator (Lawn and Garden products Inc., Fresno, CA) to break dormancy on 17 May 2019. Forty-eight hours later, seeds were planted in #123 7.62 cm round x 11.43 cm deep Jiffy pots (Harris Seeds, Rochester, NY) filled with Promix growth medium (Premier Tech Horticulture, Quakertown, PA). The plants were transplanted to the Gibbs Farm in Tift County, GA on 6 April 2019.

An *IpaCor*^{4x} BC_1F_2 population was created due to segregation of seed dormancy in the (*A. hypogaea* x *IpaCor*^{4x})_F_1 seeds. Out of 56 F_1 seeds, 42 (75%) germinated just 58 days after peg formation. Germinated F_1 hybrids were transplanted in the greenhouse and crossed with the breeding lines to produce BC_1F_1 individuals as detailed above, meaning the *IpaCor*^{4x} F_1 and BC_1F_1 were produced in the same summer. The BC_1F_2 population was made of 624 individuals total and was comprised of four subpopulations all made with the same *IpaCor*^{4x} allotetraploid (Table 7.1). The BC_1F_2 population was derived from two F_1 progeny, one from the cross (13-1014 x *IpaCor*^{4x}_$S_{0:5}$) and the other from (13-2113 x *IpaCor*^{4x}_$S_{0:5}$). These two F_1 progeny, (13-1014 x *IpaCor*)_$F_{1:4}$) and (13-2113 x *IpaCor*)_$F_{1:3}$), were both backcrossed to 13-1014. Two BC_1F_1 plants from each cross were progressed to the BC_1F_2; therefore, the BC_1F_2 population is comprised of four subpopulations detailed in Table 7.1. The BC_1F_2 population and three parental

controls, 13-1014, 13-1014 *IpaCor*^{4x}_$S_{0:5}$_S_1 (a descendent from self-pollination of the original allotetraploid used to make this population) seeds were coated in Vitavax PC and treated overnight in 0.5% Florel Growth Regulator to break dormancy on 3 April 2019. The BC_1F_2 plants and three control plants were transplanted into the NESPAL field in Tift County, GA on 23 April 2019.

Phenotyping of BC_1F_1 individuals

One hundred and twenty-four days after transplanting, the BC_1F_1 plants were phenotyped for growth habit, plant size, main stem apparentness, main stem color, flowering on main stem, and leaf color following the rating scales of Pittman's "United States Peanut Descriptors" (1995). Each plant was also evaluated for the percent of its canopy exhibiting tomato spotted wilt orthotospovirus symptoms, indicated by leaves with yellow, mosaic patterns. The plants were also given a late leaf spot rating using the Florida rating scale, which takes into account both lesion coverage and percent defoliation (Chiteka et al., 1988). The rating scale ranged from 1, indicating no disease, to 10, indicating total plant death.

On the day of harvest, 184 days after transplanting, the length of 6 randomly chosen pegs connected to a branch no more than 25 cm away from the main stem were measured. After harvest, total pod weight was recorded for all plants and 100-pod weight, shell-out ratio, 100-seed weight, and seed coat color were recorded for plants that had 10% or less of their canopy showing symptoms of tomato spotted wilt orthotospovirus.

Phenotyping of *IpaCor*^{4x} BC_1F_2 population

Flower Count and Size

Flower count was performed from 43 to 121 days after planting between 8:30 to 11:00 am. Data collection was performed twice a week. On four days between 56 and 71 days after

transplanting, four flowers were collected from primary lateral branch(es) from each plant in 15 ml falcon tubes (Corning CoStar, Corning, NY) containing a moist Kimwipe (Kimerly-Clark, Neenah, WI) to keep flowers from wilting during collection. Flowers were dissected and scanned; measurements on hypanthium area (cm^2) and left wing area (cm^2) were taken with ASSESS 2.0 software (APS Press). Banner area (cm^2) was not measured, since it was found to be highly correlated with left wing area in a field study with allotetraploids in the same field in NESPAL in 2018 (Levinson et al., 2021). *IpaCor*^{4x}_$S_{0:5}$_S_1 developed tomato spotted wilt orthotospovirus 88 days after transplanting, so it was included in flower size measurements but not for flower counts.

Leaf Hairs

Between 58 and 88 days after transplanting, the most distal fully expanded leaflet was collected from two n+1 branches from each plant. The number of leaf hairs at the edge of the mature peanut leaflet within 1,000 µm was counted under the microscope.

Growth Habit, Tomato Spotted Wilt Orthotospovirus, and Flowering on Main Stem

One hundred and twenty-eight days after transplanting, the BC_1F_2 population and parental controls were phenotyped for growth habit and flowering on the main stem following the rating scale described by Pittman (1995). The plants were also given a rating on whether their growth was more similar to cultivated peanut or more similar to the branching, sprawling growth of wild *Arachis* species (Fig. 7.1). Each plant was also evaluated for the percent of its canopy exhibiting tomato spotted wilt orthotospovirus symptoms, including leaves with yellow, mosaic patterns.

Peg length, and Pod and Seed Traits

On the day of harvest, 133 days after transplanting, the length of 6 randomly chosen pegs connected to a branch no more than 25 cm away from the main stem were measured. After harvest, total pod weight, 100-pod weight, kernel weight from the 100 pods, shell-out ratio, 100-seed weight, and seed coat color were recorded. Cultivated peanut control 13-2113 died 105 days after transplanting due to deer, so post-harvest phenotyping was not performed on this parental control. However, data were taken for both parental controls 13-1014 and *IpaCor*^{4x}_$S_{0:5}$_S_1 for all postharvest traits.

DNA Extraction and Affymetrix Genotyping

Plant samples were collected at the seedling stage and stored at -80 °C for DNA isolation. The genomic DNA was extracted using the modified CTAB method (Porebski et al. 1997). Purified DNA was dissolved in TE buffer for subsequent analysis. The quantity and quality of the DNA were evaluated on 1% agarose gel electrophoresis and by Quant-iT Picogreen dsDNA assay kit (Thermo Fisher Scientific, Waltham, MA). All DNAs were genotyped using the Axiom_Arachis2 SNP array (Clevenger et al., 2018; Korani et al., 2019) consisting of 47,000 features (ThermoFisher Scientific). Using the Affymetrix data, BC_1F_1 hybrids were confirmed. SNP calling was performed with Axiom Analysis Suite (Version 1.2). Genetic markers were grouped into six categories by the software depending on the quality and separation of markers 1) Monomorphic, 2) PolyHighResolution, 3) NoMinorHom, 4) OfftargetVariant, 5) CallRateBelowThreshold, and 6) Other. The markers in the PolyHighResolution class were used for analysis since the grouping of samples was unambiguous and all of the samples passed quality control.

JoinMap and MapQTL

Genetic maps of the *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} BC_1F_1 groups and the *IpaCor*^{4x} population were constructed using JoinMap 4.1 software (Van Ooijen, 2006). Population nodes were created for *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} with 2,521, 2,384, 3,054, and 4,453 loci, respectively, and 25, 80, 60, and 37 individuals, respectively. The multipoint recombination frequency estimation mapping algorithm (Jansen et al., 2001) was used to calculate locus genotype frequency, and only SNPs with chi-square p-value ≤0.05 (1 degree of freedom) were kept. Linkage groupings were determined based on independent LOD ranging from two to 10; the optimal LOD would produce 20 linkage groups with markers separated by assigned marker positions from the Axiom Arachis2 SNP array. Map distances were estimated using Haldane's (1919) mapping function. After filtering and linkage group determination, the *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} BC_1F_1 groups had genetic maps with 568, 1,074, 1,023, and 1,126 loci, respectively.

Four genetic maps for the *IpaCor*^{4x} BC_1F_2 population were created for each subpopulation (Table 7.1) following the same methods to create the BC_1F_1 genetic maps. Population nodes were created for each subpopulation with 3,687 loci for the number of individuals listed in Table 7.1. The subpopulations, [13-1014 x ((13-1014 x *IpaCor*)_$F_{1:4}$)]_$BC_{1:4}$, [13-1014 x ((13-1014 x *IpaCor*)_$F_{1:4}$)]_$BC_{1:5}$, [13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:1}$, and [13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:2}$ had genetic maps with 1,530, 1,213, 2,127, and 2,069 loci, respectively.

QTL mapping analyses were performed with MapQTL 6 (Van Ooijen, 2004). For the BC_1F_1 groups, QTL mapping was performed for the following traits: growth habit, plant size, main stem apparentness, main stem color, leaf color, percent of canopy exhibiting tomato spotted wilt orthotospovirus symptoms, leaf spot severity rating, peg length, total pod weight, 100-pod

weight, 100-seed weight, shell out ratio, and seed coat color. For the *IpaCor*^{4x} BC_1F_2 subpopulations, QTL mapping was performed for the following traits: average flower count, hypanthium area, left wing area, hair count, flowering on main stem, growth habit, percent of canopy exhibiting tomato spotted wilt orthotospovirus symptoms, peg length, total pod weight, 100-pod weight, 100-seed weight, shell out ratio, and seed coat color. Significant loci were detected by interval mapping using threshold levels derived from 200 permutation tests and a mapping step size of one. A LOD score of three was used to confirm putative QTL.

Construction of Bulks for QTL-Seq

QTL-Seq for tomato spotted wilt orthotospovirus was performed on each of the four groups of the BC_1F_1 individuals, including *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x}, as well as on the *IpaCor*^{4x} BC_1F_2 population (Table 7.2). Each had a resistant pool consisting of individuals that had 0% of their canopy displaying tomato spotted wilt orthotospovirus symptoms. For the BC_1F_1 resistance bulks, individuals were included if 90% or more of their canopy displayed tomato spotted wilt orthotospovirus symptoms. The number of individuals in each bulk is shown in Table 7.2. For the *IpaCor*^{4x} BC_1F_2 resistant bulk, individuals were included if 70% or more of their canopy was symptomatic. QTL-Seq for flowering on the main stem was also performed on the *IpaCor*^{4x} BC_1F_2 population (Table 7.2). Each bulk was made of the same quantity of DNA from each individual so that representation for each plant was equal.

Library Construction, Sequencing, and QTL Identification

A total of 12 Illumina libraries were prepared using the TruSeq Nano DNA Low Throughput Library Prep Kit. For each library, 200 ng of DNA was sheared for a 550 bp insert size with a Covaris E220 Evolution by Georgia Genomics and Bioinformatics Core at the University of Georgia (Athens, GA). After library construction, paired-end, whole genome

resequencing was performed with NovaSeq S4 Flow at 26X coverage by Novogene (Sacramento, CA). The QTL-seq pipeline was performed by HudsonAlpha (Huntsville, AL). Khufu was used to filter the data for high-quality reads and samtools mpileup 1.2 was used to call SNPs. Confidence interval significance lines were generated using QTLseqR (Mansfeld and Grumet, 2018).

Results

Phenotyping of BC_1F_1 individuals

All four growth habits were exhibited by individuals in the *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} BC_1F_1 groups, and prostrate growth habit was the most common (Fig. 7.2). Even a few individuals had bunch-type growth, despite all parents of the population, the *A. hypogaea* runner types and the allotetraploids, having spreading or prostate growth. Main stem apparentness segregated in the *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} BC_1F_1 plants with apparent being the most common phenotype for except for *IpaCor*^{4x} BC_1F_1 plants, in which the most common phenotype was somewhat apparent (Fig. 7.3). No BC_1F_1 plants had flowering on their main stems. For all four allotetraploid combinations, most BC_1F_1 plants had a mix of green and purple stems and green leaves (Fig. 7.4 and 7.5). For each allotetraploid-derived BC_1F_1 group, most plants had either no tomato spotted wilt orthotospovirus symptoms or had 100% of their canopy displaying symptoms (Fig. 7.6). Leaf spot severity ratings ranged from one to five, with most *IpaSten*^{4x} and *ValSten*^{4x} plants rated at two and most *IpaCor*^{4x}, *IpaDur*^{4x} plants rated at three (Fig. 7.7). The only plants with a leaf spot rating of one were *ValSten*^{4x} and the only plants with the highest rating of five were *IpaDur*^{4x} plants. The average peg length for the *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} BC_1F_1 plants was 6.6, 7.7, 8.2, and 7.8 cm, respectively. These averages were all more than twice as much as the average peg length of the cultivated

peanut parental control 13-1014, which had an average peg length of 3.25 cm. This control was grown in the NESPAL field as a control for the *IpaCor*^{4x} BC_1F_2 population and not alongside the BC_1F_1 plants in the Gibbs farm. The average peg length ranged from 3.17 cm for one *IpaCor*^{4x} and one *ValSten*^{4x} BC_1F_1 plant to 14.00 cm for one *IpaSten*^{4x} plant (Fig. 7.8).

The average total pod weight of the *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} BC_1F_1 that had less than 10 % of their canopy displaying tomato spotted wilt orthotospovirus symptoms was 86.8, 114.6, 92.7, and 105.9 g, respectively, which were all less than the total pod weight of 173.6 g of the 13-1014 peanut cultivated control included in the NESPAL field for comparison to the *IpaCor*^{4x} BC_1F_2 population. However, there were BC_1F_1 individuals from each allotetraploid that exceed the total pod weight of 13-1014, and an *IpaDur*^{4x} BC_1F_1 had the largest total pod weight of 259.4 g (Fig. 7.9). The average 100-pod and 100-seed weights were between 65 to 77 g and between 41 to 47 g for the *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} BC_1F_1 groups, both of which are much lower than the average 100 pod and seed weight of 13-1014 at 113.8 and 70.2 g. There were five BC_1F_1 plants, one *IpaCor*^{4x}, three *IpaDur*^{4x}, and one *IpaSten*^{4x}, that had higher 100-pod weight than 13-1014; the highest was the *IpaCor*^{4x} BC_1F_1 with a 100-pod weight of 128.6 g (Fig. 7.10). Only one *IpaDur*^{4x} BC_1F_1 had a 100-seed weight higher than 13-1014 at 74.1 g (Fig. 7.11). The average shell out ratio for each allotetraploid BC_1F_1 group was between .64 to .66, lower than the shell-out ratio of 13-1014 at .72. Fourteen BC_1F_1 plants had a higher shell out ratio than 13-1014, with the highest ratio of 0.79 for an *IpaDur*^{4x} BC_1F_1 (Fig. 7.12). In the BC_1F_2 population, the parents, 13-1014 and the *IpaCor* allotetraploid, both had pink seed coats immediately after shelling. About half of the *IpaCor*^{4x} BC_1F_1 had tan or pink seed coats, except one individual that had deep purple seeds right after shelling. All the *IpaDur*^{4x} BC_1F_1 seed coats were tan, except for two individuals that had pink seed coats. Similarly, all the *ValSten*^{4x}

BC_1F_1 had tan seed coats, except for three individuals that had pink seed coats. Three fourths of the *IpaSten*^{4x} BC_1F_1 had tan seed coats, while one fourth had pink seed coats.

Phenotyping of *IpaCor*^{4x} *BC_1F_2* population

Flower Count and Size

IpaCor^{4x} BC_1F_2 plants displayed a lot of diversity in flowering course. The cultivated peanut control 13-1014 had an average flower count over the 12-week period of 8.5 flowers/ day and maintained a steady flower production of less than 20 flowers/ day over the entire 12-week period with a slight peak in production between 85 and 99 days after transplanting (Fig. 7.13). The *IpaCor*^{4x}_$S_{0:5}$_S_1 parental control developed tomato spotted wilt orthotospovirus 88 days after transplanting, so it was not included in Fig. 7.13; however, in a study performed in the same NESPAL field the year before, the average flower count of *IpaCor*^{4x} allotetraploids was 47.5 flowers per day and these allotetraploids did not slow in flower production at the end of the season at 108 days after transplanting (Levinson et al., 2021). In Fig. 7.13, six BC_1F_2 plants were chosen to demonstrate the two main flowering courses observed. Three *IpaCor*^{4x} BC_1F_2 plants in grey demonstrate flowering courses similar to the 13-1014 peanut cultivated control with a steady production of a few flowers over the 12-week period; three *IpaCor*^{4x} BC_1F_2 plants showed flowering courses more similar to the *IpaCor*^{4x} allotetraploids described by Levinson et al. (2021) with a higher amount of flower production, peaking at about 140 flowers per day 106 days after transplanting (Fig. 7.13). The highest average flower count for was 55.3 flowers per day for one BC_1F_2 individual, more than six times as much as the average flower count for 13-1014.

Hypanthium area in the *IpaCor*^{4x} BC_1F_2 population ranged from 0.18 to 1.29 cm^2, and the average hypanthium area was 0.65 cm^2. None of the *IpaCor*^{4x} BC_1F_2 plants had a larger

hypanthium area than the parental control *IpaCor*^{4x}_$S_{0:5}$_S_1 at 1.34 cm^2 (Fig. 7.14). Many *IpaCor*^{4x} BC_1F_2 plants had hypanthium areas smaller than the two cultivated peanut controls, 13-1014 and 13-2113, which had hypanthium areas of 0.47 and 0.52 cm^2, respectively. Left wing area ranged from 0.18 to 0.98 cm^2, and the average area was 0.48 cm^2. There were *IpaCor*^{4x} BC_1F_2 plants that had larger left wing areas than the parental control *IpaCor*^{4x}_$S_{0:5}$_S_1 as well as smaller left wing areas than the two cultivated peanut controls, 13-1014 and 13-2113 (Fig. 7.15). All *IpaCor*^{4x} BC_1F_2 plants had yellow wings and banners with a yellow center and an orange edge, with thin red stripes originating from where the hypanthium and banner connect and spreading outwards (Fig. 7.16). However, the *IpaCor*^{4x} BC_1F_2 plants did differ in the intensity of the orange on the edge of their banners. For example, *IpaCor*^{4x} $BC_1F_{2:73}$ had an intense dark orange banner edge (Fig. 7.16D) while $BC_1F_{2:73}$ had a light orange banner edge (Fig. 7.16E).

Leaf Hairs

The number of leaf hairs at the edge of the mature peanut leaflets within 1,000 µm ranged from 4 to 23 hairs with an average of 10.5 hairs per 1,000 µm for the *IpaCor*^{4x} BC_1F_2 population. Some *IpaCor*^{4x} BC_1F_2 plants had more leaf hairs than *IpaCor*^{4x}_$S_{0:5}$_S_1 and some had less than the cultivated peanut controls (Fig. 7.17). The diversity of leaf hair density in the *IpaCor*^{4x} BC_1F_2 population is displayed in Fig. 7.18, showing contrasting images of the *IpaCor*^{4x} BC_1F_2 individual with the most leaf hairs (Fig. 7.18C) and the individual with the least (Fig. 7.18D) as compared to the parental controls.

Growth Habit, Flowering on Main Stem, and Tomato Spotted Wilt Orthotospovirus

Fifty-two point four, 36.6, and 11 % of the *IpaCor*^{4x} BC_1F_2 population had prostrate, spreading, and spreading and bunch growth, respectively (Fig. 7.19); no individuals displayed strictly bunch growth habit. About half of the *IpaCor*^{4x} BC_1F_2 population displayed cultivated-

like growth, characterized by compact and full branching, and the other half displayed wild *Arachis*-like growth, characterized by few, spreading branches (Fig. 7.1). Unlike the *IpaCor*^{4x} BC_1F_1 plants that had no flowering on their main stems, 15.7% of the *IpaCor*^{4x} BC_1F_2 population did have flowering on their main stems (Fig. 7.20). Most *IpaCor*^{4x} BC_1F_2 plants had no tomato spotted wilt orthotospovirus symptoms, but more than 100 individuals had more than 70% of their canopy displaying symptoms (Fig. 7.20).

Peg Length and Pod and Seed Traits

Peg length in the *IpaCor*^{4x} BC_1F_2 population ranged from 1.9 to 11.4 cm with an average of 5.2 cm. Many BC_1F_2 individuals had longer peg length than the parental control *IpaCor*^{4x}_$S_{0:5}$_S_1, which had an average peg length of 4.3 cm, and some BC_1F_2 individuals had peg length shorter than the cultivated peanut parental control 13-1014, which had a peg length of 3.3 cm (Fig. 7.21). Sixty-three BC_1F_2 individuals had higher total pod weights as compared to the cultivated peanut control 13-1014, which had a total pod weight of 173.6 g. One BC_1F_2 plant had a total pod weight of 326.6 g. Only two BC_1F_2 individuals had higher 100-pod weights as compared to the cultivated peanut control 13-1014, which had a 100-pod weight of 113.8 g (Fig. 7.22). The highest 100-pod weight was 129.4 g in the BC_1F_2 population. Only one BC_1F_2 individual had a higher 100-seed weight of 94.0 g as compared to the to the cultivated peanut control 13-1014, which had a 100-seed weight of 70.2 g (Fig. 7.23). Shell out ratio in the BC_1F_2 population reached as high as 89.7%, and many BC_1F_2 individuals had a higher ratio than 13-1014, which had a shell out ratio of 71.7%. Twenty-nine, 33, 34, and 5% of the BC_1F_2 plants had seed coat colors of tan, pink, tan/pink, or deep, dark purple, respectively. A new category of tan/pink was made due to many of the seed coats being tan-coral color in between tan and pink. Of the 28 BC_1F_2 individuals with dark purple seed coats, 26 were individuals made from

crossing with 13-2113 and 13-1014 and two individuals were made from two crosses with 13-1014.

MapQTL

Genetic maps with 37, 26, 25, and 24 linkage groups were created for the *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} BC_1F_1 groups. No putative QTL were identified for the *IpaCor*^{4x}, *IpaDur*^{4x}, and *IpaSten*^{4x} groups, but three putative QTL were identified for the *ValSten*^{4x} group (Fig. 7.25). A QTL for main stem color on linkage group one with the peak LOD score of 3.15 was identified; the significant markers have positions around B01 148,743,882 bp according to the assignments of the Axiom Arachis2 SNP array (Fig. 7.25A). A QTL for peg length on linkage group 12 with a peak LOD score of 3.02 was identified, and the markers in the peak of this putative QTL are associated with B02 7,812,645 bp in the Axiom Arachis2 SNP array (Fig. 7.25B). One QTL for 100-seed weight on linkage group eight with a peak LOD score of 3.11 was identified, and the markers in the peak of this putative QTL are associated with B01 135,396,794 bp in the Axiom Arachis2 SNP array (Fig. 7.25B).

Genetic maps with 20, 19, 22, and 18 linkage groups were created for the [13-1014 x ((13-1014 x *IpaCor*)_$F_{1:4}$)]_$BC_{1:4}$, [13-1014 x ((13-1014 x *IpaCor*)_$F_{1:4}$)]_$BC_{1:5}$, [13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:1}$, and [13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:2}$ subpopulations. A putative QTL for flowering on the main stem was identified in all *IpaCor*^{4x} BC_1F_2 subpopulations on A06. For the [13-1014 x ((13-1014 x *IpaCor*)_$F_{1:4}$)]_$BC_{1:4}$ subpopulation, the markers were assigned to an unknown linkage group, but the peak LOD score was 12.55 for a marker assigned to A06 83,183,757 on the Axiom Arachis2 SNP array. The [13-1014 x ((13-1014 x *IpaCor*)_$F_{1:4}$)]_$BC_{1:5}$ had markers in linkage group two with a peak LOD score of 11.31 at A06 97,904,366 (Fig. 7.26B). [13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:1}$

also had markers that were unassigned to a linkage group that had a peak LOD score of 5.38 and ranged from 4,057,858 to 105,388,502 bp on A06. For the [13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:2}$ subpopulation, markers assigned to linkage group five had a peak LOD score of 9.45 at A06 7,153,986 (Fig. 7.26A). Although the markers are grouped closely by JoinMap, the markers with a LOD above 3 in the peak in Fig. 7.25A have Axiom Arachis2 SNP array assigned positions across the entire A06 chromosome.

The only other putative QTL that was identified by three or more subpopulations was for 100-pod weight on chromosome B06. For the [13-1014 x ((13-1014 x *IpaCor*)_$F_{1:4}$)]_$BC_{1:5}$ subpopulation, markers in linkage group two had a peak LOD score of 3.02 for markers located around B06 123,448,432 bp. For the [13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:1}$ subpopulation, markers in linkage group two had a peak LOD of 3 for one marker at B06 128,287,517 bp. For the [13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:2}$ subpopulation, markers in linkage group one had a peak LOD score of 9.53 at B06 9,151,998, but markers with an LOD above 3 spanned from 6,591,604 to 135,668,437 bp, essentially the entire chromosome.

QTL-Seq

QTLs were not identified for tomato spotted wilt orthotospovirus resistance in the *IpaCor*^{4x}, *IpaDur*^{4x} nor *IpaSten*^{4x} BC_1F_1 libraries. For the *ValSten*^{4x} BC_1F_1 libraires, significant candidate QTL for tomato spotted wilt orthotospovirus resistance were found on A03 (Fig. 7.27) and B08 (Fig. 7.28). In the *IpaCor*^{4x} BC_1F_2 population, significant candidate QTL for tomato spotted wilt orthotospovirus resistance were found on B05 (Fig. 7.29) and B10 (Fig. 7.30). The QTL on B05 spans about 3.6 Mbp and is made mostly of *A. ipaensis* alleles. Two significant QTL were identified on the end of B10, with the QTL closest to the centromere spanning about 0.78 Mbp at 96,236,841 bp and the QTL further from the centromere spanning about 0.91 Mbp

at 122,931,853 bp. Both QTLs on B10 are from either *A. correntina* or *A. hypogaea*, not *A. ipaensis*. QTL for flowering on the main stem were also identified in the in the *IpaCor*^{4x} BC_1F_2 population on A02, A05, and A06. The QTL on A02 was found to span the entire chromosome (Fig. 7.28A), and the QTL on A05 spans almost the entire chromosome as well (Fig. 7.31B). The QTL on A06 is has high deltaSNP values and is located in the pericentromere (Fig. 7.31C). The rest of the chromosomes with no introgressions had deltaSNP values under 0.10; this is well below the deltaSNP values found for the QTL in A02, A05, and A06 (Fig. 7.31).

Discussion

Wild *Arachis* species have a wide range of genetic and phenotypic variability as well as strong resistances to pathogens such as tomato spotted wilt orthotospovirus (Stalker, 2017). This variability is valuable for peanut breeding programs to widen genetic diversity in peanut and to introgress strong resistances and agronomically important phenotypic traits into cultivated peanut. Species like *A. cardenasii* have already been used successfully to introgress resistances to leaf spot and nematodes into cultivated peanut, and Fonceka et al. (2012) found that introgression of wild segments into cultivated peanut improved flowering precocity, seed and pod number or length and size, and pod maturity in cultivated peanut. To introgress these desirable traits into cultivated peanut, the genetic and phenotypic diversity of these materials must be documented, and QTL associated with these traits need to be identified to promote efficient introgression and to reduce linkage drag. The goal of this study was to characterize morphological and reproductive traits of BC_1F_1 individuals made from four different allotetraploids made from underutilized *Arachis* species, *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x}, and an *IpaCor*^{4x} BC_1F_2 population, and to identify putative QTL for these traits.

In this study, we utilized both Axiom Arachis2 SNP array data with JoinMap and QTLMap 6 as well as QTL-Seq to identify putative QTL for both tomato spotted wilt orthotospovirus resistance and agronomic traits such as flowering on the main stem. The most important findings of this study were the putative QTL for tomato spotted wilt orthotospovirus resistance identified in the *IpaCor*^{4x} BC_1F_2 population by QTL-seq on top of B05 and end of B10 and in the *ValSten*^{4x} BC_1F_1 group on the end of A03 and the top of B08. These putative QTL need to be validated, but they showed promise as new sources of novel, strong, tomato spotted wilt orthotospovirus resistance. Identifying QTL for tomato spotted wilt orthotospovirus in the *IpaCor*^{4x} BC_1F_2 population complements the findings of Lyerly et al. (2002), who tested 46 *Arachis* accessions for resistance to tomato spotted wilt orthotospovirus in the greenhouse by artificial inoculation and found nine accessions, including *A. correntina* 9530 to have no disease symptoms. However, the putative QTL on B05 consisted of *A. ipaensis* alleles, but the putative QTL on B10 may be from *A. correntina.* Once validated, these QTL hold promise as new sources of alleles for tomato spotted wilt orthotospovirus resistance, since only moderate resistance has been identified in cultivated peanut and no tomato spotted wilt orthotospovirus resistance QTL have been identified on B05 and B10. Therefore, after validation, these QTL will be pyramided into peanut cultivars to improve the strength and durability of currently used resistance genes, leading to cultivars with more protected yields.

Additional putative QTL were identified for agronomic traits such as flowering on the main stem and 100-pod weight. For flowering on main stem, a putative QTL located in the pericentromere of A06 in the *IpaCor*^{4x} BC_1F_2 population was identified by both MapQTL 6 and by QTL-Seq, and putative QTL were also identified on A02 and A05 by QTL-Seq. The strong QTL on A06 may be the same QTL identified by Chopra et al. (2018), who utilized a mapping

population derived from crossing *A. duranensis* KSSc38901 and *A. cardenasii* GKP10017 and identified a QTL on A06 that explained 44% of phenotypic variation and two QTL on B08 that explained 10% of the variation each. However, this could be a novel QTL on A06 from *A. correntina* on the same chromosome. Either way, the putative QTL on A02 and A05 are novel. Flowering on the main stem only presented in the *IpaCor*^{4x} BC_1F_2 population, not in BC_1F_1 individuals, and only 15.7% of the population had this trait, indicating this trait may be caused by two recessive genes or two genes with epistasis between the loci. This two gene system matches previous findings of main stem flowering being caused by two sets of duplicate loci with epistasis among the alleles (Hammons 1971; Wynne 1975). In cultivated peanut, flowering on the main stem is one distinguishing characteristic between subsp. *hypogaea*, which does not flower on the main stem, and subsp. *fastigiata*. Flowering on the main stem may be contributed by *A. correntina* 9530, since the cultivated parents used for crossing were runner breeding lines in the subsp. *hypogaea* and *A. ipaensis* GKBSPSc 300076 does not have flowering on the main stem. Furthermore, one 1977 specimen of *A. correntina* 9530 in the unofficial NCSU peanut lab herbarium has flowering on the main stem (H.T. Stalker, personal communication, April 14, 2021) and *A. correntina* 9530 has been reported to have a high level of heterogeneity, so some individuals of this accession may flower on the main stem while others do not (Levinson et al., 2020). In addition, Levinson et al. (2021) documented reproductive nodes on the main stem of some *IpaCor*^{4x} allotetraploids.

Similar to the major putative QTL for flowering on main stem, the putative QTL for 100-pod weight on the end of chromosome B06, may have also been identified by Chavarro et al. (2020). In a recombinant inbred line population made from a cultivated by cultivated cross, 'Tifrunner' x 'NC3033,' nine QTL for 100-pod weight, 100-seed weight, pod width, pod length,

seed length, seed width, and seed length:width ratio were identified on the end of B06 between 130.49 Mpb to 150.86 Mbp; the same region where the 100-pod weight putative QTL was located in this study (Chavarro et al. 2020). It is likely that the 100-pod weight QTL originated from the *A. hypogaea* parents, 13-1014 and 13-2113, since it complemented the findings of Chavarro et al. (2020).

A wide range of phenotypic diversity was captured in the BC_1F_1 groups; although these were not true populations and comprised a small number of individuals. Despite these limitations, these individuals in all four BC_1F_1 groups (*IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x}) had all fourth growth habits (prostate, spreading, spreading and bunch, and bunch). Bunch growth habit is characteristic of subsp. *fastigiata*, so this trait was likely influenced by the wild *Arachis* species' alleles. BC_1F_1 individuals in all four groups also had all three categories of main stem apartness and stem color, showing the phenotypic diversity in this population. There were also individuals with greater total pod weight, 100 pod weight, and 100 seed weight that may have beneficial alleles that can be moved into cultivated peanut to improve yields. One *IpaDur*^{4x} BC_1F_1 had a total pod weight 86 g more than the 13-1014 breeding line control, and one *IpaCor*^{4x} BC_1F_2 individual had a total pod weight 153 g more than the breeding line. While pod weight is usually not measured per plant, but per plot, this result is still encouraging that these wild *Arachis* derived materials likely have desirable agronomic traits in addition to disease resistance.

A wide range of phenotypic diversity was also captured in the *IpaCor*^{4x} BC_1F_2 population. Variation ranging from high flower production similar to that documented in *IpaCor*^{4x} (Levinson et al., 2021) to low flower production similar to 13-2113 was demonstrated in the BC_1F_2 population. However, flower production has not been correlated to yield, so this

trait may be less valuable in peanut cultivars grown for food as opposed to ornamental varieties (Songsri et al., 2009). Variation in hypanthium size and wing size was also identified along with leaf hair density, which may be a trait that breeders focus on to increase resistance to some insect pests, since hair density has been demonstrated as one defense mechanism to some insect pests (Mohammad et al. 2019). The BC_1F_2 also segregated for growth habit, with individuals displaying all growth habits except bunch. About 16% of the BC_1F_2 population had flowering on the main stem; this may be a trait that breeders select against since these pegs do not reach the ground to form peanut pods and may reduce the fitness of the plant. A wide variation in peg length was observed, but when breeders utilize this population for desirable traits, they will select for yield, which will indirectly select against long peg length. The most promising diversity captured in this population were the individuals with higher total pod weight, 100 pod weight, and 100 seed weights as compared to the cultivated control that could be selected to directly increase yield in peanut cultivars.

The limitations in this study were that the BC_1F_1 groups were not true populations and that they, along with the *IpaCor*^{4x} population, only have a single year of data. However, these limitations will be overcome by advancing these BC_1F_1 plants into populations and testing them again for agronomic and disease resistance QTL. These populations will be evaluated for the same traits, and the identified QTL will be validated with another year repetition and with KASP markers designed in the regions found to be associated with the traits. In addition, QTL-seq will also be performed for late leaf spot resistance in addition to tomato spotted wilt orthotospovirus, since these materials showed high resistance to late leaf spot, especially in the *ValSten*^{4x} BC_1F_1 group. Although, the *IpaCor*^{4x} population only had one year of data, it was comprised of four subpopulations that yielded confidence in the putative QTL when they were identified in three or

more subpopulations. All four subpopulations will be progressed and QTL identification will be performed for agronomic traits as well as for tomato spotted wilt orthotospovirus and late leaf spot resistance.

Conclusions

Morphological, reproductive and pathogen resistance characterization of BC_1F_1 individuals derived from four unique allotetraploids and of four *IpaCor*^{4x} BC_1F_2 subpopulations were performed on field grown materials in this study. These materials showed a wide range of phenotypic diversity in flower production, flower size, leaf hair density, growth habit, flowering on the main stem, peg length, 100-pod weight, and 100-seed weight. Some of these traits can be introgressed into cultivated peanut in peanut breeding programs for cultivar improvement. QTL-seq and MapQTL 6 were utilized to identify putative QTL for tomato spotted wilt orthotospovirus resistance in the *IpaCor*^{4x} BC_1F_2 population on B05 and B10 and in the *ValSten*^{4x} BC_1F_1 group on A03 and B08. Putative QTL for flowering on the main stem were also identified in the *IpaCor*^{4x} BC_1F_2 population on A02, A05, and A06. These populations will be further studied to validate these QTL and to identify more QTL for other traits such as late leaf spot resistance. Once validated, these QTL will be used to introgress these resistances into peanut breeding lines that can be shared with peanut breeders to introgress these resistances into their best peanut cultivars. These improved cultivars will have greater yield and yield protection to increase and protect peanut production in the United States and promote global food security.

Acknowledgements

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Table 7.1. Subpopulation crossing explanations and sizes.

Crossing Explanation	Size
[13-1014 x ((13-1014 x *IpaCor*)_$F_{1:4}$)]_$BC_{1:4}$	88
[13-1014 x ((13-1014 x *IpaCor*)_$F_{1:4}$)]_$BC_{1:5}$	114
[13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:1}$	272
[13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:2}$	150

Table 7.2. Descriptions of the twelve QTL-Seq bulks made and the number of plants pooled for each library.

Population	Trait	Number of Plants Pooled
IpaCor^{4x} BC_1F_1	0% tomato spotted wilt orthotospovirus	16
IpaCor^{4x} BC_1F_1	≥ 90% tomato spotted wilt orthotospovirus	5
IpaDur^{4x} BC_1F_1	0% tomato spotted wilt orthotospovirus	42
IpaDur^{4x} BC_1F_1	≥ 90% tomato spotted wilt orthotospovirus	15
IpaSten^{4x} BC_1F_1	0% tomato spotted wilt orthotospovirus	14
IpaSten^{4x} BC_1F_1	≥ 90% tomato spotted wilt orthotospovirus	7
ValSten^{4x} BC_1F_1	0% tomato spotted wilt orthotospovirus	30
ValSten^{4x} BC_1F_1	≥ 90% tomato spotted wilt orthotospovirus	16
IpaCor^{4x} BC_1F_2	0% tomato spotted wilt orthotospovirus	100
IpaCor^{4x} BC_1F_2	≥ 70% tomato spotted wilt orthotospovirus	100
IpaCor^{4x} BC_1F_2	Flowering on main stem	100
IpaCor^{4x} BC_1F_2	No flowering on main stem	100

Figure 7.1. BC_1F_2 plants representative of the cultivated (compact and full) (A) versus wild *Arachis*-like (few spreading branches) (B) growth habit rating.

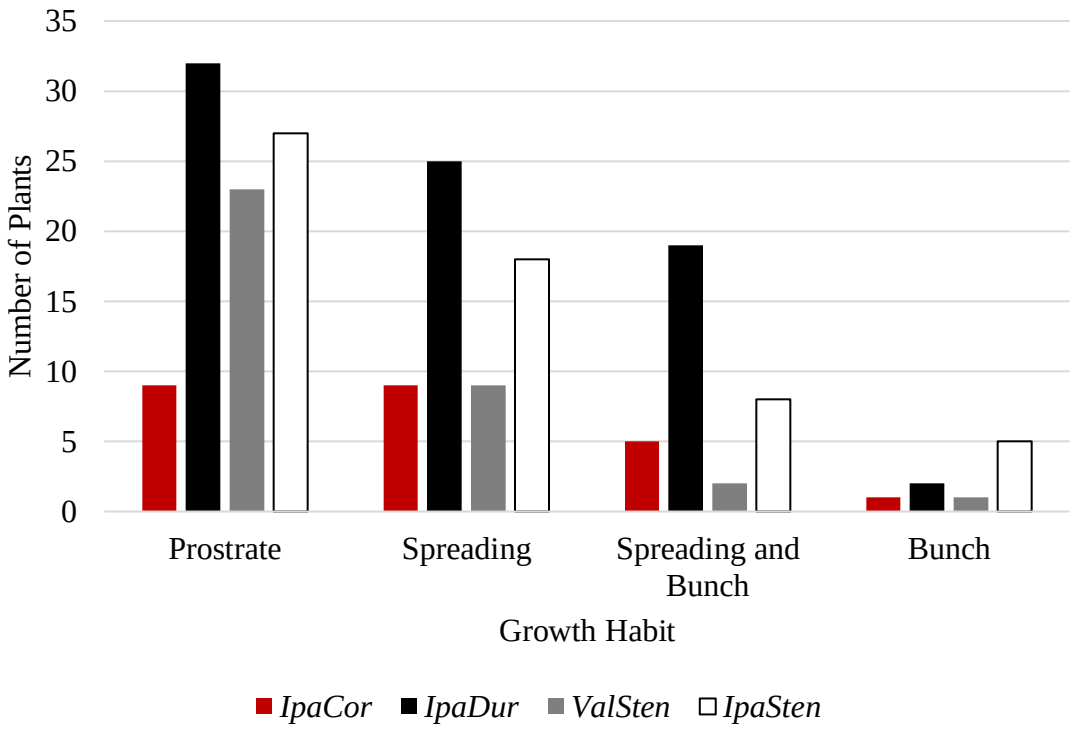

Figure 7.2. Histograms of the number of BC_1F_1 plants with each type of growth habit.

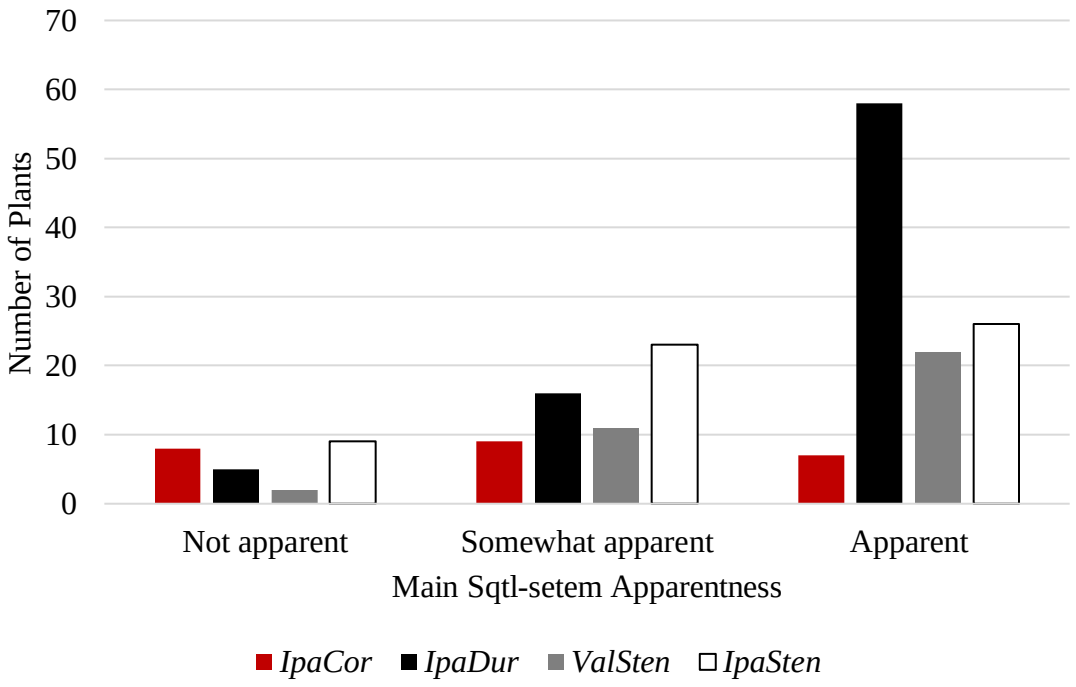

Figure 7.3. Histograms of the number of BC_1F_1 plants with main stem apparentness phenotype.

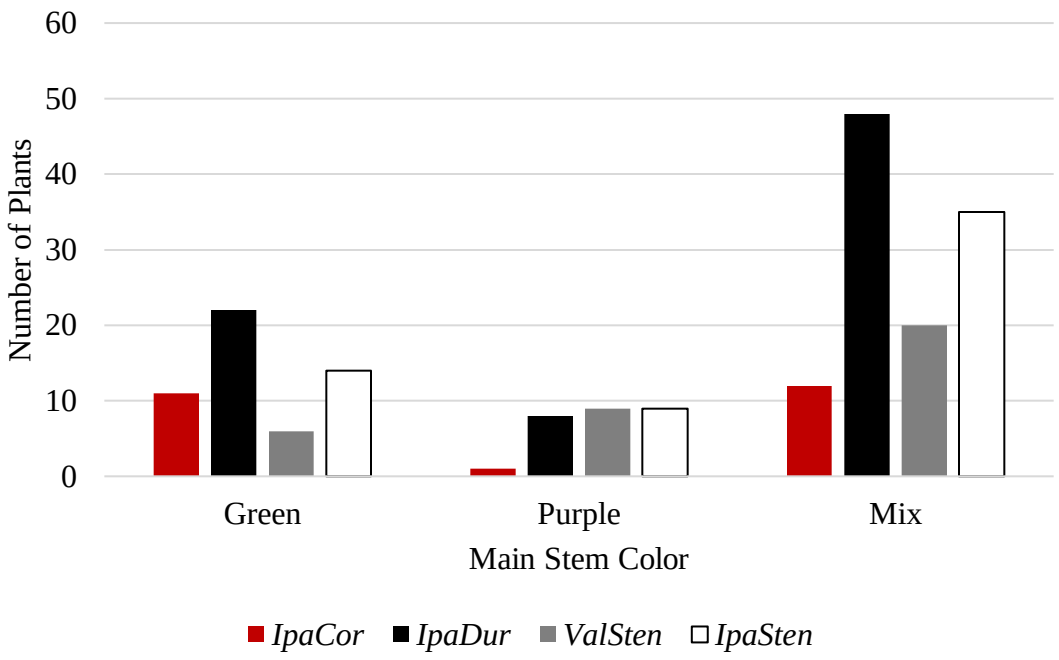

Figure 7.4. Histograms of the number of BC_1F_1 plants with each main stem color.

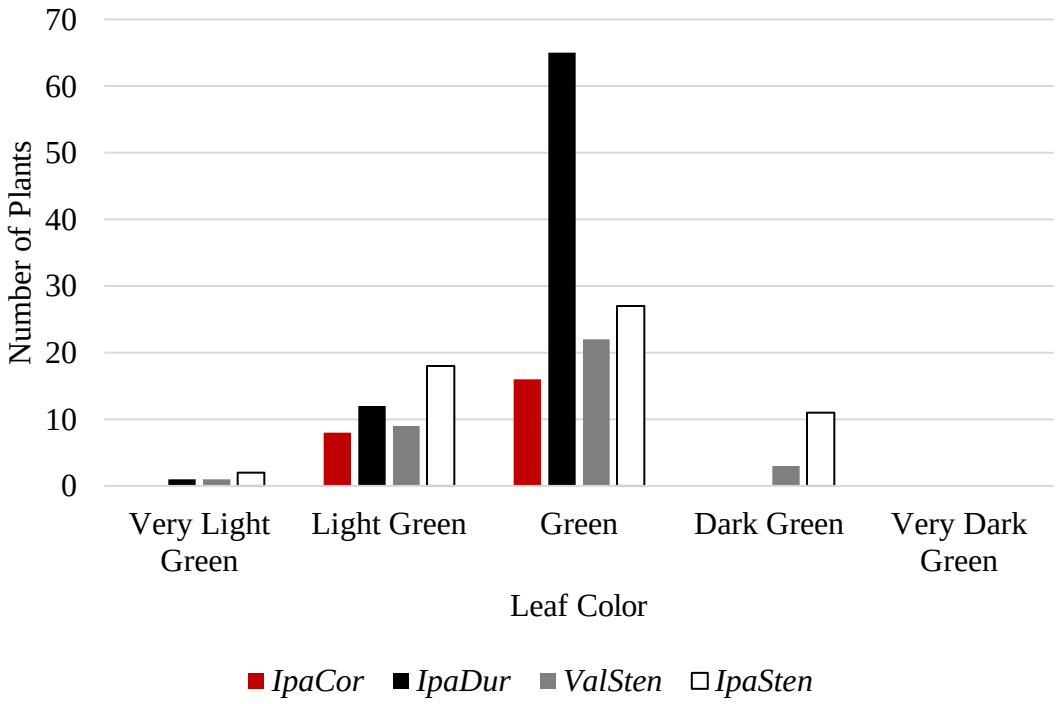

Figure 7.5. Histograms of the number of BC_1F_1 plants with each leaf color.

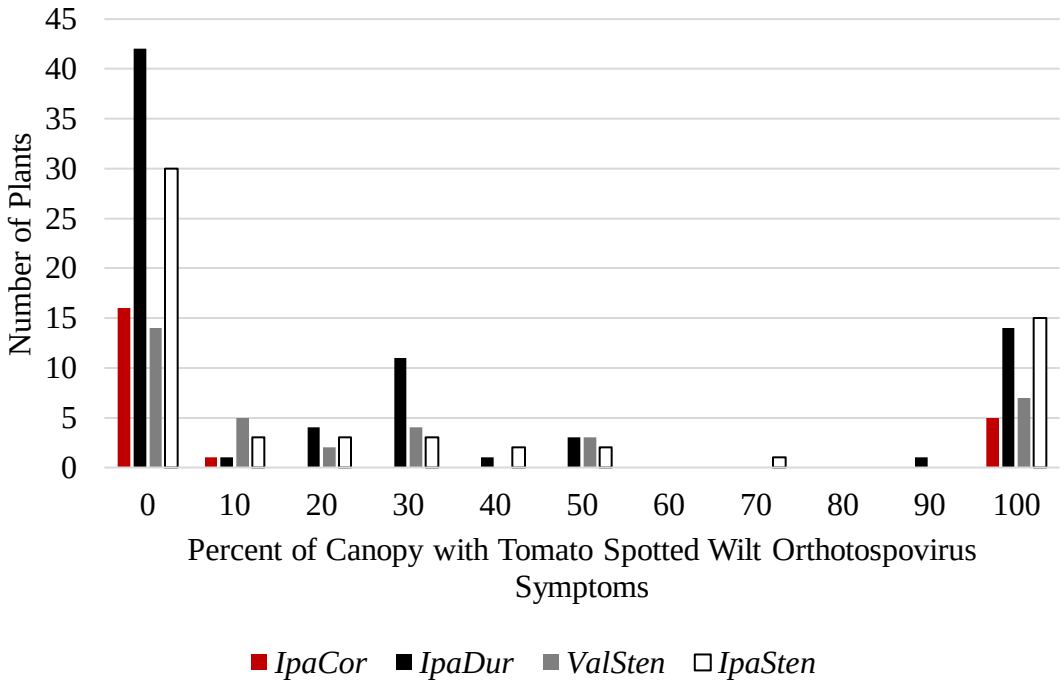

Figure 7.6. Histograms of the number of BC_1F_1 plants with different percentages of their canopy displaying tomato spotted wilt orthotospovirus symptoms.

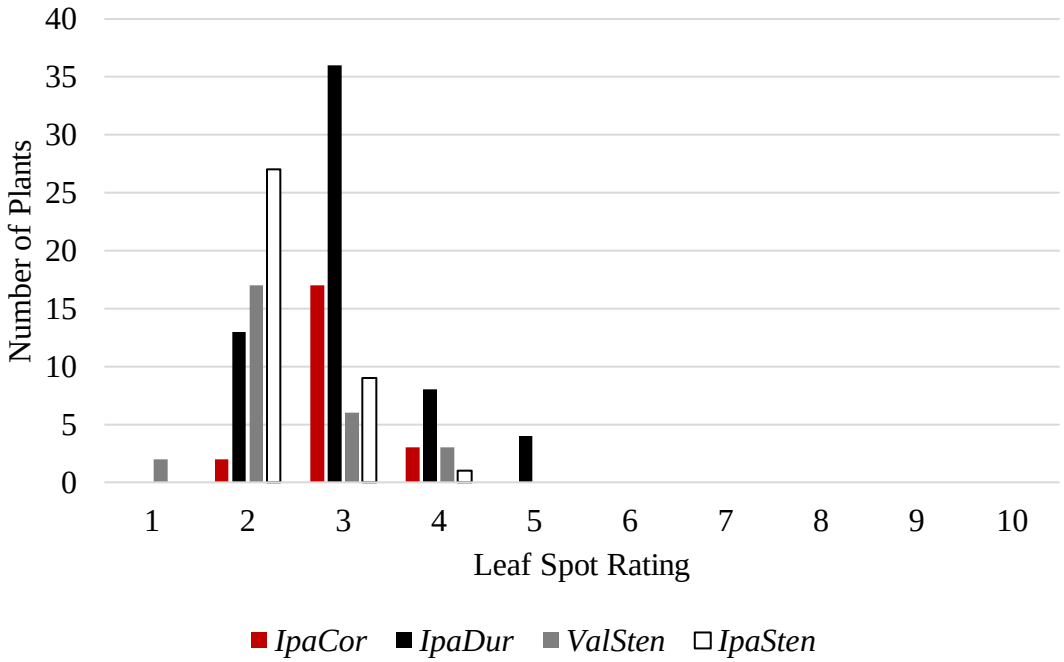

Figure 7.7. Histograms of the number of BC_1F_1 plants with each leaf spot severity rating.

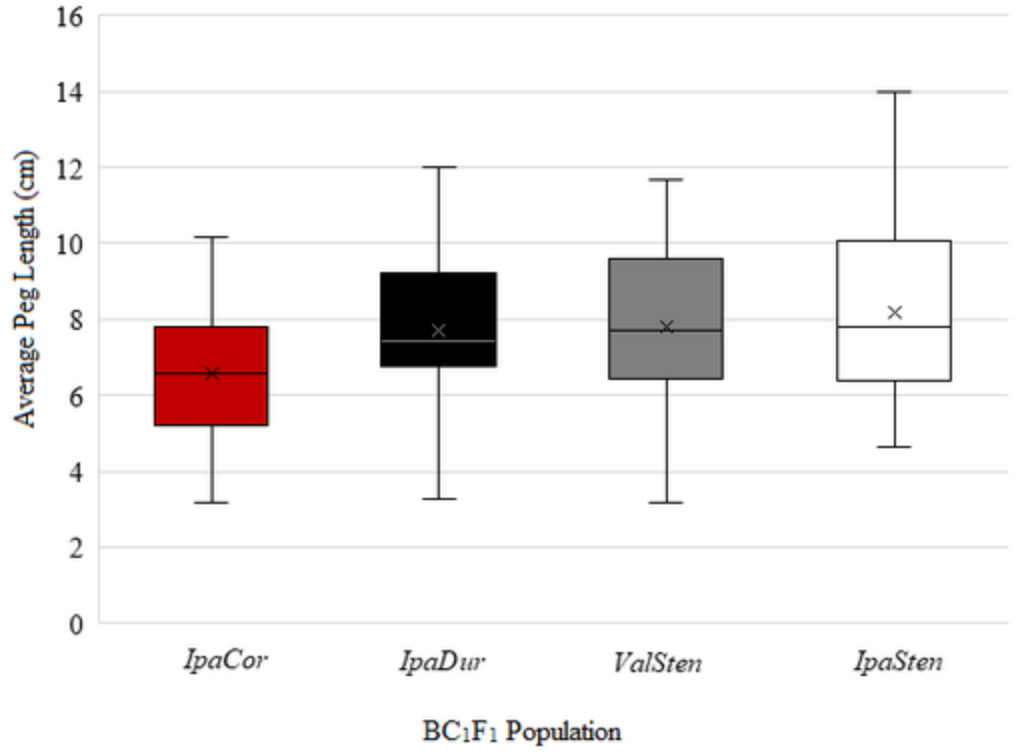

Figure 7.8. Bar and whiskers plot of peg length of BC_1F_1 plants.

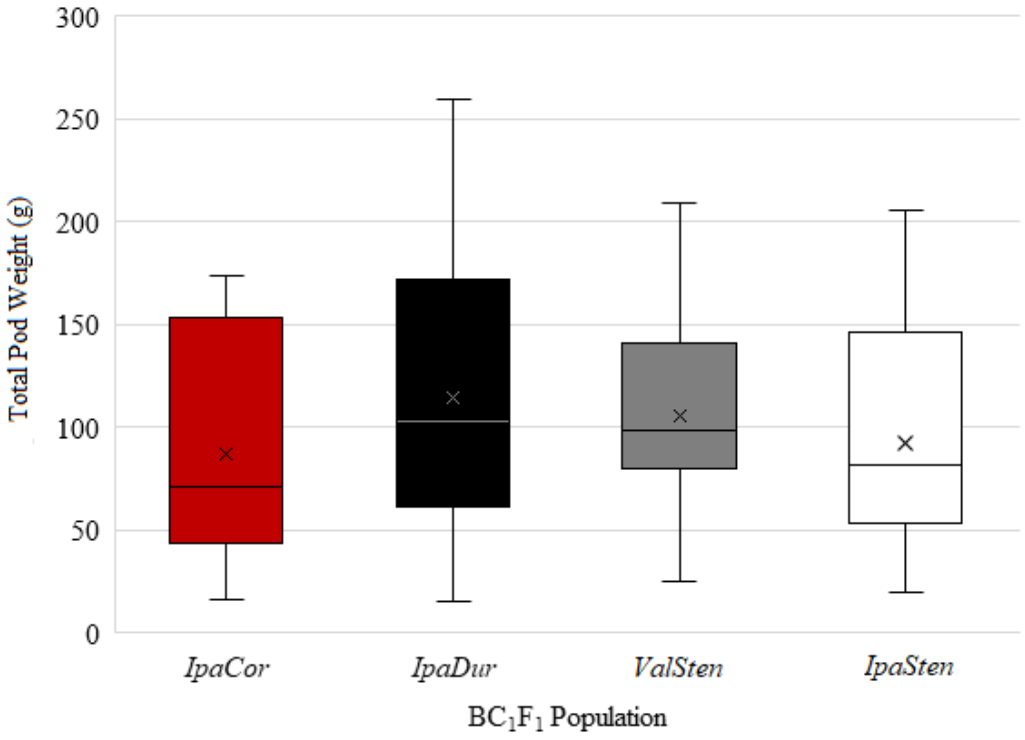

Figure 7.9. Bar and whiskers plot of total pod weight of BC_1F_1 plants.

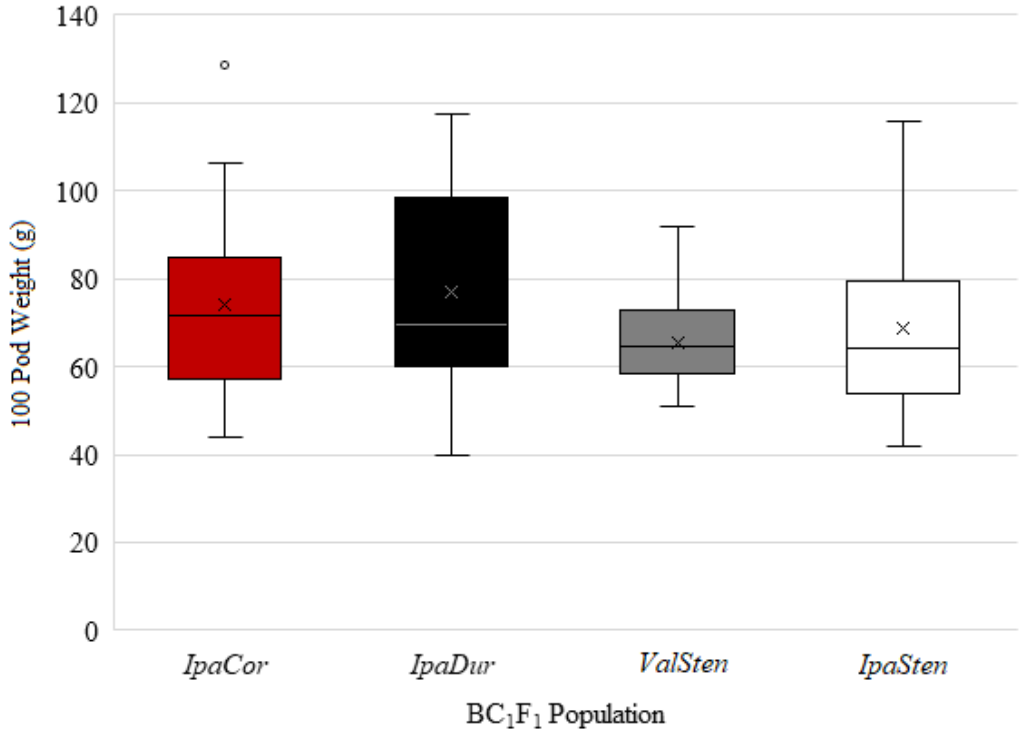

Figure 7.10. Bar and whiskers plot of 100-pod weight of BC_1F_1 plants.

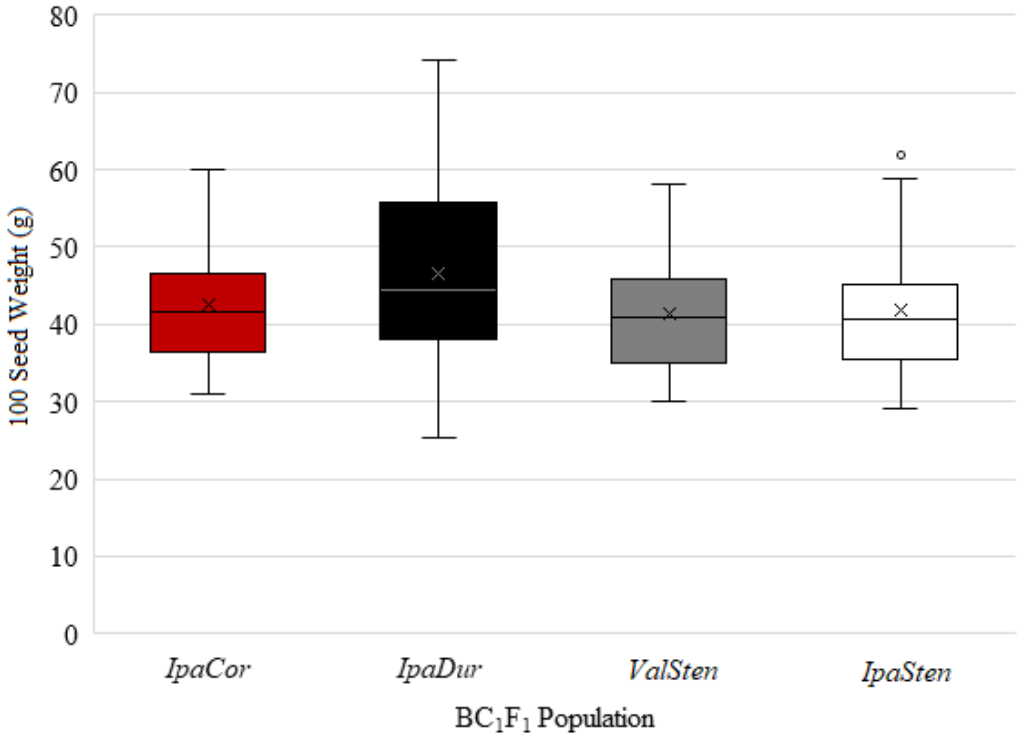

Figure 7.11. Bar and whiskers plot of 100-seed weight of BC_1F_1 plants.

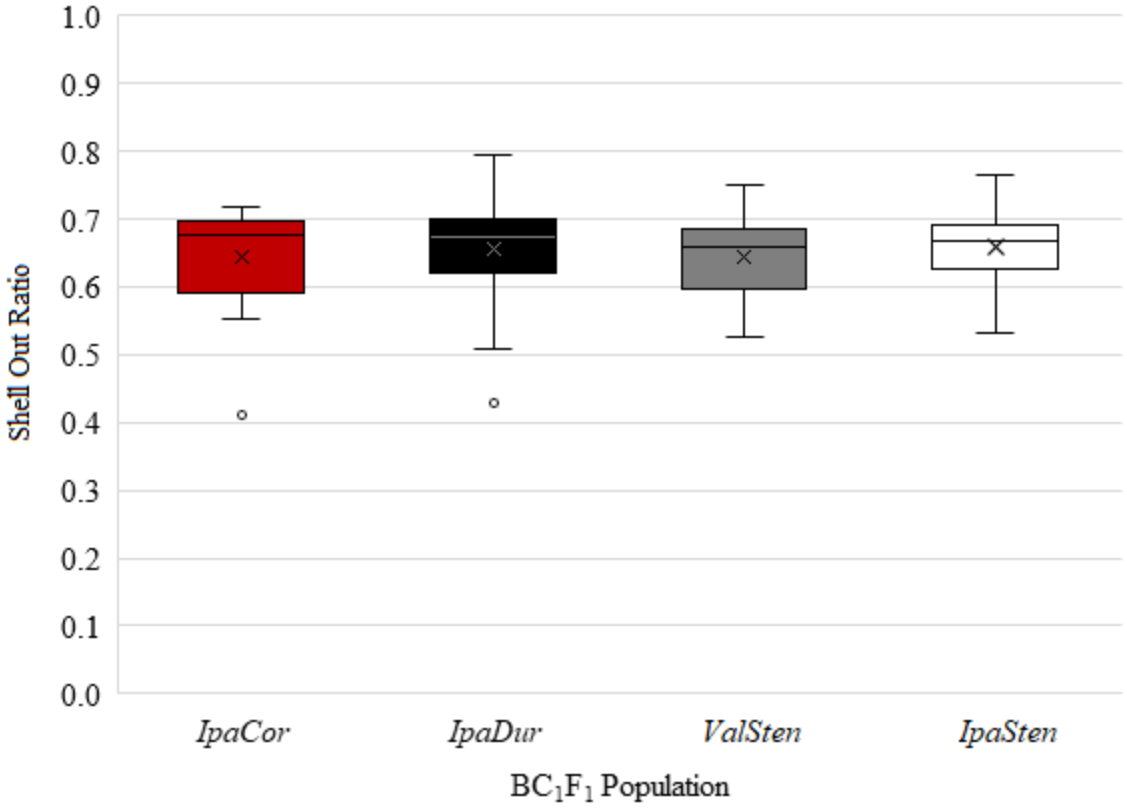

Figure 7.12. Bar and whiskers plot of shell out ratio of BC_1F_1 plants.

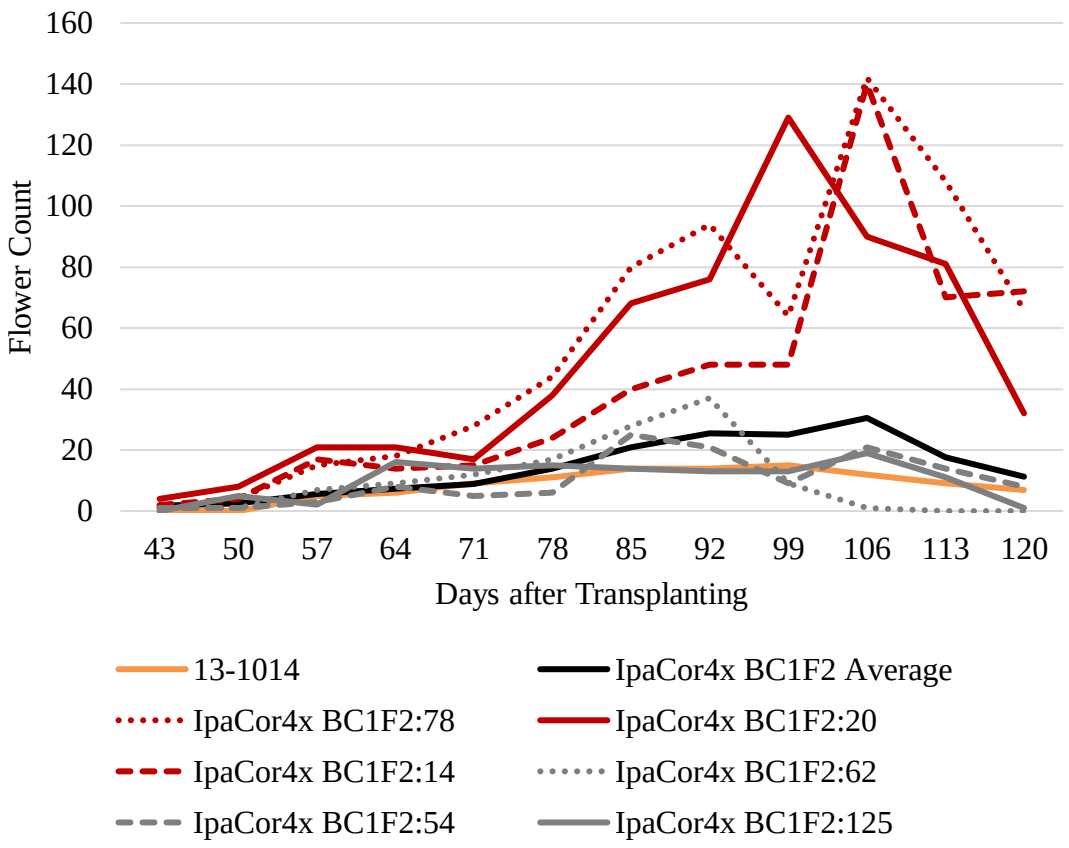

Figure 7.13. Flower count for 13-1014, the average of all *IpaCor*^{4x} BC_1F_1s, three BC_1F_1s similar to 13-1014, and three BC_1F_1s similar to *IpaCor*^{4x} from 43 to 120 days after transplanting.

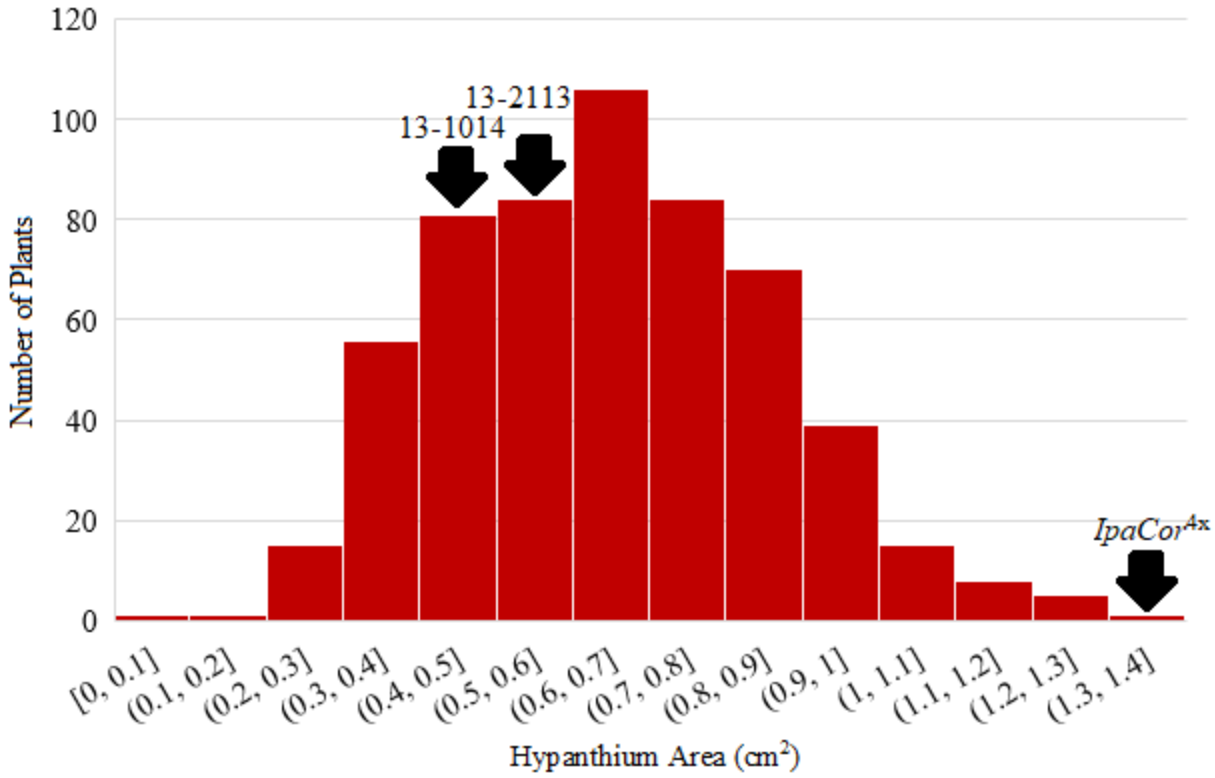

Figure 7.14. Histogram of hypanthium area of the *IpaCor*^{4x} BC_1F_2 population.

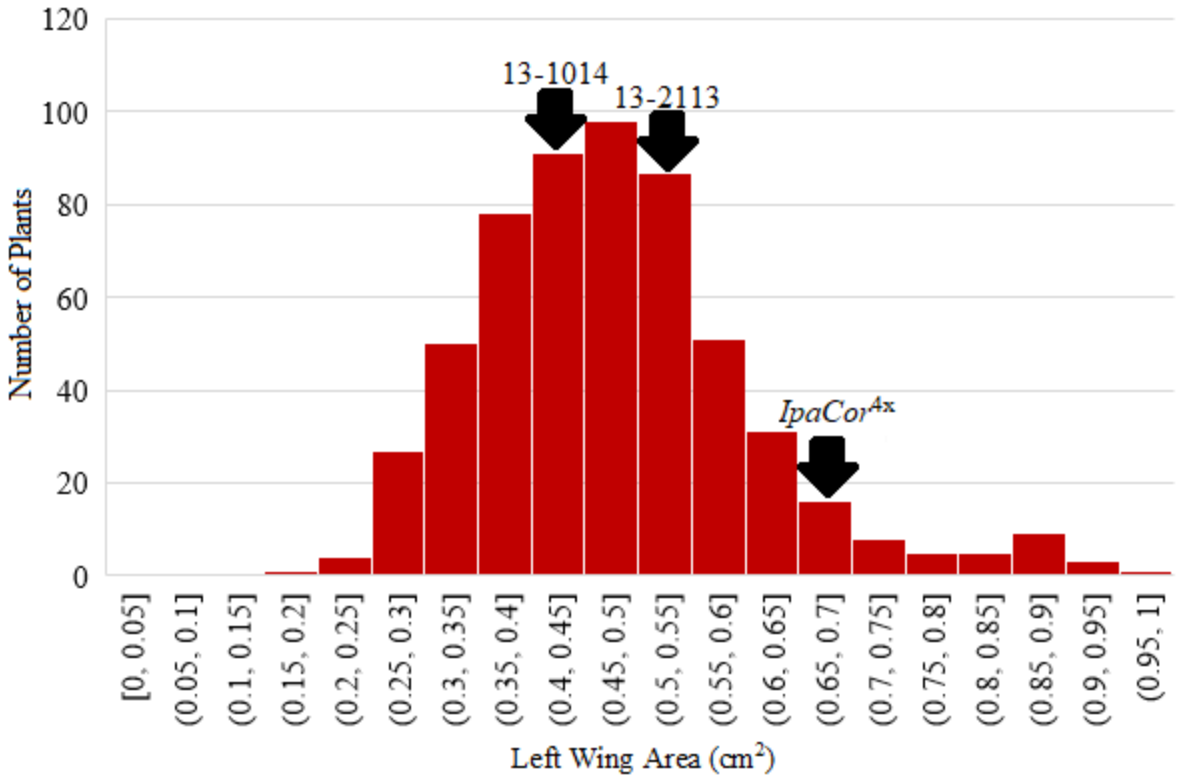

Figure 7.15. Histogram of left wing area of the *IpaCor*^{4x} BC_1F_2 population.

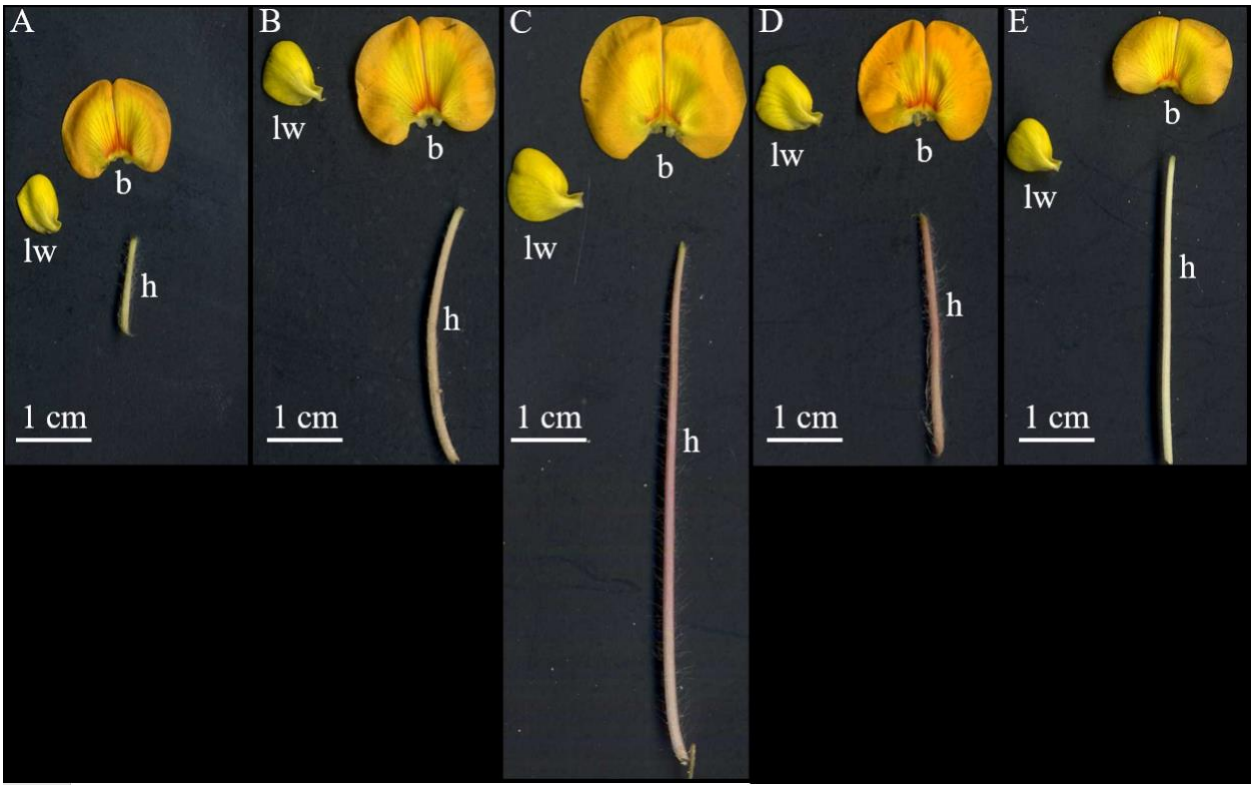

Figure 7.16. Scans of dissected flowers of A) 13-1014, B) 13-2113, C) *IpaCor*^{4x}_$S_{0:5}$_S_1, D) *IpaCor*^{4x} $BC_1F_{2:73}$, and E) *IpaCor*^{4x} $BC_1F_{2:393}$, in which banner is labeled b, hypanthium h, and left wing lw.

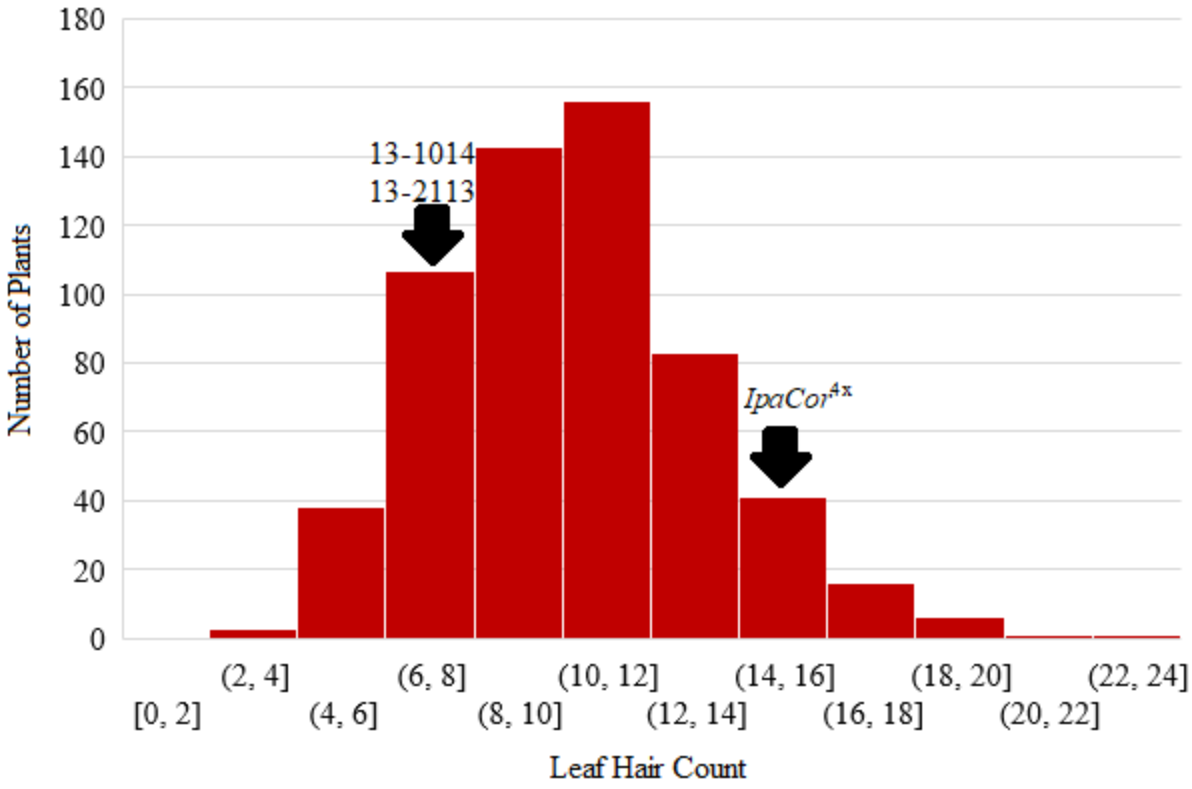

Figure 7.17. Histogram of leaf hair count of the *IpaCor*^{4x} BC_1F_2 population.

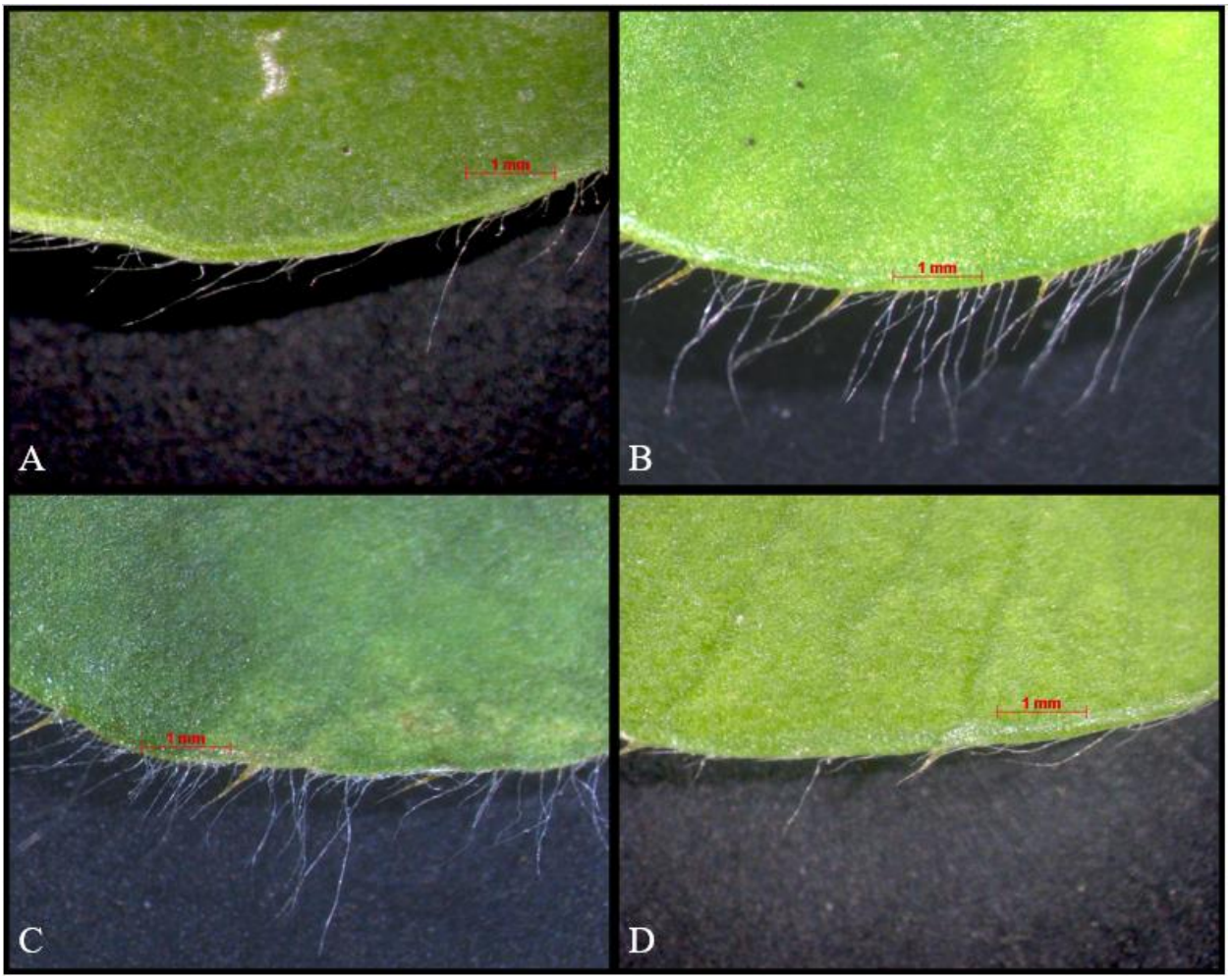

Figure 7.18. Scans of the bottom edge of the bottom, right leaflet from the most distal, fully expanded leaf excised from the primary laterals with a 1mm scale bar in red for A) 13-2113, B) *IpaCor*^{4x}_$S_{0:5}$_S_1, C) *IpaCor*^{4x} $BC_1F_{2:421}$, and D) *IpaCor*^{4x} $BC_1F_{2:30}$.

Figure 7.19. BC_1F_2 plants representative of A) prostrate, B) spreading, and C) intermediate between spreading and bunch growth habit.

Figure 7.19. *IpaCor*^{4x} BC_1F_2 plant that had flowering and pegging on main stem.

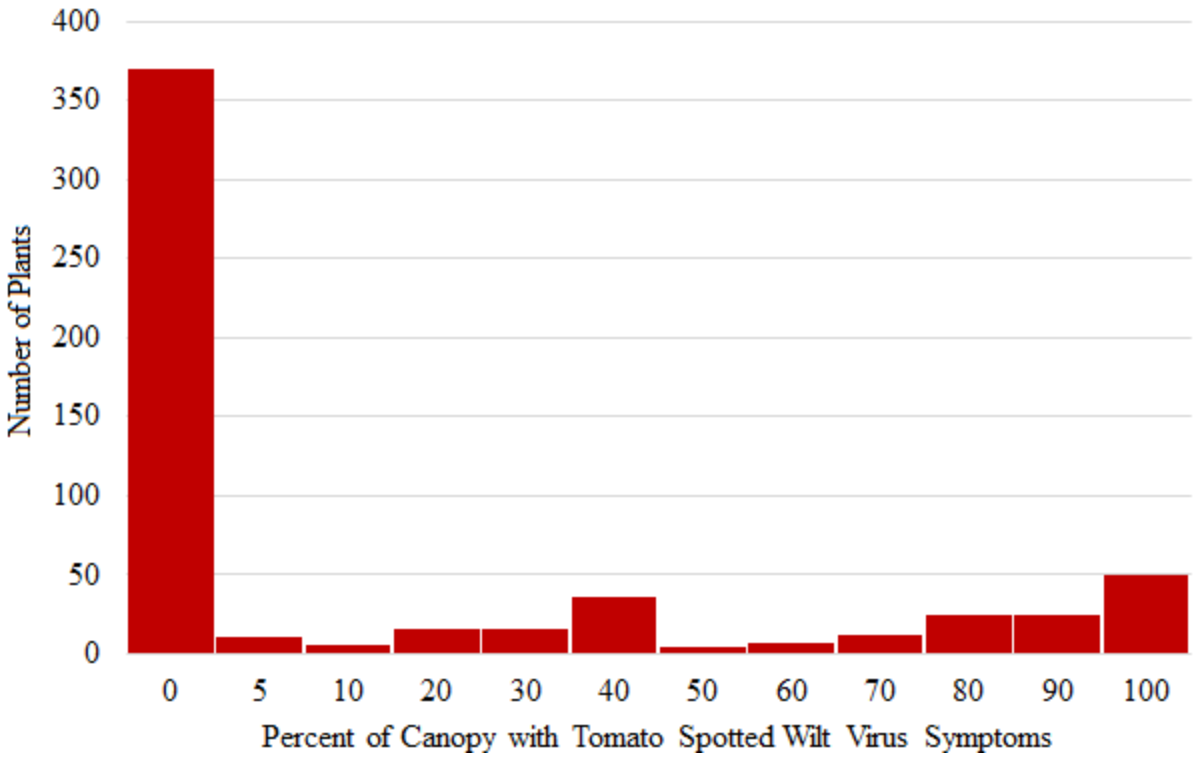

Figure 7.20. Histogram of the number of *IpaCor*^{4x} BC_1F_2 plants with different percentages of their canopy displaying tomato spotted wilt orthotospovirus symptoms.

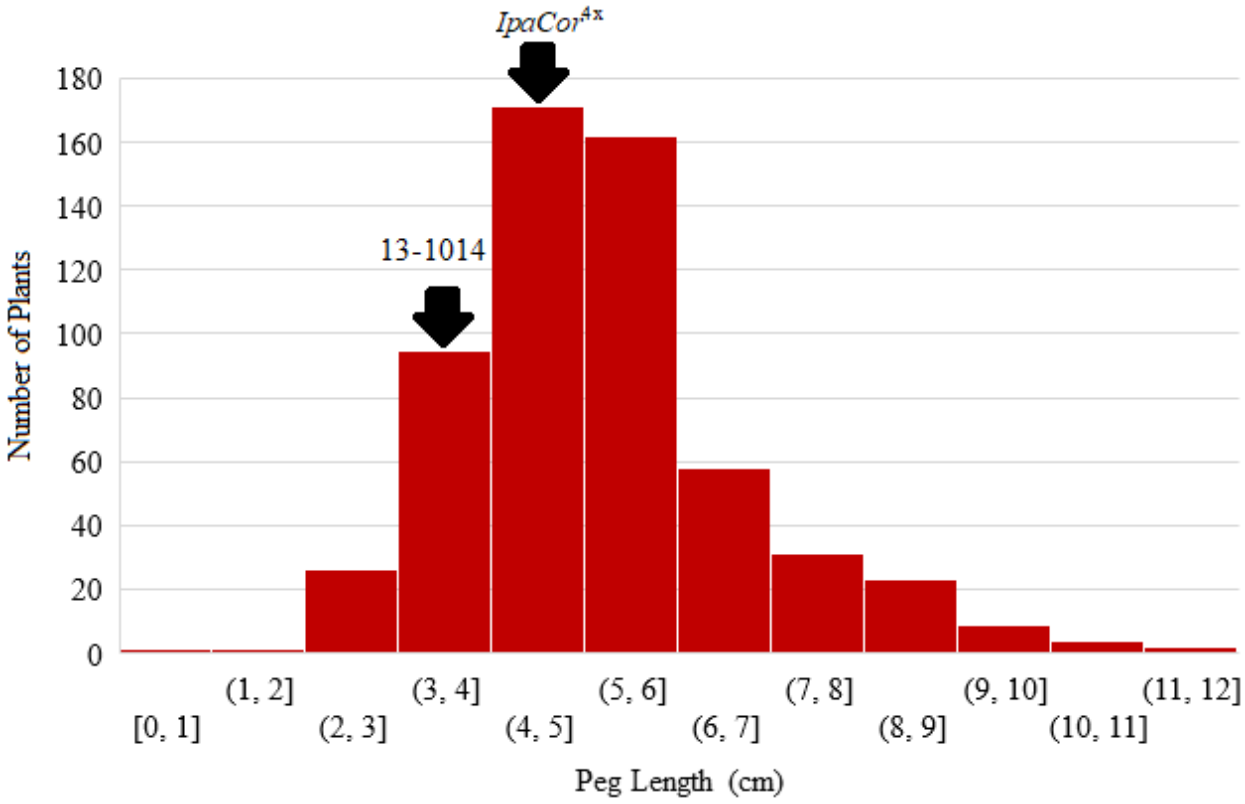

Figure 7.21. Histogram of peg length for the *IpaCor*^{4x} BC_1F_2 population.

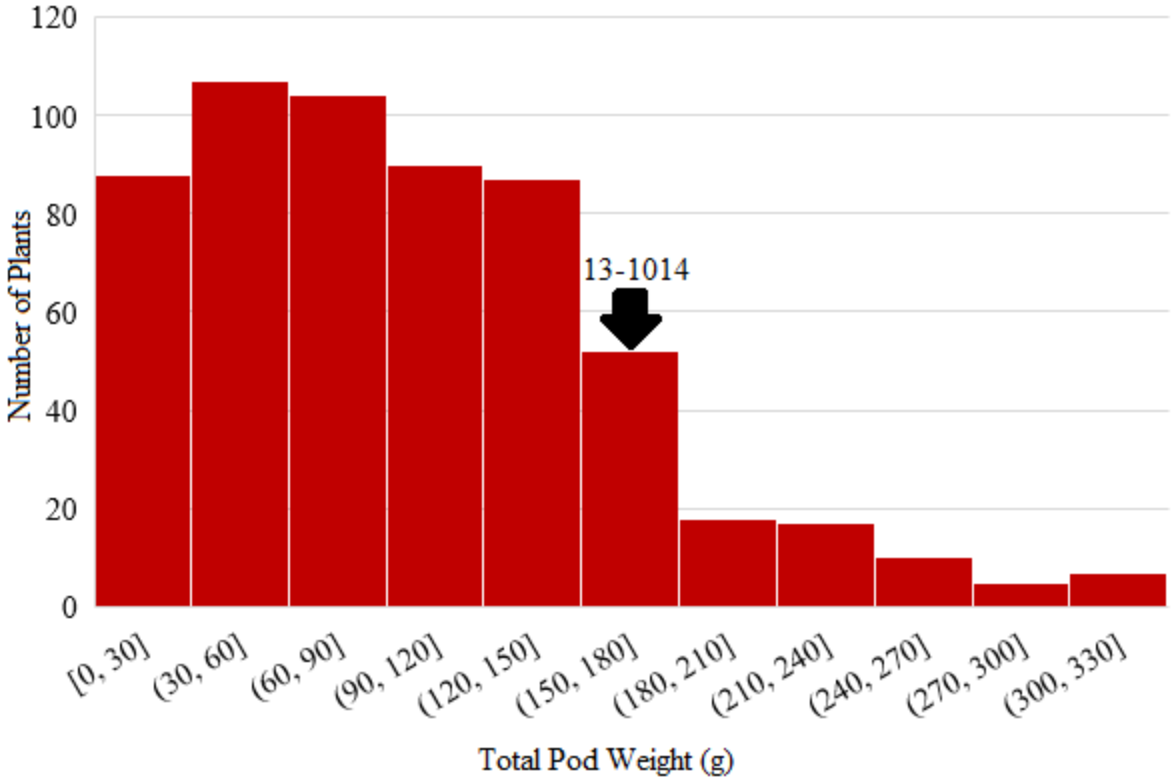

Figure 7.21. Histogram of total pod weight for the *IpaCor*^{4x} BC_1F_2 population.

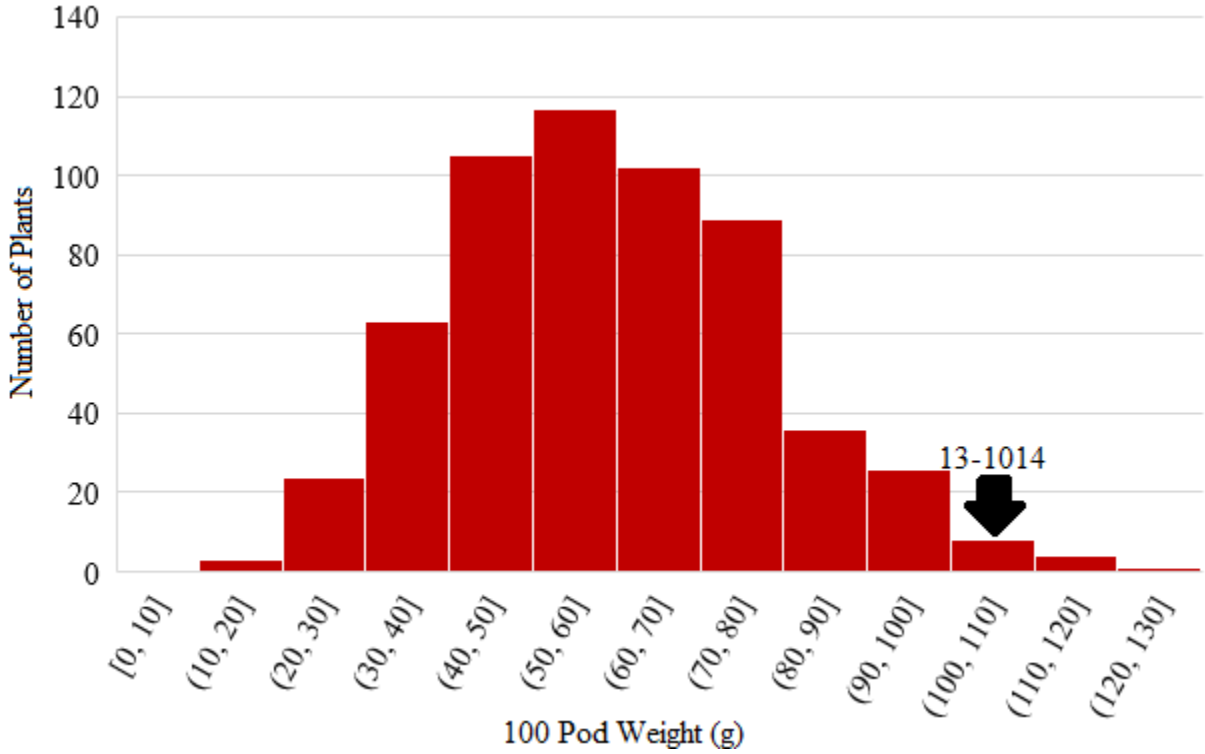

Figure 7.22. Histogram of 100-pod weight for the *IpaCor*^{4x} BC_1F_2 population.

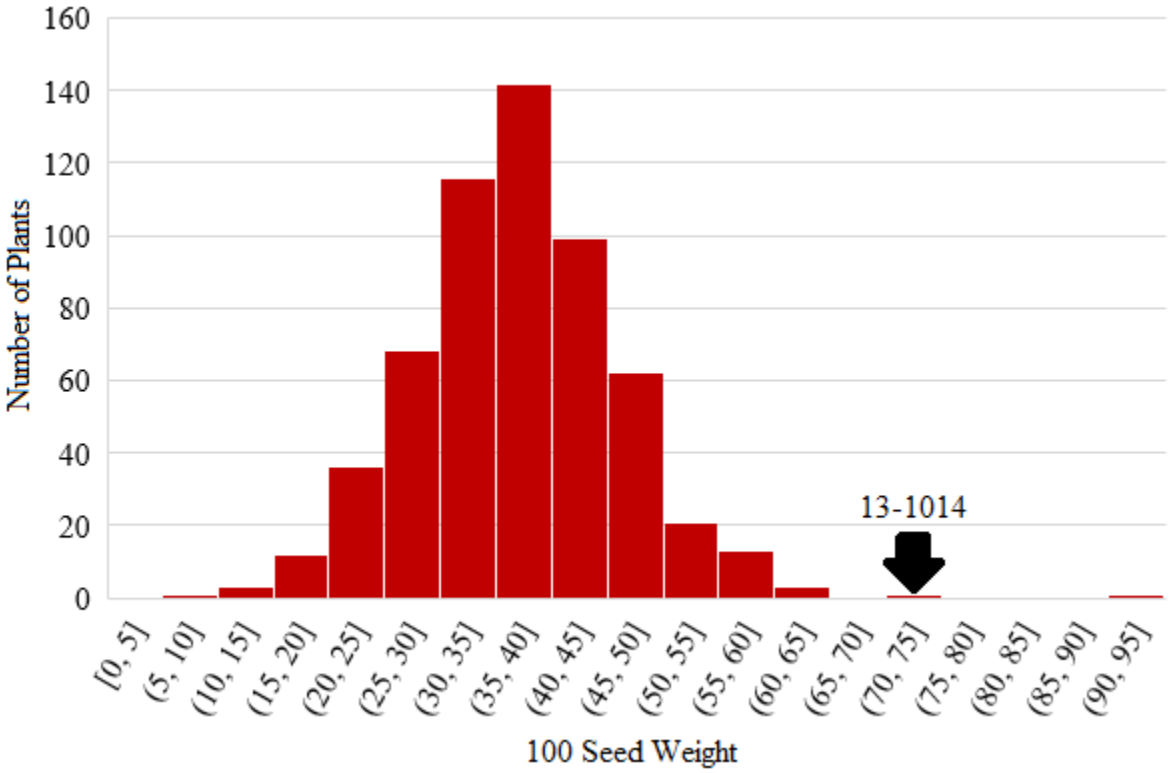

Figure 7.23. Histogram of 100-seed weight for the *IpaCor*^{4x} BC_1F_2 population.

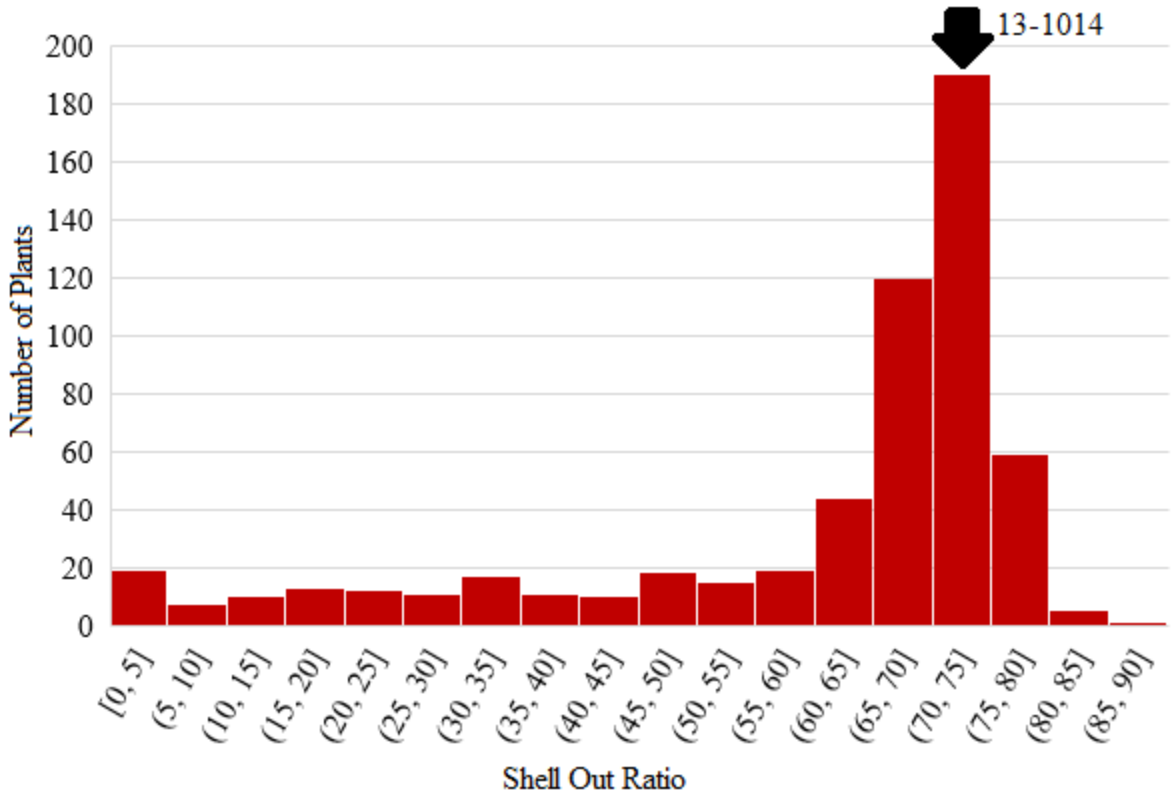

Figure 7.24. Histogram of shell-out ratio for the *IpaCor*^{4x} BC_1F_2 population.

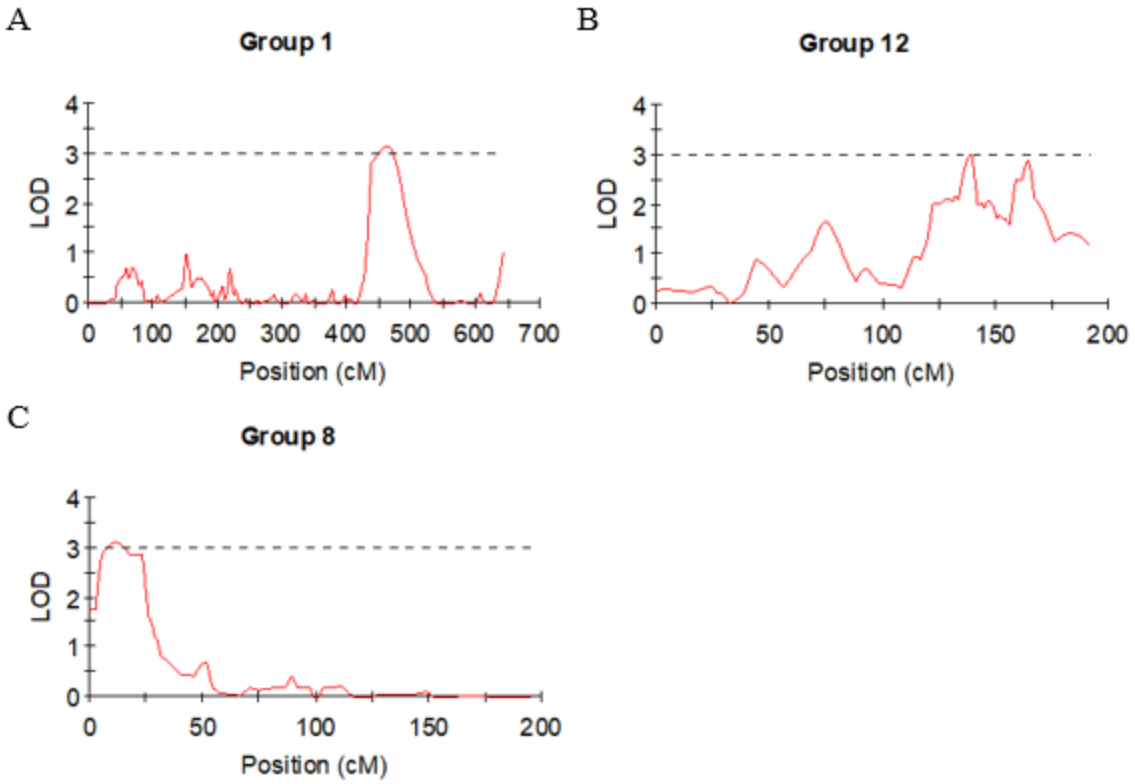

Figure 7.25. Output from MapQTL 6 showing putative QTL for A) main stem color, B) peg length, and C) 100-seed weight found with LOD scores above 3.

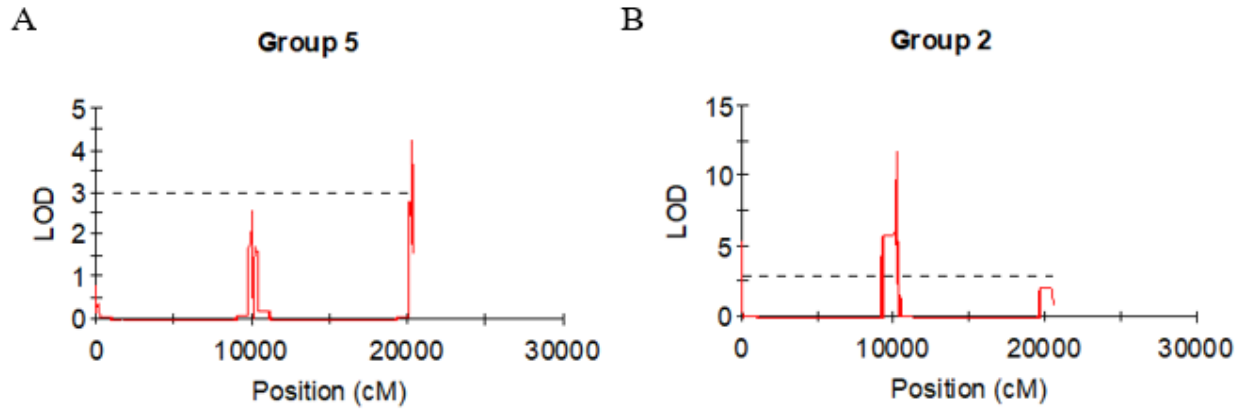

Figure 7.26. Output from MapQTL 6 showing putative QTL for flowering on main stem for A) [13-1014 x ((13-2113 x *IpaCor*)_$F_{1:3}$)]_$BC_{1:2}$ and B) [13-1014 x ((13-1014 x *IpaCor*)_$F_{1:4}$)]_$BC_{1:5}$ subpopulations.

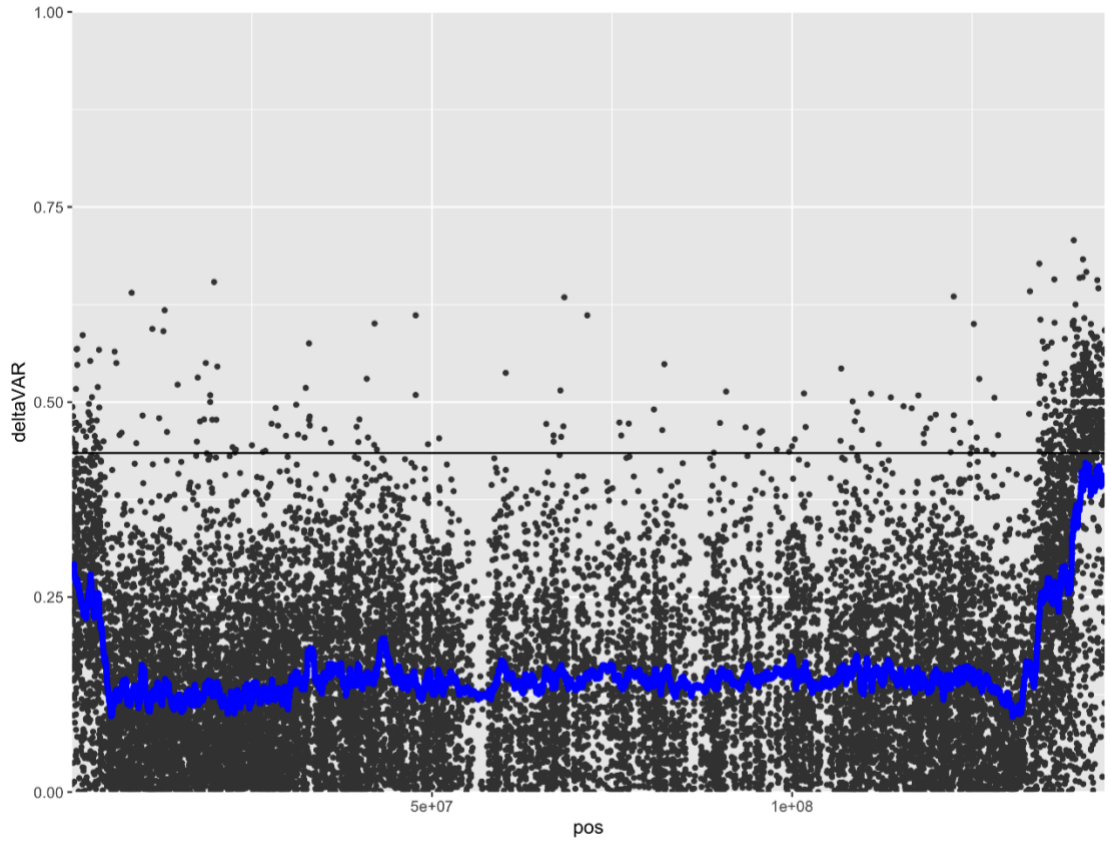

Figure 7.27. Significant QTL for tomato spotted wilt orthotospovirus resistance identified on A03 in the *ValSten*^{4x} BC_1F_1 materials.

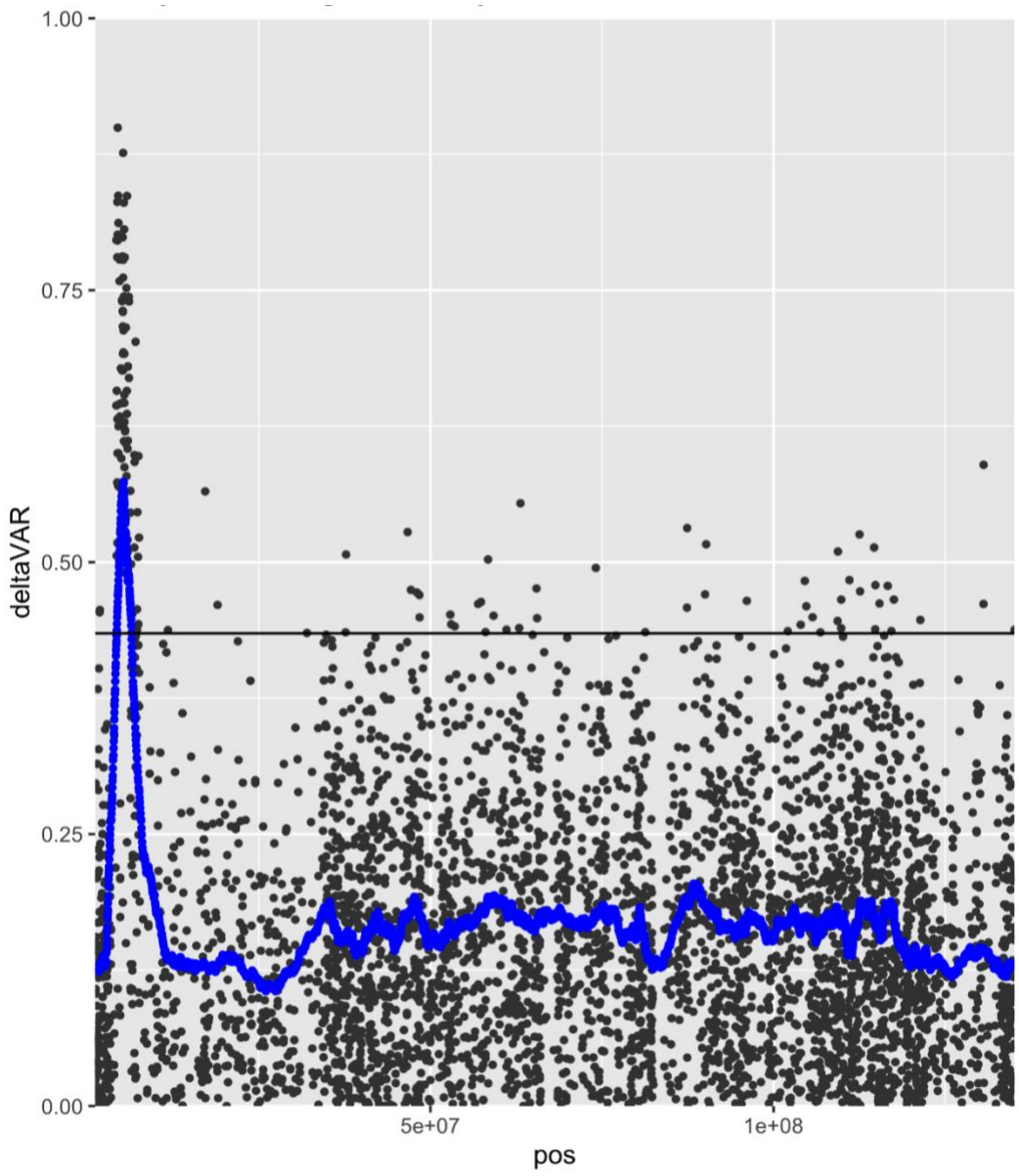

Figure 7.28. Significant QTL for tomato spotted wilt orthotospovirus resistance identified on B08 in the *ValSten*^{4x} BC_1F_1 materials.

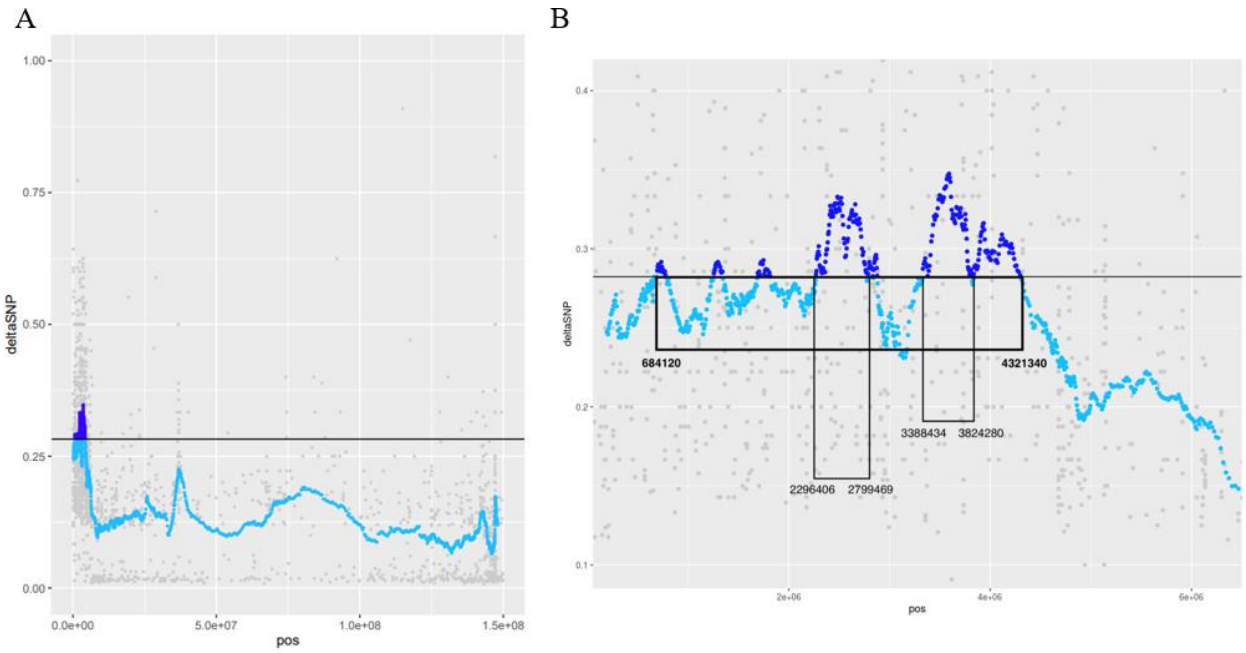

Figure 7.29. Significant QTL for tomato spotted wilt orthotospovirus resistance identified on B05 in the *IpaCor*^{4x} BC_1F_2 population, in which A) shows the entire chromosome and B) shows a close-up on the QTL significant peaks at the start of B05.

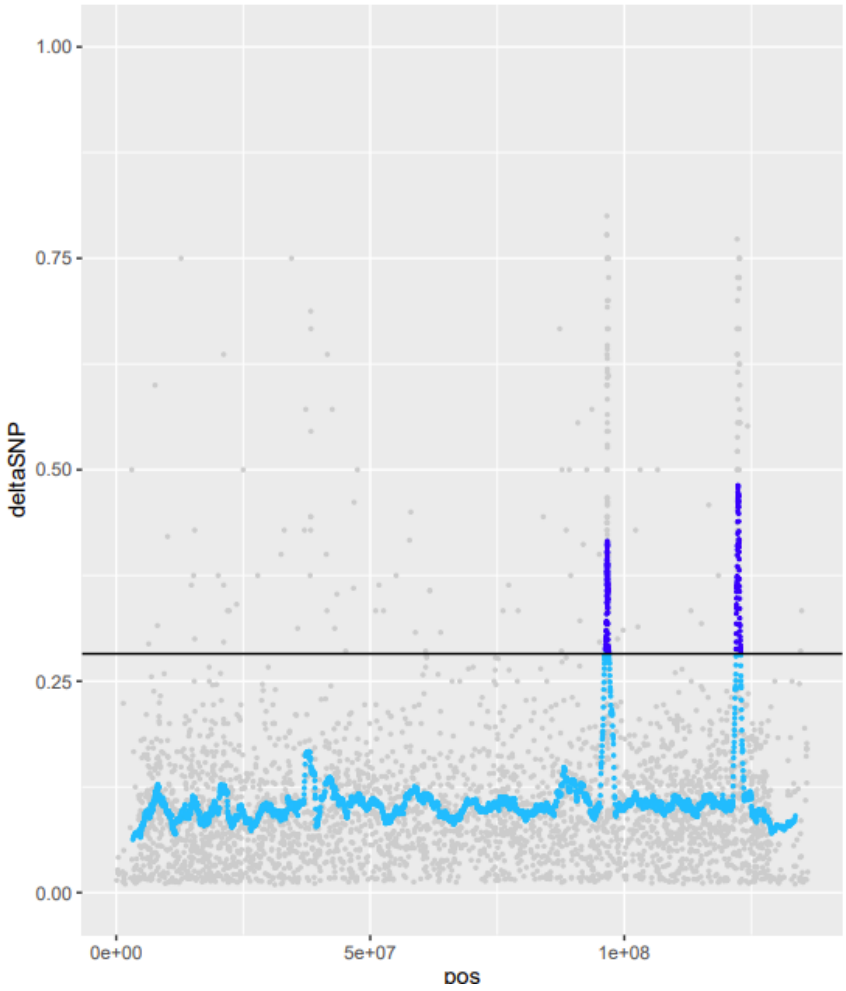

Figure 7.30. Significant QTLs for tomato spotted wilt orthotospovirus resistance identified on B10 in the *IpaCor*^{4x} BC_1F_2 population.

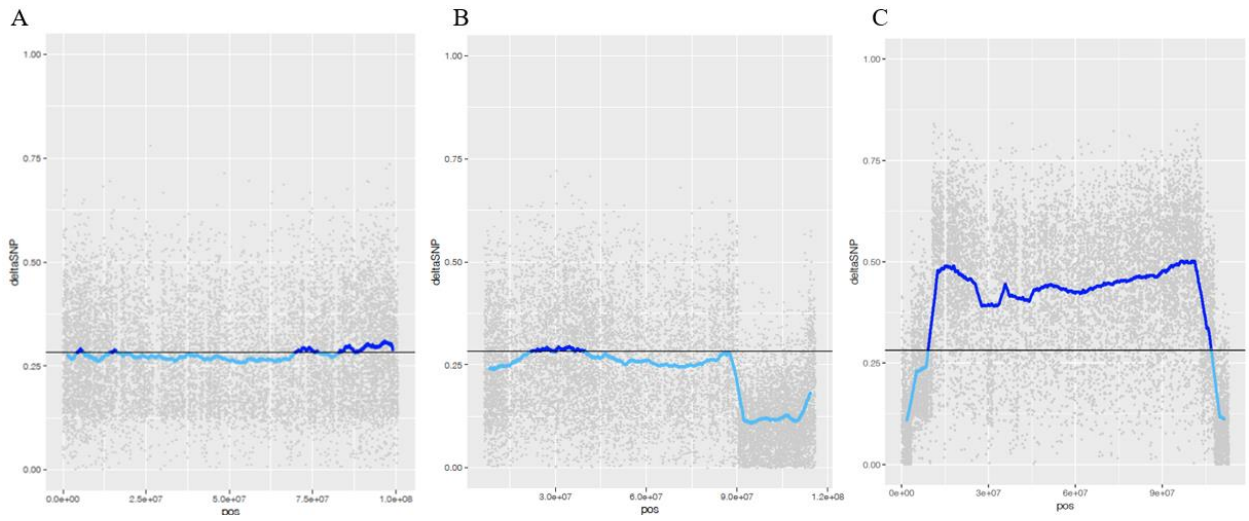

Figure 7.31. Significant QTLs for flowering on main stem identified on A) A02, B) A05, and C) A06 in the *IpaCor*^{4x} BC_1F_2 population.

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Appendix 7

Supplemental Tables

Table S7.1. Crossing identification of the *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x} derived BC_1F_1 individuals that were produced and evaluated in this study.

IpaCor BC_1F_1 Pedigree	Number of BC_1F_1 Plants from Combination
13-1014 x (13-1014 x *IpaCor*^{4x}_$S_{0:5}$)_$F_{1:4}$	1
13-1014 x (13-2113 x *IpaCor*^{4x}_$S_{0:5}$)_$F_{1:1}$	2
13-1014 x (13-2113 x *IpaCor*^{4x}_$S_{0:5}$)_$F_{1:3}$	5
13-1014 x (13-2113 x *IpaCor*^{4x}_$S_{0:5}$)_$F_{1:4}$	3
5-646-10 x (13-1014 x *IpaCor*^{4x}_$S_{0:6}$)_$F_{1:4}$	4
13-1014 x (13-1014 x *IpaCor*^{4x}_$S_{0:2}$)_$F_{1:4}$	7
13-2113 x (13-1014 x *IpaCor*^{4x}_$S_{0:5}$)_$F_{1:3}$	2
13-2113 x (13-2113 x *IpaCor*^{4x}_$S_{0:5}$)_$F_{1:7}$	1
IpaDur BC_1F_1 Individuals	Number of BC_1F_1 Plants from Combination
13-1014 x (13-1014 x *IpaDur*^{4x}_$S_{0:1}$)_$F_{1:2}$	6
13-1014 x (13-1014 x *IpaDur*^{4x}_$S_{0:1}$)_$F_{1:3}$	1
13-1014 x (13-1014 x *IpaDur*^{4x}_$S_{0:5}$)_$F_{1:3}$	1
13-1014 x (13-1014 x *IpaDur*^{4x}_$S_{0:6}$)_$F_{1:1}$	7
13-1014 x (13-1014 x *IpaDur*^{4x}_$S_{0:6}$)_$F_{1:2}$	9
13-1014 x (13-1014 x *IpaDur*^{4x}_$S_{0:6}$)_$F_{1:3}$	8
13-2113 x (13-2113 x *IpaDur*^{4x}_$S_{0:1}$)_$F_{1:2}$	13
13-2113 x (13-2113 x *IpaDur*^{4x}_$S_{0:1}$)_$F_{1:3}$	9
13-2113 x (13-2113 x *IpaDur*^{4x}_$S_{0:5}$)_$F_{1:1}$	7
13-2113 x (13-2113 x *IpaDur*^{4x}_$S_{0:5}$)_$F_{1:2}$	7
13-2113 x (13-2113 x *IpaDur*^{4x}_$S_{0:6}$)_$F_{1:1}$	7
IpaSten BC_1F_1 Individuals	Number of BC_1F_1 Plants from Combination
13-1014 x (13-1014 x *IpaSten*^{4x}_$S_{0:1}$)_$F_{1:1}$	16
13-1014 x (13-1014 x *IpaSten*^{4x}_$S_{0:1}$)_$F_{1:2}$	22
13-2113 x (13-2113 x *IpaSten*^{4x}_$S_{0:1}$)_$F_{1:1}$	7
13-2113 x (13-2113 x *IpaSten*^{4x}_$S_{0:1}$)_$F_{1:2}$	14
ValSten BC_1F_1 Individuals	Number of BC_1F_1 Plants from Combination
13-1014 x (13-1014 x *ValSten*^{4x}_$S_{0:2}$)_$F_{1:2}$	6
13-1014 x (13-1014 x *ValSten*^{4x}_$S_{0:2}$)_$F_{1:3}$	7
13-1014 x (13-1014 x *ValSten*^{4x}_$S_{0:5}$)_$F_{1:1}$	3
13-2113 x (13-2113 x *ValSten*^{4x}_$S_{0:2}$)_$F_{1:1}$	4
13-2113 x (13-2113 x *ValSten*^{4x}_$S_{0:2}$)_$F_{1:2}$	1
13-2113 x (13-2113 x *ValSten*^{4x}_$S_{0:4}$)_$F_{1:1}$	5
13-2113 x (13-2113 x *ValSten*^{4x}_$S_{0:5}$)_$F_{1:1}$	7
13-2113 x (13-2113 x *ValSten*^{4x}_$S_{0:5}$)_$F_{1:1}$	9

CHAPTER 8

DEVELOPMENT AND APPLICATIONS OF KASP MARKERS DISTINGUISHING A- AND B/K-GENOMES OF *ARACHIS*

[1] C.M. Levinson, D. Bertioli, Y. Chu, A.K. Culbreath, H.T. Stalker, and P. Ozias-Akins. To be submitted to Euphytica.

Abstract

Peanut is an important global food crop with a narrow genetic base due to its domestication bottleneck and the ploidy barrier between it and most of its wild, diploid relatives. Increasingly, peanut breeders have been introgressing beneficial alleles from its diploid relatives into the cultigen to improve agronomic traits along with as its pathogen and insect resistance. This process of introgression can be carried out by making crosses between wild A- and B/K-genome *Arachis* species and doubling the chromosomes of these diploid hybrids to produce synthetic allotetraploids cross-compatible with peanut. The Axiom_Arachis SNP array is an incredible genomic tool that can be used on allotetraploid-derived peanut populations to map desirable QTL for introgression, among other applications. However, the high resolution of this array is not always necessary for applications such as 1) confirming true hybrids made from crossing A- and B/K-genome wild *Arachis* species and 2) confirmation and tracking of the inheritance of previously discovered homoeologous recombination events, which commonly occur in synthetic allotetraploid-derived materials. In this study, 105 KASP markers distinguishing A- and B/K-genomes were designed to span the entire peanut genome that can be used as a cost-efficient and time-efficient alternative to the array for these two applications.

Introduction

Peanut (*Arachis hypogaea* L.) is an important food crop with an annual production of about 49 million tons (FAOSTAT, 2019). The *Arachis* genus includes over 80 named species, including peanut, that originated in South America (Stalker et al., 2016). It has been suggested that around 9,400 years ago, *A. ipaensis* (BB; 2*n*=2*x*=20) was transported by humans to the

eastern foothills of the Andes mountains and into the range of *A. duranensis* (AA; 2*n*=2*x*=20) (Bertioli et al., 2016). The two wild species likely were crossed by insect pollination, producing sterile AB hybrid offspring (AB; 2*n*=2*x*=20). Either through spontaneous doubling or fusion of unreduced gametes, one or a few fertile tetraploids (AB; 2*n*=4*x*=40) were produced and later gave rise to cultivated peanut through domestication (Stalker et al., 2016).

Peanut's single origin, small number of progenitors, and the ploidy barrier between it and the mostly diploid, wild *Arachis* species, as well as its domestication, caused a severe bottleneck that greatly restricted the genetic diversity among peanut landraces and cultivars (Kochert et al., 1996; Seijo et al., 2007; Grabiele et al., 2012). Despite this genetic bottleneck, peanut is morphologically diverse with thousands of landraces and cultivars having distinguishing seed color and size, pod shape and reticulation, and growth habit, among other characteristics (Krapovickas and Gregory, 2007). While morphologically diverse, peanut's limited genetic base impinges through lack of strong and durable abiotic and biotic stress resistance. Fortunately, high levels of resistance to multiple pathogens and insect pests as well as abiotic stresses such as drought have been identified in wild *Arachis* species and can be introgressed into cultivated peanut to protect peanut production (Stalker et al., 2017). Furthermore, introgression of wild *Arachis* segments into cultivated peanut also was found to strengthen a number of agronomic traits such as flowering precocity, seed and pod number or length and size, and pod maturity (Fonceka et al., 2012; Moretzsohn et al., 2013; Stalker et al., 2016).

Alleles from wild *Arachis* species have been underutilized for peanut production. The use of *Arachis* species in peanut breeding programs is hindered by many obstacles, such as ploidy barriers, linkage drag of desirable traits with undesirable traits such as weak peg strength, small seed size, and low yield, and difficulties in tracking introgressions (Stalker et al., 2016).

Additionally, maintaining seed from *Arachis* species with low seed set and sprawling, weed-like growth is challenging. Introgressing alleles from the wild, diploid *Arachis* species also takes additional years of work. The most efficient introgression pathway requires diploid A and B/K-genome species to be crossed; the resulting diploid, AB progeny to be planted; and finally, cuttings from these plants to be treated with colchicine to double the chromosome number before crosses can be made with tetraploid peanut. Not only is this a time-consuming process but crossing efficiencies between different wild *Arachis* species vary from 0 to 31%, depending on the species crossed, and doubling success of colchicine treatment for increasing ploidy level varies for different interspecific hybrids (Fávero et al., 2019). All these obstacles to using wild species make breeders reluctant to pursue wild allele introgression into peanut cultivars, yet these strong resistances are worth the effort if these obstacles can be minimized.

Utilizing molecular tools can improve the ease and speed of introgression of wild *Arachis* alleles and can be used to monitor the size of introgressed segments to reduce linkage drag. For example, SSR markers have been used to study the genetic diversity of peanut and to map resistance and agronomic QTL (Foncéka et al., 2012). However, SNPs offer the advantage of abundance; 58,233 SNPs have been tiled on the Axiom_Arachis1 SNP array (Clevenger et al., 2017; Pandey et al. 2017). The array was updated to version 2 upon improvement of SNP calling accuracy (Clevenger et al., 2018; Korani et al., 2019). SNP array data has been used for high resolution genetic mapping of various resistances, yet, for some applications, the high resolution of the SNP chip is unnecessary and other types of markers could be more cost efficient. Other markers may also be more efficient if a sample slot on an array is not open, since the SNP chip requires 384 samples to be submitted at a time and costs $28 per sample, $10,750 for one submission. Two applications that do not always require high marker resolution are 1)

determination of true hybrids made from crosses between A- and B/K-genome species before or after advancement to colchicine treatment and 2) confirmation of homoeologous recombination events, which occurs commonly in newly synthesized allotetraploids used for introgression of wild *Arachis* alleles into cultivated peanut (Chu et al., 2021). Determination of true hybrids has previously relied on morphological observations and male fertility levels measured by infertile pollen grain counts (Stalker, 1981; Stalker et al., 1991). Both applications can be achieved with markers that can distinguish allele dosage between A- and B/K-genomes. This study sought to design low-cost molecular markers distinguishing A- and B/K-genomes distributed across the 20 chromosomes of peanut as tools for peanut breeding.

Materials and Methods

Marker Design

DNA was extracted from sixteen wild *Arachis* species using the Qiagen DNeasy Plant mini kit (Qiagen, Germantown, MD) (Table 8.1). The diploid species were genotyped using the Affymetrix Axiom_Arachis1 SNP array (Clevenger et al., 2017; Pandey et al., 2017) consisting of 58,233 features (ThermoFisher Scientific). SNP calling was performed with Axiom Analysis Suite (Version 1.2). Genetic markers were grouped into six categories by the software depending on the quality and separation of markers 1) Monomorphic 2) PolyHighResolution 3) NoMinorHom 4) OfftargetVariant 5) CallRateBelowThreshold 6) Other. Only the markers in the PolyHighResolution class were used for analysis since the grouping of samples was unambiguous and all the samples passed quality control. Of these markers, 3,359 distinguished 11 of the A-genome species from 5 of the B/K-genome species. The flanking sequences of these markers were used to design KASP allele-specific and allele-flanking primers with BatchPrimer3 (You et al., 2008). The default settings in BatchPrimer3 were used except a 58-62 °C melting

temperature, 60-120 bp product size, and 20-80% GC content were specified. BatchPrimer3 marker sets were only considered for selection if they had a Q score of 70 or higher. Six markers per chromosome were selected for each of the 20 A and B chromosomes (120 markers total). An emphasis was placed on evenly distributing the markers, with three on the top and three on the bottom of each chromosome; however, even distance between markers was affected by gaps in the Affymetrix Axiom_Arachis1 SNP array as well as gaps in available markers after being filtered by BatchPrimer3. For the selected primer sets, the sequences for the fluorescent tags FAM (5′-GAAGGTGACCAAGTTCATGCT-3') and VIC (5'-GAAGGTCGGAGTCAACGGATT-3') were added to the beginning of each allele-specific primer.

Marker Validation

The markers were validated by running KASP (KBioscience Ltd., Hoddesdon, UK) with multiple A-genome and B/K-genome species as well as AB/K hybrids such as a synthetic allotetraploid [*A. ipaensis* (PI 468322) x *A. duranensis* (PI 468197)]^{4x} (abbreviated as *IpaDur*^{4x}) and *A. hypogaea* cultivars 'Tifguard' (Holbrook et al., 2008) and 'Tifrunner' (Holbrook and Culbreath, 2007) (Table 8.2). Sequences of allele-specific and allele-flanking primers are listed in Table S8.1. The primer mix comprised of 46 µl of distilled water, 30 µl of the allele-flanking primer (100 µM), and 12 µl of each allele-specific primer (100 µM). For each KASP reaction, 0.07 µl of the primer mix, 2.5 µl of 2x KASP PCR mix, 0.5 µl of 20x diluted DNA (extracted by the Qiagen DNeasy Plant mini kit), and 1.93 µl of distilled water was combined. Thermocycling and endpoint genotyping was performed with a Roche LightCycler 480 II following the protocol used by Chu et al. (2016). After marker validation, a BLASTn search of the Tifrunner genome with the FASTA sequences of the allele-flanking primers was conducted in PeanutBase (Dash et

al., 2016) to determine if these markers were in conserved regions. To identify where A-genome markers were located in the B-genome and vice versa, the FASTA sequences of the validated allele-flanking markers were aligned using BLASTn (with the default settings, except for an e-value threshold raised to 1) of *A. ipaensis* K30076, *A. duranensis* V14167, and Tifrunner on PeanutBase (Bertioli et al., 2016, 2019).

Validation of Markers for Confirming Allotetraploids and Interspecific, Diploid Hybrids

Affymetrix Validation

Seven of the 105 successful markers designed using data from the Affymetrix Axiom_Arachis1 SNP array were also included on the Affymetrix Axiom_Arachis2 SNP array (Table S8.1). Fifty-six synthetic interspecific AB-genome allotetraploids were genotyped on the version 2 SNP array, including 12 *IpaCor*2, 11 *IpaDur*4, 12 *IpaSten*1, and 21 *ValSten*1 allotetraploids (Table 8.3). SNP calling was performed with Axiom Analysis Suite (Version 1.2) using default polyploid threshold configurations for each interspecific allotetraploid combination along with its corresponding parental wild diploid species. The seven markers were validated for each allotetraploid combination if the wild diploid species showed the alternate alleles and if the confirmed allotetraploids were clustered between the wild parental controls.

KASP Validation

The five validated markers designed for chromosome A01 (Table S8.1) were used to confirm 77 potential, newly synthesized, diploid AB hybrids, representing 25 different interspecific hybrid combinations between 27 different wild *Arachis* species (Table 8.4). KASP assays were carried out following the method previously described in the "Marker Validation" section with DNA from each potential hybrid as well as control DNA, including DNA from the

wild *Arachis* parents and *A. hypogaea* cultivars, Tifguard, 'Florunner,' Tifrunner, and 'Georgia Green.'

Validation of Markers for Detecting Homoeologous Recombination

Affymetrix Validation

Clevenger et al. (2017) used the Axiom_Arachis1 SNP array to identify 1,193 SNPs that illuminated genomic regions with homoeologous recombination in the 109 accessions of the USDA mini core collection and 64 additional released cultivars. SNP calling was performed on this same array data from Clevenger et al. (2017) along with six A-genome *Arachis* species (*A. stenosperma* V10309, *A. duranensis* V14167, *A. duranensis* K7988, *A. cardenasii* GKP10017, *A. villosa* V121812, and *A. correntina* 9530) and four B-genome *Arachis* species (*A. ipaensis* KG30076, *A. magna* 30097, *A. gregoryi* V6389, and *A. batizocoi* K9484) as controls in Axiom Analysis Suite (Version 1.2) using default polyploid threshold configurations. The loci of the markers designed in this study were viewed to determine if segregation of the individuals matched what was reported in Supplementary Table 4 in Clevenger et al. (2017).

KASP Validation

Chu et al. (2021) documented homoeologous recombination between selfed progenies from the synthetic allotetraploid (*A. ipaensis* x *A. correntina*)^{4x} as well as F_1 hybrids and over 900 F_2 progenies from crosses between *A. hypogaea* x (*A. ipaensis* x *A. correntina*)^{4x} with Axiom_Arachis2 SNP array data. Almost half of the F_2 lines were found to have at least one event of homoeologous recombination, with most events occurring in chromosomes A03/B03, A04/B04, A05/B05, and A06/B06. The Axiom array data for these F_2 hybrids were found in Supplementary Table 5 in Chu et al. (2021). To demonstrate that the A- and B/K-genome distinguishing markers were useful as a quick method of homoeologous recombination

confirmation, one KASP marker designed for each of these previously identified chromosomes was tested with DNA from the *A. hypogaea* x (*A. ipaensis* x *A. correntina*)^{4x} F_2 progeny. These markers were: A03-123 Mbp, A04-7 Mbp, A05-11 Mbp, A06-6 Mbp, B03-2 Mbp, B04-6 Mbp, B05-16 Mbp, and B06-7 Mbp. Each marker was tested with control DNA: *A. hypogaea* '13-1014' and Tifrunner (homoeologous controls), *A. ipaensis* (30076) and *A. magna* (30092) (B-genome controls), *A. correntina* 9530 and *A. duranensis* (V14167) (A-genome controls), and six [*A. hypogaea* 13-1014 x (*A. ipaensis* (30076) x *A. correntina* (9530))^{4x}]_$F_{1:4}$_F_2 hybrids (labeled $F_{2:3}$, $F_{2:4}$, $F_{2:5}$, $F_{2:99}$, $F_{2:100}$, $F_{2:101}$ by Chu et al. (2021)) that did not have homoeologous recombination on these chromosomes. Each marker was also tested with DNA from 12 F_2 hybrids, in which six hybrids had homoeologous recombination where an *A. ipaensis* allele replaced *A. correntina* an allele and the other six hybrids had the opposite event occur (Table 8.5).

Results

Marker Validation

One hundred and five of the 120 designed markers amplified the polymorphic loci as expected (Table S8.1). KASP results showed alternate allele clusters for A-genome and B/K-genome species with an AB genotype cluster of cultivated peanut in the middle (Fig. S8.1). All 105 markers were found to be in conserved, gene-encoding regions. While six markers were designed per chromosome, these markers detected alleles from orthologous/ homoeologous loci in both the A- and B/K-genomes so there are up to 12 markers per chromosome. Given the high similarity of the *A. ipaensis* K30076 and *A. duranensis* V14167 genomes, most of the markers fall on the expected corresponding chromosome position (i.e., the marker on the beginning of A01 at 2 Mbp was aligned to the beginning of B01 at 12 Mbp) (Table S8.1). However, there

were 20 markers that did not map to the corresponding chromosome. Three of these 20 markers, A02-12 Mbp, A04-3 Mbp, and A06-109 Mbp did not map to the *A. ipaensis* K30076 genome, yet the markers amplify an allele from *A. ipaensis* DNA in the KASP reaction. In addition, these three markers mapped to the corresponding B-genome chromosomes of the *A. hypogaea* Tifrunner 1.0 alignment, indicating they may have been excluded from the *A. ipaensis* assembly. One marker, B06-125, did not map to the *A. duranensis* V14167 genome, yet the marker amplifies an allele from *A. duranensis* DNA in the KASP reaction. In addition, this marker mapped to the corresponding A-genome chromosome of the *A. hypogaea* Tifrunner 1.0 alignment, indicating they may have been excluded from the *A. duranensis* assembly. Five markers, B05-135 Mbp, B06-129 Mbp, A04-95 Mbp, A06-104 Mbp, and A08-25 Mbp, did not map to a chromosome in the genome for which they were designed nor to the corresponding genome in the Tifrunner 1.0 alignment but did map to the corresponding chromosome in the alternative genome (i.e., marker B05-135 Mbp was designed from Affymetrix sequence data for A05, but only mapped to Araip.B05 and Arahy.15). Seven of the 19 markers that did not map to the corresponding chromosomes in the complementing genome were located on A07, A08, B07, and B08. This is due to a translocation that has been described in detail by Bertioli et al. (2019). The remaining four markers, A01-102 Mbp, A03-2 Mpb, A08-49 Mbp, and B02-4 Mbp mapped to completely different chromosomes in the alternate genome and mapped to the same noncorresponding position in the *A. hypogaea* Tifrunner 1.0 alignment (i.e. A01-102 Mbp mapped to Araip.B10 9 Mbp and Arahy.20 9 Mbp) (Table S8.1). Lastly, there were three markers, B03-5 Mbp, B10 7 Mbp, and B10-12 Mbp, that mapped to additional locations at the same e-value, identity score, and query match (i.e., B03-5 Mbp mapped to Araip.B05 and

Arahy.15 in addition to the expected locations on Aradu.A03, Araip.B03, Arahy.03, and Arahy.13).

Application of Markers to Confirm Allotetraploids and Interspecific, Diploid Hybrids

Hybrid Confirmation with Affymetrix Genotyping

The seven markers that are on both the version one and two of the Affymetrix Axiom_Arachis SNP array are A04-118 Mbp, A07-11 Mbp, A10-41 Mbp, A10-45 Mbp, B03-2 Mbp, B03-130 Mbp, and B06-135 Mbp. All seven of these markers reconfirmed the Affymetrix Axiom_Arachis2 SNP array data, that the 56 synthetic allotetraploids (12 *IpaCor*2, 11 *IpaDur*4, 12 *IpaSten*1, and 21 *ValSten*1) were true interspecific hybrids (Fig. 8.1A-D). True allotetraploids cluster in the middle as AB calls, while selfed progeny from the mother plant would cluster with the *Arachis* species used as the recipient parent.

Hybrid Confirmation with KASP Markers

All five of the validated markers designed for A01 confirmed that 59 of the 77 diploid, potential interspecific hybrids were true (Fig. 8.2). All 18 (*A. gregoryi* V6389 x *A. stenosperma* V10309) putative hybrids grouped with the wild *Arachis* A-genome species. After this unexpected result, the same A01 markers were run with DNA from ten plants labeled as *A. gregoryi* V6389, all of which grouped with A-genome species DNA (Fig. S8.2). Given *A. gregoryi* V6389 is a B-genome species, this seed source had been incorrectly labeled.

Validation of Markers for Confirming Homoeologous Recombination

Affymetrix Validation

Clevenger et al. (2017) identified 1,193 SNPs in genomic regions with homoeologous recombination, and the density of SNPs per chromosome varied. For instance, A01 and B01 had 11 and 8 reported SNPs, respectively, while B06 had 171 SNPs, with 158 of those SNPs in the

small region spanning 105 Mbp to 111 Mbp. Due to low SNP density on chromosomes A01, A04, A07, B02, and B05, the segregation of only 68 of the 105 loci representing the markers made in this study could be verified; 54 showed segregation that matched the results found by Clevenger et al. (2017). Of these 54 loci, 50 correctly grouped all the mini core collection and historical cultivars together with no individuals displaying homoeologous recombination.

Four loci, A02-0.3 Mbp, A10-45 Mbp, B03-2 Mbp, and B03-7 Mbp correctly identified individuals with homoeologous recombination. A02-0.3 Mbp grouped PI 339960 with the A-genome controls, and PI 471952 was called as unknown but grouped closest to the A-genome species (Fig. 8.3A). Both PI 339960 and PI 471952 were reported to have homoeologous recombination at the top of A02, including the locus of marker A02-0.3 Mbp (Clevenger et al., 2017). A02-311,724 bp also had PI 157542 called as unknown, but it grouped between the B-genome species and the cluster of cultivated peanuts, suggesting that it may have three B-genome alleles at this locus. Due to the low density of markers in Clevenger et al. (2017) in this region, it is unknown if this is true detection of homoeologous recombination. A10-45 Mbp clustered PI 468197 and PI 371521 with the A-genome species, and Virginia Bunch 67 was called as unknown but was grouped close to the A-genome species, confirming the homoeologous recombination found in this location for these individuals in Clevenger et al. (2017) (Fig. 8.3C). With B03-2 Mbp, PI 295250 was called AB but was located between the B-genome species and the cluster of cultivated peanut DNA, indicating it may have three B-genome alleles at this locus, matching the report of Clevenger et al. (2017) that PI 295250 had a homoeologous recombination event spanning the entire chromosome of B03 (Fig. 8.3D). B03-2 Mbp also grouped ‘Hanoch’, ‘Harari’, ‘Altika’, and ‘Jenkins Jumbo’ with the B-genome species, indicating possible homoeologous recombination, but the gap of SNPs between 1,671,090 to

14,617,119 bp in Clevenger et al. (2017) means this cannot be confirmed. Like B03-2 Mbp, B03-7 Mbp had PI 295250 called as unknown, but the call was located between the B-genome species and the cluster of cultivated peanut DNA, consistent with homoeologous recombination with three B-genome alleles, which confirms the findings of Clevenger et al. (2017) (Fig. 8.3E).

Of the remaining 14 loci that did not show segregation that matched the results found by Clevenger et al. (2017), five missed one or more individuals reported to have homoeologous recombination. A02-81 Mbp did not report 'Katie SARI,' A09-20 Mbp did not report Tifguard, B03-126 Mbp did not report PI 295250, B04-6 Mbp did not report 'SSD6-2' or PI 331334, and B09-145 Mbp did not identify Tifguard to have homoeologous recombination. Two loci reported individuals with homoeologous recombination that Clevenger et al. (2017) did not report to have homoeologous recombination. A10-41 Mbp identified seven individuals ('Virginia Bunch 67,' PI 371521, PI 1442768, PI 147395, PI 1270907, PI 274193, and PI 1497318) as having homoeologous recombination which matched the findings of Clevenger et al. (2017) but identified another seven individuals (PI 1502040, PI 1502120, PI 476025, PI 497639, PI 494034, PI 1478850, PI 338338) as having homoeologous recombination that Clevenger et al. (2017) did not report to have homoeologous recombination. B06-135 Mbp identified 'NC3033' and PI 270905 as having homoeologous recombination as Clevenger et al. (2017) did but also identified PI 331334, PI 157542, SSD6-S, PI 295730, PI 319768, and PI 313129 having homoeologous recombination which were not reported by Clevenger et al. (2017) as having homoeologous recombination. The remaining seven loci did not show intergenomic variation in cultivated peanut. Instead of having three clusters of A-genome species, the cultivated peanut lines, and the B-genome species, these loci (A06-105 Mbp, B03-5 Mbp, B03-134 Mbp, B06-125 Mbp, B09-145 Mbp, B10- 7 Mbp, and B10-11 Mbp) had two clusters, in which all the cultivated lines

clustered with one group of *Arachis* species. Three of these markers, B05- 134 Mbp, B09-145 Mbp, and B10-11 Mbp mapped to the expected chromosomes in the *A. duranensis*, *A. ipaensis,* and Tifrunner reference genomes. Marker A06-105 did not map to A-genome chromosomes, B06-125 Mbp did not map to the *A. duranensis* reference, and B03-5 Mbp and B10-7 Mbp mapped to additional locations.

KASP Validation

The eight tested markers A03-123 Mbp, A04-7 Mbp, A05-11 Mbp, A06-6 Mbp, B03-2 Mbp, B04-6 Mbp, B05-16 Mbp, and B06-7 Mbp all successfully distinguished the six [*A. hypogaea* 13-1014 x (*A. ipaensis* (30076) x *A. correntina* (9530))^{4x}]_$F_{1:4}$_F_2 hybrid DNA with no homoeologous recombination, the six F_2 hybrids with three *A. ipaensis* alleles, and the six F_2 hybrids with three *A. correntina* alleles from one another (Fig. 8.4A-H). The individuals with homoeologous recombination grouped between the middle cluster of individuals with no homoeologous recombination (AABB) and the control DNA of the parent with which it had the most alleles in common. If the progeny of these F_2 individuals were genotyped with these markers, then it is likely some segregating individuals could be found with all four alleles of one parent and these individuals would group with the *Arachis* species DNA.

Discussion

Creation of genetic and genomic resources for peanut has increased tremendously over the last five years beginning with the release of genome sequences of the diploid ancestors (Bertioli et al., 2016). One highly beneficial resource has been the Axiom_Arachis SNP arrays (Clevenger et al., 2017, 2018; Pandey et al. 2017; Korani et al., 2019), which has tens of thousands of SNPs that enable more efficient QTL mapping and introgression in breeding populations than previously used markers (Pandey et al. 2012; Ozias-Akins et al. 2017). In some

instances, however, submission of samples with the SNP chip is not the most cost-efficient or time-efficient method. This study sought to utilize markers curated on the Axiom_Arachis1 SNP chip to design KASP markers that could be used to quickly and cheaply validate true hybrids made from crosses between A- and B/K-genome species and could be used to confirm homoeologous recombination. The former application is important to the peanut breeding community, because crossing wild species is the first step to introgressing beneficial alleles from wild *Arachis* species into cultivated peanut. Confirmation of and tracking the inheritance of homoeologous recombination is important to the peanut genetics and breeding community because it has been found to occur in cultivated peanut (Clevenger et al., 2017) as well as newly synthesized allotetraploids and it can create additional genetic diversity in the form of allele dosage as well as lead to destabilization of some traits found in these regions of homoeologous recombination (Leal-Bertioli et al., 2015; Chu et al., 2021).

One hundred and five KASP markers spanning all 20 chromosomes were created and validated as successfully being able to distinguish A- and B/K-genome diploid species. While only 12 markers were tested with both Affymetrix data or KASP to distinguish true hybrids made from crosses between A- and B/K-genome species from possible selfs produced from the mother parent, any of these 105 validated markers can be used for this purpose. When using these markers to determine true hybrids, only about 5 markers run with parental control DNA are necessary. Using more markers will yield redundant results. These markers also revealed incorrect cataloging of one seed source.

In addition to being able to distinguish A- and B/K-genomes, these markers were able to distinguish allele dosages from A- and B/K-genomes, enabling confirmation of homoeologous recombination in both cultivated peanut and neoallotetraploids and their derivatives. This was

determined by two different methods. The first method was using Affymetrix data to compare segregation at the loci used to design the A- and B/K-genome distinguishing markers with the findings of Clevenger et al. (2017). Most markers matched the results reported by Clevenger et al. (2017), but a small portion of these markers did not. This result highlights an important consideration; these markers are best for confirmation of large blocks of homoeologous recombination, since there is only a maximum of 12 A- and B/K-genome distinguishing markers per chromosome. These markers can be used to track the inheritance of homoeologous recombination in early-generation derivatives in neoallotetraploid-derived populations, which have been reported to have frequent events of homoeologous recombination often spanning entire chromosomes (Leal-Bertioli et al., 2015; Chu et al., 2021). The usefulness of these markers to track inheritance of homoeologous recombination may decline in later generations when additional recombination can reduce these homoeologous events to smaller blocks. The second method of validating these markers as tools for detecting homoeologous recombination was by running KASP on DNA from the *A. hypogaea* x (*A. ipaensis* x *A. correntina*)^{4x} F_2 progeny that Chu et al. (2021) reported to have homoeologous recombination on chromosomes A03, B03, A04, B04, A05, B05, A06, and B06. These markers confirmed 12 reported events of homoeologous recombination on all tested chromosomes.

Conclusions

Over one hundred A- and B/K-genome distinguishing KASP markers that span the entire peanut genome were created and can be used to confirm progeny from crosses between *Arachis* A-genome and B/K-genome species as well as to confirm homoeologous recombination. Costing about $30 per marker mix, in which one marker mix can test tens of thousands of samples, these KASP markers can be more cost-efficient than running samples on the Axiom_Arachis SNP

array and can be more time-efficient if a spot on the array is not open. The sequences for these markers are provided in the Table S8.1, allowing the public to order these markers for use in their own peanut breeding programs. These markers will be most useful for peanut breeding and genetics programs working with wild *Arachis* species and populations with large homoeologous recombination events.

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Table 8.1. Genetic materials used to develop A- and B/K-genome distinguishing KASP markers.

Genotype	Identification Number	DNA Abbreviation	Genome
A. batizocoi	PI 298639, K 9484	*Bat1*	K
A. cardenasii	PI 289640, GKP 10017	*Card1*	A
A. correntina	PI 262808, GKP 9530	*Cor1*	A
A. diogoi	PI 331200, INTA 10602	*Dio1*	A
A. duranensis	V14167	*Dur1*	A
A. duranensis	sesn2848	*Dur2*	A
A. duranensis	K7988	*Dur3*	A
A. ipaensis	PI 468322, GKBSPSc 30076	*Ipa1*	B
A. magna	PI 468337, GKSSc 30092	*Mag1*	B
A. magna	PI 468340, GKSSc 30097	*Mag2*	B
A. stenosperma	PI 666100, V 10309	*Sten1*	A
A. stenosperma	PI 338280, 410	*Sten2*	A
A. stenosperma	V 15076	*Sten3*	A
A. valida	PI 468154, GK 30011	*Val1*	B
A. villosa	V 12812	*Vill1*	A
A. villosa	PI 298636	*Vill2*	A

Table 8.2. DNA used to validate the KASP markers.

Markers Tested	A-genome species	B/K-genomes species	AB/K Hybrids
A01 markers	*Card1*	*Bat1*	*IpaDur*^{4x}
	Cor1	*Ipa1*	Tifrunner
	Dio (PI 276235)	*Mag2*	
	Dur1	*Val1*	
	Sten1		
	Vill1		
A02 markers	*Card1*	*Bat1*	Tifguard
	Cor1	*Ipa1*	Tifrunner
	Dur1	*Mag1*	
	Dio1		
	Vill1		
A03-B10 markers	*Card1*	*Bat1*	Tifguard
	Dio1	*Ipa1*	Tifrunner
	Dur1	*Mag1*	

Table 8.3. Synthetic interspecific AB-genome allotetraploids validated with Affymetrix data and their abbreviations.

Genotype	Abbreviation
A. ipaensis (PI 468322) x *A. correntina* (PI 262808)	*IpaCor*2
A. ipaensis (PI 468322) x *A. duranensis* (PI 468197)	*IpaDur*4
A. ipaensis (PI 468322) x *A. stenosperma* (PI 666100)	*IpaSten*1
A. valida (PI 468154) x *A. stenosperma* (PI 666100)	*ValSten*1

Table 8.4. Potential diploid, interspecific hybrids tested with A- and B/K-genome distinguishing markers. Bolded genotypes are displayed in Fig. 8.4.

Genotype	Number of Plants
A. batizocoi K 9484 x *A. correntina* 9530	2
A. batizocoi K 9484 x *A. diogoi* 10602	4
***A. batizocoi* K 9484 x *A. kempff-mercadoi* PI 468330**	**2**
***A. batizocoi* K 9484 x *A. kempff-mercadoi* PI 468334**	**3**
***A. batizocoi* PI 468325 x *A. kempff-mercadoi* PI 468330**	**1**
***A. batizocoi* PI 468352 x *A. kempff-mercadoi* PI 468330**	**1**
***A. cruziana* Grif 14257 x *A. simpsonii* Grif 14534**	**5**
***A. cruziana* PI 476003 x *A. simpsonii* Grif 14534**	**4**
A. gregoryi V 6389 x *A. stenosperma* V 10309	18
A. ipaensis KG 30076 x *A. correntina* 9530	12
A. ipaensis KG 30076 x *A. diogoi* 10602	3
***A. ipaensis* K 30076 x *A. duranensis* V 14167**	**1**
A. ipaensis KG 30076 x *A. duranensis* 30060	2
A. ipaensis KG 30076 x *A. stenosperma* V10309	1
A. ipaensis KG 30076 x *A. stenosperma* 410	1
A. magna K 30092 x *A. cardenasii* 10017	2
A. magna K 30092 x *A. diogoi* 10602	3
A. magna K 30092 x *A. hoehnei* PI 666086	2
***A. magna* PI 599184 x *A. kuhlmanni* PI 476108**	**1**
***A. magna* V 13752 x *A. hoehnei* V 9140**	**1**
***A. magna* V 13752 x *A. hoehnei* PI 666086**	**1**
A. magna V 13752 x *A. hoehnei* V 9094	1
A. valida PI 468152 x *A. stenosperma* V 10309	3
***A. valida* PI 666102 x *A. microsperma* Grif 15116**	**2**
***A. valida* PI 666103 x *A. microsperma* Grif 15116**	**1**

Table 8.5. [*A. hypogaea* 13-1014 x (*A. ipaensis* (30076) x *A. correntina* (9530))^{4x}]_$F_{1:4}$_F_2 hybrids identified by Chu et al. (2021) to have three *A. ipaensis* alleles or *A. correntina* alleles for most loci along chromosome A03/B03. A04/B04, A05/B05, and A06/B06. These DNAs were used for KASP assays with A- and B/K-genome distinguishing markers designed for the corresponding chromosome.

Individuals with three *A. ipaensis* alleles	Individuals with three *A. correntina* alleles
A03/ B03	
$F_{2:19}$	$F_{2:31}$
$F_{2:106}$	$F_{2:52}$
$F_{2:117}$	$F_{2:73}$
$F_{2:142}$	$F_{2:98}$
$F_{2:161}$	$F_{2:121}$
$F_{2:166}$	$F_{2:130}$
A04/ B04	
$F_{2:43}$	$F_{2:49}$
$F_{2:82}$	$F_{2:67}$
$F_{2:97}$	$F_{2:69}$
$F_{2:135}$	$F_{2:146}$
$F_{2:142}$	$F_{2:149}$
$F_{2:144}$	$F_{2:152}$
A05/B05	
$F_{2:82}$	$F_{2:61}$
$F_{2:184}$	$F_{2:102}$
$F_{2:280}$	$F_{2:107}$
$F_{2:187}$	$F_{2:186}$
$F_{2:239}$	$F_{2:220}$
$F_{2:251}$	$F_{2:467}$
A05/B05	

$F_{2:1}$	$F_{2:2}$
$F_{2:30}$	$F_{2:11}$
$F_{2:48}$	$F_{2:93}$
$F_{2:75}$	$F_{2:166}$
$F_{2:152}$	$F_{2:205}$
$F_{2:170}$	$F_{2:225}$

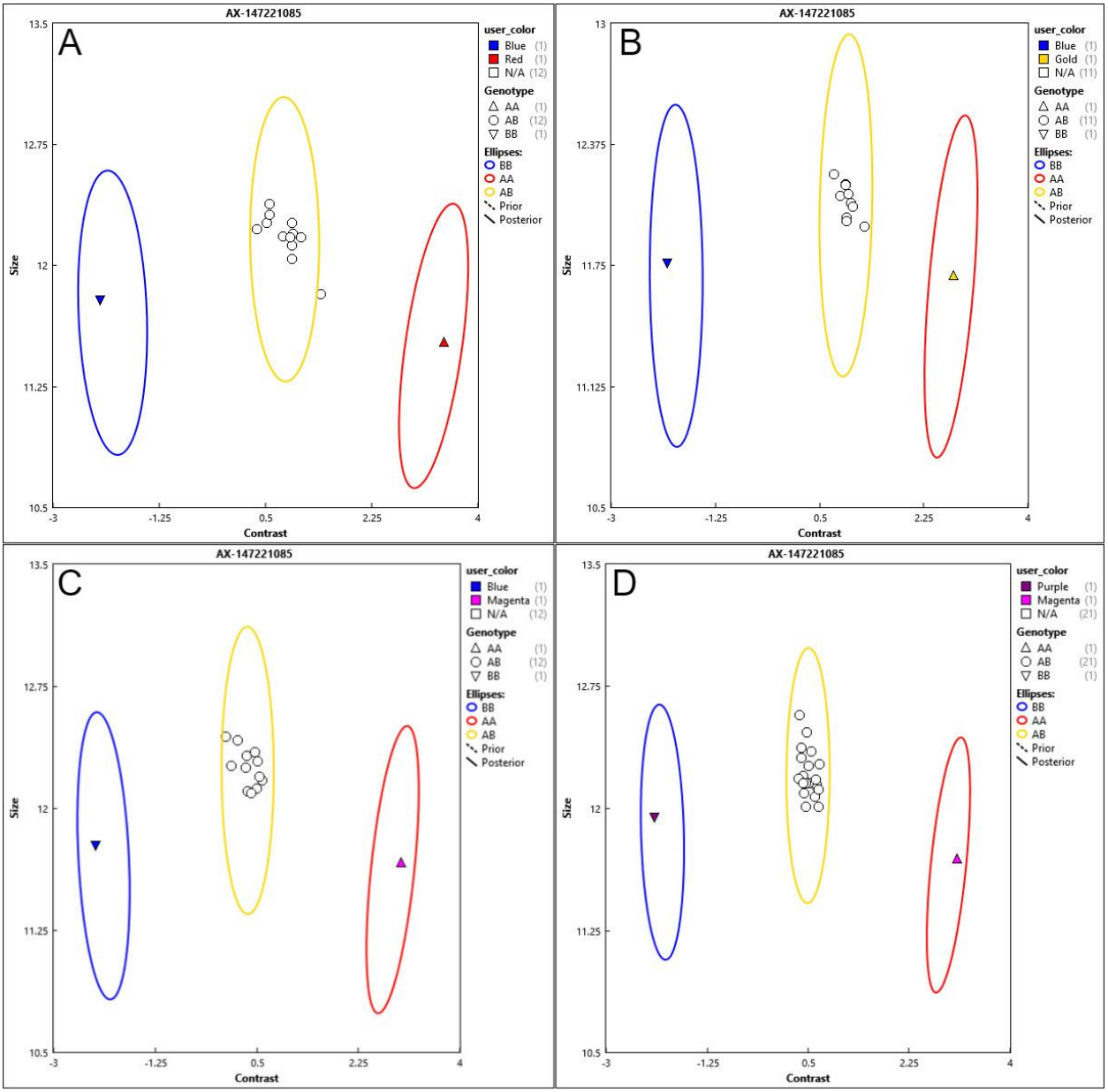

Figure 8.1. Example Affymetrix array data for marker A04-118 Mbp confirming A) *IpaCor*2, B) *IpaDur*4, C) *IpaSten*1 and D) *ValSten*1 hybrids, in which the parental controls *A. ipaensis* (PI 468322) is blue, *A. correntina* (PI 262808) is red, *A. duranensis* (PI 468197*)* is gold, *A. stenosperma* (PI 666100) is magenta, and *A. valida* (PI 468154*)* is purple.

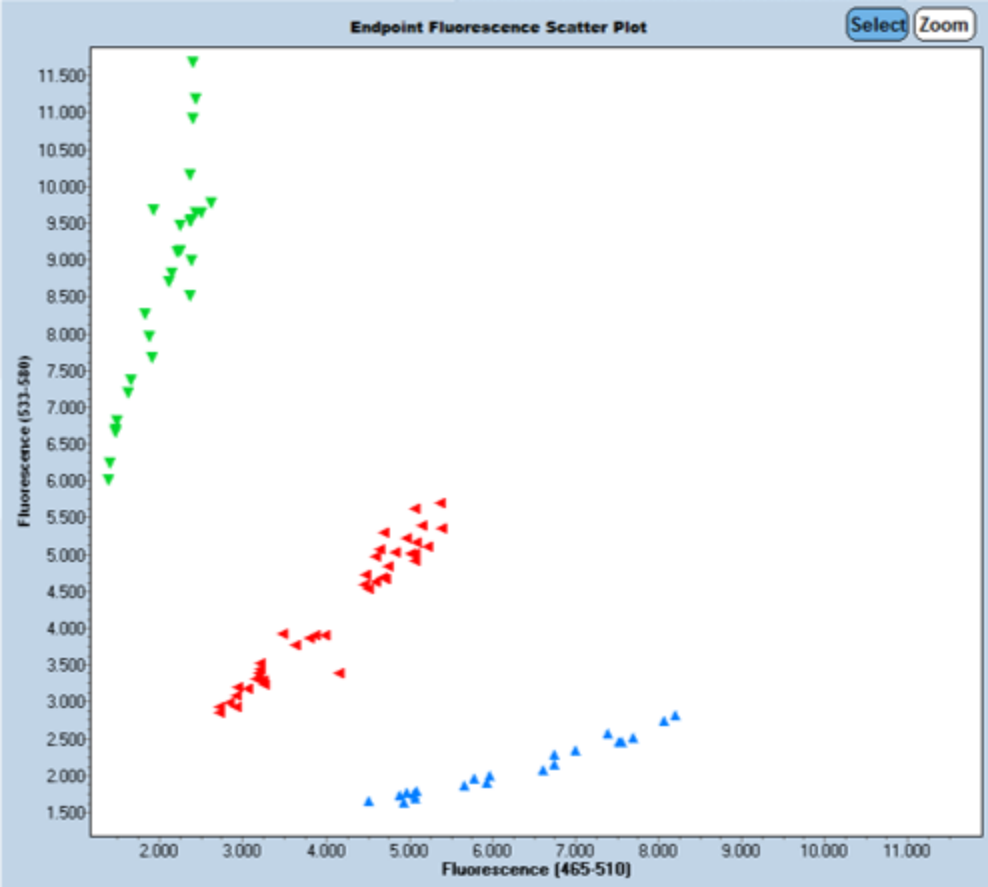

Figure 8.2. KASP result for marker A01-102 Mbp confirming the bolded hybrids in Table 4, in which the A-genome parental controls, *Dio*, *Dur1*, *A. hoehnei* (accessions PI 666068, V 9094, and , V 9140), *A. kempff-mercadoi* (accessions PI 468330 and PI 468334), *A. microsperma* Greif 15116, *A. simpsonii* Grif 14534, and *A valida* (accessions PI 666102 and 666103), are green, the confirmed hybrids are grouped with the cultivated peanut control DNA in red, and the B-genome parental controls, *Bat1*, *A. cruziana* (accessions Grif 14257 and PI 476003), and *Ipa1*, *A. kuhlmanii* PI 476108, and *A. magna* accessions (*Mag1*, Pi 599184, and V 13752) in blue. All DNA had two technical replicates.

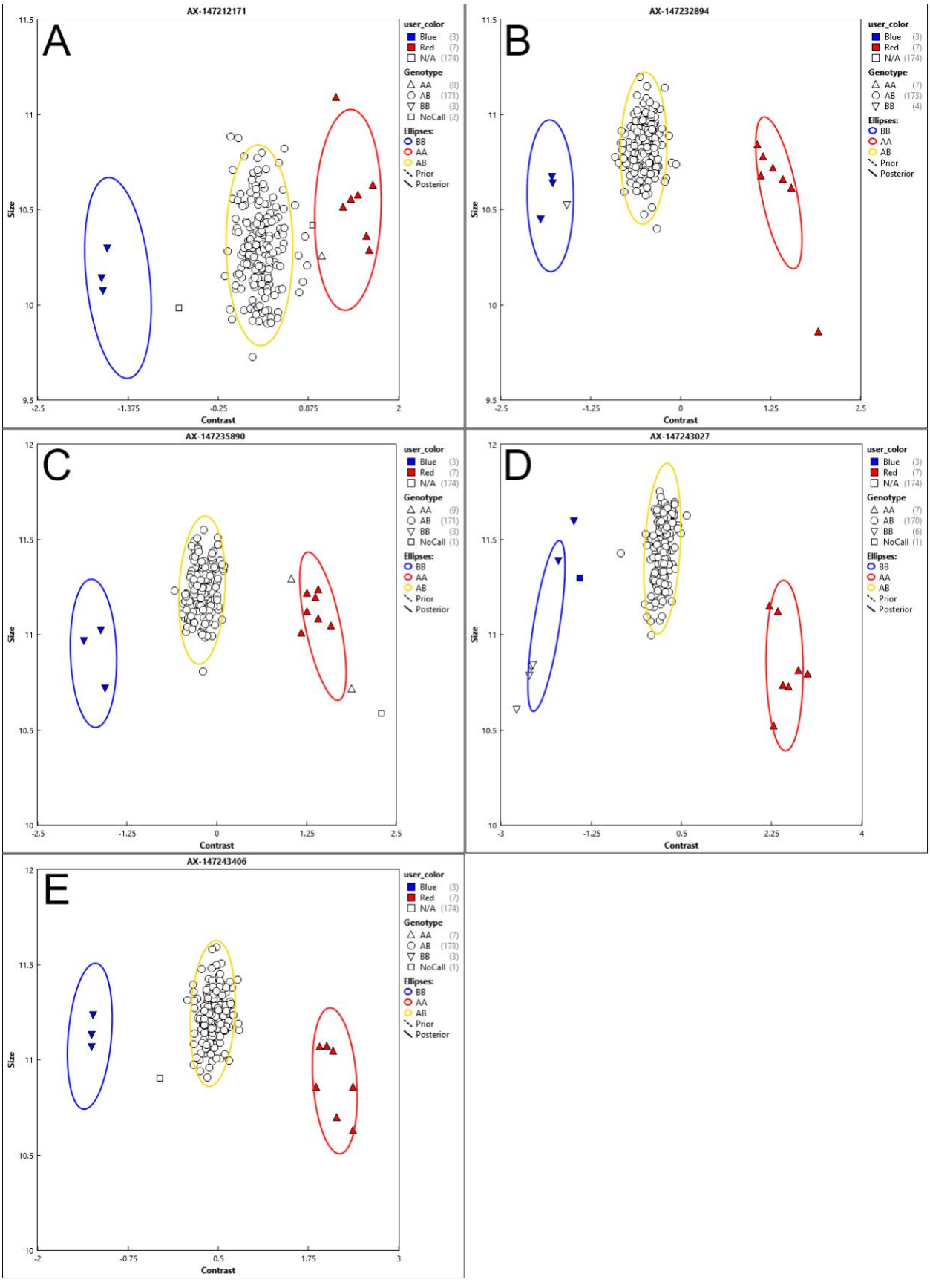
AX-147212171
A
AX-147232894
B
AX-147235890
C
AX-147243027
D
AX-147243406
E
Size
Contrast
user_color
Blue (3)
Red (7)
N/A (174)
Genotype
AA (8)
AB (171)
BB (3)
NoCall (2)
Ellipses:
BB
AA
AB
Prior
Posterior

Figure 8.3. Affymetrix array data of five markers that correctly identified individuals reported to have homoeologous recombination by Clevenger et al. (2017). B-genome species are blue, upside-down triangles, A-genome species are red triangles, cultivated peanut lines are unfilled circles or triangles, and unknown calls are unfilled squares. A) A02-0.3 Mbp grouped PI 339960 with the A-genome species, PI 471952 was called unknown but grouped closely with the A-genome species, and PI 157542 was called unknown and was located between the B-genome cluster and cultivated peanut cluster (circled in yellow). B) A09-14 Mbp grouped Tifguard (white, upside down triangle) with the *Arachis* B-genome species. C) A10-45 Mbp clustered PI 468197 and PI 371521 (white triangles) with *Arachis* A-genome species, and Virginia Bunch 67 was called unknown but grouped closely with the A-genome species. D) B03-2 Mbp called PI 295250 AB (white circle closest to the blue B-genome species) and called Hanoch, Harari, Altika, and Jenkins Jumbo (white, upside down triangles) with the B-genome species. E) B03-7 Mbp called PI 295250 as unknown, but the call was located between the B-genome species and the cluster of cultivated peanut DNA.

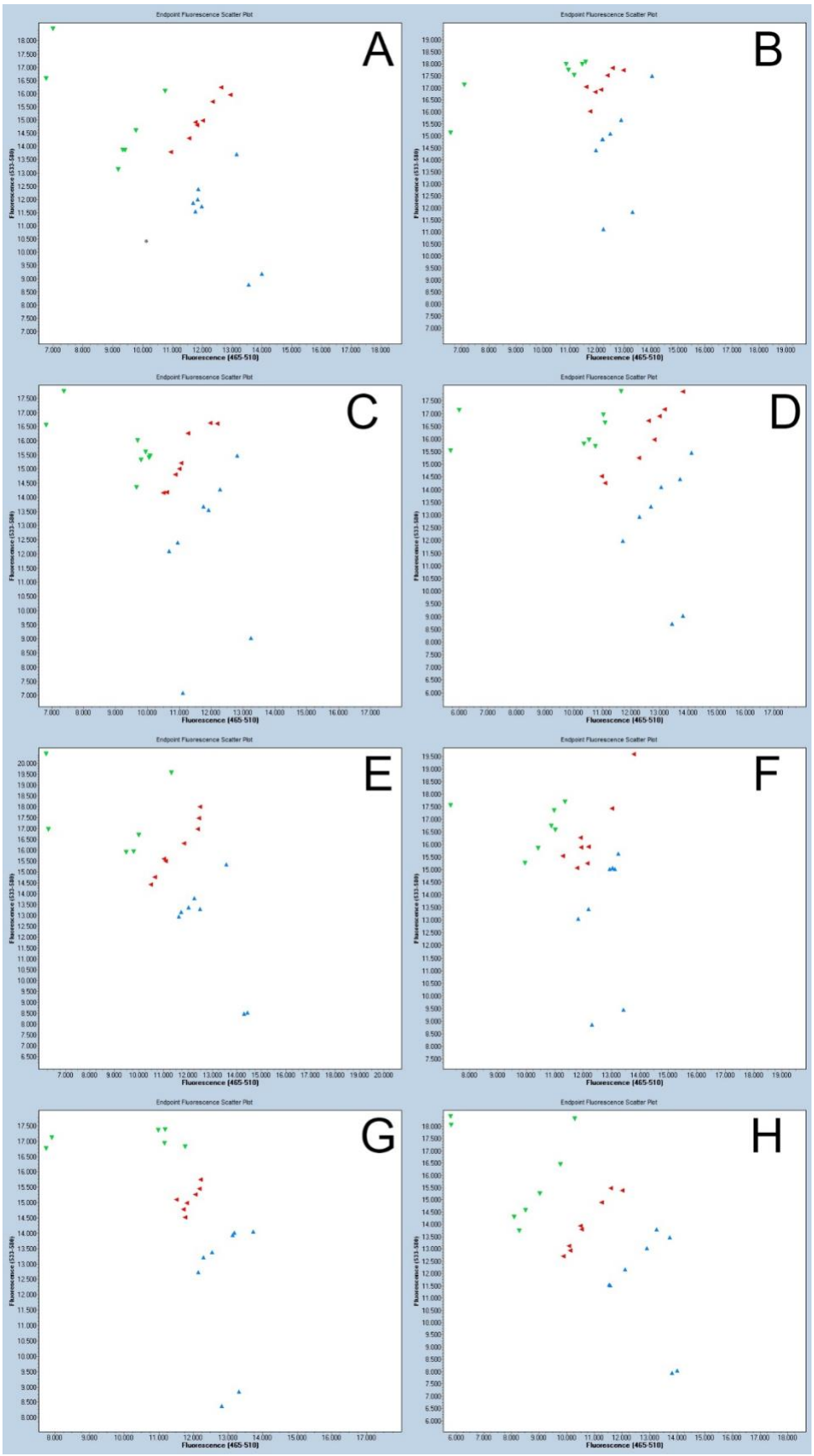
Endpoint Fluorescence Scatter Plot
A
Fluorescence (533-580)
Fluorescence (465-510)
Endpoint Fluorescence Scatter Plot
B
Fluorescence (533-580)
Fluorescence (465-510)
Endpoint Fluorescence Scatter Plot
C
Fluorescence (533-580)
Fluorescence (465-510)
Endpoint Fluorescence Scatter Plot
D
Fluorescence (533-580)
Fluorescence (465-510)
Endpoint Fluorescence Scatter Plot
E
Fluorescence (533-580)
Fluorescence (465-510)
Endpoint Fluorescence Scatter Plot
F
Fluorescence (533-580)
Fluorescence (465-510)
Endpoint Fluorescence Scatter Plot
G
Fluorescence (533-580)
Fluorescence (465-510)
Endpoint Fluorescence Scatter Plot
H
Fluorescence (533-580)
Fluorescence (465-510)

Figure 8.4. KASP assay results for A) A03-123 Mbp, B) A04-7 Mbp, C) A05-11 Mbp, D) A06-6 Mbp, E) B03-2 Mbp, F) B04-6 Mbp, G) B05-16 Mbp, and H) B06-7 Mbp, in which two A-genome species are clustered together in green, six F_2 progeny with three *A. correntina* alleles are clustered in green, two cultivated peanut and six F_2 progeny with no homoeologous recombination are clustered in red, six F_2 progeny with three *A. ipaensis* alleles are clustered in blue, and two B-genome species are clustered in blue.

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Appendix 8A

Supplemental Tables

Table S8.1. Details of 105 validated A- and B/K-genome distinguishing markers, A) including marker name, probeset identification on the Affymetrix Axiom_Arachis array, the version of the array the loci can be found on, the A- and B-genome chromosome and position of the marker with the lowest e-value, highest identity, and highest query match (in which Aradu stands for A. duranensis, Araip stands for A. ipaensis, and Arahy stands for A. hypogaea), B) the primer ID of the allele-flanking and allele specific primers (in which F stands for forward, R stands for reverse, FF stands for forward FAM, FV stands for forward VIC, RF stands for reverse FAM, and RV stands for reverse VIC, and the sequence of each primer.

A) All details of 105 validated A- and B/K-genome distinguishing markers except primer ID and sequence.

Marker Name	Probeset ID	Affymetrix version	Aradu. Chr.	Aradu. Chr. Position (bp)	Araip. Chr.	Araip. Position (bp)	Arahy. Chrs.	Arahy. Positions (bp)
01-2 Mbp	Affx-152075270	1	Aradu.A01	1,953,813	Araip.B01	11,835,030	Arahy.01 Arahy.11	2,025,019 12,452,120
A01-4 Mbp	Affx-152075215	1	Aradu.A01	3,647,636	Araip.B01	7,929,386	Arahy.01 Arahy.11	3,892,038 8,426,208
A01-5 Mbp	Affx-152069527	1	Aradu.A01	5,465,381	Araip.B01	4,985,138	Arahy.01 Arahy.11	5,864,303 5,258,269
A01-93 Mbp	Affx-152027001	1	Aradu.A01	93,311,229	Araip.B01	134,526,782	Arahy.01 Arahy.11	98,085,814 146,201,782

A01-102 Mbp	Affx-152068622	1	Aradu.A01	102,164,597	Araip.B10	8,545,299	Arahy.01 Arahy.20	107,081,729 8,919,936
A02-0.3 Mbp	Affx-152038856	1	Aradu.A02	311,689	Araip.B02	565,228	Arahy.02 Arahy.12	354,716 1,698,262
A02-7 Mbp	Affx-152035822	1	Aradu.A02	7,227,785	Araip.B02	9,563,284	Arahy.02 Arahy.12	12,474,598 16,397,129
A02-12 Mbp	Affx-152047047	1	Aradu.A02	11,789,648	-	-	Arahy.02 Arahy.12	12,474,596 16,397,127
A02-81 Mbp	Affx-152075699	1	Aradu.A02	81,266,923	Araip.B02	93,121,343	Arahy.02 Arahy.12	89,752,385 104,103,882
A02-87 Mbp	Affx-152036157	1	Aradu.A02	86,990,872	Araip.B02	100,153,992	Arahy.02 Arahy.12	95,442,437 111,525,437
A02-91 Mbp	Affx-152047513	1	Aradu.A02	91,014,119	Araip.B02	104,818,575	Arahy.02 Arahy.12	99,527,560 116,361,603
A03-2 Mbp	Affx-152084394	1	Aradu.A03	2,194,952	Araip.B02	103,322,649	Arahy.03 Arahy.12	2,553,230 114,863,855
A03-5 Mbp	Affx-152065904	1	Aradu.A03	4,940,611	Araip.B03	7,648,624	Arahy.03 Arahy.13	6,675,602 7,874,605

A03-9 Mbp	Affx-152044930	1	Aradu.A03	8,835,768	Araip.B03	12,052,927	Arahy.03 Arahy.13	9,191,377 12,484,762
A03-123 Mbp	Affx-152035789	1	Aradu.A03	122,594,484	Araip.B03	123,234,346	Arahy.03 Arahy.13	131,142,498 123,234,346
A03-129 Mbp	Affx-152030759	1	Aradu.A03	128,796,707	Araip.B03	129,514,389	Arahy.03 Arahy.13	129,514,389 140,169,325
A04-0.6 Mbp	Affx-152056145	1	Aradu.A04	562,030	Araip.B04	997,442	Arahy.04 Arahy.14	576,555 1,043,261
A04-3 Mbp	Affx-152061930	1	Aradu.A04	2,945,301	-	-	Arahy.04 Arahy.14	3,061,554 4,394,980
A04-7 Mbp	Affx-152060455	1	Aradu.A04	7,270,298	Araip.B04	8,744,128	Arahy.04 Arahy.14	7,570,158 9,048,439
A04-94 Mbp	Affx-152030122	1	Aradu.A04	93,979,145	Araip.B04	102,848,850	Arahy.04 Arahy.14	101,236,543 110,114,442
A04-109 Mbp	Affx-152031697	1	Aradu.A04	109,023,112	Araip.B04	117,451,787	Arahy.04 Arahy.14	114,987,64 126,668,942
A04-118 Mbp	Affx-152081925	1&2	Aradu.A04	118,413,691	Araip.B04	147,226,682	Arahy.04 Arahy.14	123,882,594 138,114,730

A05-7 Mbp	Affx-152029549	1	Aradu.A05	6,922,573	Araip.B05	7,161,692	Arahy.05 Arahy.15	7,409,173 7,403,635
A05-9 Mbp	Affx-152050940	1	Aradu.A05	8,614,561	Araip.B05	9,061,984	Arahy.05 Arahy.15	8,614,561 9,374,185
A05-11 Mbp	Affx-152063640	1	Aradu.A05	10,960,255	Araip.B05	11,848,330	Arahy.05 Arahy.15	11,616,839 12,233,363
A05-90 Mbp	Affx-152059805	1	Aradu.A05	89,848,949	Araip.B05	138,876,968	Arahy.05 Arahy.15	95,982,036 149,581,342
B05-135 Mbp	Affx-152078904	1	-	-	Araip.B05	135,159,498	Arahy.15	145,730,312
A05-102 Mbp	Affx-152082693	1	Aradu.A05	102,388,926	Araip.B05	118,302,712	Arahy.05 Arahy.15	108,230,608 128,045,910
A06-0.2 Mbp	Affx-152050077	1	Aradu.A06	226,633	Araip.B06	26,727,421	Arahy.06 Arahy.16	265,627 31,476,939
A06-4 Mbp	Affx-152040840	1	Aradu.A06	4,031,544	Araip.B06	14,832,729	Arahy.06 Arahy.16	4,110,496 18,914,484
A06-6 Mbp	Affx-152034819	1	Aradu.A06	6,211,613	Araip.B06	10,807,769	Arahy.06 Arahy.16	6,433,260 14,774,035

A06-97 Mbp	Affx-152041797	1	Aradu.A06	97,228,532	Araip.B06	120,319,602	Arahy.06 Arahy.16	102,497,389 133,821,568
B06-129 Mbp	Affx-152043126	1	-	-	Araip.B06	129,306,381	Arahy.16	143,449,189
A06-109 Mbp	Affx-152083209	1	Aradu.A06	109,219,574	-	-	Arahy.06 Arahy.16	111,634,129 150,675,322
A07-6 Mbp	Affx-152045038	1	Aradu.A07	5,510,845	Araip.B07	5,074,430	Arahy.07 Arahy.17	4,546,286 6,198,462
A07-11 Mbp	Affx-152081780	1&2	Aradu.A07	11,237,250	Araip.B07	10,837,454	Arahy.07 Arahy.17	10,309,319 12,344,037
A07-57 Mbp	Affx-152053954	1	Aradu.A07	57,271,180	Araip.B07	64,178,212	Arahy.07 Arahy.17	54,327,351 65,281,587
A07-66 Mbp	Affx-152053653	1	Aradu.A07	65,769,603	Araip.B07	38,955,195	Arahy.07 Arahy.17	63,598,313 42,410,855
A07-72 Mbp	Affx-152044100	1	Aradu.A07	72,383,581	Araip.B08	26,706,048	Arahy.07 Arahy.18	79,221,544 28,361,010
A08-2 Mbp	Affx-152077759	1	Aradu.A08	1,556,980	Araip.B07	104,849,530	Arahy.08 Arahy.17	1,550,366 112,200,175

A08-5 Mbp	Affx-152058562	1	Aradu.A08	5,359,032	Araip.B07	108,521,329	Arahy.08 Arahy.17	51,660,434 116,229,398
A08-9 Mbp	Affx-152074187	1	Aradu.A08	9,001,341	Araip.B07	115,649,227	Arahy.08 Arahy.17	8,782,040 123,816,669
A08-41 Mbp	Affx-152079038	1	Aradu.A08	41,154,136	Araip.B08	116,709,617	Arahy.08 Arahy.18	43,185,644 121,576,029
A08-46 Mbp	Affx-152052606	1	Aradu.A08	45,855,950	Araip.B08	126,006,130	Arahy.08 Arahy.18	47,783,551 131,271,524
A08-49 Mbp	Affx-152074798	1	Aradu.A08	49,409,852	Araip.B03	45,768	Arahy.08 Arahy.13	51,500,571 50,467
A09-2 Mbp	Affx-152038974	1	Aradu.A09	2,200,680	Araip.B09	2,848,596	Arahy.09 Arahy.19	2,022,718 2,572,222
A09-14 Mbp	Affx-152055946	1	Aradu.A09	14,093,144	Araip.B09	18,420,062	Arahy.09 Arahy.19	14,282,331 18,721,768
A09-20 Mbp	Affx-152075843	1	Aradu.A09	20,294,874	Araip.B09	25,591,717	Arahy.09 Arahy.19	20,421,306 26,183,638
A09-111 Mbp	Affx-152075881	1	Aradu.A09	111,439,592	Araip.B09	145,592,838	Arahy.09 Arahy.19	110,891,871 157,100,306

A09-117 Mbp	Affx-152043716	1	Aradu.A09	116,718,755	Araip.B09	139,946,215	Arahy.09 Arahy.19	116,347,246 151,123,663
A09-119 Mbp	Affx-152051265	1	Aradu.A09	118,569,589	Araip.B09	136,707,611	Arahy.09 Arahy.19	118,196,666 147,863,227
A10-30 Mbp	Affx-152053164	1	Aradu.A10	30,387,048	Araip.B10	38,699,542	Arahy.10 Arahy.20	28,180,500 42,148,746
A10-41 Mbp	Affx-152044688	1&2	Aradu.A10	40,987,560	Araip.B10	60,635,009	Arahy.10 Arahy.20	71,195,469 81,104,406
A10-45 Mbp	Affx-152073289	1&2	Aradu.A10	44,830,416	Araip.B10	55,018,910	Arahy.10 Arahy.20	69,066,765 85,104,100
A10-105 Mbp	Affx-152035171	1	Aradu.A10	105,336,463	Araip.B10	131,999,995	Arahy.10 Arahy.20	112,956,796 139,543,201
B01-0.2 Mbp	Affx-152068415	1	Aradu.A01	11,767,173	Araip.B01	242,759	Arahy.01 Arahy.11	9,641,225 1,660,978
B01-5 Mbp	Affx-152030107	1	Aradu.A01	5,505,972	Araip.B01	4,916,513	Arahy.01 Arahy.11	5,906,067 5,196,497
B01-26 Mbp	Affx-152057635	1	Aradu.A01	20,288,394	Araip.B01	26,280,127	Arahy.01 Arahy.11	22,073,685 30,612,879

B01-126 Mbp	Affx-152075122	1	Aradu.A01	99,577,260	Araip.B01	125,577,716	Arahy.01 Arahy.11	104,467,474 136,316,102
B01-131 Mbp	Affx-152029833	1	Aradu.A01	95,579,690	Araip.B01	131,389,675	Arahy.01 Arahy.11	100,684,260 142,888,979
B01-137 Mbp	Affx-152079210	1	Aradu.A01	89,687,535	Araip.B01	137,133,552	Arahy.01 Arahy.11	94,401,263 148,997,438
B02-4 Mbp	Affx-152060971	1	Aradu.A08	47,005,156	Araip.B02	4,192,914	Arahy.08 Arahy.12	48,948,406 4,481,130
B02-10 Mbp	Affx-152055900	1	Aradu.A02	7,227,449	Araip.B02	9,562,948	Arahy.02 Arahy.12	7,500,341 10,134,158
B02-13 Mbp	Affx-152036249	1	Aradu.A02	10,014,970	Araip.B02	13,241,884	Arahy.02 Arahy.12	10,686,937 14,130,225
B02-89 Mbp	Affx-152062099	1	Aradu.A02	77,558,224	Araip.B02	88,724,965	Arahy.02 Arahy.12	85,767,628 99,474,592
B02-103 Mbp	Affx-152046468	1	Aradu.A02	89,017,121	Araip.B02	102,562,197	Arahy.02 Arahy.12	97,436,046 114,054,424
B03-2 Mbp	Affx-152047226	1&2	Aradu.A03	59,821	Araip.B03	2,045,552	Arahy.03 Arahy.13	40,140 2,079,829

B03-5 Mbp	Affx-152066956	1	Aradu.A03	2,574,777	Araip.B03 Araip.B05	4,663,765 28,981,956	Arahy.03 Arahy.13 Arahy.15	2,956,113 4,744,787 30,239,973
B03-7 Mbp	Affx-152055144	1	Aradu.A03	4,270,770	Araip.B03	7,044,145	Arahy.03 Arahy.13	4,740,363 7,220,960
B03-126 Mbp	Affx-152029772	1	Aradu.A03	125,351,340	Araip.B03	126,182,473	Arahy.03 Arahy.13	133,909,543 136,656,195
B03-130 Mbp	Affx-152047679	1&2	Aradu.A03	128,884,209	Araip.B03	129,622,396	Arahy.03 Arahy.13	137,430,948 140,282,981
B03-134 Mbp	Affx-152045022	1	Aradu.A03	132,683,899	Araip.B03	133,813,144	Arahy.03 Arahy.13	141,444,701 144,391,851
B04-2 Mbp	Affx-152071722	1	Aradu.A04	1,187,482	Araip.B04	1,728,114	Arahy.04 Arahy.14	1,215,311 1,754,890
B04-6 Mbp	Affx-152058035	1	Aradu.A04	4,780,568	Araip.B04	6,100,431	Arahy.04 Arahy.14	5,053,468 6,317,258
A04-95 Mbp	Affx-152028848	1	Aradu.A04	95,442,593	-	-	Arahy.04	102,808,569
B04-115 Mbp	Affx-152059933	1	Aradu.A04	106,621,264	Araip.B04	114,777,420	Arahy.04 Arahy.14	112,651,932 123,882,537

B04-129 Mbp	Affx-152033432	1	Aradu.A04	118,551,690	Araip.B04	128,510,519	Arahy.04 Arahy.14	124,002,473 138,245,754
B05-11 Mbp	Affx-152067776	1	Aradu.A05	10,256,691	Araip.B05	11,103,552	Arahy.05 Arahy.15	10,932,825 11,518,671
B05-16 Mbp	Affx-152049046	1	Aradu.A05	14,722,929	Araip.B05	15,568,302	Arahy.05 Arahy.15	41,775,430 16,091,399
B05-20 Mbp	Affx-152077784	1	Aradu.A05	20,436,217	Araip.B05	20,485,513	Aradu.A05 Aradu.A15	36,021,763 21,217,818
B05-145 Mbp	Affx-152029970	1	Aradu.A05	82,077,732	Araip.B05	145,087,644	Arahy.05 Arahy.15	87,398,430 156,056,493
B05-148 Mbp	Affx-152038899	1	Aradu.A05	108,133,771	Araip.B05	148,473,621	Arahy.05 Arahy.15	114,387,271 159,400,741
B05-150 Mbp	Affx-152039609	1	Aradu.A05	109,264,824	Araip.B05	149,728,328	Arahy.05 Arahy.15	115,679,174 160,692,197
B06-7 Mbp	Affx-152054738	1	Aradu.A06	8,853,317	Araip.B06	7,052,883	Arahy.06 Arahy.16	9,079,797 10,727,064
B06-125 Mbp	Affx-152036729	1	-	-	Araip.B06	124,836,962	Arahy.06 Arahy.16	105,905,237 138,661,933

A06-104 Mbp	Affx-152078255	1	Aradu.A06	104,789,158	-	-	Arahy.16	145,781,007
B06-135 Mbp	Affx-152029967	1&2	Aradu.A06	109,959,294	Araip.B06	134,703,628	Arahy.06 Arahy.16	112,446,434 151,663,772
B07-11 Mbp	Affx-152082107	1	Aradu.A07	11,241,241	Araip.B07	10,841,410	Arahy.07 Arahy.17	10,313,379 12,347,837
B07-23 Mbp	Affx-152048437	1	Aradu.A07	21,909,424	Araip.B07	22,820,684	Arahy.07 Arahy.17	20,151,690 25,022,309
B07-27 Mbp	Affx-152075989	1	Aradu.A07	25,451,838	Araip.B07	27,259,201	Arahy.07 Arahy.17	27,480,153 29,730,255
B07-115 Mbp	Affx-152061943	1	Aradu.A08	8,661,301	Araip.B07	115,226,079	Arahy.08 Arahy.17	8,436,638 123,347,252
B07-121 Mbp	Affx-152050616	1	Aradu.A08	12,458,371	Araip.B07	120,609,577	Arahy.08 Arahy.17	12,427,348 129,031,013
B07-126 Mbp	Affx-152075606	1	Aradu.A07	28,154,525	Araip.B07	126,178,442	Arahy.7 Arahy.17	72,022,222 134,783,748
A08-25 Mbp	Affx-152046389	1	Aradu.A08	25,481,591	-	-	Arahy.08	27,734,151

B08-96 Mbp	Affx-152031689	1	Aradu.A07	43,506,342	Araip.B08	96,296,986	Arahy.07 Arahy.18	40,466,273 99,695,251
B08-126 Mbp	Affx-152064428	1	Aradu.A08	45,609,403	Araip.B08	125,650,941	Arahy.08 Arahy.18	47,536,178 130,903,342
B08-127 Mbp	Affx-152061412	1	Aradu.A08	46,444,251	Araip.B08	127,337,594	Arahy.08 Arahy.18	48,414,260 132,656,006
B09-0.7 Mbp	Affx-152036558	1	Aradu.A09	646,720	Araip.B09	731,771	Arahy.09 Arahy.19	416,462 384,734
B09-7 Mbp	Affx-152033828	1	Aradu.A09	5,913,094	Araip.B09	7,368,949	Arahy.09 Arahy.19	5,870,830 7,333,704
B09-9 Mbp	Affx-152040114	1	Aradu.A09	7,414,251	Araip.B09	9,226,819	Arahy.09 Arahy.19	7,516,138 9,101,264
B09-145 Mbp	Affx-152068655	1	Aradu.A09	112,519,049	Araip.B09	144,754,017	Arahy.09 Arahy.19	112,040,357 156,247,747
B09-147 Mbp	Affx-152066908	1	Aradu.A09	109,534,524	Araip.B09	146,819,700	Arahy.09 Arahy.19	108,991,661 158,419,582
B10-7 Mbp	Affx-152035468	1	Aradu.A10	4,915,438	Araip.B10 Araip.B10	6,963,233 16,787,683	Arahy.04 Arahy.10 Arahy.20 Arahy.20	109,742,010 4,557,007 7,199,803 17,591,982

B10-11 Mbp	Affx-152035885	1	Aradu.A10	6,345,490	Araip.B10	10,669,873	Arahy.10 Arahy.20	6,037,632 11,166,512
B10-12 Mbp	Affx-152036647	1	Aradu.A02	16,754,057	Araip.B02 Araip.B10	19,360,043 12,359,318	Arahy.02 Arahy.12 Arahy.20	18,095,580 20,876,123 12,989,808

B) Primer ID and sequence of 105 validated A- and B/K-genome distinguishing markers.

Marker Name	Primer ID	Sequence
A01-2 Mbp	A01_873824F	GAGTCACACAATATGCCAGAAAA
	A01_873824RF	GAAGGTGACCAAGTTCATGCTATCTGTACCCTTTGGAGCAGAC
	A01_873824RV	GAAGGTCGGAGTCAACGGATTTCTGTACCCTTTGGAGCAGAT
A01-4 Mbp	A01_3647671F	CAGGGGTTTAGAGGGAATGA
	A01_3647671RF	GAAGGTGACCAAGTTCATGCTATTCCAGATCCACCAGTACCC
	A01_3647671RV	GAAGGTCGGAGTCAACGGATTCTAATTCCAGATCCACCAGTACCT
A01-5 Mbp	A01_5465413F	GAGAACCGTGGAACTTTGTTG
	A01_5465413RV	GAAGGTCGGAGTCAACGGATTAGAAAGCTAAACTCTCTCCAACCA
	A01_5465413RF	GAAGGTGACCAAGTTCATGCTGAAAGCTAAACTCTCTCCAACCG
A01-93 Mbp	A01_93311263F	TCCTTCCAATGGTTTCGTACA
	A01_93311263RF	GAAGGTGACCAAGTTCATGCTAAAGCTAGATGGTTCTGATTATCCC
	A01_93311263RV	GAAGGTCGGAGTCAACGGATTAAAAGCTAGATGGTTCTGATTATCCT
A01-102 Mbp	A01_102164570R	ATGTTATTGACCACACCATCG
	A01_102164570FV	GAAGGTCGGAGTCAACGGATTGAGGAGCTTGTTGGAATTCTTT
	A01_102164570FF	GAAGGTGACCAAGTTCATGCTGAGGAGCTTGTTGGAATTCTTC

A02-0.3 Mbp	A02_311724F	TGCAACATCTGGGTCTTCTG
	A02_311724RV	GAAGGTCGGAGTCAACGGATTGTTTTTCTTATGTTGGTCTCTCTCAGT
	A02_311724RF	GAAGGTGACCAAGTTCATGCTTTTTCTTATGTTGGTCTCTCTCAGG
A02-7 Mbp	A02_7227811F	CGTCATCGCTATGGACCAC
	A02_7227811RV	GAAGGTCGGAGTCAACGGATTAAAGAATCATCGAAGTGAGGCTA
	A02_7227811RF	GAAGGTGACCAAGTTCATGCTAGAATCATCGAAGTGAGGCTG
A02-12 Mbp	A02_11789681F	CAAAAGCACACACGTGGAAA
	A02_11789681RF	GAAGGTGACCAAGTTCATGCTTGCAACCTTTATCTTGCTGTATTC
	A02_11789681RV	GAAGGTCGGAGTCAACGGATTCTGCAACCTTTATCTTGCTGTATTT
A02-81 Mbp	A02_81266957F	AAGCAGCTGTTGCAGATGTC
	A02_81266957RF	GAAGGTGACCAAGTTCATGCTCACAACCGAAGTAGCAGTTGAC
	A02_81266957RV	GAAGGTCGGAGTCAACGGATTACACAACCGAAGTAGCAGTTGAT
A02-87 Mbp	A02_86990839R	TTCACACAGAAGATAACGTTTAATGA
	A02_86990839FF	GAAGGTGACCAAGTTCATGCTCATTCATTTGATTCTGTCTTCCAG
	A02_86990839FV	GAAGGTCGGAGTCAACGGATTCATTCATTTGATTCTGTCTTCCAT
A02-91 Mbp	A02_91014088R	GCTTCTTCCTTTTATCGGAACC
	A02_91014088FV	GAAGGTCGGAGTCAACGGATTTGACCGTGAAGTGTTTGGTTAT
	A02_91014088FF	GAAGGTGACCAAGTTCATGCTTGACCGTGAAGTGTTTGGTTAC
A03-2 Mbp	A03_2194952R	GGCCATGAAGTCCCATTTG
	A03_2194952FV	GAAGGTCGGAGTCAACGGATTTCCGGGACCTTGAGAAGAAT
	A03_2194952FF	GAAGGTGACCAAGTTCATGCTCCGGGACCTTGAGAAGAAC
A03-5 Mbp	A03_4940640F	TTCAGAACACTATCAACAAATACAACA
	A03_4940640RV	GAAGGTCGGAGTCAACGGATTCTGTGTTGACACCCTTCTTTGA
	A03_4940640RF	GAAGGTGACCAAGTTCATGCTTGTGTTGACACCCTTCTTTGG
A03-9 Mbp	A03_8835735R	CAAAATGGTTACTTCCAAACCTG
	A03_8835735FV	GAAGGTCGGAGTCAACGGATTTGCTAGTTTTGATCCTTTTTGATCT
	A03_8835735FF	GAAGGTGACCAAGTTCATGCTGCTAGTTTTGATCCTTTTTGATCC

A03-123 Mbp	A03_122594519F	GATCATGGGATACAAGTTGCTT
	A03_122594519RV	GAAGGTCGGAGTCAACGGATTGGGAAAGTTGCTCCTGTGAA
	A03_122594519RF	GAAGGTGACCAAGTTCATGCTGGGAAAGTTGCTCCTGTGAG
A03-129 Mbp	A03_128796739F	TGGTGATAGAACAAGCATCACTT
	A03_128796739RV	GAAGGTCGGAGTCAACGGATTGCTTTCCACTCCAAACTTCCT
	A03_128796739RF	GAAGGTGACCAAGTTCATGCTCTTTCCACTCCAAACTTCCG
A04-0.6 Mbp	A04_561995R	TATGATGGCGATCGGTTTCT
	A04_561995FF	GAAGGTGACCAAGTTCATGCTTTGACCAAGAAATGGGCTG
	A04_561995FV	GAAGGTCGGAGTCAACGGATTCTTGACCAAGAAATGGGCTT
A04-3 Mbp	A04_2945332F	CTTCCCGTGGTAAACAATCC
	A04_2945332RV	GAAGGTCGGAGTCAACGGATTCGGGAATGTGATCGGAAA
	A04_2945332RF	GAAGGTGACCAAGTTCATGCTCGGGAATGTGATCGGAAG
A04-7 Mbp	A04_7270331F	AAAGTTTCTGATTTTGGACTCTCC
	A04_7270331RF	GAAGGTGACCAAGTTCATGCTCGTAACCTTTTTCACCATCTCC
	A04_7270331RV	GAAGGTCGGAGTCAACGGATTGTAACGTAACCTTTTTCACCATCTCT
A04-94 Mbp	A04_93979118R	AAAAATCATTCATTAGTTATGCCAGTC
	A04_93979118FF	GAAGGTGACCAAGTTCATGCTTACAGAGTTTCACGATGCCG
	A04_93979118FV	GAAGGTCGGAGTCAACGGATTTTACAGAGTTTCACGATGCCA
A04-109 Mbp	A04_109023083R	TCTAGAGTGGAAGCTGTTATTCAA
	A04_109023083FF	GAAGGTGACCAAGTTCATGCTCTGTCTGTAAAAGAGGACACTCAAAG
	A04_109023083FV	GAAGGTCGGAGTCAACGGATTCTGTCTGTAAAAGAGGACACTCAAAA
A04-118 Mbp	A04_118413726F	GGTTTTCTAGAACTTCAGGGAAGA
	A04_118413726RV	GAAGGTCGGAGTCAACGGATTCATCGGTTCTGCATCGTCT
	A04_118413726RF	GAAGGTGACCAAGTTCATGCTTCGGTTCTGCATCGTCG
A05-7 Mbp	A05_6922538R	GCAGAATCAAGTTCTCTCTTGAA
	A05_6922538FF	GAAGGTGACCAAGTTCATGCTAGGCTTTGATAGGGAGAGGG
	A05_6922538FV	GAAGGTCGGAGTCAACGGATTGAGGCTTTGATAGGGAGAGGA

A05-9 Mbp	A05_8614530R	TGAGAGACGCACCTTCAAGA
	A05_8614530FV	GAAGGTCGGAGTCAACGGATTCTACATCCATTCATTTCAGCTCAT
	A05_8614530FF	GAAGGTGACCAAGTTCATGCTCTACATCCATTCATTTCAGCTCAC
A05-11 Mbp	A05_10960288F	ATTTTTGCATCCTGCTCCAC
	A05_10960288RV	GAAGGTCGGAGTCAACGGATTCAAATATGGCCGTTGCTGTAA
	A05_10960288RF	GAAGGTGACCAAGTTCATGCTCAAATATGGCCGTTGCTGTAG
A05-90 Mbp	A05_89848949F	GAAGGAGGGGGTTTGCAC
	A05_89848949RF	GAAGGTGACCAAGTTCATGCTAAGAAGAGGAACATGACGGC
	A05_89848949RV	GAAGGTCGGAGTCAACGGATTCAAAGAAGAGGAACATGACGGT
B05-135 Mbp	A05_93188374F	CACATGGGGGTGATCAGAG
	A05_93188374RF	GAAGGTGACCAAGTTCATGCTGCAAAACACTTTGGAACTTTGAC
	A05_93188374RV	GAAGGTCGGAGTCAACGGATTGCAAAACACTTTGGAACTTTGAT
A05-102 Mbp	A05_102388961F	GCACATGCCACAGACAAAAA
	A05_102388961RF	GAAGGTGACCAAGTTCATGCTTGCAAGCAGCACAAACTCTC
	A05_102388961RV	GAAGGTCGGAGTCAACGGATTTTGCAAGCAGCACAAACTCTA
A06-0.2 Mbp	A06_226664F	ACCAAGATGATGGAGGAGGA
	A06_226664RF	GAAGGTGACCAAGTTCATGCTATCATGCACCTTGTCGAGC
	A06_226664RV	GAAGGTCGGAGTCAACGGATTCATCATGCACCTTGTCGAGT
A06-4 Mbp	A06_4031510R	CATGCCTATCTACTGATAAGATGTGG
	A06_4031510FV	GAAGGTCGGAGTCAACGGATTAACAGATTTGCTTCAGAAAGAGGT
	A06_4031510FF	GAAGGTGACCAAGTTCATGCTCAGATTTGCTTCAGAAAGAGGC
A06-6 Mbp	A06_6211589R	ATCAGGTTGTCGGAGAATGC
	A06_6211589FV	GAAGGTCGGAGTCAACGGATTAAGCAATTGCCACCTATCGT
	A06_6211589FF	GAAGGTGACCAAGTTCATGCTGCAATTGCCACCTATCGC
A06-97 Mbp	A06_97228498R	CACTTCGTCTTCAATATCAGAAAAA
	A06_97228498FV	GAAGGTCGGAGTCAACGGATTGACAGGTCATTAGTTCTCCCTGA
	A06_97228498FF	GAAGGTGACCAAGTTCATGCTACAGGTCATTAGTTCTCCCTGC

B06-129 Mbp	A06_104969920R	CTTACAAAGAAAAGAGACAGCTCA
	A06_104969920FV	GAAGGTCGGAGTCAACGGATTAAAGTTCTTTTTGCCATGCACT
	A06_104969920FF	GAAGGTGACCAAGTTCATGCTAGTTCTTTTTGCCATGCACC
A06-109 Mbp	A06_109219545R	TTGAGTAATGTCAAATCAAACCTTG
	A06_109219545FV	GAAGGTCGGAGTCAACGGATTCCGTGGCTTAGTAAGGGTTTT
	A06_109219545FF	GAAGGTGACCAAGTTCATGCTCCGTGGCTTAGTAAGGGTTTC
A07-6 Mbp	A07_5510815R	AGCTGGATCAAGTGTCAGCA
	A07_5510815FF	GAAGGTGACCAAGTTCATGCTGATCGTCATGCTCTGGAATTG
	A07_5510815FV	GAAGGTCGGAGTCAACGGATTTGATCGTCATGCTCTGGAATTA
A07-11 Mbp	A07_11237222R	TGACAATACCTAATAGCAGTTGGA
	A07_11237222FV	GAAGGTCGGAGTCAACGGATTATTAACAAGGAAGGCACACGAT
	A07_11237222FF	GAAGGTGACCAAGTTCATGCTTTAACAAGGAAGGCACACGAC
A07-57 Mbp	A07_57271145R	TGCGCTGGGATATCTTGC
	A07_57271145FF	GAAGGTGACCAAGTTCATGCTAATTCGAAATCCATCAAAAACG
	A07_57271145FV	GAAGGTCGGAGTCAACGGATTAAATTCGAAATCCATCAAAAACA
A07-66 Mbp	A07_65769632F	GGTTATGATGGTGTAGGTGTGCTT
	A07_65769632RV	GAAGGTCGGAGTCAACGGATTAAGTCCTTCATCAGCATAAGATTGA
	A07_65769632RF	GAAGGTGACCAAGTTCATGCTGTCCTTCATCAGCATAAGATTGG
A07-72 Mbp	A07_72383552R	CGTCTCAGATACAGAAGCTGTTGA
	A07_72383552FF	GAAGGTGACCAAGTTCATGCTTTATAGTGATGGAGAGGGCAGG
	A07_72383552FV	GAAGGTCGGAGTCAACGGATTAATTATAGTGATGGAGAGGGCAGA
A08-2 Mbp	A08_1556945R	CTTAACTCTTTGAAGATGAAAATGTCT
	A08_1556945FV	GAAGGTCGGAGTCAACGGATTCAAGATTCATCTGGCATACTCCT
	A08_1556945FF	GAAGGTGACCAAGTTCATGCTAAGATTCATCTGGCATACTCCC
A08-5 Mbp	A08_5359006R	AACTCATCCCCGTCGGAGT
	A08_5359006FV	GAAGGTCGGAGTCAACGGATTAAAGTGGCAAAGAAGATTGTGAAT
	A08_5359006FF	GAAGGTGACCAAGTTCATGCTAAGTGGCAAAGAAGATTGTGAAC

A08-9 Mbp	A08_9001307R	TGCCAAGAGCAATGAATCAG
	A08_9001307FV	GAAGGTCGGAGTCAACGGATTGCTTGTTGTTCCAATCAAAGGT
	A08_9001307FF	GAAGGTGACCAAGTTCATGCTCTTGTTGTTCCAATCAAAGGC
A08-41 Mbp	A08_41154169F	CACAAGAAGAGCATTATACAAGGTG
	A08_41154169RV	GAAGGTCGGAGTCAACGGATTGTTCACCAAGTAAATCAATGGCT
	A08_41154169RF	GAAGGTGACCAAGTTCATGCTCACCAAGTAAATCAATGGCG
A08-46 Mbp	A08_45855985F	AACAAAATTAGACACCAATCAAAATG
	A08_45855985RF	GAAGGTGACCAAGTTCATGCTTTCAGCCATGTGCTTTGTTC
	A08_45855985RV	GAAGGTCGGAGTCAACGGATTGTTCAGCCATGTGCTTTGTTT
A08-49 Mbp	A08_49409817R	CCCAACAAAGAAGTTTAGCAA
	A08_49409817FF	GAAGGTGACCAAGTTCATGCTGAGCTTGAGGAGGAGCAAAG
	A08_49409817FV	GAAGGTCGGAGTCAACGGATTGAGCTTGAGGAGGAGCAAAA
A09-2 Mbp	A09_2200713F	TGCCAAGTTTCTTGATGAGG
	A09_2200713RV	GAAGGTCGGAGTCAACGGATTGTGGCCGAAGAGAACATGA
	A09_2200713RF	GAAGGTGACCAAGTTCATGCTTGGCCGAAGAGAACATGG
A09-14 Mbp	A09_14093120R	AACCTTGATAATACTGCCGTCTG
	A09_14093120FF	GAAGGTGACCAAGTTCATGCTTATTTGAGCAGGGAGGATCG
	A09_14093120FV	GAAGGTCGGAGTCAACGGATTCTATTTGAGCAGGGAGGATCA
A09-20 Mbp	A09_20294840R	TCAATACAATTTCCCTCAAGAATTT
	A09_20294840FF	GAAGGTGACCAAGTTCATGCTACCGAAATGATAAAGCACTCG
	A09_20294840FV	GAAGGTCGGAGTCAACGGATTCACCGAAATGATAAAGCACTCA
A09-111 Mbp	A09_111439627F	GGACTTCTTTCCACCAAGATT
	A09_111439627RV	GAAGGTCGGAGTCAACGGATTCAAATCTGATGAGATGGTTGAGAA
	A09_111439627RF	GAAGGTGACCAAGTTCATGCTCAAATCTGATGAGATGGTTGAGAG
A09-117 Mbp	A09_116718782F	AATTTCGTACCAAACTCAATAAATCA
	A09_116718782RV	GAAGGTCGGAGTCAACGGATTTGTGCATAATTGAATTCCTTGTAACTA
	A09_116718782RF	GAAGGTGACCAAGTTCATGCTGTGCATAATTGAATTCCTTGTAACTG

A09-119 Mbp	A09_118569556R	CAGACTAAGGATGGTCAATGAGG
	A09_118569556FF	GAAGGTGACCAAGTTCATGCTCCTTGGGAGATGAGCAAAAG
	A09_118569556FV	GAAGGTCGGAGTCAACGGATTCCTTGGGAGATGAGCAAAAA
A10-30 Mbp	A10_30387024R	CTGTGGCTGCCAGCACTT
	A10_30387024FV	GAAGGTCGGAGTCAACGGATTAACATTTTGACTCTCCTCAAACCT
	A10_30387024FF	GAAGGTGACCAAGTTCATGCTCATTTTGACTCTCCTCAAACCC
A10-41 Mbp	A10_40987534R	CTGCAAGAAATATCGCTAGCAAAC
	A10_40987534FF	GAAGGTGACCAAGTTCATGCTTGATCATCAGGCACAGCG
	A10_40987534FV	GAAGGTCGGAGTCAACGGATTTTGATCATCAGGCACAGCA
A10-45 Mbp	A10_44830451F	CAGTTCTTGAACCGTTACGAC
	A10_44830451RF	GAAGGTGACCAAGTTCATGCTGGGACATGGAGGAGGAGC
	A10_44830451RV	GAAGGTCGGAGTCAACGGATTAGGGACATGGAGGAGGAGA
A10-105 Mbp	A10_105336430R	GAAAAATGGACTAGAGTCTCTTGATG
	A10_105336430FV	GAAGGTCGGAGTCAACGGATTAAGAGTTACCATTGTTCAATTTGCT
	A10_105336430FF	GAAGGTGACCAAGTTCATGCTGAGTTACCATTGTTCAATTTGCC
B01-0.2 Mbp	B01_242787F	GAATTTATGCCATGCCTGCT
	B01_242787RF	GAAGGTGACCAAGTTCATGCTCCCTGTTGTTTGTTGCTGC
	B01_242787RV	GAAGGTCGGAGTCAACGGATTATCCCTGTTGTTTGTTGCTGT
B01-5 Mbp	B01_4916482R	CCCAACAATGAACCTTTCCA
	B01_4916482FF	GAAGGTGACCAAGTTCATGCTCTGGTTTCTCTTGTGGGGTG
	B01_4916482FV	GAAGGTCGGAGTCAACGGATTTCTGGTTTCTCTTGTGGGGTA
B01-26 Mbp	B01_26280096R	GCTGTGAAGACATAGGAGAAGATG
	B01_26280096FV	GAAGGTCGGAGTCAACGGATTGCTGCAAGTGTTCTCCTTGTT
	B01_26280096FF	GAAGGTGACCAAGTTCATGCTGCTGCAAGTGTTCTCCTTGTC
B01-126 Mbp	B01_125577744F	AAACAATCCGGGAGGAAGAT
	B01_125577744RF	GAAGGTGACCAAGTTCATGCTACAGCCTTCAAGTAGGGAAGC
	B01_125577744RV	GAAGGTCGGAGTCAACGGATTCACAGCCTTCAAGTAGGGAAGT

B01-131 Mbp	B01_131389709F	TCCAGTTTTATTTACCCTGGTCTT
	B01_131389709RF	GAAGGTGACCAAGTTCATGCTATGTGAAGTTTCGCAAGACAAC
	B01_131389709RV	GAAGGTCGGAGTCAACGGATTGATGTGAAGTTTCGCAAGACAAT
B01-137 Mbp	B01_137133587F	AATTTCTGGTTAACAGGGATTTTG
	B01_137133587RF	GAAGGTGACCAAGTTCATGCTGCCATTACAATGAGTTTTTCCAC
	B01_137133587RV	GAAGGTCGGAGTCAACGGATTAGCCATTACAATGAGTTTTTCCAT
B02-4 Mbp	B02_4192887R	CCCTGATATCAATGAATTCCAA
	B02_4192887FV	GAAGGTCGGAGTCAACGGATTATCGCACAAAGAACAAAAACATT
	B02_4192887FF	GAAGGTGACCAAGTTCATGCTTCGCACAAAGAACAAAAACATC
B02-10 Mbp	B02_9562914R	GCTGACACTACGAGAAAATATAAGCA
	B02_9562914FV	GAAGGTCGGAGTCAACGGATTTGTCCTGTCCTCTGCTCCTT
	B02_9562914FF	GAAGGTGACCAAGTTCATGCTTGTCCTGTCCTCTGCTCCTC
B02-13 Mbp	B02_13241860R	TTAGCCTCATTAGTGCTTGTCG
	B02_13241860FV	GAAGGTCGGAGTCAACGGATTGACATCTTGGCTGGGTAGTGT
	B02_13241860FF	GAAGGTGACCAAGTTCATGCTACATCTTGGCTGGGTAGTGC
B02-89 Mbp	B02_88724993F	GCATTCATAATTTGCCAAAAACA
	B02_88724993RV	GAAGGTCGGAGTCAACGGATTTTAGGACATTGATTGTTAATGGGA
	B02_88724993RF	GAAGGTGACCAAGTTCATGCTAGGACATTGATTGTTAATGGGG
B02-103 Mbp	B02_102562170R	TGACCTCATTAGACCTGCTTCA
	B02_102562170FF	GAAGGTGACCAAGTTCATGCTTTGATTCAGGAACCACCAAAG
	B02_102562170FV	GAAGGTCGGAGTCAACGGATTTTGATTCAGGAACCACCAAAA
B03-2 Mbp	B03_2045587F	CATGATTGGTTAATGGATTTGC
	B03_2045587RF	GAAGGTGACCAAGTTCATGCTATTCTCCTGCCTTATGGTTCC
	B03_2045587RV	GAAGGTCGGAGTCAACGGATTAATTCTCCTGCCTTATGGTTCA
B03-5 Mbp	B03_4663794F	ACTATGTCAATGGTTTGGATTTTG
	B03_4663794RF	GAAGGTGACCAAGTTCATGCTAGTGAGTTCGAAAATGATGTCAAC
	B03_4663794RV	GAAGGTCGGAGTCAACGGATTAGTGAGTTCGAAAATGATGTCAAA

B03-7 Mbp	B03_7044145F	AAAAATGTGTTTGATCCATTCTCC
	B03_7044145RV	GAAGGTCGGAGTCAACGGATTAACCAGCACAGAAGGTGGAA
	B03_7044145RF	GAAGGTGACCAAGTTCATGCTAACCAGCACAGAAGGTGGAG
B03-126 Mbp	B03_126182438R	CAGCTAGAATGTTTGTCTCAGAATA
	B03_126182438FF	GAAGGTGACCAAGTTCATGCTCCTGTTACACAAAATCCTTCCAG
	B03_126182438FV	GAAGGTCGGAGTCAACGGATTCCTGTTACACAAAATCCTTCCAA
B03-130 Mbp	B03_129622425F	GGTAACAGCCTGAGAGAGTCG
	B03_129622425RF	GAAGGTGACCAAGTTCATGCTTGTTTGCCTCGCTGAGTTC
	B03_129622425RV	GAAGGTCGGAGTCAACGGATTTGTTTGCCTCGCTGAGTTT
B03-134 Mbp	B03_133813176F	GCATCAGTATGTCTGATGAATGC
	B03_133813176RV	GAAGGTCGGAGTCAACGGATTCAATGATCTTGCATTTTCCCA
	B03_133813176RF	GAAGGTGACCAAGTTCATGCTAATGATCTTGCATTTTCCCG
B04-2 Mbp	B04_1728086R	ATGAATCACTATGCACTACCCATC
	B04_1728086FF	GAAGGTGACCAAGTTCATGCTTCAGTCATACTCAGGGTTATGGG
	B04_1728086FV	GAAGGTCGGAGTCAACGGATTAATTCAGTCATACTCAGGGTTATGGT
B04-6 Mbp	B04_6100403R	AAAGGAACGCCACCAGACT
	B04_6100403FV	GAAGGTCGGAGTCAACGGATTGCTTGGATTTGGCTATGGAGT
	B04_6100403FF	GAAGGTGACCAAGTTCATGCTCTTGGATTTGGCTATGGAGC
A04-95 Mbp	B04_104604988R	CCAACCAGCAACTGACACTG
	B04_104604988FV	GAAGGTCGGAGTCAACGGATTGTAATAATCAAACTGCACCAAACATT
	B04_104604988FF	GAAGGTGACCAAGTTCATGCTGTAATAATCAAACTGCACCAAACATC
B04-115 Mbp	B04_114777391R	TGTCACCTAGGACACGGATG
	B04_114777391FF	GAAGGTGACCAAGTTCATGCTAAGCCCGTAATATGGCAAGTAG
	B04_114777391FV	GAAGGTCGGAGTCAACGGATTAAGCCCGTAATATGGCAAGTAA
B04-129 Mbp	B04_128510484R	TCTAAGCAGTGGAGAGACATAGC
	B04_128510484FF	GAAGGTGACCAAGTTCATGCTGTTGGAACAAAGACATTTCCATAAG
	B04_128510484FV	GAAGGTCGGAGTCAACGGATTTTGGAACAAAGACATTTCCATAAA

B05-11 Mbp	B05_11103581F	TTCTTCTCACGAGAAAGCAGTG
	B05_11103581RF	GAAGGTGACCAAGTTCATGCTAGCGAAGGTTTCTTGCCC
	B05_11103581RV	GAAGGTCGGAGTCAACGGATTCAGCGAAGGTTTCTTGCCT
B05-16 Mbp	B05_15568334F	CACACCGATCTCATGTCCAC
	B05_15568334RV	GAAGGTCGGAGTCAACGGATTGAGTTAGAGAAGTTACAAGGGGCA
	B05_15568334RF	GAAGGTGACCAAGTTCATGCTAGTTAGAGAAGTTACAAGGGGCG
B05-20 Mbp	B05_20485479R	TTTCACCTCTCCCTGTGTCC
	B05_20485479FV	GAAGGTCGGAGTCAACGGATTTTTGCTGTGCACTGCTTGT
	B05_20485479FF	GAAGGTGACCAAGTTCATGCTTGCTGTGCACTGCTTGC
B05-145 Mbp	B05_145087676F	GAGTTCGAAAGCGATTCGTG
	B05_145087676RV	GAAGGTCGGAGTCAACGGATTCTAGAAAGGTTATCGCTGCCA
	B05_145087676RF	GAAGGTGACCAAGTTCATGCTTAGAAAGGTTATCGCTGCCG
B05-148 Mbp	B05_148473590R	AAAGAGCATCAAGCTGGTGAA
	B05_148473590FV	GAAGGTCGGAGTCAACGGATTAGAGGCCTCAAGATTCAAAGACT
	B05_148473590FF	GAAGGTGACCAAGTTCATGCTAGGCCTCAAGATTCAAAGACC
B05-150 Mbp	B05_149728363F	CAATGAAAAATGGCTGGGATA
	B05_149728363RV	GAAGGTCGGAGTCAACGGATTATAATGGGGGAAGGCATACA
	B05_149728363RF	GAAGGTGACCAAGTTCATGCTTAATGGGGGAAGGCATACG
B06-7 Mbp	B06_7052911F	GCAAAGAGTTGTAGTATGATTGTGC
	B06_7052911RV	GAAGGTCGGAGTCAACGGATTCAAATGTTGAGATTAGCCGGA
	B06_7052911RF	GAAGGTGACCAAGTTCATGCTAAATGTTGAGATTAGCCGGG
B06-125 Mbp	B06_124836997F	GACAATGAGAGGGATTTAAGCAA
	B06_124836997RV	GAAGGTCGGAGTCAACGGATTGTTTCTCCAGATACTCAAGAACACAA
	B06_124836997RF	GAAGGTGACCAAGTTCATGCTGTTTCTCCAGATACTCAAGAACACAG
A06-104 Mbp	B06_129105472F	TATAGAGGCATGGGCTTGG
	B06_129105472RF	GAAGGTGACCAAGTTCATGCTTTCATCCTCACCATTTGTTCC
	B06_129105472RV	GAAGGTCGGAGTCAACGGATTACTTTCATCCTCACCATTTGTTCT

B06-135 Mbp	B06_134703663F	GACTCTTTCCTTTGTGAAGCA
	B06_134703663RV	GAAGGTCGGAGTCAACGGATTTCAATGGCGAAGAAGTCACA
	B06_134703663RF	GAAGGTGACCAAGTTCATGCTCAATGGCGAAGAAGTCACG
B07-11 Mbp	B07_10841443F	GGGGAGAGTGCGTCAGATT
	B07_10841443RF	GAAGGTGACCAAGTTCATGCTTTCTTCACCTCTAAATCGGAAATC
	B07_10841443RV	GAAGGTCGGAGTCAACGGATTGATTCTTCACCTCTAAATCGGAAATA
B07-23 Mbp	B07_22820655R	TCCAGACAGTGTTTTCTGATGG
	B07_22820655FV	GAAGGTCGGAGTCAACGGATTACAGCTTCATTCACCAAGAACAT
	B07_22820655FF	GAAGGTGACCAAGTTCATGCTCAGCTTCATTCACCAAGAACAC
B07-27 Mbp	B07_27259232F	TTCGTGACTACACAAAGATAATCCA
	B07_27259232RV	GAAGGTCGGAGTCAACGGATTTCAAGTCGATTATCCTTGCCA
	B07_27259232RF	GAAGGTGACCAAGTTCATGCTCAAGTCGATTATCCTTGCCG
B07-115 Mbp	B07_115226111F	CGAATGATGGAATGTCTTATCC
	B07_115226111RF	GAAGGTGACCAAGTTCATGCTAGATTCCGGCTATCCACCTAC
	B07_115226111RV	GAAGGTCGGAGTCAACGGATTAAGATTCCGGCTATCCACCTAT
B07-121 Mbp	B07_120609612F	CCTCCAGACTGCAAGCATATAA
	B07_120609612RF	GAAGGTGACCAAGTTCATGCTTCATTTGATGTTGAGGTACTGACC
	B07_120609612RV	GAAGGTCGGAGTCAACGGATTGTTCATTTGATGTTGAGGTACTGACT
B07-126 Mbp	B07_126178475F	TTTTCTCCTCGTCGTCATCC
	B07_126178475RV	GAAGGTCGGAGTCAACGGATTTTTAGGAGGCTTGTAACTTTTCTGA
	B07_126178475RF	GAAGGTGACCAAGTTCATGCTTTAGGAGGCTTGTAACTTTTCTGG
A08-25 Mbp	B08_2938899F	CCGGGGCAGTTTGTTGAT
	B08_2938899RF	GAAGGTGACCAAGTTCATGCTCCCTTCAGGGACATCGC
	B08_2938899RV	GAAGGTCGGAGTCAACGGATTGACCCTTCAGGGACATCGT
B08-96 Mbp	B08_96297017F	TCATCGAGGACAAGAGAAGAGA
	B08_96297017RF	GAAGGTGACCAAGTTCATGCTGCTTCCTTTTTCCTTCAAAACTAAC
	B08_96297017RV	GAAGGTCGGAGTCAACGGATTGCTTCCTTTTTCCTTCAAAACTAAT

B08-126 Mbp	B08_125650913R	GTCTCTATGCAGAGGCAATGG
	B08_125650913FV	GAAGGTCGGAGTCAACGGATTTGAGCATCATCGCCTGTTACT
	B08_125650913FF	GAAGGTGACCAAGTTCATGCTGAGCATCATCGCCTGTTACC
B08-127 Mbp	B08_127337559R	AAAAGGTGGCCTTGCTTGTT
	B08_127337559FF	GAAGGTGACCAAGTTCATGCTCCAATTTGCAATGGCTGAG
	B08_127337559FV	GAAGGTCGGAGTCAACGGATTCCAATTTGCAATGGCTGAA
B09-0.7 Mbp	B09_731802F	GCAATGGGAGTGAGATTCTTG
	B09_731802RF	GAAGGTGACCAAGTTCATGCTTGGTTTTTGGGGATGAGC
	B09_731802RV	GAAGGTCGGAGTCAACGGATTCTTTGGTTTTTGGGGATGAGT
B09-7 Mbp	B09_7368978F	GAATCAGCTGTCTCAGGGTTCT
	B09_7368978RF	GAAGGTGACCAAGTTCATGCTCCATATCAAACCCAGCAAGC
	B09_7368978RV	GAAGGTCGGAGTCAACGGATTATCCATATCAAACCCAGCAAGT
B09-9 Mbp	B09_9226786R	TTCACAGCACAAAGCTCGTT
	B09_9226786FF	GAAGGTGACCAAGTTCATGCTGAATTGGAGGTGGCCAATAG
	B09_9226786FV	GAAGGTCGGAGTCAACGGATTGAATTGGAGGTGGCCAATAA
B09-145 Mbp	B09_144754045F	TCTTGTTGAGAGCATGAAAGC
	B09_144754045RV	GAAGGTCGGAGTCAACGGATTGCACAAATCCGGGACAAA
	B09_144754045RF	GAAGGTGACCAAGTTCATGCTGCACAAATCCGGGACAAG
B09-147 Mbp	B09_146819670R	GGAGGAGGGGATGGTAAGG
	B09_146819670FV	GAAGGTCGGAGTCAACGGATTCTCTAAATGCTTGGAGGAAGAACT
	B09_146819670FF	GAAGGTGACCAAGTTCATGCTCTAAATGCTTGGAGGAAGAACC
B10-7 Mbp	B10_6963233F	GCGAGTTCCTCAAGGAGTGA
	B10_6963233RV	GAAGGTCGGAGTCAACGGATTATCTGCTTGCTACAAGTGACCA
	B10_6963233RF	GAAGGTGACCAAGTTCATGCTCTGCTTGCTACAAGTGACCG
B10-11 Mbp	B10_10669908F	TTCTTTTGTAAGCCTCTATTACAAACT
	B10_10669908RF	GAAGGTGACCAAGTTCATGCTCTAATTTGACGGAAGGGGC
	B10_10669908RV	GAAGGTCGGAGTCAACGGATTGCTAATTTGACGGAAGGGGT

B10-12 Mbp	B10_12359285R	TGGAGTTGAGTGCCAATTCA
	B10_12359285FF	GAAGGTGACCAAGTTCATGCTTGGATTTCACTGGCTGTGTG
	B10_12359285FV	GAAGGTCGGAGTCAACGGATTTTGGATTTCACTGGCTGTGTT

Appendix 8B

Supplemental Figures

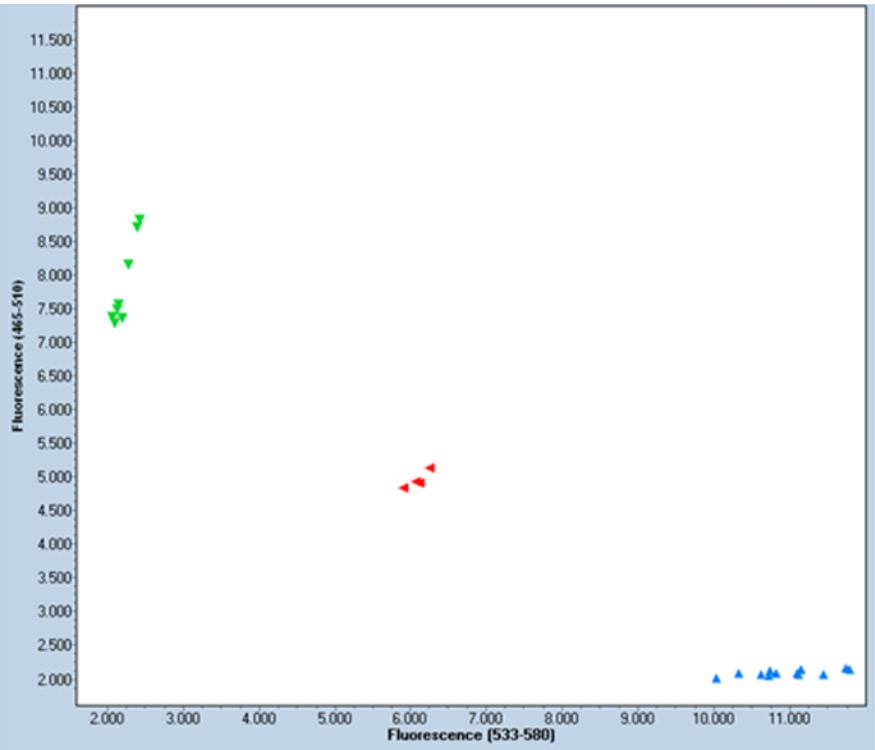

Figure S8.1. Example KASP results for a validated marker on the end of A01 (position, 93 Mbp). B/K- species DNA is green, A-genome species are blue, and AB DNA from cultivated controls is red; all DNA has two technical replicates.

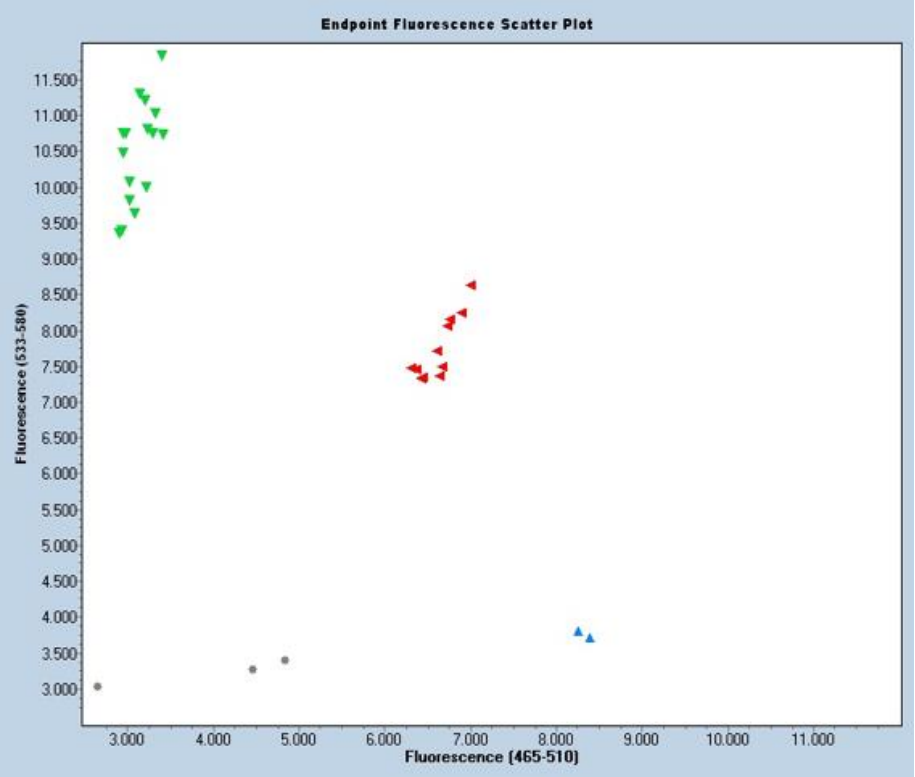

Figure S8.2. KASP assay result for marker A01-102 Mbp, in which DNA from 10 different plants labeled *A. gregoryi* V 6389 grouped with five A-genome species (green). Ten AB hybrid DNAs including two cultivars and eight confirmed AB allotetraploids were correctly clustered in the middle (red), and one B-genome species and one K-genome species grouped together (blue).

CHAPTER 9

SUMMARY

Genus *Arachis* has numerous, highly diverse species with beneficial alleles conferring insect and pathogen resistance, abiotic and biotic stress tolerance, and important agronomic traits that can be introgressed into cultivated peanut for cultivar improvement. Apart from *A. cardenasii*, these species have been underutilized for peanut crop improvement largely due to limitations such as ploidy barriers, low multiplication rates, low ploidy doubling success rates, and until recently, lack of molecular tools to analyze and track introgressions from wild *Arachis* species in peanut breeding materials. The release of the sequences of the diploid progenitors of peanut, *A. ipaensis* and *A. duranensis*, of the sequence of cultivated peanut, and of the Axiom_*Arachis* SNP chip has opened the door to harness the variability in wild *Arachis* species to create higher yielding, more resistant peanut cultivars that can improve global agriculture.

The goal of this work was to create genetic and molecular resources for peanut breeders to integrate beneficial alleles from wild *Arachis* species into their peanut breeding programs. To do this, novel allotetraploids, *IpaCor*^{4x}, *IpaDur*^{4x}, *IpaSten*^{4x}, and *ValSten*^{4x}, cross-compatible with cultivated peanut, were created. For breeders to efficiently utilize these allotetraploids, knowledge of the genetic and phenotypic diversity present in the materials was crucial. Therefore, comprehensive morphological and reproductive characterization on these materials was performed. Most of these allotetraploids produced more flowers over the growing season, larger flowers, larger and hairier leaves, taller main stems, longer primary laterals, longer internodes, lower percentages of reproductive nodes, heavier plant body masses, and smaller

seeds and pods than cultivated peanut, although there were exceptions for certain allotetraploids. These traits will likely need to be selected against while desirable traits such as pathogen and insect resistance are maintained. These allotetraploids were released (Chu et al., 2021) and deposited in the USDA Plant Genetic Resources and Conservation Unit (Griffin, GA) and the National Laboratory for Genetic Resources Preservation (Fort Collins, CO) so that breeders can access them for use in their breeding programs.

While wild, *Arachis* species are a great source genetic diversity for peanut breeding programs, these species also have undesirable characteristics such as small seed size, low yield, and weak peg strength. The peg strength and anatomy of wild *Arachis* species, allotetraploids, and allotetraploid-derived materials were evaluated to assess if peanut breeders need to take peg strength into account when using these materials in their breeding programs. In general, cultivated breeding lines had higher peg strength than allotetraploids and *Arachis* species; however, peg strength comparable to the peanut breeding lines was recovered in F_1 hybrids. This indicated that only a few backcrosses may be needed to select against this trait. Furthermore, there may be positive alleles for peg strength in some wild, *Arachis* species such as *A. valida* (PI 468154); although, breeders have an upper limit of desirable peg strength as pegs must disconnect from the plants during harvest. Yet, peanut breeders should be encouraged that the weak peg strength of *Arachis* species should not hinder the introgression of beneficial alleles from wild, *Arachis* species into peanut cultivars.

This study built upon previous reports by testing fall armyworm and rust resistance in allotetraploids instead of wild *Arachis* species alone. The tested allotetraploids showed strong fall armyworm resistance, donated primarily by *A. ipaensis*, making them more useful than peanut cultivars previously found to have only moderate levels of fall armyworm resistance.

Likewise, nine allotetraploids demonstrated immunity to rust, making them a better source of rust resistance than cultivated peanut germplasm, which has only been found to have moderate levels of resistance. These allotetraploids are publicly available in germplasm banks, so breeders can access them to introgress fall armyworm and rust resistance into their elite peanut cultivars. Fall armyworm and rust resistant cultivars can increase yields for farmers that cannot afford, or do not have access to, costly pesticides, and can protect yields and decrease reliance on pesticides in the United States.

While identifying resistance in allotetraploids is great first step to harnessing the genetic variability of wild *Arachis* species for peanut cultivar improvement, identifying QTL associated to resistance and other agronomic traits is essential for pyramiding desirable traits into peanut cultivars and for minimizing linkage drag. Therefore, morphological, reproductive and disease resistance characterization and QTL mapping of these traits was performed on BC_1F_1 individuals derived from four unique allotetraploids and on an *IpaCor*^{4x} BC_1F_2 population. These materials showed a wide range of phenotypic diversity, exceeding the averages of the allotetraploids and peanut breeding controls in flower production and other traits: flower size, leaf hair density, growth habit, flowering on the main stem, peg length, 100-pod weight, and 100-seed weight. Novel, putative QTLs for tomato spotted wilt orthotospovirus resistance were identified in the *IpaCor*^{4x} BC_1F_2 population on chromosomes B05 and B10 and in the *ValSten*^{4x} BC_1F_1 individuals on chromosomes A03 and B08. Putative QTL for flowering on the main stem were also identified in the *IpaCor*^{4x} BC_1F_2 population on A02, A05, and A06. Once validated, these novel tomato spotted wilt orthotospovirus QTL will be used to introgress resistance into peanut breeding lines that can be shared with peanut breeders to introgress these resistances into their best peanut cultivars.

The Axiom_Arachis SNP chip is an incredible genomic tool that can be used on allotetraploid-derived peanut populations to map important QTL desirable for introgression as was done in this study. However, the high resolution of this array is not always necessary for applications such as confirming true hybrids made from crossing A- and B/K-genome wild *Arachis* species, crosses which are necessary for producing allotetraploids. This study produced over one hundred A- and B/K-genome distinguishing KASP markers that span the entire peanut genome that can be used instead of the Axiom_Arachis SNP chip for this application. Each marker mix costs $30 and can be used to test tens of thousands of samples, making these KASP markers more cost- and time-efficient than the Axiom_Arachis SNP array, especially if a spot on the array is not open. The sequences of these markers are provided, enabling the public to order these markers for use in their own peanut breeding programs.

This work created novel allotetraploids and generated morphological, reproductive, and pathogen and insect resistance characterization data for these materials to promote their efficient utilization in peanut breeding programs. However, future work will focus on mapping QTL in mapping populations derived from these materials for more agronomic and pathogen and insect pest resistance to allow breeders to pyramid QTLs into elite peanut cultivars while minimizing linkage drag. Specifically, the *IpaDur*^{4x}, *IpaSten*^{4x} and *ValSten*^{4x} BC_1F_1 individuals will be progressed into mapping populations and the *IpaCor*^{4x} BC_1F_1 population will be progressed and utilized for validation of the tomato spotted wilt orthotospovirus resistance QTLs, flowering on main stem QTLs, and 100 pod weight QTL. These populations will also be used for mapping additional morphological and reproductive QTL including seed dormancy in the *IpaCor*^{4x} derived populations, and for mapping tomato spotted wilt orthotospovirus, late leaf spot, rust, and smut QTL. Identifying these QTL will allow breeders to pyramid all these desirable traits

from wild *Arachis* species into peanut cultivars. These high yielding, resistant peanut cultivars will improve global agriculture and increase global food security.

References

Chu, Y., Stalker, H.T., Marasigan, K., Levinson, C.M., Gao, D., Bertioli, D.J., Leal-Bertioli, S.C.M., Holbrook, C.C., Jackson, S.A., and P. Ozias-Akins. 2021. Registration of three peanut allotetraploid interspecific hybrids resistant to leaf spot diseases. J. Plant Regist.