A NEW SOURCE OF PEANUT RUST (*PUCCINIA ARACHIDIS*) RESISTANCE FROM WILD PEANUT SPECIES *ARACHIS BATIZOCOI*

by

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(Under the Direction of SORAYA BERTIOLI)

ABSTRACT

Cultivated peanut, *Arachis hypogaea*, is an allotetraploid species with low genetic diversity that is susceptible to the pathogen peanut rust, caused by the fungus *Puccinia arachidis*. Research has shown that wild peanut species *A. stenosperma* and *A. batizocoi* harbor resistance to various pests and pathogens, including *P. arachidis*. Previously, a synthetic allotetraploid created from a cross between *A. stenosperma* and *A. batizocoi* was utilized in a marker-assisted backcrossing scheme to introgress wild-derived root-knot nematode resistance into *A. hypogaea*. The resulting BC_3 population showed possible resistance to other pathogens. Select BC_3 families were screened and subsequent genotyping lead to the identification of an introgression of wild DNA on linkage group B02, from *A. batizocoi*, related to rust resistance. Primers were also developed and validated for this segment of wild alleles. We expect that the lines and markers developed here will be used for breeding cultivars with rust resistance using marker-assisted selection.

INDEX WORDS: *Puccinia arachidis*, *Arachis hypogaea*, wild peanut species, *Arachis* sp., *Arachis stenosperma*, *Arachis batizocoi*, marker-assisted backcross, SNP genotyping, KASP, B-subgenome, K-subgenome, A-subgenome, synthetic allotetraploid

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Cultivated peanut— *Arachis hypogaea* L.— is an allotetraploid species with low genetic diversity due to its unique polyploid origin, subsequent domestication, and sexual isolation from its wild diploid relatives (Bertioli et al., 2019; Halward et al., 1992; Holbrook & Stalker, 2003; Husted, 1936; Kochert et al., 1996; Leal-Bertioli et al., 2021). Some sources of resistance have been identified in cultivated peanut; however, no complete resistance has been found in the cultivated germplasm (Bertioli et al., 2019; Hammons, 1977; Husted, 1936; Kochert et al., 1996; Mondal et al., 2012; Mondal & Badigannavar, 2015; Moretzsohn et al., 2013; Subrahmanyam et al., 1983). Wild peanut species contain many sources of resistance to pests and diseases and are adapted to a wide variety of environments, so they are an important source of resistance in breeding programs (Bertioli et al., 2011; Leal-Bertioli et al., 2015a; Leal-Bertioli et al., 2018).

Peanut rust, *Puccinia arachidis,* is a fungal disease that affects peanut crops worldwide. *P. arachidis* can lead to early pod senescence, reduced seed size, and a low seed oil content which can lead up to a 50% reduction in yield (Leal-Bertioli et al., 2010; Mondal et al., 2014; Subrahmanyam et al., 1997; Subrahmanyam et al., 1985b). Several methods of cultural and chemical control can be used to reduce the effect of rust on crop yield but utilizing pathogen-resistant peanut cultivars as an alternative is recognized to be a more effective strategy for managing pathogens in the field for a myriad of reasons (Culbreath et al., 2002; Daudi et al., 2021;

Francis et al., 2020; Knauft & Ozias-Akins, 1995; Levinson et al., 2021; Subrahmanyam et al., 1997). For this purpose, multiple synthetic allotetraploids with resistance to rust have been created and various rust-resistant breeding lines have been released by breeding those synthetic allotetraploids with *A. hypogaea* (Bertioli et al., 2021b; Clevenger et al., 2018; Gao et al., 2021; Gowda et al., 2002; Levinson et al., 2021; Moss et al., 1998; Moss et al., 1997; Nigam et al., 1998; Reddy et al., 1996; Singh, 1986; Singh et al., 2003; Stalker, 2017; Varman, 1999). However, to combat the constantly evolving nature of plant pathogens, it is important to identify and utilize novel sources of resistance when breeding new lines of cultivated peanut. To this purpose, this study aimed to discover a new source of rust resistance from wild peanut species that will be useful to researchers that are breeding pathogen-resistant lines of *A. hypogaea*. A segment of wild alleles on the B/K-subgenome, derived from *A. batizocoi*, was successfully identified as a new source of resistance to peanut rust.

LITERATURE REVIEW

Peanut (*Arachis hypogaea*)

The peanut— *Arachis hypogaea* L.— is an important crop used for food, oil, and fodder that is mostly cultivated in tropical and sub-tropical regions of the world. In 2020, peanut was cultivated on a total of 31.6 million ha worldwide with an annual production of 53.8 million tons. The main producers of peanut in 2020 were: Asia at 58% (percentage of worldwide production in tons) with China producing 34% of the total and India producing 18%, Africa at 26% with Nigeria producing 8%, and the Americas at 8% with the United States production accounting for 5%. Yields around the world vary widely, in Africa averaging about 1 t/ha, in China just under 4 t/ha and the United States about 4.25 t/ha (FAOSTAT, 2021). In 2021, the United States planted more

than half a million ha (0.65 Mha) of peanut. The state of Georgia produced 52% of the total U.S. peanut production in 2021, followed by Alabama, Florida, Texas, North Carolina, South Carolina, Arkansas, Virginia, Mississippi, Oklahoma, and New Mexico (NASS, 2021) (Figure 1.1).

There are four primary commercial classes of *A. hypogaea* that are commonly grown: Virginia, Spanish, Valencia, and Runner (Holbrook & Stalker, 2003; Smith, 1950). Of the peanuts grown in the United States, about 70% are runner type, 20% are Virginia type, 10% are Spanish type, and less than 1% are Valencia type (Holbrook & Stalker, 2003). Peanut is used both for oil production due to its high oil content and for direct consumption of the grain (Arya et al., 2016). It can be consumed raw, boiled, roasted, mixed with other dishes, or used as a peanut butter spread. It provides vital dietary calories, nutrients, and proteins that are especially important in developing countries such as Sub-Saharan Africa. Peanut is often used as a weaning food for children in these countries, who are especially vulnerable to malnutrition (Arya et al., 2016; Mupunga et al., 2017; Rami et al., 2013; Settaluri et al., 2012; Singh & Singh, 1991).

Peanut origin and genetics

Taxonomically, *Arachis hypogaea* L. belongs in the genus *Arachis*, in the family Fabaceae, and the subfamily Faboidea. The genus *Arachis* likely originated in the highlands in the southwestern Mato Grosso do Sul region of Brazil close to Gran Pantanal, which is where most wild species with three leaflets (*A. guaranitica* Chodat. and Hassl. and *A. tuberosa* Bong. Ex Benth.), an ancestral phenotype, are found (Gregory et al., 1980; Simpson, 2001a; Stalker, 2017). The genus is comprised of approximately 83 described species that are divided into nine sections based on geographic distribution, reproductive compatibility, and morphology (Del Pilar De Souza et al., 2005; Krapovickas & Gregory, 1994; Krapovickas et al., 2007; Valls & Simpson, 2010). Cultivated peanut (*A. hypogaea* L.) is part of the section *Arachis* and is one of two tetraploid (2n

= 4x =40) species, the other being the wild species *A. monticola*. The section *Arachis* also includes 29 wild diploid species (2n = 2x =20) including its progenitors *A. ipaënsis* and *A. duranensis.* (Chu et al., 2021; Krapovickas & Gregory, 1994; Krapovickas et al., 2007; Leal-Bertioli et al., 2021; Rami et al., 2013; Seijo et al., 2021; Ballén-Taborda, 2019; Valls & Simpson, 2010).

A. hypogaea is an allotetraploid species (AABB, 2n = 4x =40) that evolved in South America approximately between five and 10 thousand years ago (Bertioli et al., 2020; Bertioli et al., 2019). This occurred due to the hybridization of two wild diploid species, *Arachis duranensis* (A genome donor) and *Arachis ipaënsis* (B genome donor) and then a spontaneous chromosome doubling (Bertioli et al., 2016; Bertioli et al., 2019; Cuc et al., 2008; Grabiele et al., 2012; Halward et al., 1992; Husted, 1936; Kochert et al., 1996; Lavia et al., 2011; Moretzsohn et al., 2013; Ramos et al., 2006; Robledo et al., 2009; Robledo & Seijo, 2010; Seijo et al., 2004). Human movement brought *A. ipaënsis* into the range of *A. duranensis* which allowed these wild species to hybridize (Bertioli et al., 2016; Simpson, 2001a). Cultivated peanut's genome size (~2.7 Gb) is roughly the size of the genomes of *A duranensis* (1.25 Gb) and *A. ipaënsis* (1.56 Gb) combined (Bertioli et al., 2016; Bertioli et al., 2019).

It has been shown that an induced allotetraploid IpaDur ([*A. ipaënsis* K30076 x *A. duranensis* 14167]$^{(2n=4x=40)}$) crossed with cultivated peanut produced highly fertile, vigorous, and phenotypically normal progeny (Fávero et al., 2006). These progenies were used—along with two other synthetic allotetraploid crosses created with A and B genome wild peanut relatives—to create a linkage map that further supports the close relationship between the genomes of the wild progenitors and current cultivated peanut (Shirasawa et al., 2013). High DNA identity (modal values of 99.98%) makes it likely that one accession of *A. ipaënsis* (K 30076) represents the B genome donor (Bertioli et al., 2016; Yin et al., 2020). Accessions of *A. duranensis* from Rio Seco

(with modal DNA identity of about 99.85%) are genetically very close to the A genome donor (Bertioli et al., 2020). Utilizing the availability of these close representatives of the original peanut ancestors, Leal-Bertioli et al., 2021 were able to recreate the events of 5-10 thousand years ago. This study concluded that two genetic bottlenecks occurred leading to *Arachis hypogaea*. First was the hybridization and polyploidization events, which lead to sexual reproductive isolation from its wild diploid relatives (Bertioli et al., 2016; Bertioli et al., 2021b; Leal-Bertioli et al., 2021; Yin et al., 2020); the second bottleneck occurred where only a few lineages with stronger mechanisms for limiting genomic instability were able to survive (Leal-Bertioli et al., 2021).

Peanut rust (*Puccinia arachidis*)

Peanut rust, *Puccinia arachidis*, is a major disease of peanut that is found worldwide. *P. arachidis* coevolved with peanut in South America, and the first record was in a collection from Surinam in 1827 (Hennen et al., 1976; Leppik, 1971). It was originally recognized as *Uredo arachidis* by Nils Gustav Lagerheim, then as *Uromyces arachidis* by Paul Hennings in 1896, then as *Bullaria arachidis* in 1922 by Arthur and Mains. It was then collected from Paraguay by Benedict Balansa and subsequently identified by Spegazzini as a new species and renamed as *P. arachidis* (Hennen et al., 1976; Mondal & Badigannavar, 2015). *P. arachidis* was mainly confined to South America until 1969, with the exception of a few outbreaks in the southern U.S. (Hammons, 1977; Subrahmanyam et al., 1989). Since 1969, *P. arachidis* has been reported around the world wherever peanut is grown (Bromfield & Bailey, 1972; Leal-Bertioli et al., 2015a; Subrahmanyam et al., 1997; Subrahmanyam et al., 1983). The full taxonomic classification of *P. arachidis* is phylum: Basidiomycota (basidial fungi), subphylum: Pucciniomycotina, class: Pucciniomycetes, order: *Pucciniales*, family: *Pucciniaceae*, genus: *Puccinia* (Aime et al., 2014; EOL, 2022; Kirk et al., 2008; Mondal & Badigannavar, 2015).

Puccinia arachidis prefers warm and humid conditions and can spread very quickly throughout the field. It primarily spreads through the repeated life cycle of the fungal urediniospores in the field. Symptoms start 8-10 days after infection. First, small white flecks appear on the abaxial leaf surface, which develop into red/brown colored pustules/uredinia. These pustules are usually circular and are raised up from the leaf surface, and measure from 0.3-2.0 mm in diameter. The pustules rupture and the urediniospores emerge from under the leaf surface. The pustules are red when they first emerge, and then become brown as they mature. Most of the pustules occur on the abaxial leaf surface, although they can form on the adaxial surface as well. Necrosis occurs around the pustules and can lead to defoliation. Rust can lead to early pod senescence, reduced seed size, and a low seed oil content which has an economic impact on the value of the crop (Leal-Bertioli et al., 2010; Mondal & Badigannavar, 2015; Subrahmanyam et al., 1997).

Puccinia arachidis urediniospores are the primary inoculum source for rust outbreaks in the field. Disease development is favored by long periods of warm temperature of 22°C or higher in conjunction with wet weather or high humidity of at least 78%. The pathogen moves within the field via wind, rain splash, and insects, and can persist from season to season if volunteer peanut plants are present (Mondal & Badigannavar, 2015; Sunkad & Kulkarni, 2007). However, the urediniospores are unlikely to survive if the field is fallow for more than 1 month (Subrahmanyam et al., 1997).

If *P. arachidis* is not prevented or controlled, it can cause as much as 50% yield loss (Subrahmanyam et al., 1985b). Late leaf spot (*Nothopassalora personata*) and early leaf spot (*Passalora arachidicola*) are often found in fields with peanut rust, and yield losses of up to 70% can occur when the diseases occur simultaneously (Khedikar et al., 2010; Levinson et al., 2021;

Subrahmanyam et al., 1997; Subrahmanyam et al., 1985a). In 2019, it was estimated that leaf spots caused a total of $44.05 million in economic losses in Georgia, caused by a 1.5% reduction in peanut crop value (Kemerait, 2021).

Puccinia arachidis management

There are several management strategies for rust control and for reducing the spread of the disease in the field. Reducing inoculum, intercropping, crop rotation, mulching, and soil amendments can be effective (Francis et al., 2020; Ghosh & Dayal, 1998; Levinson et al., 2021; Sill, 1982; Subrahmanyam et al., 1997; Thurston, 2019). Leaving the field fallow for one month in between successive peanut crops and ensuring there are no volunteer peanuts present to harbor inoculum is also recommended (Levinson et al., 2021; Subrahmanyam et al., 1997). Adjusting the time of sowing and controlling weeds in order to avoid the environmental conditions that favor the fungus can also be useful (Subramanyam & McDonald, 1984). Ensuring strict plant quarantine practices can be effective in keeping rust from spreading to disease-free areas (Francis et al., 2020). These practices are especially important in countries where chemical controls are less readily available due to financial constraints (Levinson et al., 2021; Subrahmanyam et al., 1997).

Rust often occurs with leaf spots; therefore, it is important that the fungicides used are effective against leaf spots as well as rust (Subrahmanyam et al., 1997). Several different chemicals are known to be effective, including dust formulations of copper, sulfur, or copper and sulfur. Using spray Bordeaux mixture plus dithiocarbamates can be even more effective (Subrahmanyam et al., 1997). Applying Calixin at 0.5 ml/liter of water or Kavach at 2 g/liter of water can also be effective (Culbreath et al., 2002). Tridemorph can be effective against *P. arachidis* but is not useful for controlling leaf spots (Chiteka et al., 1988; Subrahmanyam et al., 1997). The application of high doses of phosphorous fertilizers (60-75kg phosphate/ha) has also been reported to slow down

the development of rust (Mayee, 1987). However, nowadays, the most commonly used fungicides are chlorothalanil 0.2%, tebuconazole, mancozeb 0.25%, or Hexaconazole/propiconazole applied 30 days after germination until 15 days before harvesting at regular 10–15-day intervals (Culbreath et al., 2002; Francis et al., 2020; Subrahmanyam et al., 1997). Standard fungicide spraying regimes in Georgia account for about 10% of farmer costs, and for more than 10% peanut cultivation's greenhouse gas emissions (UGA Extension, 2022).

Despite the availability of chemical control, small-holder farmers in developing countries rarely follow the recommended application standards. This can lead to lowered efficacy of the chemical controls and to the possible development of pathogen resistance (Monyo et al., 2009; Smith & Littrell, 1980). The development of pathogen resistance to chemical control along with the natural evolution of plant pathogens that leads to resistance against various forms of management could cause additional problems for farmers. Therefore, it is important for farmers in developing countries as well as the United States that rust resistant, high-yielding peanut cultivars are developed (Daudi et al., 2021; Khedikar et al., 2010; Levinson et al., 2021; Mondal & Badigannavar, 2015).

Arachis genetic resources

As described in a previous section, *A. hypogaea* likely went through two genetic bottlenecks that, along with domestication by humans, led to the cultivated peanut we know today (Bertioli et al., 2020; Bertioli et al., 2016; Bertioli et al., 2021b; Bertioli et al., 2019; Leal-Bertioli et al., 2021; Yin et al., 2020). While *A. hypogaea* is morphologically diverse, resistances to pathogens and pests are limited due to its lack of genetic diversity and reproductive isolation from its diploid wild relatives (Bertioli et al., 2016; Bertioli et al., 2019; Halward et al., 1992; Husted, 1936; Kochert et al., 1996; Leal-Bertioli et al., 2015b; Moretzsohn et al., 2013). Strong resistance

against *P. arachidis* and other important peanut pathogens and pests have been found in the secondary, and tertiary gene pool of the genus *Arachis*. However only the secondary gene pool (which includes the section *Arachis*) is useful due to its compatibility with cultivated peanut, albeit with special manipulations of ploidy (Mondal & Badigannavar, 2015; Rami et al., 2013).

Rust resistance in cultivated peanut

Some rust resistance has been found in cultivated peanut, mainly in the subspecies *fastigiata* (Hammons, 1977; Mehan et al., 1996; Singh et al., 1997). The resistance available in cultivated peanut is often called the "slow rusting type" because the incubation period is longer, the pustules are smaller, the uridinia often fail to rupture or have reduced urediniospore production, and the germinability is lowered (Bromfield, 1971; Cook, 1980; Power et al., 2019; Subrahmanyam et al., 1983; Subrahmanyam et al., 1997; Wynne et al., 1991).

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) previously screened more than 13,000 cultivated germplasm accessions for resistance to rust and found about 150 resistant lines (Mondal & Badigannavar, 2015; Subrahmanyam et al., 1980; Subrahmanyam et al., 1997; Subrahmanyam et al., 1983; Subrahmanyam et al., 1995). Several rust-resistant mutants have also been reported in cultivated species, mainly in Peru, Bolivia, and India (Badigannavar et al., 2005; Bromfield, 1971; Mondal & Badigannavar, 2015; Power et al., 2013; Power et al., 2019; Rajgopal et al., 2002; Shokes & Melouk, 1995; Wynne et al., 1991). Various rust-resistant lines have also been bred using cultivated peanut and released as germplasm. Some examples include Tifrust1-14 (Hammons et al., 1982a; Hammons et al., 1982b) and ICG 12625, 2381, 12697, and 11426 (Upadhyaya et al., 2014). Research is ongoing into breeding more resistant cultivars and finding resistance in commonly used cultivars (Power et al., 2019).

Rust resistance in wild peanut

Wild peanut species (*Arachis* sp.) are adapted to a large variety of habitats and can be found in xerophytic forests, in temporarily flooded areas, in grasslands, and in sub-tropical rainforest. They can also survive on a wide range of soil types, from rocky soils to sandy soils with a variety of drainage types (Bertioli et al., 2011; Krapovickas et al., 2007) *Arachis* species are indigenous to South America and can be found widely distributed across the whole continent (Moss & Feakin, 1985; Stalker, 2017).

Wild peanuts contain many sources of resistance to pests and diseases that plague *A. hypogaea* (Bertioli et al., 2003; Bertioli et al., 2011; Stalker, 2017). Within the section *Arachis*, 29 wild diploid species harbor resistance to rust, late leaf spot, early leaf spot, and other important peanut pathogens (Stalker, 2017). Various studies have identified which of these wild species are resistant to diseases that commonly affect peanut, including: *A. batizocoi, A. cardenasii, A. duranensis, A. ipaënsis, A. stenosperma, A. valida, A. monticola, A. diogoi,* and *A. correntina* (Ballén-Taborda et al., 2021; Bera et al., 2018; Bertioli et al., 2003; Bertioli et al., 2011; Leal-Bertioli et al., 2010; Fávero et al., 2009; Holbrook & Stalker, 2003; Kumar & Kirti, 2015; Leal-Bertioli et al., 2009; Leal-Bertioli et al., 2015a; Leal-Bertioli et al., 2015b; Leal-Bertioli et al., 2017; Morgante et al., 2013; Mota et al., 2018; Nelson et al., 1989; Nelson et al., 1990; Nigam et al., 1991; Pande & Rao, 2001; Rami et al., 2013; Sharma et al., 2003; Simpson et al., 2003; Stalker, 1984; Stalker, 2017; Subrahmanyam et al., 1983; Subrahmanyam et al., 1985a; Subramanyam & McDonald, 1984; Upadhyaya et al., 2011). It was recently discovered that a single accession of *A. cardenasii* (GKP 10017) that was utilized in breeding programs in the 1960s (Stalker et al., 1979) has resulted in current commercial lines with resistance to *P. arachidis* as well as to other important peanut diseases (Bertioli et al., 2021a).

Transferring wild alleles into peanut

While it is possible to use chemical and cultural control to deal with diseases in the field, utilizing pathogen-resistant peanut cultivars is a more economically viable, technically feasible, environmentally friendly, and socially acceptable strategy (Daudi et al., 2021; Knauft & Ozias-Akins, 1995; Mondal & Badigannavar, 2015). In the past, incorporating wild species disease resistance into breeding programs tended to decrease the yield because most wild alleles are agronomically maladapted. However, in recent years molecular techniques and other methods have allowed researchers to increase disease resistance while also increasing yield and other beneficial characteristics (Holbrook et al., 2016). Compared to conventional breeding, molecular breeding allows researchers to better handle traits which are difficult to manage through conventional phenotypic selection as well as increasing the efficiency and accuracy of pyramiding traits or QTLs for a specific trait (Ribaut and Hossington, 1998; Xu and Crouch, 2008).

Most wild species are diploid, so transferring genes/alleles from the wild species to the tetraploid *A. hypogaea* is not a simple process (Bertioli et al., 2021b; Rami et al., 2013; Simpson, 2001; Simpson, 1991; Stalker, 2017). However, there are methods for creating wild-derived synthetic allotetraploids that can then be bred with cultivated peanut (Bertioli et al., 2021b; Rami et al., 2013; Simpson, 2001; Simpson, 1991). The first method is called the "hexaploid" route. First the tetraploid *A. hypogaea* (2n=40) is hybridized with a diploid wild relative (2n =20) to produce a sterile triploid. The hybrid is then chromosome-doubled to create a hexaploid (6x=60) which is crossed and then selfed or back-crossed with *A. hypogaea.* Selection can be attempted for desired traits, but not every combination of cultivated and wild peanut is viable, as some produce sterile offspring (Simpson, 2001; Simpson and Starr, 2001). A second method is known as the "tetraploid" route: a diploid A-genome wild peanut species is crossed with a diploid B-genome

species resulting in a sterile hybrid that is then treated with colchicine. This induces doubling of the chromosomes to create a tetraploid hybrid that can be successfully crossed with cultivated peanut (Simpson, 2001; Simpson and Starr, 2001). Researchers have focused their efforts on the tetraploid route in recent years because of its greater ease and higher fertility of progeny (Ballén-Taborda et al., 2021; Chu et al., 2021; Fávero et al., 2006; Foncéka et al., 2009; Gao et al., 2021; Leal-Bertioli et al., 2018; Levinson et al., 2021; Mondal & Badigannavar, 2015; Stalker, 2017).

Breeding for rust resistance

Researchers have been successful utilizing wild peanut species to introgress resistance to peanut pathogens and pests, and specifically to *P. arachidis.* Synthetic allotetraploids have been created by crossing different combinations of wild peanut species. Allotetraploids that are sexually compatible with *A. hypogaea* can be used to introgress resistance alleles by utilizing conventional breeding methods as well as marker-assisted selection to advance progeny with selected QTL that have been tested for resistance to rust (Gao et al., 2021; Khedikar et al., 2010; Leal-Bertioli et al., 2015a; Levinson et al., 2021; Stalker and Moss, 1987; Stalker and Wynne, 1982; Stalker et al., 1982; Stalker, 2017; Stalker et al., 1979; Sujay et al., 2012). Various wild species containing rust resistant breeding lines have been released for use, including VG 9514 (Varman, 1999), GPBD 4 (Clevenger et al., 2018; Gowda et al., 2002), ICGV 86699 (Reddy et al., 1996), and ICGV 99001-99005 (Singh et al., 2003) and more (Moss et al., 1998; Moss et al., 1997; Nigam et al., 1998; Stalker, 2017).

In more recent years, wild peanuts species have been used to introgress rust resistance into cultivated peanut including *A. batizocoi, A. correntina, A. duranensis, A. stenosperma, A. gregoryi, A. magna, A. cardenasii, A. diogoi,* and *A. valida*. Multiple accessions of these synthetic allotetraploids have been found to be resistant to rust, including: BatCor, BatDur1, BatDur2,

BatSten1, GregSten, MagCard, MagDio, MagDur, and ValSten1 (Gao et al., 2021; Holbrook et al., 2016; Khedikar et al., 2010; Leal-Bertioli et al., 2015b; Levinson et al., 2021; Rami et al., 2013; Singh, 1986; Sujay et al., 2012). It has been found that the specific accessions within the wild peanut species is also important when trying to utilize that species for breeding, as resistance can vary between individual accessions in a species (Pande & Rao, 2001; Rami et al., 2013; Singh et al., 1996) so induced allotetraploids have been created using multiple accessions of the same species. Rust resistant germplasm has also been released from more complicated crosses, like the [*A.batizocoi* (*A. cardenasii* × *A. diogoi*)]^{4x} cross as well as a line from its backcross with cultivated *A. hypogaea* Florunner (Simpson et al., 1993). However, only a few accessions have been used so far for breeding rust resistance, so it is important that research continues to identify and use new sources of resistance.

OBJECTIVES

The main objective of this project was to utilize a population of BC_3 plants from a marker-assisted backcrossing scheme to identify the wild-derived source of resistance to peanut rust. The BC_3 population had originally been created to introgress resistance to root-knot nematode from the wild donor parents (*A. batizocoi* and *A. stenosperma*) but the wild parents have previously been found to have resistance to *P. arachidis* as well. In order to utilize this source of resistance to help create rust resistant lines, selected BC_3 families were first screened in order to identify a family that segregates for rust resistance and then single-nucleotide polymorphism (SNP)-based genotyping was used to identify the linkage group containing the segment of wild DNA related to the resistance.

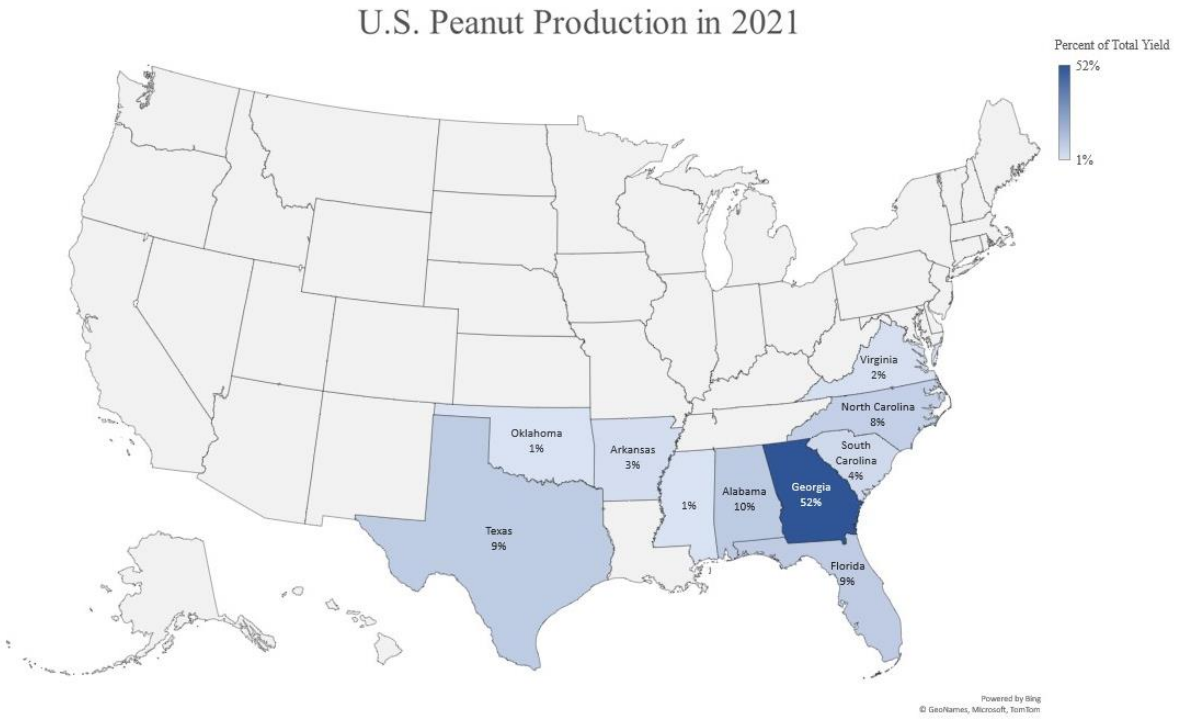

Figure 1.1: U.S. Peanut Production 2021. Percentages of peanut produced in 2021 (based on the total lb produced per state) in the U.S (NASS, 2021). Map created with Microsoft Excel.

LITERATURE CITED

Aime, M. C., Toome, M., & McLaughlin, D. J. (2014). Pucciniomycotina. In D. J. McLaughlin & J. W. Spatafora (Eds.), *Systematics and Evolution: Part A* (pp. 271-294). Springer Berlin Heidelberg.

Arya, S. S., Salve, A. R., & Chauhan, S. (2016). Peanuts as functional food: a review. *Journal of Food Science Technology*, *53*(1), 31-41. https://doi.org/10.1007/s13197-015-2007-9

Badigannavar, A., Kale, D., Mondal, S., & Murty, G. (2005). Trombay groundnut recombinants resistant to foliar diseases. *Mutat Breed Newsl Rev*, *1*, 11-12.

Ballén-Taborda. (2019). *Exploration of Arachis stenosperma as a source of nematode resistance for peanut.* [University of Georgia].

https://esploro.libs.uga.edu/esploro/outputs/doctoral/Exploration-of-Arachis-stenosperma-as-a/9949365657502959#file-0

Ballén-Taborda, C., Chu, Y., Ozias-Akins, P., Timper, P., Jackson, S. A., Bertioli, D. J., & Leal-Bertioli, S. C. M. (2021). Validation of resistance to root-knot nematode incorporated in peanut from the wild relative *Arachis stenosperma*. *Agronomy Journal*, *113*(3), 2293-2302. https://doi.org/https://doi.org/10.1002/agj2.20654

Bera, S. K., Manohar, S. S., Variath, M. T., Chaudhari, S., Yaduru, S., Thankappan, R., Narayana, M., Kurapati, S., Pandey, M. K., & Sudini, H. K. (2018). Assessing variability for disease resistance and nutritional quality traits in an interspecific collection of groundnut (*Arachis hypogaea*). *Plant Breeding*, *137*(6), 883-894.

Bertioli, D. J., Abernathy, B., Seijo, G., Clevenger, J., & Cannon, S. B. (2020). Evaluating two different models of peanut's origin. *Nature Genetics*, *52*(6), 557-559.

Bertioli, D. J., Cannon, S. B., Froenicke, L., Huang, G., Farmer, A. D., Cannon, E. K. S., Liu, X., Gao, D., Clevenger, J., Dash, S., Ren, L., Moretzsohn, M. C., Shirasawa, K., Huang, W., Vidigal, B., Abernathy, B., Chu, Y., Niederhuth, C. E., Umale, P., Araújo, A. C. G., Kozik, A., Do Kim, K., Burow, M. D., Varshney, R. K., Wang, X., Zhang, X., Barkley, N., Guimarães, P. M., Isobe, S., Guo, B., Liao, B., Stalker, H. T., Schmitz, R. J., Scheffler, B. E., Leal-Bertioli, S. C. M., Xun, X., Jackson, S. A., Michelmore, R., & Ozias-Akins, P. (2016). The genome sequences of *Arachis duranensis* and *Arachis ipaënsis*, the diploid ancestors of cultivated peanut. *Nature Genetics*, *48*(4), 438-446. https://doi.org/10.1038/ng.3517

Bertioli, D. J., Clevenger, J., Godoy, I. J., Stalker, H. T., Wood, S., Santos, J. F., Ballén-Taborda, C., Abernathy, B., Azevedo, V., Campbell, J., Chavarro, C., Chu, Y., Farmer, A. D.,

Fonceka, D., Gao, D., Grimwood, J., Halpin, N., Korani, W., Michelotto, M. D., Ozias-Akins, P., Vaughn, J., Youngblood, R., Moretzsohn, M. C., Wright, G. C., Jackson, S. A., Cannon, S. B., Scheffler, B. E., & Leal-Bertioli, S. C. M. (2021a). Legacy genetics of *Arachis cardenasii* in the peanut crop shows the profound benefits of international seed exchange. *Proceedings of the National Academy of Sciences*, *118*(38), e2104899118. https://doi.org/doi:10.1073/pnas.2104899118

Bertioli, D. J., Gao, D., Ballén-Taborda, C., Chu, Y., Ozias-Akins, P., Jackson, S. A., Holbrook, C. C., & Leal-Bertioli, S. C. M. (2021b). Registration of GA-BatSten1 and GA-MagSten1, two induced allotetraploids derived from peanut wild relatives with superior resistance to leaf spots, rust, and root-knot nematode. *Journal of Plant Registrations*, *15*(2), 372-378. https://doi.org/https://doi.org/10.1002/plr2.20133

Bertioli, D. J., Jenkins, J., Clevenger, J., Dudchenko, O., Gao, D., Seijo, G., Leal-Bertioli, S. C. M., Ren, L., Farmer, A. D., Pandey, M. K., Samoluk, S. S., Abernathy, B., Agarwal, G., Ballén-Taborda, C., Cameron, C., Campbell, J., Chavarro, C., Chitikineni, A., Chu, Y., Dash, S., El Baidouri, M., Guo, B., Huang, W., Kim, K. D., Korani, W., Lanciano, S., Lui, C. G., Mirouze, M., Moretzsohn, M. C., Pham, M., Shin, J. H., Shirasawa, K., Sinharoy, S., Sreedasyam, A., Weeks, N. T., Zhang, X., Zheng, Z., Sun, Z., Froenicke, L., Aiden, E. L., Michelmore, R., Varshney, R. K., Holbrook, C. C., Cannon, E. K. S., Scheffler, B. E., Grimwood, J., Ozias-Akins, P., Cannon, S. B., Jackson, S. A., & Schmutz, J. (2019). The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. *Nature Genetics*, *51*(5), 877-884. https://doi.org/10.1038/s41588-019-0405-z

Bertioli, D. J., Leal-Bertioli, S. C. M., Lion, M. B., Santos, V. L., Pappas, G., Cannon, S. B., & Guimarães, P. M. (2003). A large scale analysis of resistance gene homologues in *Arachis.*

Molecular Genetics and Genomics, *270*(1), 34-45. https://doi.org/10.1007/s00438-003-0893-4

Bertioli, D. J., Seijo, G., Freitas, F. O., Valls, J. F. M., Leal-Bertioli, S. C. M., & Moretzsohn, M. C. (2011). An overview of peanut and its wild relatives. *Plant Genetic Resources*, *9*(01), 134-149. https://doi.org/10.1017/s1479262110000444

Bromfield, K., & Bailey, W. (1972). Inheritance of resistance to *Puccinia arachidis* in peanut. Phytopathology,

Bromfield, K. R. (1971). Peanut rust: a review of literature. *Journal, American Peanut Research and Education Association, Inc.*, *3*(1), 111-121.

Chiteka, Z., Gorbet, D., Shokes, F., Kucharek, T., & Knauft, D. (1988). Components of resistance to late leafspot in peanut. I. Levels and variability-implications for selection. *Peanut Science*, *15*(1), 25-30.

Chu, Y., Bertioli, D., Levinson, C. M., Stalker, H. T., Holbrook, C. C., & Ozias-Akins, P. (2021). Homoeologous recombination is recurrent in the nascent synthetic allotetraploid *Arachis ipaënsis* × *Arachis correntina*^{4x} and its derivatives. *G3 Genes|Genomes|Genetics*, *11*(4). https://doi.org/10.1093/g3journal/jkab066

Clevenger, J., Chu, Y., Chavarro, C., Botton, S., Culbreath, A., Isleib, T. G., Holbrook, C. C., & Ozias-Akins, P. (2018). Mapping late leaf spot resistance in peanut (*Arachis hypogaea*) using QTL-seq reveals markers for marker-assisted selection. *Frontiers in Plant Science*, *9*.

https://doi.org/10.3389/fpls.2018.00083

Cook, M. (1980). Host-parasite relations in uredial infections of peanut by *Puccinia arachidis*. *Phytopathology*, *70*(8), 822-826.

Cuc, L. M., Mace, E. S., Crouch, J. H., Quang, V. D., Long, T. D., & Varshney, R. K. (2008). Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). *BMC Plant Biology*, *8*(1), 1-11.

Culbreath, A., Stevenson, K., & Brenneman, T. (2002). Management of late leaf spot of peanut with benomyl and chlorothalonil: A study in preserving fungicide utility. *Plant Disease*, *86*(4), 349-355.

Daudi, H., Shimelis, H., Mathew, I., Rathore, A., & Ojiewo, C. O. (2021). Combining ability and gene action controlling rust resistance in groundnut (*Arachis hypogaea* L.). *Scientific Reports*, *11*(1), 16513. https://doi.org/10.1038/s41598-021-96079-z

Del Pilar De Souza, P. E., Aloza, A., Valls, J. O. S., & Francisco, M. (2005). Chromosome number and satellited chromosome morphology of eleven species of *Arachis* (leguminsae)*Bonplandia*, *14*(1/2), 65-72. http://www.jstor.org/stable/41941267

EOL. (2022). *Puccinia arachidis Speg. 1884*. National Museum of Natural History. https://eol.org/pages/154780

FAOSTAT. (2021). *Crops and livestock products- Groundnuts* FAO. http://faostat.fao.org/

Fávero, A. P., Moraes, S. A. d., Garcia, A. A. F., Valls, J. F. M., & Vello, N. A. (2009). Characterization of rust, early and late leaf spot resistance in wild and cultivated peanut germplasm. *Scientia Agricola*, *66*(1), 110-117.

Fávero, A. P., Simpson, C. E., Valls, J. F. M., & Vello, N. A. (2006). Study of the evolution of cultivated peanut through crossability studies among *Arachis ipaënsis, A. duranensis,* and *A. hypogaea*. *Crop Science*, *46*(4), 1546-1552. https://doi.org/https://doi.org/10.2135/cropsci2005.09-0331

Foncéka, D., Hodo-Abalo, T., Rivallan, R., Faye, I., Sall, M. N., Ndoye, O., Fávero, A. P., Bertioli, D. J., Glaszmann, J.-C., & Courtois, B. (2009). Genetic mapping of wild introgressions into cultivated peanut: a way toward enlarging the genetic basis of a recent allotetraploid. *BMC Plant Biology*, *9*(1), 1-13.

Francis, M., Chacha, M., Mbega, E., & Ndakidemi, P. (2020). Groundnut rust disease epidemiology and potential sustainable management strategies. *International Journal of Bioscience*, *16*, 524-536.

Gao, D., Araujo, A. C. G., Nascimento, E. F. M. B., Chavarro, M. C., Xia, H., Jackson, S. A., Bertioli, D. J., & Leal-Bertioli, S. C. M. (2021). ValSten: a new wild species derived allotetraploid for increasing genetic diversity of the peanut crop (*Arachis hypogaea* L.). *Genetic Resources and Crop Evolution*, *68*(4), 1471-1485. https://doi.org/10.1007/s10722-020-01076-2

Ghosh, P., & Dayal, D. (1998). Effect of varying levels of nitrogen in three groundnut-based intercropping systems. Proc. Int. Conf. on Food Security and Crop Sci., CCS Haryana Agricultural University, Hissar-125

Gowda, M., Motagi, B., Naidu, G., Diddimani, S., & Sheshagiri, R. (2002). GPBD 4: a spanish bunch groundnut genotype resistant to rust and late leaf spot. *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)*.

Grabiele, M., Chalup, L., Robledo, G., & Seijo, G. (2012). Genetic and geographic origin of domesticated peanut as evidenced by 5S rDNA and chloroplast DNA sequences. *Plant Systematics and evolution*, *298*(6), 1151-1165.

Gregory, W., Krapovickas, A., & Gregory, M. P. (1980). Structure, variation, evolution, and classification in Arachis. *Structure, variation, evolution, and classification in Arachis.*, 469-481.

Halward, T., Stalker, T., LaRue, E., & Kochert, G. (1992). Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). *Plant Molecular Biology*, *18*(2), 315-325. https://doi.org/10.1007/bf00034958

Hammons, R. (1977). Groundnut rust in the United States and the Caribbean. *Pans*, *23*(3), 300-304.

Hammons, R. O., Branch, W., Bromfield, K., Subrahmanyam, P., Rao, V., Nigam, S., & Gibbons, R. (1982a). Registration of Tifrust-14 Peanut Germplasm1 (Reg. No. GP31). *Crop Science*, *22*(3), 697-698.

Hammons, R. O., Subrahmanyam, P., Rao, V., Nigam, S., & Gibbons, R. (1982b). Registration of peanut germplasms Tifrust-1 to Tifrust-4 (Reg. No. GP18 to GP21). *Crop Science*, *22*(2), 453-453.

Hennen, J. F., Figueiredo, M. B., Ribeiro, I. J. A., & Soave, J. (1976). The occurrence of teliospores of *Puccinia arachidis* (Uredinales) on *Arachis hypogaea* in Sao Paulo State, Brazil. *Summa Phytopathologica*, *2*, 44-46.

Holbrook, C. C., Burow, M. D., Chen, C. Y., Pandey, M. K., Liu, L., Chagoya, J. C., Chu, Y., Ozias-Akins, P. (2016). Recent Advances in Peanut Breeding and Genetics. Pp. 111-145. In: H. T. Stalker and R. F. Wilson (editors). Peanut: Genetics, Processing, and Utilization. Academic Press and AOCS Press.

Holbrook, C. C., & Stalker, H. T. (2003). Peanut Breeding and Genetic Resources.In: J. Janick (ed.). *Plant Breeding Reviews*, *22*, 297-356.

Husted, L. (1936). Cytological studies on the peanut, *Arachis.* II Chromosome number, morphology and behavior, and their application to the problem of the origin of the cultivated forms. *Cytologia*, *7*(3), 396-423.

Kemerait, R. (2021). 2019 Georgia Plant Disease Loss Estimates. *Georgia Plant Disease Loss Estimates*. University of Georgia Department of Plant Pathology Extension. May 2022.

Khedikar, Y., Gowda, M., Sarvamangala, C., Patgar, K., Upadhyaya, H., & Varshney, R. (2010). A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). *Theoretical and applied genetics*, *121*(5), 971-984.

Kirk, P. M., Ainsworth, G. C., Bisby, G. R., & International, C. A. B. (2008). *Ainsworth & Bisby's Dictionary of the Fungi*. CABI. https://books.google.com/books?id=IFD4_VFRDdUC

Knauft, D. A., & Ozias-Akins, P. (1995). Recent methodologies for germplasm enhancement and breeding. *Advances in peanut science*, 54-94.

Kochert, G., Stalker, H. T., Gimenes, M., Galgaro, L., Lopes, C. R., & Moore, K. (1996). RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (Leguminosae). *American Journal of Botany*, *83*(10), 1282-1291.

Krapovickas, A., & Gregory, W. C. (1994). Taxonomia del genero “Arachis (Leguminosae)". *Bonplandia*, *8*(1/4), 1-186. http://www.jstor.org/stable/41941177

Krapovickas, A., Gregory, W. C., Williams, D. E., & Simpson, C. E. (2007). Taxonomy of the genus *Arachis* (Leguinoasae). *Bonplandia*, *16*, 7-205. http://www.jstor.org/stable/41941433

Kumar, D., & Kirti, P. B. (2015). Transcriptomic and proteomic analyses of resistant host responses in *Arachis diogoi* challenged with late leaf spot pathogen, *Phaeoisariopsis personata*. *PloS one*, *10*(2), e0117559.

Lavia, G. I., Ortiz, A. M., Robledo, G., Fernández, A., & Seijo, G. (2011). Origin of triploid *Arachis pintoi* (Leguminosae) by autopolyploidy evidenced by FISH and meiotic behavior. *Annals of Botany*, *108*(1), 103-111.

Leal-Bertioli, S. C., De Farias, M. P., Silva, P. Í. T., Guimarães, P. M., Brasileiro, A. C. M., Bertioli, D. J., & De Araujo, A. C. G. (2010). Ultrastructure of the initial interaction of *Puccinia arachidis* and *Cercosporidium personatum* with leaves of *Arachis hypogaea* and *Arachis stenosperma*. *Journal of Phytopathology*, *158*(11-12), 792-796.

Leal-Bertioli, S., José, A. C. V., Alves-Freitas, D. M., Moretzsohn, M. C., Guimarães, P. M., Nielen, S., Vidigal, B. S., Pereira, R. W., Pike, J., & Fávero, A. P. (2009). Identification of candidate genome regions controlling disease resistance in *Arachis. BMC Plant Biology*, *9*(1), 1-12.

Leal-Bertioli, S. C. M., Cavalcante, U., Gouvea, E. G., Ballén-Taborda, C., Shirasawa, K., Guimarães, P. M., Jackson, S. A., Bertioli, D. J., & Moretzsohn, M. C. (2015a). Identification of QTLs for rust resistance in the peanut wild species *Arachis magna* and the development of KASP markers for marker-assisted selection. *G3 Genes|Genomes|Genetics*, *5*(7), 1403-1413. https://doi.org/10.1534/g3.115.018796

Leal-Bertioli, S. C. M., Moretzsohn, M. C., Roberts, P. A., Ballén-Taborda, C., Borba, T. C. O., Valdisser, P. A., Vianello, R. P., Araujo, A. C. G., Guimaraes, P. M., & Bertioli, D. J. (2015b). Genetic Mapping of Resistance to *Meloidogyne arenaria* in *Arachis stenosperma*:

A New Source of Nematode Resistance for Peanut. G3: Genes, Genomes, Genetics 6(2):377-390.https://doi.org/10.1534/g3.115.023044

Leal-Bertioli, S. C. M., Moretzsohn, M. C., Santos, S. P., Brasileiro, A. C. M., Guimarães, P. M., Bertioli, D. J., & Araujo, A. C. G. (2017). Phenotypic effects of allotetraploidization of wild *Arachis* and their implications for peanut domestication. *American Journal of Botany*, *104*(3), 379-388. https://doi.org/10.3732/ajb.1600402

Leal-Bertioli, S. C. M., Nascimento, E. F. M. B., Chavarro, M. C. F., Custódio, A. R., Hopkins, M. S., Moretzsohn, M. C., Bertioli, D. J., & Araújo, A. C. G. (2021). Spontaneous generation of diversity in *Arachis* neopolyploids (*Arachis ipaënsis* × *Arachis duranensis*)^{4x} replays the early stages of peanut evolution. *G3 Genes|Genomes|Genetics*, *11*(11). https://doi.org/10.1093/g3journal/jkab289

Leal-Bertioli, S. C. M., Santos, S. P., Dantas, K. M., Inglis, P. W., Nielen, S., Araujo, A. C. G., Silva, J. P., Cavalcante, U., Guimarães, P. M., Brasileiro, A. C. M., Carrasquilla-Garcia, N., Penmetsa, R. V., Cook, D., Moretzsohn, M. C., & Bertioli, D. J. (2015c). *Arachis batizocoi*: a study of its relationship to cultivated peanut (*A. hypogaea*) and its potential for introgression of wild genes into the peanut crop using induced allotetraploids. *Annals of Botany*, *115*(2), 237-249. https://doi.org/10.1093/aob/mcu237

Leal-Bertioli, S. C., Godoy, I. J., Santos, J. F., Doyle, J. J., Guimarães, P. M., Abernathy, B. L., Jackson, S. A., Moretzsohn, M. C., & Bertioli, D. J. (2018). Segmental allopolyploidy in action: increasing diversity through polyploid hybridization and homoeologous recombination. *American Journal of Botany*, *105*(6), 1053-1066.

Leppik, E. E. (1971). Assumed gene centers of peanuts and soybeans. *Economic Botany*, *25*(2), 188-194.

Levinson, C. M., Antepenko, E., Leal-Bertioli, S., Chu, Y., Culbreath, A. K., Stalker, H. T., Gao, D., & Ozias-Akins, P. (2021). Resistance to rust (*Puccinia arachidis* Speg.) identified in nascent allotetraploids cross-compatible with cultivated peanut (*Arachis hypogaea* L.). *Peanut Science*, *48*(2), 123-130.

Mayee, C. (1987). Rust disease of groundnut in Maharashtra state of India. Citation: ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1987. Groundnut rust disease. Proceedings of a Discussion Group Meeting, 24-28 Sep 1984, ICRISAT Center, India. Patancheru, AP 502324, India: ICRISAT.

Mehan, V., Reddy, P., Subrahmanyam, P., McDonald, D., & Singh, A. (1996). Identification of new sources of resistance to rust and late leaf spot in peanut. *International Journal of Pest Management*, *42*(4), 267-271.

Mondal, S., & Badigannavar, A. (2015). Peanut rust (*Puccinia arachidis* Speg.) disease: its background and recent accomplishments towards disease resistance breeding. *Protoplasma*, *252*(6), 1409-1420.

Mondal, S., Hande, P., & Badigannavar, A. M. (2014). Identification of transposable element markers for a rust (*Puccinia arachidis* Speg.) resistance gene in cultivated peanut. *Journal of Phytopathology*, *162*(7-8), 548-552.

Monyo, E and Osiru, M and Kadyampakeni, D and Mponda, O and Chinyamunyamu, B (2009) *Improving Food Security and Nutrition in Malawi and Tanzania through Research on Edible Legumes.* Other. International Crops Research Institute for the Semi-Arid Tropics. Moretzsohn, M. C., Gouvea, E. G., Inglis, P. W., Leal-Bertioli, S. C., Valls, J. F., & Bertioli, D. J. (2013). A study of the relationships of cultivated peanut (*Arachis hypogaea*)

and its most closely related wild species using intron sequences and microsatellite markers. *Annals of Botany, 111*(1), 113-126.

Morgante, C. V., Brasileiro, A. C., Roberts, P. A., Guimaraes, L. A., Araujo, A. C., Fonseca, L. N., Leal-Bertioli, S. C., Bertioli, D. J., & Guimaraes, P. M. (2013). A survey of genes involved in *Arachis stenosperma* resistance to *Meloidogyne arenaria* race 1. *Functional Plant Biology*, *40*(12), 1298-1309.

Moss, B., & Feakin, S. D. (1985). Proceedings of an International Workshop on Cytogenetics of *Arachis.*

Moss, J., Singh, A., Nigam, S., Hildebrand, G., Govinden, N., Ismael, F., Subrahmanyam, P., & Reddy, L. (1998). Registration of ICGV-SM 86715 peanut germplasm. *Crop Science*, *38*(2), 572-572.

Moss, J. P., Singh, A. K., Reddy, L. J., Nigam, S. N., Subrahmanyam, P., McDonald, D., & Reddy, A. G. S. (1997). Registration of ICGV 87165 peanut germplasm line with multiple resistance. *Crop Science*, *37*(3), cropsci1997.0011183X003700030089x. https://doi.org/https://doi.org/10.2135/cropsci1997.0011183X003700030089x

Mota, A. P. Z., Vidigal, B., Danchin, E. G., Togawa, R. C., Leal-Bertioli, S. C., Bertioli, D. J., Araujo, A. C. G., Brasileiro, A. C. M., & Guimaraes, P. M. (2018). Comparative root transcriptome of wild *Arachis* reveals NBS-LRR genes related to nematode resistance. *BMC Plant Biology*, *18*(1), 1-16.

Mupunga, I., Mngqawa, P., & Katerere, D. R. (2017). Peanuts, aflatoxins and undernutrition in children in Sub-Saharan Africa. *Nutrients*, *9*(12), 1287.

NASS. (2021). *Quick Stats- Groundnut 2021*. USDA- National Agricultural Statistics Service https://quickstats.nass.usda.gov/

Nelson, S., Simpson, C., & Starr, J. (1989). Resistance to Meloidogyne arenaria in *Arachis* spp. germplasm. *Journal of Nematology*, *21*(4S), 654.

Nelson, S., Starr, J., & Simpson, C. (1990). Expression of resistance to *Meloidogyne arenaria* in *Arachis* batizocoi and A. cardenasii. *Journal of Nematology*, *22*(3), 423.

Nigam, S., Hildebrand, G., Bock, K., Ismael, F., Govinden, N., Subrahmanyam, P., & Reddy, L. (1998). Registration of ICGV-SM 85048 peanut germplasm. *Crop Science*, *38*(2), 572-573.

Nigam, S. N., Dwivedi, S. L., & Gibbons, R. W. (1991). Groundnut breeding: constraints, achievements, and future possibilities. *Plant Breeding Abstracts*, *61*, 1127-1136.

Pande, S., & Rao, J. N. (2001). Resistance of wild *Arachis* species to late leaf spot and rust in greenhouse trials. *Plant Disease*, *85*(8), 851-855.

Power, I. L., Culbreath, A. K., & Tillman, B. L. (2013). Characterization of resistance of peanut to *Puccinia arachidis*. *Plant Health Progress*, *14*(1), 31.

Power, I. L., Tillman, B. L., Brenneman, T. B., Kemerait, R. C., Stevenson, K. L., & Culbreath, A. K. (2019). Field evaluation and components of peanut rust resistance of newly developed breeding lines. *Peanut Science*, *46*(1), 22-36. https://doi.org/10.3146/ps18-4.1

Rajgopal, K., Manival, P., Bandyopadhyay, A., Chandran, K., Ghewande, M., Misra, J., Lalwani, H., Ghetia, N., & Bhalodia, P. (2002). Characterization of released groundnut (*Arachis hypogaea* L.) cultivars. *Comprehensive descriptor*. *National Research Centre for Groundnut, Junagadh, Gujarat*, 1-156.

Rami, J. F., Leal-Bertioli, S., Foncéka, D., Moretzsohn, M., & Bertioli, D. (2013). Groundnut. *Alien Gene Transfer in Crop Plants*, *2*, 253-279. https://doi.org/10.1007/978-1-4614-9572-7_12

Ramos, M. L., Fleming, G., Chu, Y., Akiyama, Y., Gallo, M., & Ozias-Akins, P. (2006). Chromosomal and phylogenetic context for conglutin genes in *Arachis* based on genomic sequence. *Molecular Genetics and Genomics*, *275*(6), 578-592.

Reddy, L., Nigam, S., Moss, J., Singh, A., Subrahmanyam, P., McDonald, D., & Reddy, A. (1996). Registration of ICGV 86699 peanut germplasm line with multiple disease and insect resistance. *Crop Science*, *36*(3), 821-821.

Ribaut, J.-M., & Hoisington, D. (1998). Marker-assisted selection: new tools and strategies. *Trends in Plant Science*, *3*(6), 236-239.

Robledo, G., Lavia, G., & Seijo, G. (2009). Species relations among wild *Arachis* species with the A genome as revealed by FISH mapping of rDNA loci and heterochromatin detection. *Theoretical and applied genetics*, *118*(7), 1295-1307.

Robledo, G., & Seijo, G. (2010). Species relationships among the wild B genome of *Arachis* species (section *Arachis)* based on FISH mapping of rDNA loci and heterochromatin detection: a new proposal for genome arrangement. *Theoretical and applied genetics*, *121*(6), 1033-1046.

Seijo, G. J., Atahuachi, M., Simpson, C. E., & Krapovickas, A. (2021). *Arachis inflata. Bonplandia*, *30*(2), 169-174.

Seijo, J. G., Lavia, G. I., Fernández, A., Krapovickas, A., Ducasse, D., & Moscone, E. A. (2004). Physical mapping of the 5S and 18S-25S rRNA genes by FISH as evidence that *Arachis duranensis* and *A. ipaënsis* are the wild diploid progenitors of *A. hypogaea* (Leguminosae). *American Journal of Botany*, *91*(9), 1294-1303. https://doi.org/10.3732/ajb.91.9.1294

Settaluri, V., Kandala, V., Puppala, N., & S, J. (2012). Peanuts and Their Nutritional Aspects—A Review. *Food and Nutrition Sciences*, *03*, 1644-1650.

https://doi.org/10.4236/fns.2012.312215

Sharma, H., Pampapathy, G., Dwivedi, S., & Reddy, L. (2003). Mechanisms and diversity of resistance to insect pests in wild relatives of groundnut. *Journal of economic entomology*, *96*(6), 1886-1897.

Shirasawa, K., Bertioli, D. J., Varshney, R. K., Moretzsohn, M. C., Leal-Bertioli, S. C., Thudi, M., Pandey, M. K., Rami, J. F., Foncéka, D., Gowda, M. V., Qin, H., Guo, B., Hong, Y., Liang, X., Hirakawa, H., Tabata, S., & Isobe, S. (2013). Integrated consensus map of cultivated peanut and wild relatives reveals structures of the A and B genomes of *Arachis* and divergence of the legume genomes. *DNA Res*, *20*(2), 173-184. https://doi.org/10.1093/dnares/dss042

Shokes, F., & Melouk, H. (1995). Plant health management in peanut production. *Peanut Health Management. Amer. Phytopath. Soc., St Paul, MN*, 1-6.

Sill, W. H. (1982). *Plant protection; an integrated interdisciplinary approach*. Ames, Iowa: Iowa State University Press.

Simpson, C. (2001a). Use of wild *Arachis* species/introgression of genes into *A. hypogaea* L. *Peanut Science*, *28*(2), 114-116.

Simpson, C., Nelson, S., Starr, J., Woodard, K., & Smith, O. (1993). Registration of TxAG-6 and TxAG-7 peanut germplasm lines. *Crop Science*, *33*(6), 1418-1418.

Simpson, C., & Starr, J. (2001b). Registration of ‘COAN' Peanut. *Crop Science*, *41*(3), 918-918.

Simpson, C., Starr, J., Church, G., Burow, M., & Paterson, A. (2003). Registration of 'NemaTAM' peanut. (Registrations Of Cultivars). *Crop Science*, *43*(4), 1561-1562.

Simpson, C. E. (1991). Pathways for introgression of pest resistance into *Arachis hypogaea* L. *Peanut Science*, *18*(1), 22-26.

Singh, A. (1986). Utilization of wild relatives in the genetic improvement of *Arachis hypogaea* L. *Theoretical and applied genetics*, *72*(4), 433-439.

Singh, A., Dwivedi, S., Pande, S., Moss, J., Nigam, S., & Sastri, D. (2003). Registration of rust and late leaf spot resistant peanut germplasm lines. *Crop Science*, *43*(1), 440-441.

Singh, A K and Mehan, V K and Nigam, S N (1997) *Sources of resistance to groundnut fungal and bacterial diseases: an update and appraisal*. Technical Report. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. Singh, A., Subrahmanyam, P., & Gurtu, S. (1996). Variation in a wild groundnut species, *Arachis duranensis* Krapov. & WC Gregory. *Genetic Resources and Crop Evolution*, *43*(2), 135-142.

Singh, B., & Singh, U. (1991). Peanut as a source of protein for human foods. *Plant Foods Hum Nutr*, *41*(2), 165-177. https://doi.org/10.1007/bf02194085

Smith, B. W. (1950). *Arachis hypogaea*. Aerial flower and subterranean fruit. *American Journal of Botany*, *37*(10), 802-815. https://doi.org/https://doi.org/10.1002/j.1537-2197.1950.tb11073.x

Smith, D. H., & Littrell, R. H. (1980). Management of peanut foliar diseases with fungicides. *Plant Disease*, *64*, 356-361.

Stalker, H. (1984). Utilizing *Arachis cardenasii* as a source of *Cercospora* leafspot resistance for peanut improvement. *Euphytica*, *33*(2), 529-538.

Stalker, H., & Moss, J. (1987). Speciation, cytogenetics, and utilization of *Arachis* species. In *Advances in Agronomy* (Vol. 41, pp. 1-40). Elsevier.

Stalker, H., & Wynne, J. (1982). Cytology and leafspot resistance in *Arachis hypogaea* x wild species hybrids. *Euphytica*, *31*(3), 885-893.

Stalker, H. T. (2017). Utilizing wild species forp improvement. *Crop Science*, *57*(3), 1102-1120. https://doi.org/https://doi.org/10.2135/cropsci2016.09.0824

Stalker, H. T., Wynne, J. C., & Company, M. (1979). Variation in progenies of an *Arachis hypogaea* x diploid wild species hybrid. *Euphytica*, *28*(3), 675-684. https://doi.org/10.1007/BF00038934

Subrahmanyam, P., Gibbons, R., Nigam, S., & Rao, V. (1980). Screening methods and further sources of resistance to peanut rust. *Peanut Science*, *7*(1), 10-12.

Subrahmanyam, P., Hammons, R., Nigam, S., McDonald, D., Gibbons, R., Fan, M.-Y., & Yeh, W.-L. (1983). International cooperative screening for resistance of peanut to rust and late leaf spot. *Plant Disease*, *67*(10), 1108-1111.

Subrahmanyam, P., Kokalis-Burelle, N., Porter, D., Rodriguez-Kabana, R., & Smith, D. (1997). *Compendium of peanut diseases* (Vol. 2). American Phytopathological Society.

Subrahmanyam, P., McDonald, D., Gibbons, R., & Rao, P. S. (1983). Components of resistance to *Puccinia arachidis* in peanuts. *Phytopathology*, *73*(2), 253-256.

Subrahmanyam, P, McDonald, D, Waliyar, F, Reddy, L J, Nigam, S N, Gibbons, R W, Rao, V R, Singh, A K, Pande, S, Reddy, P M and Rao, P V S (1995) *Screening methods and sources of resistance to rust and late leaf spot of groundnut. Information Bulletin no. 47.* Technical Report. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. Subrahmanyam, P., Moss, J., McDonald, D., Rao, P. S., & Rao, V. (1985a). Resistance to leaf spot caused by *Cercosporidium personatum* in wild *Arachis* species. *Plant Disease*, *69*(11), 951-954.

Subrahmanyam, P., Rao, V. R., McDonald, D., Moss, J. P., & Gibbons, R. W. (1989). Origins of resistances to rust and late leaf spot in peanut (*Arachis hypogaea*, Fabaceae). *Economic Botany*, *43*(4), 444-455. http://www.jstor.org/stable/4255188

Subrahmanyam, P., Reddy, L., Gibbons, R., & McDonald, D. (1985b). Peanut rust: a major threat to peanut production in the semiarid tropics. *Plant Disease*, *69*(9), 813-819.

Subramanyam, P., & McDonald, D. (1984). Groundnut Rust Disease: Epidemiology and Control. ICRISAT (International Crops Research Institute for Semi-Arid Tropics), 1987. Groundnut Rust Disease. Proceedings of a Discussion Group Meeting,

Sujay, V., Gowda, M., Pandey, M., Bhat, R., Khedikar, Y., Nadaf, H., Gautami, B., Sarvamangala, C., Lingaraju, S., & Radhakrishan, T. (2012). Quantitative trait locus analysis and construction of consensus genetic map for foliar disease resistance based on two recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). *Molecular breeding*, *30*(2), 773-788.

Sunkad, G., & Kulkarni, S. (2007). Studies on perpetuation and carry over of groundnut rust (Puccinia arachidis Speg.) in northern Karnataka. *Karnataka Journal of Agricultural Sciences*, *20*(2), 297-300.

Thurston, H. D. (2019). *Sustainable practices for plant disease management in traditional farming systems*. CRC Press.

UGA Extension, D. o. A. a. A. E. (2022). *Budgets*. https://agecon.uga.edu/extension/budgets.html

Upadhyaya, H., Sharma, S., Dwivedi, S., & Kole, C. (2011). *Arachis. In: Wild crop relatives: genomic and breeding resources: millets and grasses*. Springer.

Upadhyaya, H. D., Dwivedi, S. L., Vadez, V., Hamidou, F., Singh, S., Varshney, R. K., & Liao, B. (2014). Multiple resistant and nutritionally dense germplasm Iidentified from mini core collection in peanut. *Crop Science*, *54*(2), 679-693. https://doi.org/https://doi.org/10.2135/cropsci2013.07.0493

Valls, J., & Simpson, C. (2010 New species of Arachis (Leguminosae) from Brazil, Paraguay and Bolivia Nuevas especies de *Arachis* (Leguminosae) de Brasil, Paraguay y Bolivia. *Bonplandia*, *14*. https://doi.org/10.30972/bon.141-21387

Vindhiya Varman, P. (1999). A foliar disease resistant line developed through interspecific hybridization in groundnut (*Arachis hypogaea*). *Indian journal of agricultural science*, *69*(1), 67-68.

Wynne, J., Beute, M., & Nigam, S. (1991). Breeding for disease resistance in peanut (*Arachis hypogaea* L.). *Annual Review of phytopathology*, *29*(1), 279-303.

Xu, Y., & Crouch, J. H. (2008). Marker-assisted selection in plant breeding: From publications to practice. *Crop Science*, *48*(2), 391-407.

Yin, D., Ji, C., Song, Q., Zhang, W., Zhang, X., Zhao, K., Chen, C. Y., Wang, C., He, G., & Liang, Z. (2020). Comparison of *Arachis monticola* with diploid and cultivated tetraploid genomes reveals asymmetric subgenome evolution and improvement of peanut. *Advanced Science*, *7*(4), 1901672

CHAPTER 2

IDENTIFYING A SOURCE OF WILD-DERIVED RESISTANCE TO PEANUT RUST (*PUCCINIA ARACHIDIS*) IN A BC_3 POPULATION

INTRODUCTION

Cultivated peanut— *Arachis hypogaea* L.— is an allotetraploid species with low genetic diversity due to its unique polyploid origin and subsequent domestication (Bertioli et al., 2019; Halward et al., 1992; Husted, 1936; Leal-Bertioli et al., 2021). Cultivated peanut is sexually isolated from its diploid wild peanut relatives, leading to an absence of gene-flow from wild peanut species which also contributes to the susceptibility of cultivated peanut to various pathogens and pests (Holbrook & Stalker, 2003; Husted, 1936; Kochert et al., 1996; Leal-Bertioli et al., 2021). Some sources of resistance have been identified in cultivated peanut; however, no complete resistance has been found in the cultivated germplasm (Bertioli et al., 2019; Hammons, 1977; Husted, 1936; Kochert et al., 1996; Mondal & Badigannavar, 2015; Moretzsohn et al., 2013; Subrahmanyam et al., 1983a). Due to this, it is important to find and utilize wild peanut species, which contain many sources of resistance to pests and diseases and are adapted to a wide variety of environments (Bertioli et al., 2011; Leal-Bertioli et al., 2018; Stalker, 2017).

Peanut rust, caused by the fungus *Puccinia arachidis*, is a fungal disease that affects crops worldwide. *Puccinia arachidis* can lead to early pod senescence, reduced seed size, and a low seed oil content which can reduce yield by up to 50% (Mondal et al., 2014; Subrahmanyam et al., 1997; Subrahmanyam et al., 1985b). Yield can be reduced up to 70% when *P. arachidis* and the fungal

diseases early leaf spot (*Passalora arachidicola*) and late leaf spot (*Nothopassalora personata*) occur simultaneously in the field (Subrahmanyam et al., 1985b). Several methods of cultural and chemical control can be used to manage *P. arachidis* in the field (Culbreath et al., 2002; Francis et al., 2020; Levinson et al., 2021; Subrahmanyam et al., 1997). However, fungicides increase the cost considerably, and, in many developing countries where peanut is an important protein source, peanut farmers rarely have the resources to afford the chemical controls in the dosages necessary to be effective, which can lead to the development of pathogen resistance (Khedikar et al., 2010). Utilizing pathogen-resistant peanut cultivars can be a more economically viable, technically feasible, environmentally friendly, and socially acceptable strategy for managing pathogens in the field (Daudi et al., 2021; Knauft & Ozias-Akins, 1995).

Cultivated peanut (*A. hypogaea*) is tetraploid (2n = 4x =40) while the related wild peanut species (*Arachis sp*.) are diploid (2n = 2x =20) (Bertioli et al., 2016; Bertioli et al., 2019; Bertioli et al., 2011; Krapovickas & Gregory, 1994). To overcome the issue of cultivated and wild peanut's sexual incompatibility, researchers can create wild-derived synthetic allotetraploids that can be bred with cultivated peanut (Bertioli et al., 2021b; Chu et al., 2021; Leal-Bertioli et al., 2021; Leal-Bertioli et al., 2015b; Nguepjop et al., 2016). Multiple synthetic allotetraploids with resistance to rust have been created for this purpose (Bertioli et al., 2021b; Fávero & Valls, 1998; Fávero et al., 2015; Fávero et al., 2006; Gao et al., 2021; Levinson et al., 2021; Singh, 1986; Singh et al., 2003). Various rust-resistant breeding lines created by crossing synthetic allotetraploids with *A. hypogaea* have also been released for use (Gowda et al., 2002; Moss et al., 1998; Moss et al., 1997; Nigam et al., 1998; Reddy et al., 1996; Singh et al., 2003; Varman, 1999). Research is ongoing to determine and utilize more sources of resistance from wild peanut species.

Previously, a marker-assisted backcrossing scheme (MABS) was utilized to introgress genes responsible for *Meloidogyne arenaria* (root-knot nematode or "RKN") resistance from wild diploid species *Arachis batizocoi* ("Bat") and *A. stenosperma* ("Sten") into cultivated peanut (Ballén-Taborda et al., 2019; Ballén-Taborda et al., 2021b; Ballén-Taborda, 2019). *A. stenosperma* was used as the source of the A-genome (Moretzsohn et al., 2013; Tallury et al., 2005) and *A. batizocoi* was the source of the B/K genome (Moretzsohn et al., 2013; Robledo et al., 2009). The wild *Arachis* relatives were obtained from the USDA-GRIN system (https://www.ars-grin.gov/) and a synthetic allotetraploid (named BatSten1) (Bertioli et al., 2021b) was created to be crossed with cultivated tetraploid peanut. *A. stenosperma* PI666100 (original collection voucher V10309) and *A. batizocoi* PI298639 (original collection voucher K9484) were crossed, and the diploid hybrid was treated with colchicine to induce chromosome doubling. BatSten1 was crossed with *A. hypogaea* cv. Runner-886 and select $F_{2:3}$ lines with RKN resistant segments previously identified on the A02 and A09 chromosomes (Leal-Bertioli et al., 2015a) were used as the donor parents (Figure 2.1) (Ballén-Taborda et al., 2019).

Three cycles of backcrossing were completed in three consecutive years (2016, 2017 and 2018) in two locations (Athens and Tifton, GA) using three breeding elite lines (*A. hypogaea* 5-646-10, 13-1014 and TifGP-2) from Tifton, GA as the recurrent parents (Ballén-Taborda et al., 2021a; Holbrook, 2016; Holbrook et al., 2012). TifGP-2 is a breeding line with good yield and grade and normal oleic content (Holbrook et al., 2012) and 5-646-10 (a [Florida-07 x Tifguard]-derived breeding line) and 13-1014 (a [C1805-617-1 (Florida-07 x Tifguard) x Georgia-06G]-derived line) both have good yield and grade and high oleic/linoleic oil ratio (Holbrook, 2016). Twenty-two SNP markers were used for selection of lines with the desired chromosome segments at each cycle (Ballén-Taborda et al., 2021b; Ballén-Taborda et al., 2019). This resulted in a BC_3F_2

population of peanut with confirmed resistance to RKN from the wild peanut genome and the phenotype of cultivated peanut *A. hypogaea* (Ballén-Taborda et al., 2021b).

Along with RKN resistance, *A. batizocoi* and *A. stenosperma* have been found to be resistant to peanut rust and other peanut diseases late leaf spot, early leaf spot, and tomato-spotted wilt virus (Ballén-Taborda et al., 2019; Ballén-Taborda et al., 2021b; Bera et al., 2018; Fávero et al., 2009; Leal-Bertioli et al., 2015a; Stalker, 2017; Subrahmanyam et al., 1983b; Subrahmanyam et al., 1985a). Therefore, it was expected that cryptic alleles that confer resistance to other pathogens were also selected into some of the BC_3 lines. In this study, selected families from the BC_3F_3 and BC_3F_4 generations of this MABS population were screened for possible resistance to rust conferred by segments of wild DNA from donor parent BatSten1 not yet characterized (Figure 2.1). The resistant genotypes identified from the screening were then used to identify the segment of wild DNA conferring rust resistance. This research will contribute to future production of peanut lines with resistance to RKN along with a new source of resistance to peanut rust.

MATERIALS AND METHODS

Plant materials

Select families from the MABS BC_3F_2 population created by Ballén-Taborda et al. (2019) were used in this project. As described above, this population was created by crossing the induced allotetraploid BatSten1 with cultivated susceptible *A. hypogaea* lines 13-1014 and 5-646-10, and TifGP-2 in order to introgress RKN resistance (Figure 2.1). Previously, phenotypic variation was found in the BC_3F_2 population by evaluating for the following variables: seed size (weight (g), length and width (mm)), leaf spot incidence (presence or absence), architecture (scored from 1-5, with 1 for erect and 4 for prostrate growth habit, and 5 for dwarf plants), fertility (number of pegs),

and flower color (cultivated orange phenotype or wild yellow phenotype) (Table 2.1) (Ballén-Taborda et al., 2021a). The BC_3F_2 population had an average seed weight of 0.72 g, an average seed length of 16.94 mm, and an average seed width of 10.11 mm (Ballén-Taborda et al., 2021a). Seeds from the selected BC_3F_2 families (BC_3F_3 genotypes) were grown under greenhouse conditions, allowed to self-pollinate, and then the progeny (BC_3F_4 genotypes) were also grown in the greenhouse (Figure 2.1).

Three characterized families from the MABS population were chosen to be screened for rust resistance. These families were selected using the percentage of Batsten1 DNA still present on the genome and the presence of certain segments of wild DNA of interest based on previous research. None of these segments of interest had previously been connected to rust resistance. Two families (BC_3F_1_Seed8_S15 and BC_3F_1_Seed7_S3) were selected with a low percentage of BatSten1 (wild) DNA (6.7% and 9.6% respectively), and one family (BC_3F_1_Seed11), with a higher percentage of BatSten1 (15.5%), based on the results from genotyping using the Thermofisher "Axiom_Arachis v 2.0" 48K SNP array (Ballén-Taborda et al., 2021a; Korani et al., 2019). These three families will herein be referred to as Seed8, Seed7 and Seed 11, respectively. Seed8 and Seed7 were bred in Tifton and Athens, GA using recurrent female parents *A. hypogaea* Runner 886 and 5-646-10. Seed8 had segments of wild DNA on LGs A04 and A06, and both Seed7 and Seed8 had a segment on LG B02 (Figure 2.2). These segments of wild DNA on LG A04, A06, and B02 have previously been linked to late leaf spot (LLS) resistance but this has not been validated (Ballén-Taborda et al., 2021a; Bertioli et al., 2022). In 2020, plants from the BC_3F_2 population were planted in the field in Midville, GA and two BC_3F_3 seeds from one Seed8 plant that performed well in the field (showed *A. hypogaea* morphology and little disease) were also screened for rust resistance. Seed11 was bred in Tifton, GA using recurrent female parents *A.*

hypogaea Runner 886, 13-1014, and 5-646-10. Along with this, Seed11 had a wild segment on LG A02, which has been found to be related to RKN resistance (Ballén-Taborda et al., 2019; Ballén-Taborda et al., 2021b), as well as wild segments on LG A06 and B02 that might be related to LLS resistance (Figure 2.2) (Ballén-Taborda et al., 2021a; Bertioli et al., 2022).

Rust resistance evaluation

Rust isolates were gathered from infected peanut plants in the field in Tifton, Georgia. Rust urediniospores were scraped off the leaf surface using a scalpel and placed in 2 mL microcentrifuge tubes. Because rust is an obligate biotroph, the rust culture was maintained on leaves from susceptible cultivars *A. hypogaea* cv. Runner-886, *A. hypogaea* Tifrunner, *A. hypogaea* 5-646-10, and *A. hypogaea* 13-1014. To maintain the necessary quantity and virulence of the rust, the spores were scraped off and used to inoculate new leaves every 14-28 days.

Eighty-two BC_3F_3 and 94 BC_3F_4 genotypes were evaluated for resistance to peanut rust (176 genotypes total). The control genotypes used were susceptible cultivated lines (*A. hypogaea* cv. Runner-886, *A. hypogaea* Tifrunner, *A. hypogaea* 5-646-10, and *A. hypogaea* 13-1014) and the resistant BatSten1 (Bertioli et al., 2021b). Evaluation was done using detached leaf assays essentially as previously described (Moraes & Salgado, 1982). Three to six of the first fully expanded lateral leaves were removed from 55 to 251-day-old plants grown under greenhouse conditions. The selection of the first expanded leaf helped to counter the differences in age between some of the plants. Most bioassays included plants that had been grown from seed, but cuttings from six BC_3F_3 plants were grown as well in order to run additional detached-leaf bioassays on those genotypes. Each leaf was rinsed in water, rubbed gently, and dried with a paper towel. The petiole of each leaf was submerged in water, cut diagonally with scissors, wrapped in wet cotton wool, and placed in an individual petri dish. Each petri dish contained a cotton pad, filter paper,

and a glass slide to keep the chamber moist but allow the leaf to rest above the water. Each leaf was inoculated on the abaxial surface using a soft brush with a suspension of $5x10^5$ spores/ml on 0.005% Tween 20. Petri dishes were maintained in a growth chamber at 24°C with a 12 hour light: dark photoperiod and kept moist using sterilized water sprayed onto the cotton every one or two days. A stereomicroscope assisted in counting pustules and evaluating sporulation. The leaves were then scanned, and leaf area was calculated using the software APS Assess 2.0.

The leaves were observed every one to two days for signs of rust pustules. Pustules typically began to emerge after approximately 14 days and the leaves were observed every day following that. The following components of resistance were recorded: incubation period (the number of days after inoculation when pustules were first observed) (IP), total number of pustules/leaf area (cm^2) (TPA), the number of sporulated pustules/leaf area (cm^2) (SPA), the susceptibility index (I), and the susceptibility index/leaf area (cm^2) (IA). The susceptibility index (I), developed by Leal-Bertioli et al. (2015a), is a measure of the number and size of pustules, using a modified scale developed by Savary et al (1989). The formula $I = \int(s * n)/LA$ was used, where s= pustule size (1 = microscopic unsporulated pustule, 2-5 = sporulated pustules from 0.5 to 3 mm, 6 = sporulated pustule more than 3mm in size) (Figure 2.3), n = number of pustules of a particular size, and LA = leaf area (mm^2). The IP was the only score recorded while the leaves were still in the growth chamber. The petri dishes containing individual leaves were placed in a -4°C refrigerator to inhibit further fungal growth and the number and size of pustules were recorded using a stereomicroscope within seven days of the end of the bioassay. To account for the variation between each bioassay caused by factors such as the age of the plants and the virulence of the rust inoculation, the data was then standardized using z-scores (IAZ) (Andrade, 2021). A formula was then used to transform the z-scores to a scale of 0-1 (Standardized IA (IAS) = $(X-X_{min})/ (X_{max}-$

X_{min})). The same method was used to standardize the IP, TPA, and SPA, and the standardized data are called IPS, TPAS, and SPAS, respectively.

Whole genome SNP genotyping, analysis, and data filtering

Genomic DNA from the 149 BC_3F3 and BC_3F_4 genotypes from the Seed7, Seed8, and Seed11 families that had been screened for rust, along with controls (*A. hypogaea* cv. Runner-886, *A. hypogaea* Tifrunner, *A. hypogaea* 5-646-10, *A. hypogaea* 13-1014, and BatSten1), was extracted from leaves using the DNeasy Plant Mini Kit (QIAGEN) according to manufacturer's instructions. DNA concentration was determined using Qubit Fluorometric Quantification and samples were then submitted for genotyping with the Thermofisher "Axiom_Arachis v 2.0" 48K SNP array (Korani et al., 2019). The genotypic data were extracted, processed, and analyzed using Axiom Analysis Suite 2.0 software and then filtered using Unix scripts (Supplemental Script 2.S1) developed by Ballén-Taborda et al. (Ballén-Taborda et al., 2019). The polymorphic SNP markers were filtered and assigned to the A-subgenome (*A. stenosperma*-specific markers) or the B/K-subgenome (*A. batizocoi*-specific markers). The positions of the A-genome marker homologues in the *A. duranensis* pseudomolecule determined their physical location on the chromosome, and the physical location of the B/K-genome markers were determined based on the *A. ipaënsis* pseudomolecules (https://www.peanutbase.org/) (Bertioli et al., 2016). The SNP markers were further divided into 20 linkage groups (LG): 10 on the A-subgenome (named A01 – A10) and 10 on the B/K-subgenome (named B01-B10). The genetic distances between the markers (in cM) were created using previous data from Ballén-Taborda, et al using Joinmap v 4.0 (Ballén-Taborda et al., 2019; Van Ooijen, 2006). Further, each SNP marker showed one of four results after filtering: no call, homozygous for the cultivated (*A. hypogaea*) allele (A1A1 or BB) (-/-),

heterozygous (A1A2 or BK) (-/+), or homozygous for the wild (*A. stenosperma* or *A. batizocoi*) allele (A2A2 or KK) (+/+).

SNP primer design and Kompetetive Allele Specific PCR (KASP) reaction

In this project, Kompetetive Allele Specific PCR (KASP) was used to validate the results from the Axiom_Arachis v 2.0 array by identifying wild segments on LG B02 using SNP-specific primers (Semagn et al., 2014). For this purpose, four primer sets consisting of three PCR primers each was developed for the segment of interest, on LG B02. The primers were designed using the web-based program BatchPrimer3 (https://wheat.pw.usda.gov/demos/BatchPrimer3/) using the "Allele-specific primers and allele flanking primers" option (You et al., 2008). The parameters were set as described by Ballén-Taborda et al. (2019):60−120 bp in size, GC content of 30-80% and Tm between 58 and 60°. Four KASP primer assays were designed for the segment at LG B02. The KASP primer assay mix comprised of 12 µl of each allele-specific primer, 30 µl of flanking marker and 46 µl of H_2O. The KASP plate was filled with 4 µl of primer assay and 1 µl of genomic DNA from individual samples in each well. Plates were read using the LightCycler® 480 System using endpoint genotyping (Hoffmann et al., 2008). 103 individuals from the Seed8 family were genotyped using KASP.

Statistical analysis

In order to analyze the results from each individual bioassay, the Shapiro-Wilk test was used to determine the normality of the phenotypic data (Shapiro & Wilk, 1972). Within each individual bioassay, a non-parametric one-way Kruskal-Wallis test was then used to assess the differences in the phenotypic data at a 5% level of significance ($P < 0.05$) (Kruskal & Wallis, 1952). The Fisher's Least Significant Differences (LSD) post hoc test was used for genotype grouping within each individual bioassay (Fisher, 1936). Both the Kruskal-Wallis and Fisher's

LSD tests were performed using the R-package "agricolae" (Mendiburu, 2021). To compare the bioassays to each other, the Skillings-Mack test was used to assess the differences in the phenotypic data at a 5% level of significance (Skillings & Mack, 1981). The R-package PMCMRplus was used for the Skillings-Mack test (Pohlert, 2022).

In order to validate that the wild segment on LG B02 is significantly correlated to rust resistance, Pearson's Correlation Coefficient (Pearson, 1895) was used with the R-package "ggcorrplot" (Kassambara, 2019). The Pearson's Correlation Coefficient was calculated using all individuals from Seed7, Seed8, and Seed11 families from all nine detached-leaf bioassays. To compare genotypes with the presence or absence of the segment on LG B02 over all nine bioassays, multiple comparisons were performed using the Kruskal Wallis method at a 5% level of significance (Kruskal & Wallis, 1952) and to test pairwise comparisons between genotypes the Wilcoxon test 5% level of significance (Woolson, 2007) was used. Finally, the same analysis was performed for the Seed8 genotypes over all nine bioassays specifically to analyze the difference in the scores from individuals with the wild segment at LG B02, heterozygous at that segment, or without the wild segment at LG B02. Both the the Kruskal Wallis and Wilcoxon tests were performed using the function stat_compare_means of "ggpubr" R package version 0.4.0 (Kassambara, 2019).

RESULTS

Selection of a BC_3 family that segregates for rust resistance

In order to identify a family that segregated for rust resistance, three families from the MABS BC_3F_2 population derived from *A. batizocoi* and *A. stenosperma* (Seed7, Seed8, and

Seed11) were screened in two detached-leaf bioassays (Assay 1 and Assay 2). Seventy BC_3F_3 genotypes were assayed: 24 genotypes each from the Seed7 and Seed11 families, and 22 genotypes from the Seed8 family. Each genotype was screened at least one time. Four susceptible cultivars (*A. hypogaea* cv. Runner-886, *A. hypogaea* Tifrunner, *A. hypogaea* 5-646-10, and *A. hypogaea* 13-1014) and one resistant allotetraploid (BatSten1) were used as controls. Although there was some variability between bioassays, BatSten1 was consistently asymptomatic and susceptible controls were consistently symptomatic

The overall average Index/Area (cm^2) (IA) for all genotypes including controls, was 3.30 and 1.07 for Assay 1 and 2, respectively. The BC_3F_3 families (Seed7, Seed8, and Seed11) screened showed variable resistance to rust (Tables 2.2-2.3, Figure 2.4). Seed7 was highly resistant to rust. In Assay 1, out of the 12 genotypes screened, 11 were asymptomatic (91.67%), and in Assay 2, nine out of 12 were asymptomatic and the symptomatic leaves had few pustules. On the other hand, Seed11 was highly susceptible to rust. In Assay 1, all 12 genotypes were symptomatic and in Assay 2, 11 out of 12 were symptomatic with many large pustules. Seed8 genotypes segregated for rust resistance. In Assay 1, six out of the 11 genotypes screened were asymptomatic, and in Assay 2, eight out of 11 were asymptomatic. Within both Assay 1 and 2, the IP, TPA, SPA, and IA values were non-normal and significantly different at $P<0.05$ (Table 2.4). Seed8 was then chosen for further analysis.

Screening Seed8 genotypes for rust resistance

The Seed8 family segregated for rust resistance therefore it was chosen for further rust screening. BC_3F_3 and BC_3F_4 individuals from Seed8 were tested for rust resistance to correlate phenotyping with genotyping results to identify the wild segments that confer resistance. Ninety-

four BC_3F_4 Seed8 genotypes and 20 BC_3F_3 Seed8 genotypes were screened for rust resistance over seven bioassays (Assays 3-9). Each genotype was screened at least two times. In all seven bioassays, BatSten1 was consistently asymptomatic, and the susceptible controls were mostly symptomatic. In Assays 3-9, the BC_3F_3 and BC_3F_4 Seed8 genotypes showed varying degrees of resistance to rust; out of the 114 Seed8 individuals screened, 40 were consistently asymptomatic over each bioassay (35.09%). Within each individual bioassay, the IP, TPA, SPA, and IA values were non-normal and significantly different at $P<0.05$ (Table 2.4). Due to the variability between the bioassays, each score was standardized so that results from different bioassays could be compared (Supplemental Tables 2.S1- 2.S3).

Results of all nine bioassays (1 and 2 using all families, and 3-9 only with Seed8) were compiled for final analyses. A total of 128 Seed8 genotypes (34 BC_3F_3 and 94 BC_3F_4) were screened over nine bioassays from 2020-2021 (called Assays 1-9). The average IAS for the susceptible controls over all nine bioassays was 0.04 for *A. hypogaea* 13-1014, 0.02 for *A. hypogaea* 5-646-10 (data lost for Assay 5), 0.07 for *A. hypogaea* Runner 886, and 0.10 for *A. hypogaea* Tifrunner. Forty-nine of the 128 Seed8 genotypes (38.28%) were consistently asymptomatic over all nine bioassays. The average IAS for all 128 Seed8 genotypes screened (not including controls) was 0.007. Within each of the nine bioassays, the frequency distributions for IAS, standardized total pustules/area (TPAS), standardized sporulating pustules/area (SPAS), and standardized incubation period (IPS) for the Seed8 genotypes (plus controls) were non-normal, with IAS, TPAS, and SPAS skewing towards resistance (example in Figure 2.5). The data variances among the genotypes (including controls) for the average IAS, TPAS, SPAS, and IPS over Assays 1-9 were also significantly different at $P<0.05$ (Table 2.5).

Whole genome SNP genotyping and identification of wild segment correlated with rust resistance

Using the Thermofisher "Axiom_Arachis" v 2.0 array (Korani et al., 2019), genomic DNA from 148 BC_3 genotypes and five controls were genotyped in two batches (herein called Affy 1 and Affy 2) and the results were then combined. In Affy 1, 67 BC_3F_3 individuals were genotyped: 22 Seed8, 23 Seed7, and 22 Seed11 individuals. After filtering, a total of 1,046 polymorphic SNP markers were identified. 618 of those SNPs were assigned to the A-subgenome (*A. stenosperma*-specific markers) and 430 to the B/K-subgenome (*A. batizocoi*-specific markers). In Affy 2, 81 individuals were genotyped: 12 BC_3F_3 Seed8 genotypes and 69 BC_3F_4 Seed8 genotypes (including two progenies from a Seed8 individual grown in the field in Midville 2020). After filtering, a total of 1,030 polymorphic SNP markers were identified. 598 of those SNPs were assigned to the A-subgenome and 432 to the B/K-subgenome. In both Affy 1 and 2, the SNPs were organized into 20 linkage groups (LGs), and the LGs ranged in size from approximately 7.84 x 10^7 base pairs (LG A07) to approximately 1.45 x 10^8 bp (LG B09). The map of filtered A- and B/K-subgenome SNP markers covered a total physical distance of about 2.46 x 10^9 bp. The distance between adjacent markers ranged from 36 bp (LG A01) to 6.27 x 10^7 bp (LG A08). The number of SNP markers in each linkage group varied from 24 (LG B04) to 162 (LG A01).

For data visualization, a color map was created from the genotyping results using Microsoft Excel. Different families had very distinct profiles (Figure 2.6A). The color map revealed that a segment of wild alleles on LG B02 was present in the resistant individuals, and absent in the susceptible ones, and therefore likely to be related to rust resistance (Figure 2.6B). Further analyses were done to confirm this. The segment consisted of six SNP markers spanning about 2.66 x 10^6 bp. Four of the markers were present in both Affy 1 and 2, while the other two were present only

in Affy 2 (Table 2.6). The full genotyping data of the presence/absence of the six SNP markers on the Seed 8 individuals is presented on Tables 2.7-2.9.

Specific SNP genotyping

Four primer sets were successfully designed to target one of the six SNP markers on the wild-derived segment on LG B02, associated with rust resistance (Table 2.10). Due to time constraints, only one of the four primer sets (the primer set targeting marker "B02_2363097_Bat") was used for KASP in this project (Figure 2.7). The KASP results confirmed the presence or absence of wild DNA segments on LG B02 as shown by Affy 1 and Affy 2, in single (heterozygosis) or double dosage (homozygosis) (Table 2.11). One hundred and three BC_3 Seed8 individuals (previously tested for rust resistance) were genotyped using KASP. Out of these, 79 individuals had already been genotyped using the Axiom_Arachis. A total of 126 BC_3 Seed8 individuals were genotyped using one or both of the SNP genotyping methods (Axiom_Arachis array and KASP) and results were consistent.

Confirmation of segment B02 as associated with rust resistance

Out of the 126 BC_3 Seed8 individuals genotyped, 100 (78.74%) presented the LG B02 segment, 42 being heterozygous (42%), and 58 homozygous (58%). The average standardized Index/Area (IAS) of BC_3 Seed8 individuals without the B02 segment was 0.031, whereas average of IAS of individuals with B02 in heterozygosis was 0.005 and in homozygosis was 0.001. Additionally, 35 out of 58 homozygotes were asymptomatic (60.34%), 12 out of 42 heterozygotes were asymptomatic (28.57%), and only one of the 29 Seed8 genotypes without the wild segment on LG B02 was asymptomatic (3.45%). All Seed8 individuals with B02 segment in homozygosis or heterozygosis had lower susceptibility traits than the individuals without the B02 segment and lower than the cultivated controls (Figure 2.8). Results showed a significant negative Pearson's

correlation between the wild SNPs at LG B02 and IAS, TPAS, and SPAS (Table 2.12). In other words, the wild segment on LG B02, derived from *A. batizocoi*, was correlated with rust resistance.

Seed7 and Seed11 families and LG B02

The Seed7 and Seed11 families were not used to identify the wild segment on LG B02, but they were genotyped alongside Seed8 on Affy1 (Figure 2.6). Of the four symptomatic Seed7 genotypes, only one had the wild segment at LG B02. Out of the 21 asymptomatic Seed7 genotypes, only one did not have the wild segment at LG B02. Twenty-three of the 24 Seed11 genotypes were symptomatic, and none had the wild segment at LG B02. The Pearson's correlation test using all families showed a strong negative correlation between the segment of wild DNA present on LG B02 and the IAS, TPAS, and SPAS, and a strong positive correlation with IPS, at a 0.001% significance (Table 2.12). These results corroborate the correlation between the wild segment on LG B02 and rust resistance.

DISCUSSION

In this study, we aimed to generate the information and tools for the introgression of genomic regions of wild species that confer rust resistance into allotetraploid cultivated peanut. The information being the definition of the segment that control disease resistance, and the tools being specific SNP markers to track the genomic regions of interest in a tetraploid genetic context. Cultivated peanut is very susceptible to rust and the knowledge and tools for introgressing wild alleles that confer resistance are of great importance for breeders and growers in regions where rust is a limiting factor for peanut production. In this project, a segment of wild alleles on linkage group (LG) B02 from the wild peanut species *Arachis batizocoi* has been identified as a new source for future *P. arachidis* resistance breeding programs.

The wild species *A. cardenasii* was recently found to be the source of disease resistance (including resistance to peanut rust) in some cultivars grown around the world (Bertioli et al., 2021a). However, in order to prevent pathogens such as *P. arachidis* from evolving to overcome the resistance provided by these sources, new diversity needs to be introduced into the peanut gene pool. Research has been done to identify and utilize sources of resistance to this pathogen from both cultivated peanut and wild peanut species. Within the last 50 years, only a few rust-resistant lines have been bred and released using only cultivated peanut. On the other hand, within the past few years, wild-derived allotetraploids GA-BatSten1 (aka BatSten1), GA-MagSten1, and ValSten1 have been registered as new sources of disease (including rust) resistance (Bertioli et al., 2021a; Chu et al., 2021; Gao et al., 2021). Here we utilized a population created from the induced allotetraploid BatSten1 as the source of resistance in a population of marker-assisted BC_3 individuals that had originally been developed for root-knot nematode (RKN) resistance.

Nine detached-leaf bioassays were performed from 2020-2021 to screen the three BC_3 families selected (Seed7, Seed8, and Seed11) from a marker-assisted backcrossing scheme (MABS) for resistance to *Puccinia arachidis.* This back-crossing scheme aimed to select for plants that had segments of wild DNA on LGs A02 and A09 that are related to RKN resistance (Ballén-Taborda et al., 2019; Ballén-Taborda et al., 2021b). Along with these segments, other wild introgressions remained in some of the families, including segments that might be related to late leaf spot (LLS) resistance (Bertioli et al., 2022). Both Seed7 and Seed8 had wild segments that might be related to LLS resistance (LGs A04, A06, and B02) and Seed11 had wild segments on LG A06 and LG B02, as well as an RKN-related segment on LG A02. While these segments are not the focus of this project, they might be useful in future breeding programs, so they were used as part of the initial family selection process. The first two bioassays were performed to identify

which of these families might segregate for rust resistance. A segregating family was needed to compare the genomes of the resistant and susceptible individuals against each other, and to use that data to determine a segment of wild DNA related to the resistance. The results for each family from the first two bioassays allowed us to determine that neither the Seed7 nor Seed11 families segregated for rust resistance, and to identify and select the Seed8 family as segregating for rust resistance. Seven more bioassays were then conducted using Seed8 individuals to create a population of resistant and susceptible plants which were then genotyped. The genotyping results were used to identify the segment related to rust resistance.

SNP Genotyping and rust resistance phenotyping led to the identification of a segment of alleles located at the top of LG B02 that was present on almost every resistant BC_3 Seed8 individual and was absent on most of the susceptible Seed8 individuals. Along with this, it was confirmed that most of the resistant individuals in the Seed7 family were homozygous or heterozygous for the LG B02 segment and that none of the individuals in the Seed11 family (susceptible to rust) contain the segment. This validated the wild segment on LG B02 as a source of rust resistance. Along with the primer set that was used in this project, three other sets of primers were designed to target the segment at LG B02 using KASP. These four primer sets will be valuable to researchers using marker-assisted selection in their breeding programs to introgress rust resistance into other peanut lines. Marker-assisted selection allows researchers to identify which plants are most likely to be resistant to the pathogen of interest without the need for extensive field testing. This can speed up the time that it takes to complete each round of breeding as well as the time it takes to identify resistant progeny after each round. Both the Seed7 and the Seed8 families have been confirmed as sources of rust resistance and can be used for breeding programs and the KASP primer sets can be used to identify other families that can be useful in the

same way. The LG B02 segment is a region that contains an abundant number of both TIR and non-TIR NB-LRR-encoding genes (Bertioli et al., 2016), which are likely to be involved in the rust resistance. In a different study, it was shown that the marker linked to a rust resistance QTL from *Arachis magna* K30097 Krapov., W.C. Greg. & C.E. Simpson maps closely to an NB-LRR–encoding gene (Araip.RV63R) (Bertioli et al., 2016; Leal-Bertioli et al., 2015a)

Previously, quantitative-trait loci (QTL) for rust resistance have been identified in the cultivated peanut genome (Khedikar et al., 2010; Sujay et al., 2012). Further, some simple sequence repeat (SSR) markers have been identified that are linked to a rust resistance gene (Mondal et al., 2012). Like the KASP primers sets that were designed to target the specific SNP markers on LG B02 in this project, these SSR markers can be used for marker-assisted selection in order to improve breeding programs (Mace et al., 2006; Mondal & Badigannavar, 2010; Mondal & Badigannavar, 2015). A QTL closely linked to SSR marker IPAHM 103 has been used in marker-assisted selection during a rust resistance breeding program (Khedikar et al. 2010; Upadhyaya et al. 2014). However, it is important to continue to find new sources of resistance to avoid the evolution of the pathogen to overcome the old resistance. Along with this, in current research, SNPs are much more specific and abundant, so the creation of primer sets to target specific SNP markers in this project will be beneficial to future researchers.

The BC_3 individuals that are homozygous or heterozygous for the segment of wild alleles on LG B02 were found to be more resistant to rust on average than the individuals without the segment. However, it was also observed that the susceptible controls (*A. hypogaea* 5-646-10, 13-1014, Tifrunner, and Runner 886) were all much more susceptible to rust than the BC_3 individuals without the segment. This suggests that there are other segments that contribute to rust resistance. The SNP genotyping revealed that along with the segment on LG B02, there were other

introgressions of wild DNA on the genomes of the three families. Discovering the location of this wild segment or segments would be beneficial for future breeding programs as either an alternate source of resistance, or to increase the resistance already being given by the segment on LG B02.

Recently, seeds from the same population of BC_3 individuals that was used in this project were sent to researchers at the Acceso Peanut Corp to be planted in a field in Haiti (Supplemental Table 2.S4). These plants were then rated for various traits, including resistance to *P. arachidis*. The disease pressure of *P. arachidis* is much higher and more predictable in Haitian fields than it is in Georgia. These individuals were genotyped using Axiom_Arachis v 2.0 array in the BC_3F_1 and BC_3F_2 generations, so the presence or absence of the wild segment on LG B02 is known for the parents of the F_3 generation that was rated in the field. The plants were rated for the presence of rust using a scale from 1-9, with one being no disease, and nine being an almost completely dead and defoliated plant (Dorzin, 2022). While this data is still preliminary, the individuals that had the segment on LG B02 in previous generations (93B, 93C, and 226C) appear to be more resistant to rust than the individuals without the segment (14D, 107B, and 214B) and the cultivated varieties (Runner Georgia-06G and Georgia Green) that were used as controls. Along with this, the families with the LG B02 segment were as susceptible or less susceptible than the lines with a rust resistance segment derived from *A. cardenasii* (IAC-321 and IAC-322) (Supplemental Table 2.S4) (Dorzin, 2022). One family in particular (226C) had a rating of 1, meaning no rust was found on the plants at all (Dorzin, 2022). While further research is needed to validate the findings from Haiti, it is promising that plants in the field with the segment on LG B02 appear to be more resistant to *P. arachidis* than cultivated peanut lines and lines with *Arachis cardenasii*-derived rust resistance. This experiment is being repeated this year to confirm these results and seeds have also been sent to Senegal for similar experiments.

Because the genome of *Arachis batizocoi* has not yet been sequenced, we do not know what QTL or genes of interest might be located on the B-subgenome or LG B02 specifically. However, because we constructed the SNP marker genomes based on pseudomolecules created using the genomes of wild ancestors *A. ipaënsis* and *A. duranensis* (Bertioli et al., 2016), we can use the corresponding genes from *A. ipaënsis* to represent the *A. batizocoi* genome. Along with this, various R genes have been identified on LG B02 (Bertioli et al., 2016). More work needs to be done to find out if the segment of wild alleles on LG B02 are linked to any resistance genes or other genes of interest.

As previously discussed, along with the segment on LG B02, there are other wild introgressions present on the genomes of some of the individuals in the three families used in this project. Many of the individuals in the Seed8 family have segments on either LG A04, LG A06, or both that might be related to late leaf spot (LLS) resistance based on preliminary data (Bertioli et al., 2022). Along with this, the segment on LG B02 has also been linked to LLS resistance using the same preliminary data. These possible wild-derived source of resistance to late leaf spot are unconfirmed and need to be researched further before being used in breeding programs. However, if this research does find that there is a relationship between these segments and LLS resistance, the Seed8 individuals with those segments would be sources of resistance to multiple pathogens. As LLS and peanut rust often occur simultaneously in the field, having a line of peanuts that has resistance to both would be useful.

This work has had significant results related to the identification of a new source of peanut rust resistance as well as these unknown segments that might be useful for rust or other disease resistance. The identification of this segment on LG B02 and the development of multiple SNP

markers that target this segment are both stepping-stones towards future MAS breeding programs for rust resistance that will have benefits for farmers around the world.

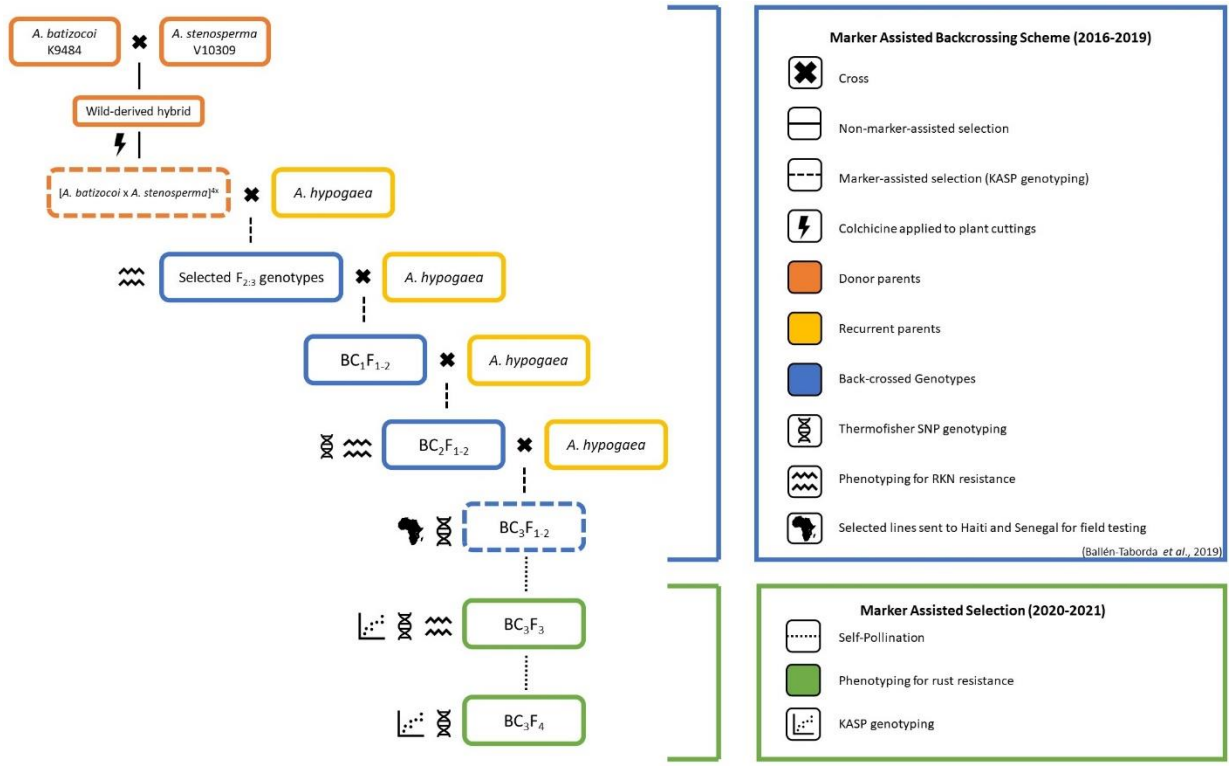

Figure 2.1. Schematic of the marker-assisted backcrossing scheme (MABS) utilized from 2016-2019 to incorporate root-knot nematode resistance from the wild species *A. stenosperma* V10309 and *A. batizocoi* K9848 into cultivated peanut (Ballén-Taborda, et al., 2019). Superior F_2-derived F_3 lines were selected as donor parents (orange box, dashed outline). Recurrent parents (yellow boxes) included TifGp-2, 5-646-10 and 13-1014. Three generation of backcrossing cycles were performed in Athens and Tifton in 2016, 2017, and 2018. In each cycle, KASP genotyping was performed to identify lines carrying RKN resistance segments. Selected progeny from each cycle were used as male parents for the next backcrossing cycle (blue boxes). In 2020, three lines (see Table 2.1) were selected from the BC_3F_2 population based on Thermofisher "Axiom_Arachis" v 2.0 SNP genotyping (blue box, dashed lines). From 2020-2021, nine detached-leaf bioassays were performed to screen these lines for resistance to *Puccinia arachidis* (green boxes). SNP genotyping via KASP and Affymetrix SNP array was performed on both generations (BC_3F_3 and BC_3F_4, green boxes).

Table 2.1. Phenotypic characterization of select BC_3F_2 (Ballén-Taborda, et al., 2019). The following traits were recorded: fertility (measured by recording the number of pegs produced by each plant), leaf spot incidence (presence '1' or absence '0') spots, plant architecture (scored from 1-5, with 1 for erect and 4 for prostrate growth habit, and 5 for dwarf plants), flower color (orange cultivated phenotype or yellow wild phenotype), seed size (weight, length, and width).

Trait	BC_3F_2_Seed7_S3	BC_3F_2_Seed8_S15	BC_3F_2_Seed11_S5
Number of Pegs	6	15	8
Leaf Spots	1	0	1
Architecture	4	3	3.5
Flower Color	Orange	Orange	Orange
Seed Weight (g)	0.83	1.255	0.52
Seed Length (mm)	16.76	18.69	14.03
Seed Width (mm)	11.23	13.42	9.57

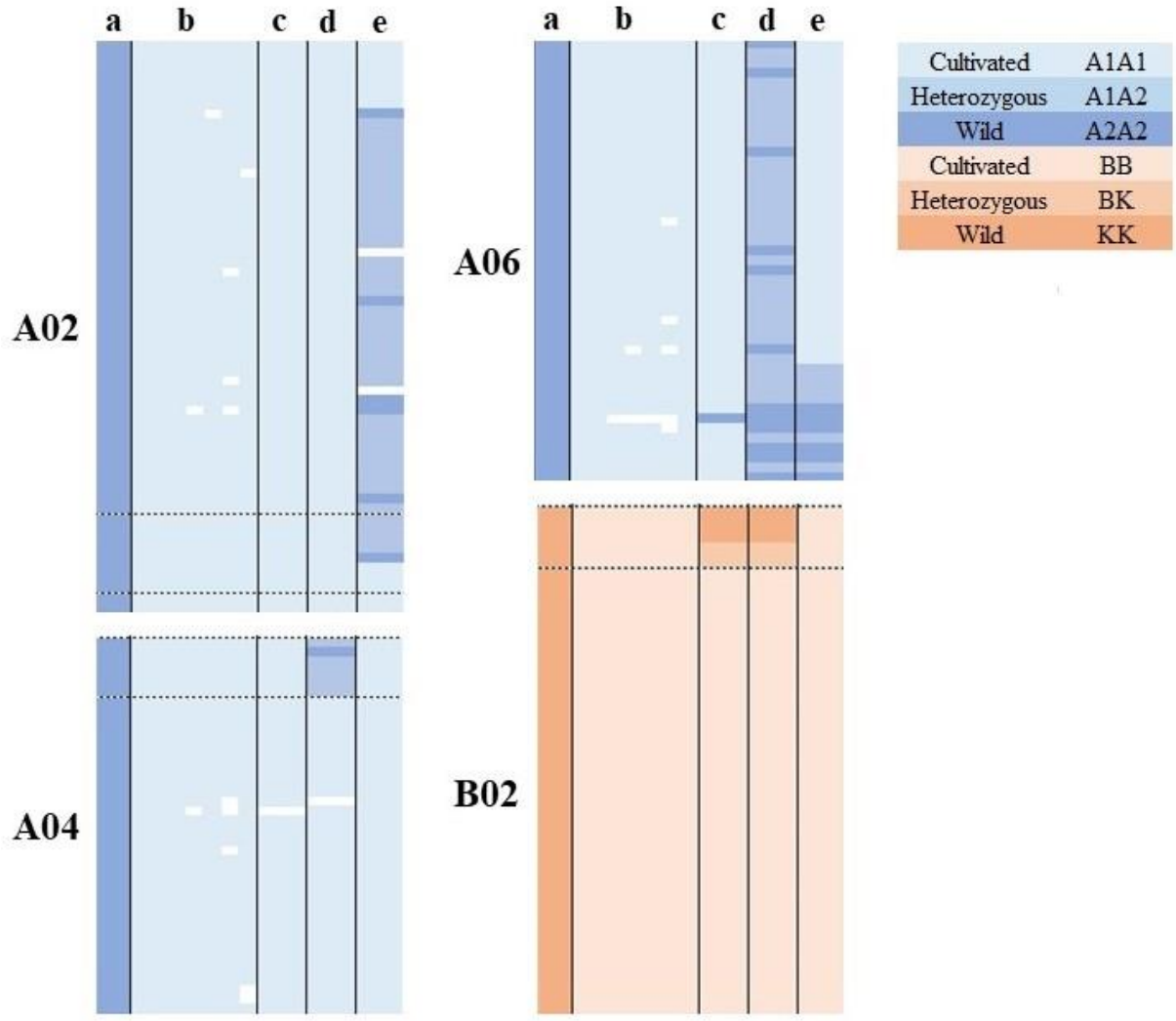

Figure 2.2. Color map of selected BC_3F_2 genotypes from the MABS population created using Excel from Axiom_Arachis 2.0 array data, courtesy of Ballén-Taborda, et al (2019). Each block represents a linkage group (LG) on the A-subgenome (blue) or the B/K-subgenome (red). Each row shows the results at a specific SNP marker and each column shows the results for a specific genotype. The rows between the dotted lines are the SNP markers that have been found to be related to disease resistance. The BC_3F_3 progeny of these three plants were then screened for rust resistance in this project.

Cultivated (A1A1), heterozygous (A1A2), and wild (A2A2) genotypes for the A-subgenome are represented by light blue, medium blue, and dark blue, respectively. Cultivated (BB), heterozygous (BK), and wild (KK) genotypes for the B/K-subgenome are represented by light orange, medium orange, and dark orange, respectively. White represents "no call".

Columns a-b show the parental controls: **a)** donor parent Batsten1, **b)** recurrent parents *A. hypogaea* Runner 886, *A. hypogaea* 5-646-10, *A. hypogaea* 13-1014, and *A. hypogaea* Tifrunner. Columns c-e show the results for the three individual BC_3F_2 plants that were seclected to test in this project: **c)** Seed7, **d)** Seed8, **e)** Seed11.

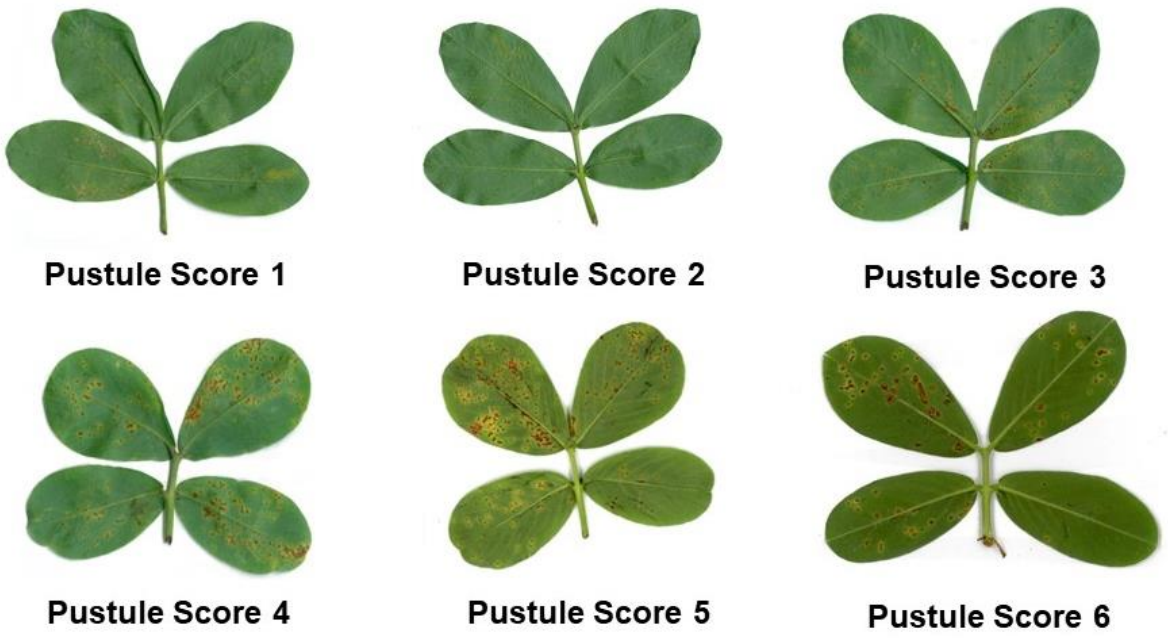

Figure 2.3. Scanned images of leaves from rust bioassays with pustule scores 1-6. Pustule score 1 = microscopic unsporulated pustule, 2-5 = sporulated pustules from 0.5 to 3 mm, 6 = sporulated pustule more than 3mm in size. The pustule scores represent individual pustules, some of these images have examples of more than one pustule score on one leaf.

Table 2.2. Overview of the results of each BC_3F_3 genotype from Assay 1. The statistical grouping was done using Fisher's LSD test. IA (Index/Area (cm^2)), TPA (Total Pustules/Area (cm^2)), SPA (Sporulating Pustules/Area (cm^2)), IP (Incubation Periods (days).

Controls	Average IA	Average TPA	Average SPA	Average IP
A. hypogaea 13-1014	3.74 defg	1.74 defg	1.4 def	17 ab
A. hypogaea 5-646-10	3.47 defg	1.87 defg	1.6 def	17.57 a
A. hypogaea Runner 886	7.94 ab	3.83 ab	2.92 abc	15.48 b
A. hypogaea Tifrunner	10.89 a	3.93 abc	3.85 ab	15.45 b
Batsten1	0.00 j	0.00 j	0.00 h	-
Genotype (BC_3F_3)				
Seed7_S1	1.76 efgh	0.96 efgh	0.8 def	18 a
Seed7_S2	0.00 j	0.00 j	0.00 h	-
Seed7_S3	0.00 j	0.00 j	0.00 h	-
Seed7_S4	0.00 j	0.00 j	0.00 h	-
Seed7_S5	0.00 j	0.00 j	0.00 h	-
Seed7_S6	0.00 j	0.00 j	0.00 h	-
Seed7_S7	0.00 j	0.00 j	0.00 h	-
Seed7_S8	0.00 j	0.00 j	0.00 h	-
Seed7_S9	0.00 j	0.00 j	0.00 h	-
Seed7_S10	0.00 j	0.00 j	0.00 h	-
Seed7_S11	0.00 j	0.00 j	0.00 h	-
Seed7_S12	0.00 j	0.00 j	0.00 h	-
Seed11_S1	3.51 abcdef	1.85 abcdef	1.66 bcde	17.17 ab
Seed11_S2	2.01 defg	1.04 defg	0.97 cdef	18.83 a
Seed11_S3	2.58 defg	1.37 defg	1.21 def	18.83 a
Seed11_S4	7.51 abcd	3.16 abcd	3.04 abcde	16.83 ab
Seed11_S5	1.36 fgh	0.79 fgh	0.56 ef	18.8 a
Seed11_S6	2.95 cdefg	1.49 cdefg	1.46 bcde	16.8 ab
Seed11_S7	3.13 bcdef	1.73 abcdef	1.41 bcde	16.4 ab
Seed11_S8	2.75 defg	1.56 defg	1.19 ef	19.17 a
Seed11_S9	2.43 gh	1.27 gh	1.16 fg	20.4 a
Seed11_S10	8.45 abc	4.01 abc	3.87 abc	16 ab
Seed11_S11	9.59 a	4.82 a	4.77 a	14.67 b
Seed11_S12	6.83 abcde	3.38 abcde	3.38 abcd	14.8 b
Seed8_S1	0.06 ij	0.06 ij	0.00 h	22 a
Seed8_S2	0.00 j	0.00 j	0.00 h	-
Seed8_S3	2.29 defg	1.16 defg	1.13 cde	17.17 ab
Seed8_S4	4.18 bcdef	2.09 bcdef	2.08 abcd	15.33 b
Seed8_S5	0.00 j	0.00 j	0.00 h	-
Seed8_S6	0.00 j	0.00 j	0.00 h	-
Seed8_S8	0.00 j	0.00 j	0.00 h	-
Seed8_S9	0.00 j	0.00 j	0.00 h	-
Seed8_S10	0.52 hi	0.36 hi	0.16 gh	20.25 a
Seed8_S11	0.00 j	0.00 j	0.00 h	-
Seed8_S12	0.02 j	0.02 j	0.00 h	22 a
Average	3.30	1.51	1.34	16.87

Table 2.3. Overview of the results of each BC_3F_3 genotype from Assay 2. The statistical grouping was done using Fisher's LSD test. IA (Index/Area (cm^2)), TPA (Total Pustules/Area (cm^2)), SPA (Sporulating Pustules/Area (cm^2)), IP (Incubation Periods (days).

Controls	Average IA	Average TPA	Average SPA	Average IP
A. hypogaea 13-1014	3.49 abc	2.16 ab	1.33 abc	13.40 bc
A. hypogaea 5-646-10	7.63 ab	3.24 a	2.59 abcd	15.20 bc
A. hypogaea Runner 886	6.98 a	2.40 a	2.40 a	13.6 bc
A. hypogaea Tifrunner	3.49 abc	2.16 ab	1.33 abc	13.40 bc
Batsten1	0.00 abc	0.00 ab	0.00 abc	-
Genotype (BC_3F_3)				
Seed7_S13	0.93 abcd	0.82 abc	0.11 def	20.33 ab
Seed7_S14	0.00 g	0.00 f	0.00 f	-
Seed7_S15	0.00 g	0.00 f	0.00 f	-
Seed7_S16	0.00 g	0.00 f	0.00 f	-
Seed7_S17	0.00 g	0.00 f	0.00 f	-
Seed7_S18	0.00 g	0.00 f	0.00 f	-
Seed7_S19	0.00 g	0.00 f	0.00 f	-
Seed7_S20	0.00 g	0.00 f	0.00 f	-
Seed7_S21	2.12 abcd	1.58 abc	0.49 bcde	20.00 ab
Seed7_S22	0.00 g	0.00 f	0.00 f	-
Seed7_S23	0.00 g	0.00 f	0.00 f	-
Seed7_S24	0.11 efg	0.11 def	0.00 f	22.00 a
Seed8_S13	0.00 g	0.00 f	0.00 f	-
Seed8_S14	1.09 abcd	1.09 abc	0.00 f	19.50 ab
Seed8_S15	0.00 g	0.00 f	0.00 f	-
Seed8_S16	0.00 g	0.00 f	0.00 f	-
Seed8_S17	0.00 g	0.00 f	0.00 f	-
Seed8_S18	0.00 g	0.00 f	0.00 f	-
Seed8_S19	0.00 g	0.00 f	0.00 f	-
Seed8_S20	0.26 g	0.13 f	0.13 ef	22.00 ab
Seed8_S22	1.45 bcd	1.04 abc	0.41 bcde	17.75 ab
Seed8_S23	0.00 g	0.00 f	0.00 f	-
Seed8_S24	0.21 g	0.21 f	0.00 f	22.00 ab
Seed11_S13	3.27 ab	1.77 a	1.47 abc	14.67 bc
Seed11_S14	0.00 g	0.00 f	0.00 f	-
Seed11_S15	1.14 abcd	0.84 abc	0.30 bcde	12.17 c
Seed11_S16	0.62 def	0.51 cde	0.11 def	19.00 ab
Seed11_S17	3.51 abc	3.01 a	0.49 abcd	15.4 bc
Seed11_S18	2.37 abcd	1.46 abc	0.90 bcde	15.4 bc
Seed11_S19	0.76 bcd	0.72 abc	0.04 def	19.2 ab
Seed11_S20	0.19 fg	0.19 ef	0.00 f	22.00 a
Seed11_S21	1.34 bcde	1.26 abc	0.08 def	17.75 ab
Seed11_S22	0.72 bcde	0.62 abcd	0.10 ef	20.00 ab
Seed11_S23	3.56 abcd	1.98 abc	1.58 ab	18.00 ab
Seed11_S24	1.28 cde	0.75 bcde	0.46 cdef	13.50 bc
Average	**1.10**	**0.66**	**0.33**	**17.14**

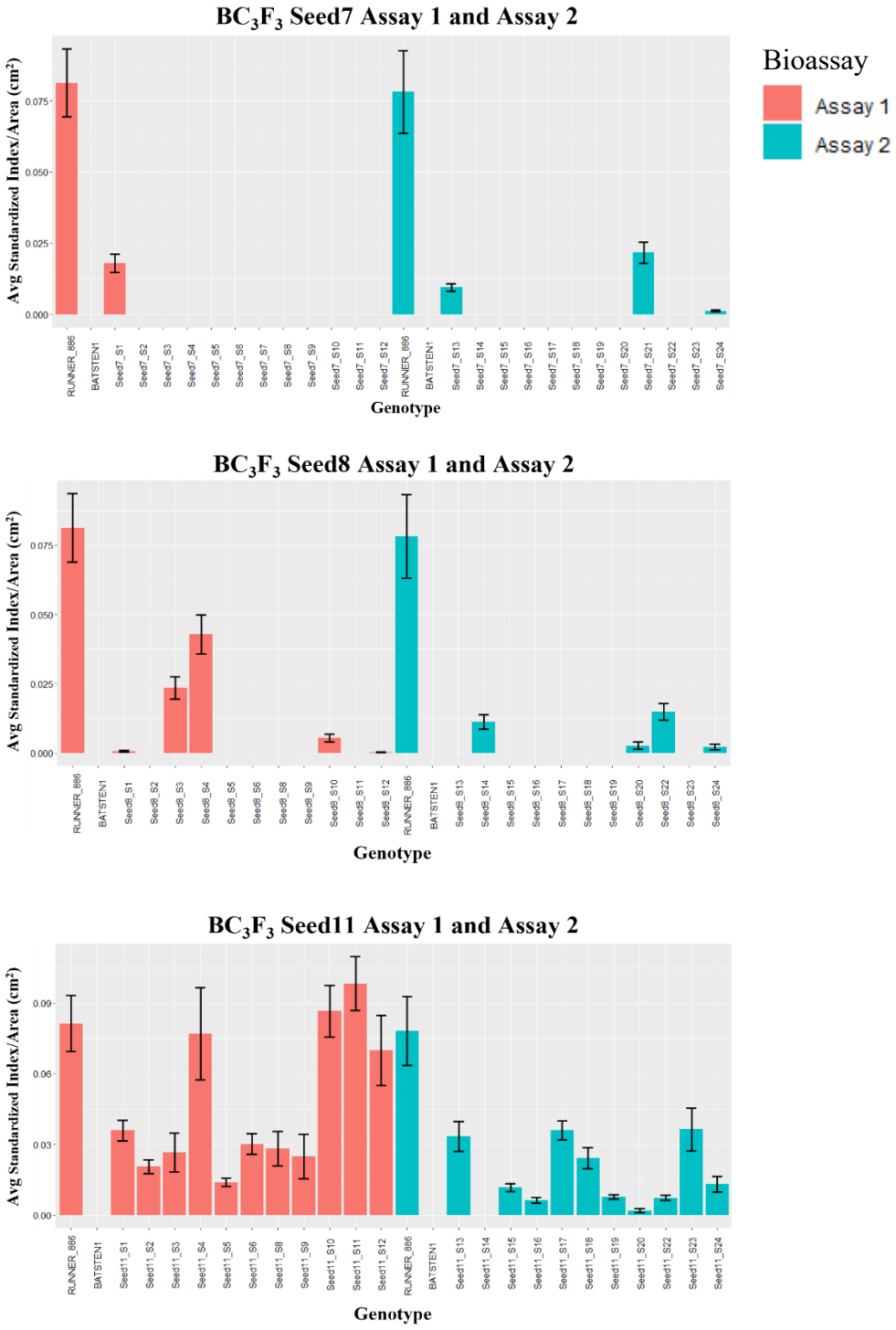

Figure 2.4. Results from Assays 1 and 2 for Seed7, Seed8, and Seed11 (BC_3F_3s) using the average standardized IA (IAS) for each genotype. Error bars represent one standard error.

Table 2.4. Overview of the chi-squared test results for each bioassay. Results are from the non-parametric Kruskal-Wallis test at P < 0.05. [a] IA (Index/Area (cm^2)), TPA (Total Pustules/Area (cm^2)), SPA (Sporulating Pustules/Area (cm^2)), IP (Incubation Periods (days)), [b] *P <0.05, **P <0.01, ***P<0.001

	Trait[a]	Chi-squared	df	p-value[b]
Assay 1	IA	268.4	39	<0.001 ***
	TPA	264.43	39	<0.001 ***
	SPA	242.88	39	<0.001 ***
	IP	49.29	21	<0.001 ***
Assay 2	IA	155.54	38	<0.001 ***
	TPA	154.72	38	<0.001 ***
	SPA	108.14	38	<0.001 ***
	IP	37.666	20	<0.001 ***
Assay 3	IA	32.48	12	<0.01 **
	TPA	30.064	12	<0.01 **
	SPA	29.522	12	<0.01 **
	IP	22.482	10	<0.05*
Assay 4	IA	83.14	20	<0.001 ***
	TPA	81.793	20	<0.001 ***
	SPA	82.141	20	<0.001 ***
	IP	30.704	14	<0.01 **
Assay 5	IA	90.328	23	<0.001 ***
	TPA	86.658	23	<0.001 ***
	SPA	101.46	23	<0.001 ***
	IP	50.467	15	<0.001 ***
Assay 6	IA	281.97	58	<0.001 ***
	TPA	275.47	58	<0.001 ***
	SPA	271.44	58	<0.001 ***
	IP	69.154	26	<0.001 ***
Assay 7	IA	216.38	67	<0.001 ***
	TPA	211.41	67	<0.001 ***
	SPA	236.23	67	<0.001 ***
	IP	82.083	48	<0.001 ***
Assay 8	IA	191.79	48	<0.001 ***
	TPA	190.54	48	<0.001 ***
	SPA	186.95	48	<0.001 ***
	IP	27.231	15	<0.05*
Assay 9	IA	134.96	38	<0.001 ***
	TPA	133.23	38	<0.001 ***
	SPA	127.14	38	<0.001 ***
	IP	**24.309**	**15**	**>0.05**

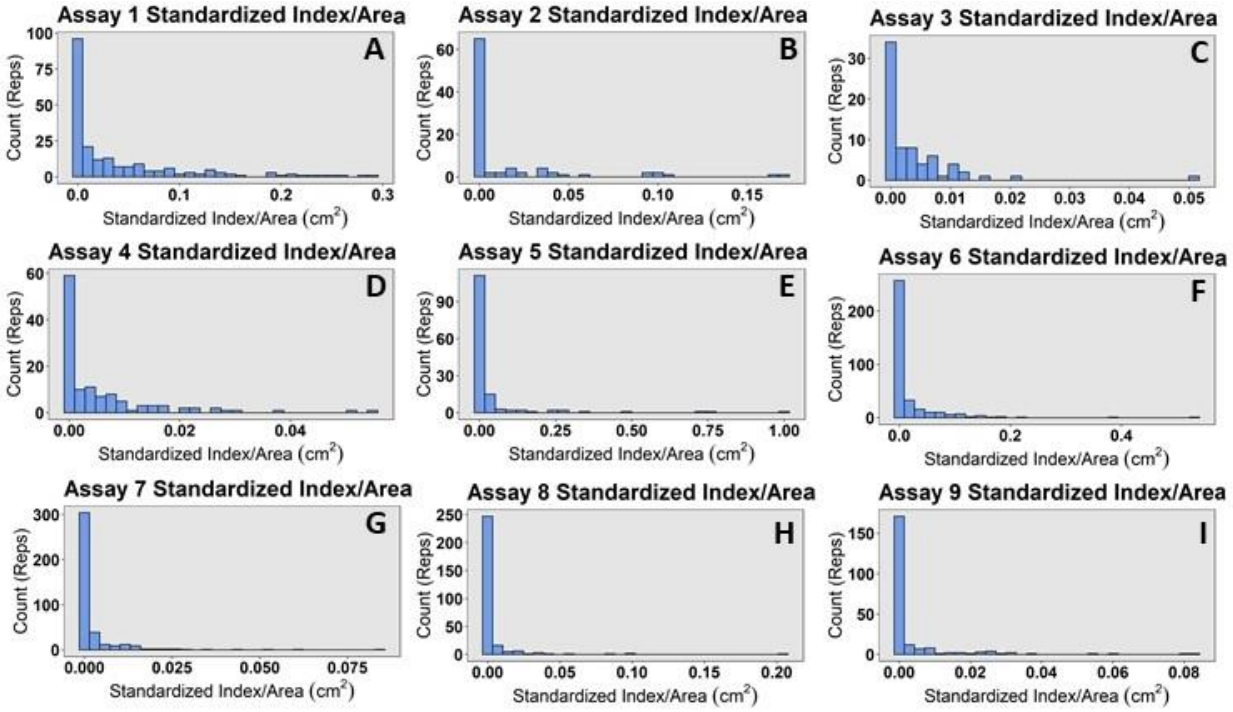

Figure 2.5. Frequency distributions of Seed8 IAS (standardized index/area (cm^2)) from each bioassay. 18 BC_3F_3 Seed8 genotypes (A, B, C, D, and G) and 94 BC_3F_4 Seed8 genotypes (C, E, F, G, H, and I) along with controls.

Table 2.5. Overview of chi-squared test results from all 9 assays. This includes all genotypes and the results from the non-parametric Skillings-Mack test at $P < 0.05$. [a] IAS (Standardized Index/Area (cm^2)), TPAS (Standardized Total Pustules/Area (cm^2)), SPAS (Standardized Sporulating Pustules/Area (cm^2)), IPS (Standardized Incubation Periods (days))
[b] * P <0.05, **P <0.01, ***P<0.001

Trait[a]	Chi-squared	df	P-value[b]
IAS	684.3	180	<0.001 ***
TPAS	670.72	180	<0.001 ***
SPAS	597.19	180	<0.001 ***
IPS	571.56	180	<0.001 ***

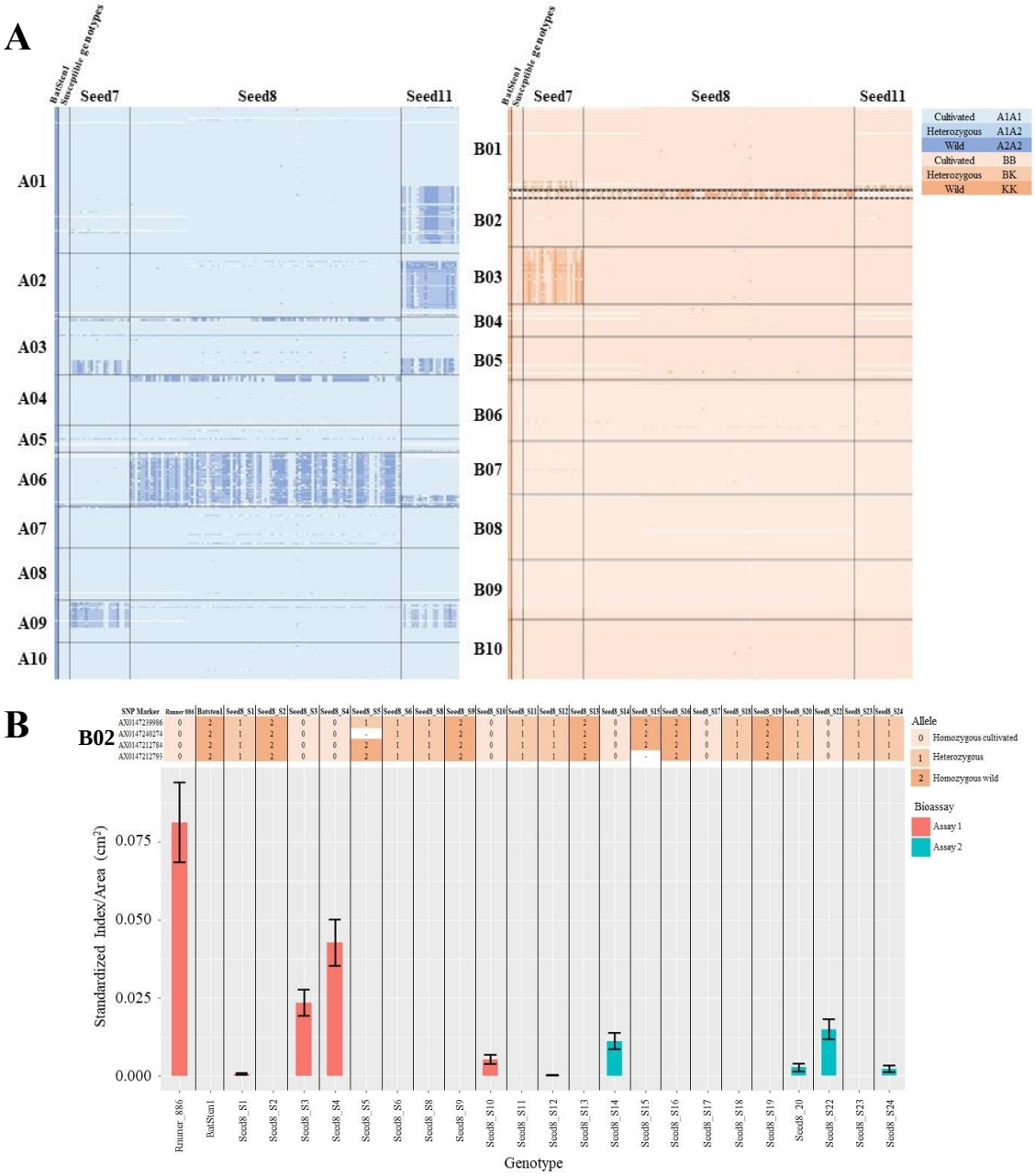

Figure 2.6: A) Color map of $BC_3F_{3\text{-}4}$ genotypes from the MABS population created using Excel from Thermofisher "Axiom_Arachis" 2.0 array data. Each block represents a linkage group (LG) on the A-subgenome (blue) or the B/K-subgenome (orange). Each row shows the results at a specific SNP marker and each column shows the results for a specific genotype. The genotypes shown (from left to right) are donor parent Batsten1, susceptible *A. hypogaea* (cultivated) genotypes, and the Seed7, Seed8, Seed11 families.

B) Top: a zoomed-in image of the genotyping of the wild LG B02 segment for some Seed 8 individuals. Top: Results for IAS of Assay 1 and 2 of the same individuals. Note that with a single exception, asymptomatic individuals possess the wild B02 segment.

Table 2.6. SNP markers on LG B02. The probeset_ID is the name assigned to the SNP marker by Thermofisher. The reassigned name can be read: linkage group, the position of the first basepair in the marker (custpos), and the wild species the marker correlates to (Bat = *A. batizocoi*). The custpos is the position of the first basepair in the marker, the numbering starts at zero at the first bp of the linkage group. * Target region used for KASP analyses in this work

Probeset_ID	Reassigned Name	custpos	Affy 1	Affy 2	KASP primer set designed	Used in KASP
AX-147239821	B02_432989_Bat	432,989	Absent	Present	No	No
AX-147239986	B02_1296859_Bat	1,296,859	Present	Present	Yes	No
AX-147240274	B02_2288084_Bat	2,288,084	Present	Present	Yes	No
AX-147240299	B02_2423971_Bat	2,423,971	Absent	Present	No	No
AX-147212793*	B02_2363097_Bat	3,023,963	Present	Present	Yes	Yes
AX-147212784	B02_2290531_Bat	3,089,814	Present	Absent	Yes	No

Table 2.7. Results from Affy 1 for LG B02 (BC3F3s). The presence or absence of the segment on the top of LG B02 on Seed8 BC_3F_3 individuals is shown, based on Thermofisher "Axiom_Arachis" v 2.0 SNP assay. Each SNP marker showed one of four results: no call, homozygous for the cultivated (*A. hypogaea*) allele (BB) (-/-), heterozygous (BK) (-/+), or homozygous for the wild (*A. batizocoi*) allele (KK) (+/+).

	Marker			
Controls	**B02_1296859_Bat**	**B02_2288084_Bat**	**B02_2290531_Bat**	**B02_2363097_Bat**
Runner 886	-/-	-/-	-/-	-/-
Batsten1	+/+	+/+	+/+	+/+
Genotype (BC_3F_3)				
Seed7_S1	-/-	-/-	-/-	-/-
Seed7_S2	-/+	-/+	-/+	-/+
Seed7_S3	-/+	-/+	-/+	-/+
Seed7_S4	-/+	-/+	-/+	-/+
Seed7_S5	-/+	-/+	-/+	-/+
Seed7_S6	-/+	-/+	-/+	-/+
Seed7_S7	-/+	-/+	-/+	-/+
Seed7_S8	-/-	-/-	-/-	-/-
Seed7_S9	-/+	-/-	-/-	-/-
Seed7_S11	+/+	+/+	+/+	+/+
Seed7_S12	-/+	-/+	-/+	-/+
Seed7_S13	-/-	-/-	-/-	-/-
Seed7_S14	-/+	-/+	-/+	-/+
Seed7_S15	+/+	+/+	+/+	+/+
Seed7_S16	-/+	-/+	-/+	-/+
Seed7_S17	+/+	-/+	-/+	-/+
Seed7_S18	-/+	-/+	-/+	-/+
Seed7_S19	-/+	-/+	-/+	-/+
Seed7_S20	-/+	-/+	-/+	-/+
Seed7_S21	-/-	-/-	-/-	-/-
Seed7_S22	-/+	-/+	-/+	-/+
Seed7_S23	No Call	+/+	+/+	+/+
Seed7_S24	-/+	-/+	-/+	-/+
Seed8_S1	-/+	-/+	-/+	-/+
Seed8_S2	+/+	+/+	+/+	+/+
Seed8_S3	-/-	-/-	-/-	-/-
Seed8_S4	-/-	-/-	-/-	-/-
Seed8_S5	-/+	No Call	+/+	+/+
Seed8_S6	-/+	-/+	-/+	-/+
Seed8_S8	-/+	-/+	-/+	-/+
Seed8_S9	+/+	+/+	+/+	+/+
Seed8_S10	-/-	-/-	-/-	-/-
Seed8_S11	-/+	-/+	-/+	-/+
Seed8_S12	-/+	-/+	-/+	-/+
Seed8_S13	+/+	+/+	+/+	+/+
Seed8_S14	-/-	-/-	-/-	-/-
Seed8_S15	+/+	+/+	+/+	No Call
Seed8_S16	+/+	+/+	+/+	+/+
Seed8_S17	-/-	-/-	-/-	-/-
Seed8_S18	-/+	-/+	-/+	-/+
Seed8_S19	+/+	+/+	+/+	+/+
Seed8_S20	-/+	-/+	-/+	-/+
Seed8_S22	-/-	-/-	-/-	-/-
Seed8_S23	-/+	-/+	-/+	-/+
Seed8_S24	-/+	-/+	-/+	-/+
Seed11_S1	-/-	-/-	-/-	-/-
Seed11_S2	-/-	-/-	-/-	-/-
Seed11_S4	-/-	-/-	-/-	-/-
Seed11_S5	-/-	-/-	-/-	-/-
Seed11_S6	-/-	-/-	-/-	-/-
Seed11_S7	-/-	-/-	-/-	-/-
Seed11_S8	-/-	-/-	-/-	-/-
Seed11_S9	-/-	-/-	-/-	-/-
Seed11_S10	-/-	-/-	-/-	-/-
Seed11_S12	-/-	-/-	-/-	-/-
Seed11_S13	-/-	-/-	-/-	-/-
Seed11_S14	-/-	-/-	-/-	-/-
Seed11_S15	-/-	-/-	-/-	-/-
Seed11_S16	-/-	-/-	-/-	-/-
Seed11_S17	-/-	-/-	-/-	-/-
Seed11_S18	-/-	-/-	-/-	-/-
Seed11_S19	-/-	-/-	-/-	-/-
Seed11_S20	-/-	-/-	-/-	-/-
Seed11_S21	-/-	-/-	-/-	-/-
Seed11_S22	-/-	-/-	-/-	-/-
Seed11_S23	-/-	-/-	-/-	-/-
Seed11_S24	-/-	-/-	-/-	-/-

Table 2.8. Results from Affy 2 for LG B02 (BC3F3s). The presence or absence of the segment on the top of LG B02 on Seed8 BC_3F_3 individuals is shown, based on Thermofisher "Axiom_Arachis" v 2.0 SNP assay. Each SNP marker showed one of four results: no call, homozygous for the cultivated (*A. hypogaea*) allele (BB) (-/-), heterozygous (BK) (-/+), or homozygous for the wild (*A. batizocoi*) allele (KK) (+/+).

	Marker					
Controls	**B02_432989_Bat**	**B02_1296859_Bat**	**B02_2288084_Bat**	**B02_2423971_Bat**	**B02_2290531_Bat**	**B02_2363097_Bat**
Runner 886	-/-	-/-	-/-	-/-	-/-	-/-
Batsten1	+/+	+/+	+/+	+/+	+/+	+/+
Genotype (BC_3F_3)						
S25	-/+	-/+	-/+	-/+	-/+	-/+
S26	-/+	-/+	-/+	-/+	-/+	-/+
S27	-/+	-/+	-/+	-/+	-/+	-/+
S29	-/+	-/+	-/+	-/+	-/+	-/+
S30	-/+	-/+	-/+	-/+	-/+	-/+
S31	-/-	-/-	-/-	-/-	-/-	-/-
S32	-/-	-/-	-/+	-/+	-/+	-/+
S34	+/+	+/+	+/+	NoCall	+/+	+/+
S35	-/+	+/+	+/+	+/+	+/+	+/+
S36	-/-	-/-	-/-	-/-	-/-	-/-
S40	-/-	-/-	-/-	-/-	-/-	-/-
S41	-/+	-/+	-/+	-/+	-/+	-/+

Table 2.9. Results from Affy 2 for LG B02 (BC3F4s). The presence or absence of the segment on the top of LG B02 on Seed8 BC_3F_3 individuals is shown, based on Thermofisher "Axiom_Arachis" v 2.0 SNP assay. Each SNP marker showed one of four results: no call, homozygous for the cultivated (*A. hypogaea*) allele (BB) (-/-), heterozygous (BK) (-/+), or homozygous for the wild (*A. batizocoi*) allele (KK) (+/+).

	Marker					
Controls	**B02_432989_Bat**	**B02_1296859_Bat**	**B02_2288084_Bat**	**B02_2423971_Bat**	**B02_2290531_Bat**	**B02_2363097_Bat**
Runner 886	-/-	-/-	-/-	-/-	-/-	-/-
Batsten1	+/+	+/+	+/+	+/+	+/+	+/+
Genotype (BC_3F_4)						
S1_S1	-/-	-/-	-/-	-/-	-/-	-/-
S1_S2	-/+	-/+	-/+	-/+	-/+	-/+
S1_S3	+/+	+/+	+/+	+/+	+/+	+/+
S1_S4	+/+	+/+	-/+	-/+	-/+	-/+
S2_S1	+/+	+/+	+/+	+/+	+/+	+/+
S2_S2	+/+	+/+	+/+	+/+	+/+	+/+
S2_S3	+/+	+/+	+/+	NoCall	+/+	+/+
S2_S4	+/+	+/+	NoCall	+/+	+/+	+/+
S3_S1	-/-	-/-	-/-	-/-	-/-	-/-
S3_S2	-/-	-/-	-/-	-/-	-/-	-/-
S4_S1	-/-	-/-	-/-	-/-	-/-	-/-
S4_S2	-/-	-/-	-/-	-/-	-/-	-/-
S5_7	-/+	-/+	+/+	+/+	+/+	+/+
S5_8	-/-	-/-	+/+	+/+	+/+	+/+
S5_S2	-/+	-/+	+/+	+/+	+/+	+/+
S5_S3	+/+	+/+	+/+	+/+	+/+	+/+
S5_S4	+/+	+/+	+/+	+/+	+/+	+/+
S6_S1	+/+	+/+	+/+	+/+	-/+	+/+
S6_S2	-/+	-/+	-/+	-/+	-/+	-/+
S6_S3	-/+	-/+	-/+	-/+	NoCall	-/+
S6_S4	+/+	+/+	+/+	+/+	+/+	+/+
S8_8	-/-	-/-	-/-	-/-	-/-	-/-
S8_S1	-/+	NoCall	-/-	-/-	-/-	-/-
S8_S2	+/+	+/+	+/+	+/+	+/+	+/+
S8_S3	-/+	-/+	-/+	-/+	-/+	-/+
S8_S4	-/+	-/+	-/+	-/+	-/+	-/+
S9_S1	+/+	+/+	+/+	+/+	+/+	+/+
S9_S2	+/+	+/+	+/+	+/+	+/+	+/+
S9_S4	+/+	+/+	+/+	+/+	+/+	+/+
S10_S1	-/-	-/-	-/-	NoCall	-/-	-/-
S10_S2	-/-	-/-	-/-	-/-	NoCall	-/-
S11_S1	+/+	+/+	+/+	+/+	+/+	+/+
S11_S2	-/+	-/+	-/+	-/+	-/+	-/+
S11_S3	+/+	+/+	+/+	+/+	+/+	+/+
S11_S4	+/+	+/+	+/+	+/+	+/+	+/+
S12_S1	-/+	-/+	-/+	-/+	-/+	-/+
S12_S2	-/-	-/-	-/-	-/-	-/-	-/-
S12_S3	-/+	-/+	NoCall	-/+	-/+	-/+
S12_S4	-/+	-/+	NoCall	-/+	-/+	-/+
S13_S1	+/+	+/+	+/+	+/+	+/+	+/+
S13_S2	+/+	+/+	+/+	+/+	+/+	+/+
S14_S1	-/-	-/-	-/-	-/-	-/-	-/-
S14_S2	-/-	-/-	-/-	-/-	-/-	-/-
S15_S1	+/+	+/+	+/+	+/+	+/+	+/+
S15_S2	+/+	+/+	+/+	+/+	+/+	+/+
S15_S5	+/+	+/+	+/+	+/+	+/+	+/+
S15_S6	+/+	+/+	+/+	+/+	+/+	+/+
S16_S1	+/+	NoCall	+/+	+/+	+/+	+/+
S18_S1	-/+	-/+	+/+	-/+	-/+	-/+
S18_S2	-/+	-/+	-/+	-/+	-/-	-/+
S18_S3	+/+	+/+	+/+	+/+	+/+	+/+
S18_S5	-/+	NoCall	-/+	-/+	-/+	-/+
S18_S6	-/-	-/-	-/-	-/-	-/-	-/-
S19_S1	+/+	+/+	+/+	+/+	+/+	+/+
S19_S2	+/+	+/+	+/+	+/+	+/+	+/+
S20_S1	-/+	-/+	-/+	-/+	-/+	-/+
S20_S2	-/+	-/+	-/+	-/+	-/+	-/+
S22_S1	-/-	-/-	-/-	-/-	-/-	-/-
S22_S2	-/-	-/-	-/-	-/-	-/-	-/-
S23_S1	-/+	-/+	-/+	-/+	NoCall	-/+
S23_S2	-/-	-/-	-/-	-/-	-/-	-/-
S23_S3	-/+	-/+	-/+	-/+	-/+	-/+
S23_S4	-/-	-/-	-/-	-/-	-/-	-/-
S24_S1	-/+	-/+	-/+	-/+	-/+	-/+
S24_S2	-/+	-/+	-/+	-/+	-/+	-/+
S24_S3	-/-	-/-	-/-	-/-	-/-	-/-
S24_S4	+/+	+/+	+/+	+/+	+/+	+/+
SA_S1	+/+	+/+	+/+	+/+	+/+	+/+
SA_S2	+/+	+/+	+/+	+/+	+/+	+/+

Table 2.10. Primer assays designed for KASP genotyping targeting SNP markers on LG B02. Each primer assay consists of two allele-specific and one allele-flanking primer. The primer names are read as follows: linkage group, SNP marker reassigned number, primer direction (forward or reverse), and dye used (FAM or VIC). KASP was run using the primer assay designed for B02_263097 (marked by * below).

Primer Name	Primer Sequence	Type	SNP
B02_2363097_Fwd*	ATGGGATCCGAATCAAAACC	Allele-flanking	
B02_2363097_Rev_FAM*	GAAGGTGACCAAGTTCATGCTCACGCTCCTGTTGCTCTCT	Allele-specific	A
B02_2363097_Rev_VIC*	GAAGGTCGGAGTCAACGGATTACGCTCCTGTTGCTCTCG	Allele-specific	C
B02_2290531_Rev	ATTTCTAATATTTATCAAGTTTGATTGAC	Allele-flanking	
B02_2290531_Fwd_VIC	GAAGGTCGGAGTCAACGGATTAATAGTAGGAATACCTTCACCAAACTTA	Allele-specific	A
B02_2290531_Fwd_FAM	GAAGGTGACCAAGTTCATGCTAGGAATACCTTCACCAAACTTG	Allele-specific	G
B02_1296859_Rev	ACAGATTATGATGCGTTGTGATTG	Allele-flanking	
B02_1296859_Fwd_VIC	GAAGGTCGGAGTCAACGGATTTCTTTTTTCAGTTCAACTCATTCGA	Allele-specific	A
B02_1296859_Fwd_FAM	GAAGGTGACCAAGTTCATGCTTCTTTTTTCAGTTCAACTCATTCGG	Allele-specific	G
B02_2288084_Fwd	ACAGATTATGATGCGTTGTGATTG	Allele-flanking	
B02_2288084_Rev_VIC	GAAGGTCGGAGTCAACGGATTTCCAGAGATTCTTTGCTACTGAATTT	Allele-specific	A
B02_2288084_Rev_FAM	GAAGGTGACCAAGTTCATGCTTCCAGAGATTCTTTGCTACTGAATTC	Allele-specific	G

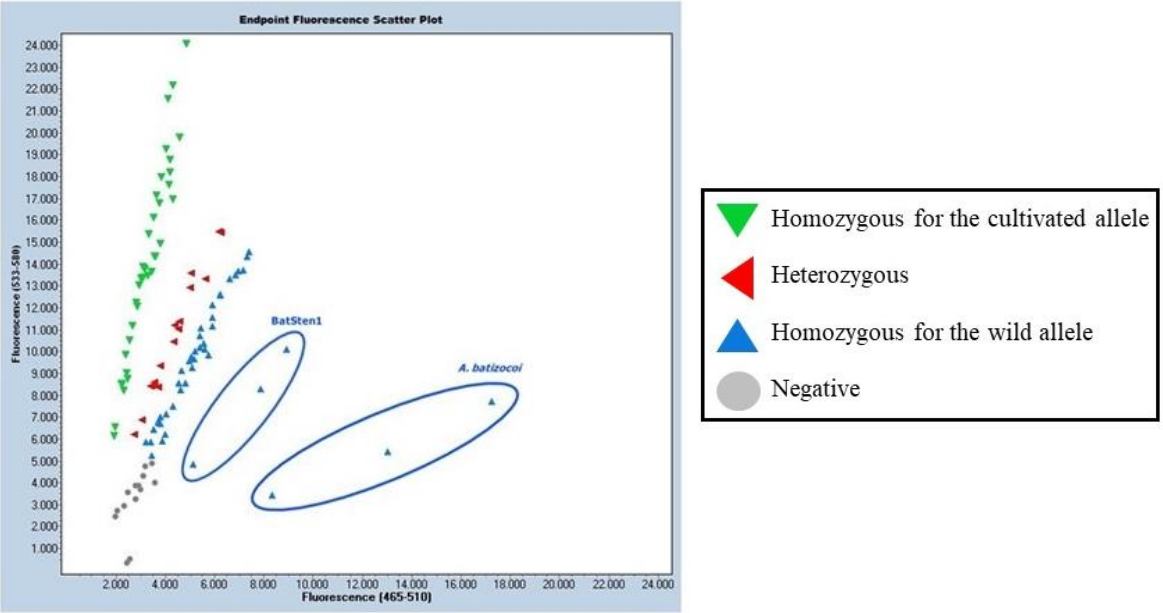

Figure 2.7 Graph of Kompetitive Allele-Specific PCR (KASP) results using a primer designed to target LG B02 (marker B02_263097). The x-axis shows the FAM fluorescence values detected by the light reader, and the y-axis shows the VIC fluorescence values. Each triangle or circle represents one DNA sample from an individual plant. The light reader detects the fluorescence from each well, and samples group on the graph as one of the two alleles, heterozygous for both, or negative. This graph shows a run with controls that represent genotypes with the wild allele at the SNP marker (blue, BatSten1 *and A. batizocoi*) and the genotypes without the wild allele (green).

Table 2.11- KASP results for SNP marker B02_2363097_Bat for BC_3F_3 and BC_3F_4 Seed8 genotypes**.** Each genotype showed one of four results: no call, homozygous for the cultivated (*A. hypogaea*) allele (BB) (-/-), heterozygous (BK) (-/+), or homozygous for the wild (*A. batizocoi*) allele (KK) (+/+).

Controls	KASP Results	Genotype (BC_3F_4)	KASP Results
Runner 886	-/-	Seed8_S13_S1	+/+
Batsten1	+/+	Seed8_S13_S3	+/+
Arachis stenosperma	-/-	Seed8_S13_S4	+/+
Arachis batizocoi	+/+	Seed8_S14_S1	-/-
Genotype (BC_3F_3)		Seed8_S15_S2	+/+
Seed8_S13	+/+	Seed8_S15_S3	+/+
Seed8_S15	+/+	Seed8_S15_S4	+/+
Seed8_S22	-/-	Seed8_S15_S5	+/+
Seed8_S25	-/+	Seed8_S15_S6	+/+
Seed8_S27	-/+	Seed8_S16_S1	+/+
Seed8_S29	-/+	Seed8_S16_S2	+/+
Seed8_S30	-/+	Seed8_S16_S3	+/+
Seed8_S31	-/-	Seed8_S16_S4	+/+
Seed8_S34	+/+	Seed8_S17_S1	-/-
Seed8_S36	-/-	Seed8_S17_S2	-/-
Seed8_S40	-/-	Seed8_S17_S4	-/-
Genotype (BC_3F_4)		Seed8_S18_S1	-/+
Seed8_S1_S2	-/+	Seed8_S18_S2	-/+
Seed8_S1_S4	-/+	Seed8_S18_S3	+/+
Seed8_S2_S4	+/+	Seed8_S18_S4	+/+
Seed8_S5_S1	+/+	Seed8_S18_S5	-/+
Seed8_S5_S2	+/+	Seed8_S18_S6	-/-
Seed8_S5_S3	+/+	Seed8_S19_S1	+/+
Seed8_S5_S4	+/+	Seed8_S19_S2	+/+
Seed8_S5_S7	+/+	Seed8_S19_S3	+/+
Seed8_S5_S8	+/+	Seed8_S19_S4	+/+
Seed8_S6_S1	+/+	Seed8_S19_S5	+/+
Seed8_S6_S2	-/+	Seed8_S19_S6	+/+
Seed8_S6_S3	-/+	Seed8_S20_S1	-/+
Seed8_S6_S4	+/+	Seed8_S20_S2	-/+
Seed8_S8_S1	-/-	Seed8_S20_S3	+/+
Seed8_S8_S2	+/+	Seed8_S20_S4	+/+
Seed8_S8_S3	-/+	Seed8_S21_S1	-/+
Seed8_S8_S4	-/+	Seed8_S21_S2	-/+
Seed8_S9_S1	+/+	Seed8_S21_S3	-/+
Seed8_S9_S2	+/+	Seed8_S21_S4	-/+
Seed8_S9_S3	+/+	Seed8_S22_S1	-/-
Seed8_S9_S4	+/+	Seed8_S22_S2	-/-
Seed8_S10_S1	-/-	Seed8_S23_S1	-/+
Seed8_S10_S2	-/-	Seed8_S23_S2	-/-
Seed8_S11_S1	+/+	Seed8_S23_S3	-/+
Seed8_S11_S2	-/+	Seed8_S23_S4	-/-
Seed8_S11_S3	+/+	Seed8_S24_S1	-/+
Seed8_S11_S4	+/+	Seed8_S24_S2	-/+
Seed8_S12_S1	-/+	Seed8_S24_S3	-/-
Seed8_S12_S2	-/-	Seed8_S24_S4	+/+
Seed8_S12_S3	-/+	Seed8_SA_S1	+/+
Seed8_S12_S4	-/+	Seed8_SA_S2	+/+

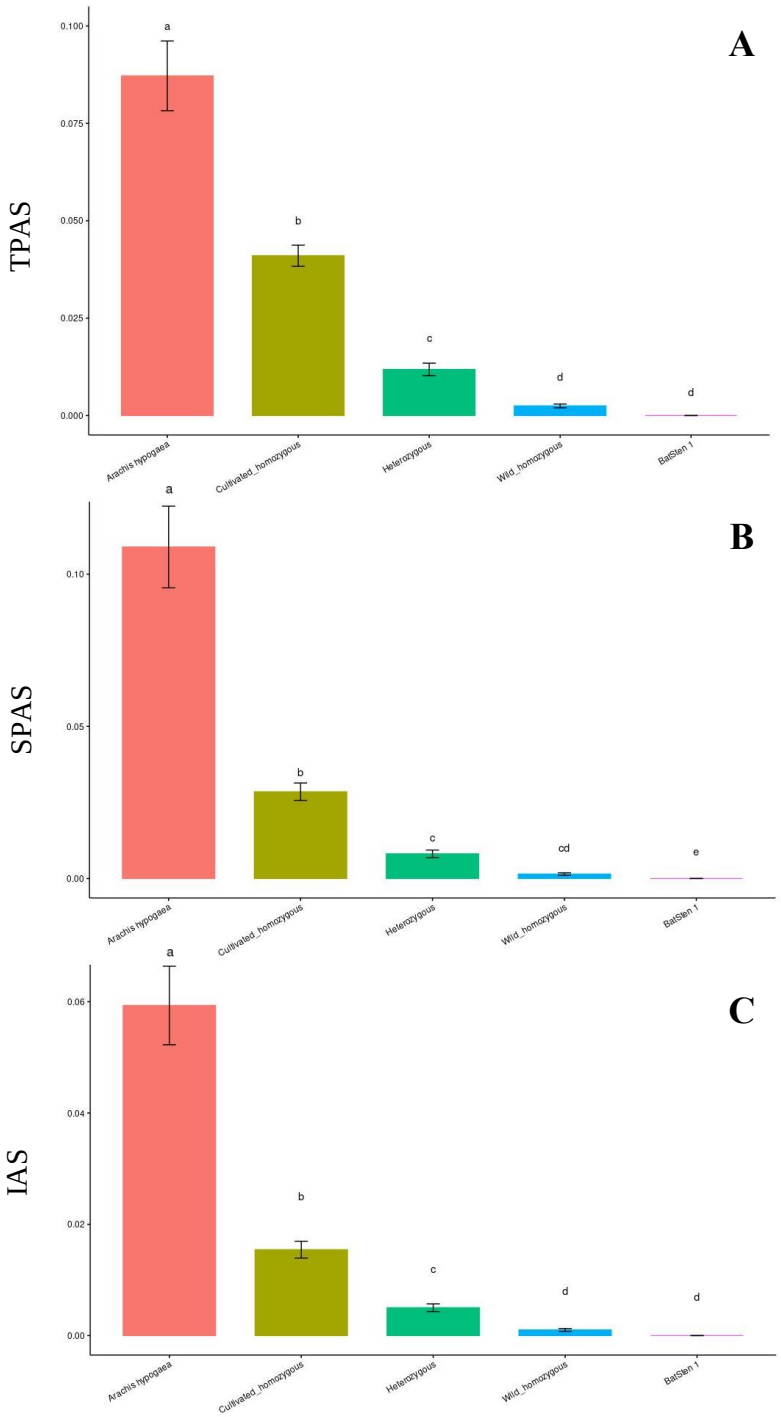

Figure 2.8. Averages of susceptibility parameters for Seed8 genotypes. **A)** Standardized total pustules per area (TPAS), **B)** Standardized sporulated pustules per area (SPAS) and **C)** standardized Index/area (IAS) of susceptible *Arachis hypogaea* cultivars, Seed8 individuals without the wild B02 segment (Cultivated_homozygous), with the segment in single js (heterozygous) and in double doses (Wild_homozygous), and the progenitor induced allotetraploid BatSten1. Error bars represent one standard error. Grouping done with the Wilcoxon test.

Table 2.12. Pearson's Correlation for all genotypes comparing the presence or absence of the wild segment on LG B02 using results from both Axiom_Arachis v 2.0 array and KASP. [a] IAS (Standardized Index/Area (cm^2)), TPAS (Standardized Total Pustules/Area (cm^2)), SPAS (Standardized Sporulating Pustules/Area (cm^2)), IPS (Standardized Incubation Periods (days))
* df=180, $\propto$ = 0.001.

Trait[a]	Correlation Coefficient
IAS	-0.285*
TPAS	-0.348*
SPAS	-0.326*
IPS	0.364*

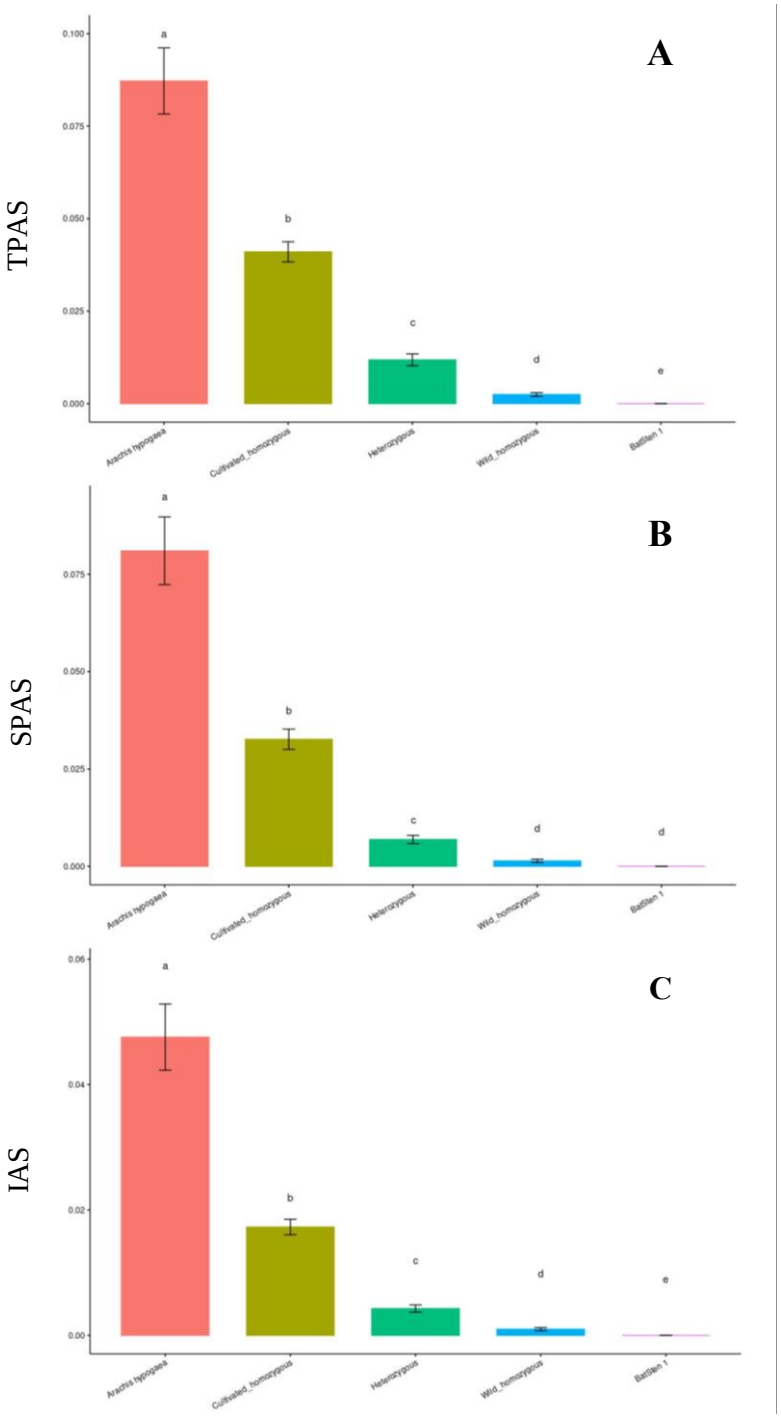

Figure 2.9. Averages of susceptibility parameters for all genotypes. **A)** Standardized total pustules per area (TPAS), **B)** Standardized sporulated pustules per area (SPAS) and **C)** standardized Index/area (IAS) of susceptible *Arachis hypogaea* cultivars, individuals from all three families without the wild B02 segment (Cultivated_homozygous), with the segment in single doses (heterozygous) and in double doses (Wild_homozygous), and the progenitor induced allotetraploid BatSten1. Error bars represent one standard error. Grouping done with the Wilcoxon test.

LITERATURE CITED

Andrade, C. (2021). Z Scores, Standard Scores, and Composite Test Scores Explained. *Indian Journal of Psychological Medicine, 43*(6), 555-557. https://doi.org/10.1177/02537176211046525

Ballén-Taborda. (2019). *Exploration of Arachis stenosperma as a source of nematode resistance for peanut.* [University of Georgia]. https://esploro.libs.uga.edu/esploro/outputs/doctoral/Exploration-of-Arachis-stenosperma-as-a/9949365657502959#file-0

Ballén-Taborda, C., Chu, Y., Ozias-Akins, P., Holbrook, C. C., Timper, P., Jackson, S. A., Bertioli, D. J., & Leal-Bertioli, S. C. (2021a). Development and genetic characterization of peanut advanced backcross lines that incorporate root-knot nematode resistance from *Arachis stenosperma*. *Frontiers in Plant Science, 12*, 785358-785358.

Ballén-Taborda, C., Chu, Y., Ozias-Akins, P., Timper, P., Holbrook, C. C., Jackson, S. A., Bertioli, D. J., & Leal-Bertioli, S. C. M. (2019). A new source of root-knot nematode resistance from *Arachis stenosperma* incorporated into allotetraploid peanut (*Arachis hypogaea*). *Scientific Reports, 9*(1), 17702. https://doi.org/10.1038/s41598-019-54183-1

Ballén-Taborda, C., Chu, Y., Ozias-Akins, P., Timper, P., Jackson, S. A., Bertioli, D. J., & Leal-Bertioli, S. C. M. (2021b). Validation of resistance to root-knot nematode incorporated in peanut from the wild relative *Arachis stenosperma*. *Agronomy Journal*, *113*(3), 2293-2302. https://doi.org/https://doi.org/10.1002/agj2.20654

Bera, S. K., Manohar, S. S., Variath, M. T., Chaudhari, S., Yaduru, S., Thankappan, R., Narayana, M., Kurapati, S., Pandey, M. K., & Sudini, H. K. (2018). Assessing variability for disease

resistance and nutritional quality traits in an interspecific collection of groundnut (*Arachis hypogaea*). *Plant Breeding*, *137*(6), 883-894.

Bertioli, D.J., Bertioli S.C. (2022). Unpublished Data.

Bertioli, D. J., Cannon, S. B., Froenicke, L., Huang, G., Farmer, A. D., Cannon, E. K. S., Liu, X., Gao, D., Clevenger, J., Dash, S., Ren, L., Moretzsohn, M. C., Shirasawa, K., Huang, W., Vidigal, B., Abernathy, B., Chu, Y., Niederhuth, C. E., Umale, P., Araújo, A. C. G., Kozik, A., Do Kim, K., Burow, M. D., Varshney, R. K., Wang, X., Zhang, X., Barkley, N., Guimarães, P. M., Isobe, S., Guo, B., Liao, B., Stalker, H. T., Schmitz, R. J., Scheffler, B. E., Leal-Bertioli, S. C. M., Xun, X., Jackson, S. A., Michelmore, R., & Ozias-Akins, P. (2016). The genome sequences of *Arachis duranensis* and *Arachis ipaënsis*, the diploid ancestors of cultivated peanut. *Nature Genetics*, *48*(4), 438-446. https://doi.org/10.1038/ng.3517

Bertioli, D. J., Clevenger, J., Godoy, I. J., Stalker, H. T., Wood, S., Santos, J. F., Ballén-Taborda, C., Abernathy, B., Azevedo, V., Campbell, J., Chavarro, C., Chu, Y., Farmer, A. D., Fonceka, D., Gao, D., Grimwood, J., Halpin, N., Korani, W., Michelotto, M. D., Ozias-Akins, P., Vaughn, J., Youngblood, R., Moretzsohn, M. C., Wright, G. C., Jackson, S. A., Cannon, S. B., Scheffler, B. E., & Leal-Bertioli, S. C. M. (2021a). Legacy genetics of *Arachis cardenasii* in the peanut crop shows the profound benefits of international seed exchange. *Proceedings of the National Academy of Sciences*, *118*(38), e2104899118. https://doi.org/doi:10.1073/pnas.2104899118

Bertioli, D. J., Gao, D., Ballén-Taborda, C., Chu, Y., Ozias-Akins, P., Jackson, S. A., Holbrook, C. C., & Leal-Bertioli, S. C. M. (2021b). Registration of GA-BatSten1 and GA-MagSten1, two induced allotetraploids derived from peanut wild relatives with superior resistance to

leaf spots, rust, and root-knot nematode. *Journal of Plant Registrations*, *15*(2), 372-378. https://doi.org/https://doi.org/10.1002/plr2.20133

Bertioli, D. J., Jenkins, J., Clevenger, J., Dudchenko, O., Gao, D., Seijo, G., Leal-Bertioli, S. C. M., Ren, L., Farmer, A. D., Pandey, M. K., Samoluk, S. S., Abernathy, B., Agarwal, G., Ballén-Taborda, C., Cameron, C., Campbell, J., Chavarro, C., Chitikineni, A., Chu, Y., Dash, S., El Baidouri, M., Guo, B., Huang, W., Kim, K. D., Korani, W., Lanciano, S., Lui, C. G., Mirouze, M., Moretzsohn, M. C., Pham, M., Shin, J. H., Shirasawa, K., Sinharoy, S., Sreedasyam, A., Weeks, N. T., Zhang, X., Zheng, Z., Sun, Z., Froenicke, L., Aiden, E. L., Michelmore, R., Varshney, R. K., Holbrook, C. C., Cannon, E. K. S., Scheffler, B. E., Grimwood, J., Ozias-Akins, P., Cannon, S. B., Jackson, S. A., & Schmutz, J. (2019). The genome sequence of segmental allotetraploid peanut *Arachis hypogaea*. *Nature Genetics*, *51*(5), 877-884. https://doi.org/10.1038/s41588-019-0405-z

Bertioli, D. J., Seijo, G., Freitas, F. O., Valls, J. F. M., Leal-Bertioli, S. C. M., & Moretzsohn, M. C. (2011). An overview of peanut and its wild relatives. *Plant Genetic Resources*, *9*(01), 134-149. https://doi.org/10.1017/s1479262110000444

Chu, Y., Bertioli, D., Levinson, C. M., Stalker, H. T., Holbrook, C. C., & Ozias-Akins, P. (2021). Homoeologous recombination is recurrent in the nascent synthetic allotetraploid *Arachis ipaënsis* × *Arachis correntina*^{4x} and its derivatives. *G3 Genes|Genomes|Genetics*, *11*(4). https://doi.org/10.1093/g3journal/jkab066

Culbreath, A., Stevenson, K., & Brenneman, T. (2002). Management of late leaf spot of peanut with benomyl and chlorothalonil: A study in preserving fungicide utility. *Plant Disease*, *86*(4), 349-355.

Daudi, H., Shimelis, H., Mathew, I., Rathore, A., & Ojiewo, C. O. (2021). Combining ability and gene action controlling rust resistance in groundnut (*Arachis hypogaea* L.). *Scientific Reports*, *11*(1), 16513. https://doi.org/10.1038/s41598-021-96079-z

Dorzin, P. (2022). Personal Communication.

Fávero, A., Valls, J. (1998). Caracterização morfológica, citogenética e molecular de acessos de germoplasma de *Arachis kuhlmannii* Krap. & Greg.(Leguminosae). Embrapa Recursos Genéticos e Biotecnologia-Resumo em anais de congresso (ALICE),

Fávero, A. P., Moraes, S. A. d., Garcia, A. A. F., Valls, J. F. M., & Vello, N. A. (2009). Characterization of rust, early and late leaf spot resistance in wild and cultivated peanut germplasm. *Scientia Agricola*, *66*(1), 110-117.

Fávero, A. P., Santos, R. F. d., Simpson, C. E., Valls, J. F. M., & Vello, N. A. (2015). Successful crosses between fungal-resistant wild species of *Arachis* (section *Arachis*) and *Arachis hypogaea*. *Genetics and Molecular Biology*, *38*, 353-365.

Fávero, A. P., Simpson, C. E., Valls, J. F. M., & Vello, N. A. (2006). Study of the Evolution of Cultivated Peanut through Crossability Studies among *Arachis ipaënsis, A. duranensis*, and *A. hypogaea*. *Crop Science*, *46*(4), 1546-1552. https://doi.org/https://doi.org/10.2135/cropsci2005.09-0331

Fisher, R. A. (1936). Design of experiments. *British Medical Journal*, *1*(3923), 554.

Francis, M., Chacha, M., Mbega, E., & Ndakidemi, P. (2020). Groundnut rust disease epidemiology and potential sustainable management strategies. *International Journal of Bioscience*, *16*, 524-536.

Gao, D., Araujo, A. C. G., Nascimento, E. F. M. B., Chavarro, M. C., Xia, H., Jackson, S. A., Bertioli, D. J., & Leal-Bertioli, S. C. M. (2021). ValSten: a new wild species derived

allotetraploid for increasing genetic diversity of the peanut crop (*Arachis hypogaea* L.). *Genetic Resources and Crop Evolution*, *68*(4), 1471-1485. https://doi.org/10.1007/s10722-020-01076-2

Gowda, M., Motagi, B., Naidu, G., Diddimani, S., & Sheshagiri, R. (2002). GPBD 4: a spanish bunch groundnut genotype resistant to rust and late leaf spot. *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)*.

Halward, T., Stalker, T., LaRue, E., & Kochert, G. (1992). Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). *Plant Molecular Biology*, *18*(2), 315-325. https://doi.org/10.1007/bf00034958

Hammons, R. (1977). Groundnut rust in the United States and the Caribbean. *Pans*, *23*(3), 300-304.

Hoffmann, M., Geulen, O., & Weilke, C. (2008). The LightCycler® 480 real-time PCR system: a versatile platform for genetic variation research. *Nature Methods*, *5*(3), i-ii. https://doi.org/10.1038/nmeth.f.207

Holbrook, C. C. (2016). Personal communication.

Holbrook, C. C., Dong, W., Timper, P., Culbreath, A. K., & Kvien, C. K. (2012). Registration of peanut germplasm line TifGP-2, a nematode-susceptible sister line of 'Tifguard'. *Journal of Plant Registrations*, *6*(2), 208-211.

Holbrook, C. C., Stalker, H. T., & Janick, J. (2002). Peanut breeding and Genetic Resources. . In: J. Janick (ed.). *Plant Breeding Reviews*, *22*, 297-356.

Husted, L. (1936). Cytological studies on the peanut, *Arachis*. II Chromosome number, morphology and behavior, and their application to the problem of the origin of the cultivated forms. *Cytologia*, *7*(3), 396-423.

Kassambara, A. (2019). *ggcorrplot: Visualization of a Correlation Matrix using 'ggplot2'*. In https://CRAN.R-project.org/package=ggcorrplot

Khedikar, Y., Gowda, M., Sarvamangala, C., Patgar, K., Upadhyaya, H., & Varshney, R. (2010). A QTL study on late leaf spot and rust revealed one major QTL for molecular breeding for rust resistance in groundnut (*Arachis hypogaea* L.). *Theoretical and applied genetics*, *121*(5), 971-984.

Knauft, D. A., & Ozias-Akins, P. (1995). Recent methodologies for germplasm enhancement and breeding. *Advances in peanut science*, 54-94.

Kochert, G., Stalker, H. T., Gimenes, M., Galgaro, L., Lopes, C. R., & Moore, K. (1996). RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, *Arachis hypogaea* (Leguminosae). *American Journal of Botany*, *83*(10), 1282-1291.

Korani, W., Clevenger, J. P., Chu, Y., & Ozias-Akins, P. (2019). Machine learning as an effective method for identifying true single nucleotide polymorphisms in polyploid plants. *The Plant Genome*, *12*(1), 180023. https://doi.org/https://doi.org/10.3835/plantgenome2018.05.0023

Krapovickas, A., & Gregory, W. C. (1994). Taxonomia del genero "*Arachis* (Leguminosae)". *Bonplandia*, *8*(1/4), 1-186. http://www.jstor.org/stable/41941177

Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. *Journal of the American statistical Association*, *47*(260), 583-621.

Leal-Bertioli, S. C., Cavalcante, U., Gouvea, E. G., Ballén-Taborda, C., Shirasawa, K., Guimarães, P. M., Jackson, S. A., Bertioli, D. J., & Moretzsohn, M. C. (2015a). Identification of QTLs for rust resistance in the peanut wild species *Arachis magna* and the development of KASP markers for marker-assisted selection. *G3: Genes, Genomes, Genetics*, *5*(7), 1403-1413.

Leal-Bertioli, S. C. M., Nascimento, E. F. M. B., Chavarro, M. C. F., Custódio, A. R., Hopkins, M. S., Moretzsohn, M. C., Bertioli, D. J., & Araújo, A. C. G. (2021). Spontaneous generation of diversity in *Arachis* neopolyploids (*Arachis ipaënsis* × *Arachis duranensis*)^{4x} replays the early stages of peanut evolution. *G3 Genes|Genomes|Genetics*, *11*(11). https://doi.org/10.1093/g3journal/jkab289

Leal-Bertioli, S. C. M., Santos, S. P., Dantas, K. M., Inglis, P. W., Nielen, S., Araujo, A. C. G., Silva, J. P., Cavalcante, U., Guimarães, P. M., Brasileiro, A. C. M., Carrasquilla-Garcia, N., Penmetsa, R. V., Cook, D., Moretzsohn, M. C., & Bertioli, D. J. (2015b). *Arachis batizocoi:* a study of its relationship to cultivated peanut (*A. hypogaea*) and its potential for introgression of wild genes into the peanut crop using induced allotetraploids. *Annals of Botany*, *115*(2), 237-249. https://doi.org/10.1093/aob/mcu237

Leal-Bertioli, S. C., Godoy, I. J., Santos, J. F., Doyle, J. J., Guimarães, P. M., Abernathy, B. L., Jackson, S. A., Moretzsohn, M. C., & Bertioli, D. J. (2018). Segmental allopolyploidy in action: increasing diversity through polyploid hybridization and homoeologous recombination. *American Journal of Botany*, *105*(6), 1053-1066.

Levinson, C. M., Antepenko, E., Leal-Bertioli, S., Chu, Y., Culbreath, A. K., Stalker, H. T., Gao, D., & Ozias-Akins, P. (2021). Resistance to rust (*Puccinia arachidis* Speg.) identified in nascent allotetraploids cross-compatible with cultivated peanut (*Arachis hypogaea* L.). *Peanut Science*, *48*(2), 123-130.

Mace, E., Phong, D., Upadhyaya, H., Chandra, S., & Crouch, J. (2006). SSR analysis of cultivated groundnut (*Arachis hypogaea* L.) germplasm resistant to rust and late leaf spot diseases. *Euphytica*, *152*(3), 317-330.

Mendiburu, F. d. (2021). *agricolae: Statistical Procedures for Agricultural Research*. In (Version R package version 1.3-5) https://CRAN.R-project.org/package=agricolae

Mondal, S., & Badigannavar, A. (2010). Molecular diversity and association of SSR markers to rust and late leaf spot resistance in cultivated groundnut (*Arachis hypogaea* L.). *Plant Breeding*, *129*(1), 68-71.

Mondal, S., & Badigannavar, A. (2015). Peanut rust (*Puccinia arachidis* Speg.) disease: its background and recent accomplishments towards disease resistance breeding. *Protoplasma*, *252*(6), 1409-1420.

Mondal, S., Badigannavar, A., & D'Souza, S. (2012). Development of genic molecular markers linked to a rust resistance gene in cultivated groundnut (*Arachis hypogaea* L.). *Euphytica*, *188*(2), 163-173.

Mondal, S., Hande, P., & Badigannavar, A. M. (2014). Identification of transposable element markers for a rust (*Puccinia arachidis* Speg.) resistance gene in cultivated peanut. *Journal of Phytopathology*, *162*(7-8), 548-552.

Moraes, S. d. A., & Salgado, C. (1982). Utilização da técnica de folhas destacadas de amendoim (*Arachis hypogaea* L.) para inoculações com *Cercospora arachidicola* Hori e *Cercospora personata* (Bert. & Curt.) Ell. & Ev. *Summa Phytopathologica*, *8*, 39-55.

Moretzsohn, M. C., Gouvea, E. G., Inglis, P. W., Leal-Bertioli, S. C., Valls, J. F., & Bertioli, D. J. (2013). A study of the relationships of cultivated peanut (*Arachis hypogaea*) and its most closely related wild species using intron sequences and microsatellite markers. *Annals of Botany*, *111*(1), 113-126.

Moss, J., Singh, A., Nigam, S., Hildebrand, G., Govinden, N., Ismael, F., Subrahmanyam, P., & Reddy, L. (1998). Registration of ICGV-SM 86715 peanut germplasm. *Crop Science, 38*(2), 572-572.

Moss, J. P., Singh, A. K., Reddy, L. J., Nigam, S. N., Subrahmanyam, P., McDonald, D., & Reddy, A. G. S. (1997). Registration of ICGV 87165 peanut germplasm line with multiple resistance. *Crop Science*, *37*(3), cropsci1997.0011183X003700030089x. https://doi.org/https://doi.org/10.2135/cropsci1997.0011183X003700030089x

Nguepjop, J. R., Tossim, H.-A., Bell, J. M., Rami, J.-F., Sharma, S., Courtois, B., Mallikarjuna, N., Sane, D., & Fonceka, D. (2016). Evidence of genomic exchanges between homeologous chromosomes in a cross of peanut with newly synthetized allotetraploid hybrids [Original Research]. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.01635

Nigam, S., Hildebrand, G., Bock, K., Ismael, F., Govinden, N., Subrahmanyam, P., & Reddy, L. (1998). Registration of ICGV-SM 85048 peanut germplasm. *Crop Science*, *38*(2), 572-573.

Pearson, K. (1895). Notes on regression and inheritance in the case of two parents. *Proceedings of the Royal Society of London,* 58, 240-242.

Pohlert, T. (2022). *PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended.* In (Version 1.9.4) https://CRAN.R-project.org/package=PMCMRplus

Reddy, L., Nigam, S., Moss, J., Singh, A., Subrahmanyam, P., McDonald, D., & Reddy, A. (1996). Registration of ICGV 86699 peanut germplasm line with multiple disease and insect resistance. *Crop Science*, *36*(3), 821-821.

Robledo, G., Lavia, G., & Seijo, G. (2009). Species relations among wild *Arachis* species with the A genome as revealed by FISH mapping of rDNA loci and heterochromatin detection. *Theoretical and Applied Genetics, 118*(7), 1295-1307.

Savary, S., Rao, P. S., & Zadoks, J. (1989). A scale of reaction types of groundnut to *Puccinia arachidi*s Speg. *Journal of Phytopathology, 124*(3), 259-266.

Semagn, K., Babu, R., Hearne, S., & Olsen, M. (2014). Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. *Molecular breeding, 33*(1), 1-14.

Shapiro, S. S., & Wilk, M. (1972). An analysis of variance test for the exponential distribution (complete samples). *Technometrics, 14*(2), 355-370.

Singh, A. (1986). Utilization of wild relatives in the genetic improvement of *Arachis hypogaea* L. *Theoretical and Applied Genetics, 72*(4), 433-439.

Singh, A., Dwivedi, S., Pande, S., Moss, J., Nigam, S., & Sastri, D. (2003). Registration of rust and late leaf spot resistant peanut germplasm lines. *Crop Science, 43*(1), 440-441.

Skillings, J. H., & Mack, G. A. (1981). On the use of a Friedman-type statistic in balanced and unbalanced block designs. *Technometrics, 23*(2), 171-177.

Stalker, H. T. (2017). Utilizing wild species for peanut improvement. *Crop Science, 57*(3), 1102-1120. https://doi.org/https://doi.org/10.2135/cropsci2016.09.0824

Subrahmanyam, P., Hammons, R., Nigam, S., McDonald, D., Gibbons, R., Fan, M.-Y., & Yeh, W.-L. (1983a). International cooperative screening for resistance of peanut to rust and late leaf spot. *Plant Disease, 67*(10), 1108-1111.

Subrahmanyam, P., Kokalis-Burelle, N., Porter, D., Rodriguez-Kabana, R., & Smith, D. (1997). *Compendium of peanut diseases* (Vol. 2). American Phytopathological Society.

Subrahmanyam, P., McDonald, D., Gibbons, R., & Rao, P. S. (1983b). Components of resistance to *Puccinia arachidis* in peanuts. *Phytopathology*, *73*(2), 253-256.

Subrahmanyam, P., Moss, J., McDonald, D., Rao, P. S., & Rao, V. (1985a). Resistance to leaf spot caused by *Cercosporidium personatum* in wild *Arachis* species. *Plant Disease*, *69*(11), 951-954.

Subrahmanyam, P., Reddy, L., Gibbons, R., & McDonald, D. (1985b). Peanut rust: a major threat to peanut production in the semiarid tropics. *Plant Disease*, *69*(9), 813-819.

Sujay, V., Gowda, M., Pandey, M., Bhat, R., Khedikar, Y., Nadaf, H., Gautami, B., Sarvamangala, C., Lingaraju, S., & Radhakrishan, T. (2012). Quantitative trait locus analysis and construction of consensus genetic map for foliar disease resistance based on two recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). *Molecular breeding*, *30*(2), 773-788.

Tallury, S., Hilu, K., Milla, S., Friend, S., Alsaghir, M., Stalker, H., & Quandt, D. (2005). Genomic affinities in *Arachis* section *Arachis* (Fabaceae): molecular and cytogenetic evidence. *Theoretical and Applied Genetics*, *111*(7), 1229-1237.

Van Ooijen, J. (2006). JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations. *Kyazma BV, Wageningen*, *33*(10.1371).

Vindhiya Varman, P. (1999). A foliar disease resistant line developed through interspecific hybridization in groundnut (*Arachis hypogaea*). *Indian Journal of Agricultural Science*, *69*(1), 67-68.

Woolson, R. F. (2007). Wilcoxon signed-rank test. *Wiley encyclopedia of clinical trials*, 1-3.

You, F. M., Huo, N., Gu, Y. Q., Luo, M.-c., Ma, Y., Hane, D., Lazo, G. R., Dvorak, J., & Anderson, O. D. (2008). BatchPrimer3: a high throughput web application for PCR and sequencing primer design. *BMC Bioinformatics*, *9*(1), 1-13.

SUPPLEMENTAL SCRIPTS

Script 2.S1. Unix script for filtering of SNP genotyping data from extracted Thermofisher "Axiom_Arachis" v 2.0 SNP genotyping results using Axiom Analysis Suite 2.0. Script written by Ballén-Taborda et al. (Ballén-Taborda et al., 2019).

#Input file:

Extracted_Affy.txt

#Filtering and replacing results by identifying cultivated alleles (A. hypogaea Runner 886), wild alleles (BatSten1), and heterozygous alleles. First filtering for markers (column2) on the A-subgenome ("Sten") and then filtering for markers on the B-subgenome ("Bat").

BatSten1 (columns 3 and 4) ≠ Runner 886 (columns 9 and 10)

AA ≠ AB

AA ≠ BB

AB ≠ AA

AB ≠ BB

BB ≠ AA

BB ≠ AB

```
awk '{if($2=="Sten" && $3=="AA" && $4=="AA" && $9== "AB" && $10== "AB")
print $0}' Extracted_Affy.txt | perl -lpe "s/AA/sten2/g" | perl -lpe "s/AB/-/g" | perl -lpe
"s/BB/sten1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_sten1.txt &
```

```
awk '{if($2=="Sten" && $3=="AA" && $4=="AA" && $9== "BB" && $10== "BB")
print $0}' Extracted_Affy.txt | perl -lpe "s/AA/sten2/g" | perl -lpe "s/BB/-/g" | perl -lpe
"s/AB/sten1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_sten2.txt &
awk '{if($2=="Sten" && $3=="AB" && $4=="AB" && $9== "AA" && $10== "AA")
print $0}' Extracted_Affy.txt | perl -lpe "s/AB/sten2/g" | perl -lpe "s/AA/-/g" | perl -lpe
"s/BB/sten1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_sten3.txt &
awk '{if($2=="Sten" && $3=="AB" && $4=="AB" && $9== "BB" && $10== "BB")
print $0}' Extracted_Affy.txt | perl -lpe "s/AB/sten2/g" | perl -lpe "s/BB/-/g" | perl -lpe
"s/AA/sten1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_sten4.txt &
awk '{if($2=="Sten" && $3=="BB" && $4=="BB" && $9== "AA" && $10== "AA")
print $0}' Extracted_Affy.txt | perl -lpe "s/BB/sten2/g" | perl -lpe "s/AA/-/g" | perl -lpe
"s/AB/sten1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_sten5.txt &
awk '{if($2=="Sten" && $3=="BB" && $4=="BB" && $9== "AB" && $10== "AB")
print $0}' Extracted_Affy.txt | perl -lpe "s/BB/sten2/g" | perl -lpe "s/AB/-/g" | perl -lpe
"s/AA/sten1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_sten6.txt

awk '{if($2=="Bat" && $3=="AA" && $4=="AA" && $9== "AB" && $10== "AB")
print $0}' Extracted_Affy.txt | perl -lpe "s/AA/bat2/g" | perl -lpe "s/AB/-/g" | perl -lpe
"s/BB/bat1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_bat1.txt &
awk '{if($2=="Bat" && $3=="AA" && $4=="AA" && $9== "BB" && $10== "BB")
print $0}' Extracted_Affy.txt | perl -lpe "s/AA/bat2/g" | perl -lpe "s/BB/-/g" | perl -lpe
"s/AB/bat1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_bat2.txt &
```

```
awk '{if($2=="Bat" && $3=="AB" && $4=="AB" && $9== "AA" && $10== "AA")
print $0}' Extracted_Affy.txt | perl -lpe "s/AB/bat2/g" | perl -lpe "s/AA/-/g" | perl -lpe
"s/BB/bat1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_bat3.txt &
awk '{if($2=="Bat" && $3=="AB" && $4=="AB" && $9== "BB" && $10== "BB")
print $0}' Extracted_Affy.txt | perl -lpe "s/AB/bat2/g" | perl -lpe "s/BB/-/g" | perl -lpe
"s/AA/bat1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_bat4.txt &
awk '{if($2=="Bat" && $3=="BB" && $4=="BB" && $9== "AA" && $10== "AA")
print $0}' Extracted_Affy.txt | perl -lpe "s/BB/bat2/g" | perl -lpe "s/AA/-/g" | perl -lpe
"s/AB/bat1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_bat5.txt &
awk '{if(($2=="Bat" && $3=="BB" && $4=="BB" && $9== "AB" && $10== "AB")
print $0}' Extracted_Affy.txt | perl -lpe "s/BB/bat2/g" | perl -lpe "s/AB/-/g" | perl -lpe
"s/AA/bat1/g" | perl -lpe "s/NoCall/-/g" > Extracted_Affy_bat6.txt
```

#Concatenate files

```
head -7 Extracted_Affy.txt > Filtered_Affy.txt
cat Extracted_Affy_sten1.txt Extracted_Affy_sten2.txt Extracted_Affy_sten3.txt
Extracted_Affy_sten4.txt Extracted_Affy_sten5.txt Extracted_Affy_sten6.txt
Extracted_Affy_bat1.txt Extracted_Affy_bat2.txt Extracted_Affy_bat3.txt
Extracted_Affy_bat4.txt Extracted_Affy_bat5.txt Extracted_Affy_bat6.txt >>
Filtered_Affy.txt
```

SUPPLEMENTAL TABLES

Supplemental Table 2.S1. Average standardized results of Seed7 and Seed11 along with the presence or absence of the wild segment on LG B02 based on SNP genotyping by Thermofisher "Axiom_Arachis" v2.0 array and KASP. Genotypes with double doses of the wild segment on LG B02 are marked "2", genotypes with one dose of the wild segment on LG B02 are marked "1", and without the segment on LG B02 marker "0". IAS (Standardized Index/Area (cm^2)), TPAS (Standardized Total Pustules/Area (cm^2)), SPAS (Standardized Sporulating Pustules/Area (cm^2)), IPS (Standardized Incubation Periods (days)).

Seed7	Avg IAS	Avg TPAS	Avg SPAS	Avg IPS	LG B02	Seed11	Avg IAS	Avg TPAS	Avg SPAS	Avg IPS	LG B02
S1	0.036	0.036	0.036	0.036	0	S1	0.018	0.043	0.041	0.483	0
S2	0.084	0.084	0.084	0.084	0	S2	0	0	0	0	1
S3	0.085	0.085	0.085	0.085	-	S3	0	0	0	0	1
S4	0.454	0.454	0.454	0.454	0	S4	0	0	0	0	1
S5	0.021	0.021	0.021	0.021	0	S5	0	0	0	0	1
S6	0.047	0.047	0.047	0.047	0	S6	0	0	0	0	1
S7	0.050	0.050	0.050	0.050	0	S7	0	0	0	0	1
S8	0.511	0.511	0.511	0.511	0	S8	0	0	0	0	0
S9	0.026	0.026	0.026	0.026	0	S9	0	0	0	0	0
S10	0.062	0.062	0.062	0.062	0	S10	0	0	0	0	-
S11	0.062	0.062	0.062	0.062	-	S11	0	0	0	0	2
S12	0.511	0.511	0.511	0.511	0	S12	0	0	0	0	1
S13	0.077	0.077	0.077	0.077	0	S13	0.009	0.037	0.005	0.563	0
S14	0.143	0.143	0.143	0.143	0	S14	0	0	0	0	1
S15	0.156	0.156	0.156	0.156	0	S15	0	0	0	0	2
S16	0.443	0.443	0.443	0.443	0	S16	0	0	0	0	1
S17	0.014	0.014	0.014	0.014	0	S17	0	0	0	0	1
S18	0.036	0.036	0.036	0.036	0	S18	0	0	0	0	1
S19	0.029	0.029	0.029	0.029	0	S19	0	0	0	0	1
S20	0.510	0.510	0.510	0.510	0	S20	0	0	0	0	1
S21	0.030	0.030	0.030	0.030	0	S21	0.022	0.072	0.025	0.552	0
S22	0.068	0.068	0.068	0.068	0	S22	0	0	0	0	1
S23	0.075	0.075	0.075	0.075	0	S23	0	0	0	0	2
S24	0.441	0.441	0.441	0.441	0	S24	0.001	0.005	0	0.621	1

Supplemental Table 2.S2. Average standardized results of BC_3F_3 Seed8 along with the presence or absence of the wild segment on LG B02 based on SNP genotyping by Thermofisher "Axiom_Arachis" v2.0 array and KASP. Genotypes with double doses of the wild segment on LG B02 are marked "2", genotypes with one dose of the wild segment on LG B02 are marked "1", and without the segment on LG B02 marker "0". IAS (Standardized Index/Area (cm^2)), TPAS (Standardized Total Pustules/Area (cm^2)), SPAS (Standardized Sporulating Pustules/Area (cm^2)), IPS (Standardized Incubation Periods (days)).

Seed8 (BC_3F_3)	Avg IAS	Avg TPAS	Avg SPAS	Avg IPS	LG B02
S1	0.001	0.003	0.000	0.621	1
S2	0.000	0.000	0.000	0.000	2
S3	0.023	0.053	0.058	0.454	0
S4	0.043	0.095	0.107	0.391	0
S5	0.000	0.000	0.000	0.000	2
S6	0.000	0.000	0.000	0.000	1
S8	0.000	0.000	0.000	0.000	1
S9	0.000	0.000	0.000	0.000	2
S10	0.005	0.016	0.008	0.560	0
S11	0.000	0.000	0.000	0.000	1
S12	0.000	0.001	0.000	0.621	1
S13	0.005	0.013	0.009	0.586	2
S14	0.011	0.050	0.000	0.534	0
S15	0.001	0.002	0.000	1.000	2
S16	0.001	0.003	0.002	0.276	2
S17	0.000	0.000	0.000	0.000	1
S18	0.001	0.002	0.003	0.701	1
S19	0.000	0.000	0.000	0.000	2
S20	0.002	0.003	0.004	0.828	1
S22	0.007	0.022	0.012	0.506	0
S23	0.000	0.000	0.000	0.000	1
S24	0.015	0.052	0.018	0.626	1
S25	0.000	0.001	0.001	0.724	1
S26	0.005	0.014	0.012	0.590	1
S27	0.000	0.002	0.000	0.628	1
S29	0.003	0.007	0.006	0.607	1
S30	0.000	0.000	0.000	0.000	1
S31	0.012	0.024	0.026	0.373	0
S32	0.014	0.028	0.028	0.357	1
S34	0.000	0.000	0.000	0.000	2
S35	0.000	0.000	0.000	0.000	2
S36	0.010	0.017	0.017	0.345	0
S40	0.007	0.015	0.017	0.326	0
S41	0.015	0.035	0.031	0.502	1

Supplemental Table 2.S3. Average standardized results of BC_3F_4 Seed8 along with the presence or absence of the wild segment on LG B02 based on SNP genotyping by Thermofisher "Axiom_Arachis" v2.0 array and KASP. Genotypes with double doses of the wild segment on LG B02 are marked "2", genotypes with one dose of the wild segment on LG B02 are marked "1", and without the segment on LG B02 marker "0". IAS (Standardized Index/Area (cm^2)), TPAS (Standardized Total Pustules/Area (cm^2)), SPAS (Standardized Sporulating Pustules/Area (cm^2)), IPS (Standardized Incubation Periods (days)). *Seed8_SA_S1 and Seed8_SA_S2 were progenies of a Seed8 plant grown in Midville, GA.

Seed8	Avg IAS	Avg TPAS	Avg SPAS	Avg IPS	LG B02	Seed8	Avg IAS	Avg TPAS	Avg SPAS	Avg IPS	LG B02
S1_S1	0.033	0.086	0.065	0.431	0	S14_S2	0.006	0.016	0.012	0.483	0
S1_S2	0.008	0.025	0.011	0.644	1	S15_S1	0	0	0	0	2
S1_S3	0	0	0	0	2	S15_S2	0	0.001	0	0.552	2
S1_S4	0.005	0.021	0	0.667	1	S15_S3	0	0.001	0	0.759	2
S2_S1	0	0	0	0	2	S15_S4	0	0	0	0.759	2
S2_S2	0.004	0.017	0	0.655	2	S15_S5	0.001	0.004	0.002	0.603	2
S2_S3	0.001	0.002	0.001	0.664	2	S15_S6	0	0	0	0	2
S2_S4	0	0.002	0	0.678	2	S16_S1	0	0	0	0	2
S2_S5	0	0	0	0	2	S16_S2	0	0	0	0	2
S3_S1	0.002	0.005	0.005	0.431	0	S16_S3	0.001	0.006	0	0.759	2
S3_S2	0.006	0.023	0.004	0.631	0	S16_S4	0.001	0.002	0.001	0.759	2
S4_S1	0.009	0.022	0.02	0.462	0	S17_S1	0.025	0.048	0.053	0.317	1
S4_S2	0.007	0.02	0.011	0.493	0	S17_S2	0.061	0.094	0.101	0.305	0
S5_S1	0	0	0	0	2	S17_S3	0.03	0.047	0.051	0.334	2
S5_S2	0	0	0	0	2	S17_S4	0.021	0.049	0.04	0.321	1
S5_S3	0	0	0	0	2	S18_S1	0.001	0.002	0.001	0.552	1
S5_S4	0.014	0.033	0.035	0.494	2	S18_S2	0.004	0.015	0.006	0.345	1
S5_S7	0	0.002	0.001	0.655	2	S18_S3	0	0	0	0	2
S5_S8	0.005	0.008	0.008	0.483	2	S18_S4	0	0	0	0	2
S6_S1	0	0	0	0	2	S18_S5	0.008	0.027	0.009	0.494	1
S6_S2	0.001	0.006	0	0.621	1	S18_S6	0.011	0.02	0.02	0.435	0
S6_S3	0.002	0.01	0	0.648	1	S19_S1	0	0	0	0	2
S6_S4	0.003	0.013	0.001	0.621	2	S19_S2	0.001	0.004	0.001	0.69	2
S8_S1	0	0	0	0	0	S19_S3	0	0	0	0	2
S8_S2	0	0	0	0	2	S19_S4	0	0	0	0	2
S8_S3	0.009	0.023	0.018	0.543	1	S19_S5	0	0	0	0	2
S8_S4	0.001	0.004	0.002	0.517	1	S19_S6	0	0	0	0	2
S8_S8	0.021	0.038	0.042	0.466	0	S20_S1	0	0	0	0	1
S9_S1	0	0	0	0	2	S20_S2	0	0	0	0	1
S9_S2	0	0	0	0.759	2	S20_S3	0	0	0	0	2
S9_S3	0	0	0	0	2	S20_S4	0	0	0	0	2
S9_S4	0	0	0	0.276	2	S21_S1	0	0	0	0	1
S10_S1	0.012	0.031	0.023	0.507	0	S21_S2	0	0	0	0	1
S10_S2	0.03	0.082	0.044	0.362	0	S21_S3	0	0	0	0	1
S11_S1	0	0	0	0	2	S21_S4	0.001	0.002	0.001	0.552	1
S11_S2	0.005	0.017	0.005	0.431	1	S22_S1	0.008	0.017	0.018	0.391	0
S11_S3	0.002	0.007	0	0.759	2	S22_S2	0.014	0.031	0.033	0.409	0
S11_S4	0	0	0	0	2	S23_S1	0	0	0	0	1
S12_S1	0.002	0.003	0.003	0.328	1	S23_S2	0.022	0.044	0.041	0.348	0
S12_S2	0.03	0.064	0.051	0.388	0	S23_S3	0.018	0.053	0.029	0.559	1
S12_S3	0.008	0.028	0.006	0.628	1	S23_S4	0.012	0.02	0.022	0.471	0
S12_S4	0.006	0.02	0.007	0.466	1	S24_S1	0.005	0.019	0.004	0.31	1
S13_S1	0	0.001	0	0.759	2	S24_S2	0.002	0.005	0.003	0.54	1
S13_S2	0	0	0	0	2	S24_S3	0.014	0.029	0.027	0.479	0
S13_S3	0	0	0	0	2	S24_S4	0	0	0	0	2
S13_S4	0	0.001	0	0.759	2	SA_S1*	0.001	0.001	0.001	1	2
S14_S1	0.01	0.025	0.021	0.414	0	SA_S2*	0	0	0	0	2

Supplemental Table 2.S4. Preliminary results from rust field trials in Haiti (Dorzin, 2022). The F_1 and F_2 generations of most of these families were genotyped using the Thermofisher "Axiom_Arachis" v 2.0 SNP Array by Ballén-Taborda et al. (Ballén-Taborda et al., 2019). Plants were rated in the field for the presence of rust using a scale from 1-9, with one being no disease, and nine being an almost completely dead and defoliated plant. 93B and 93C were not included in the Axiom_Arachis v 2.0 array. [a] Families with rust resistance derived from A. cardenasii [b] Families from the marker-assisted backcrossing scheme (MABS) created for root-knot nematode resistance [c] 216C did not have the segment by BC_3F_2, so the plants grown in Haiti did not have the segment [d] 93B and 93C were not included in the Axiom_Arachis v 2.0 array

		Segment on LG B02	
Controls	**Rust Rating**	**BC_3F_1**	**BC_3F_2**
Runner GA 06G	7.25	no	no
Georgia Green	7.5	no	no
***A. Cardenasii* families[a]**			
IAC-321	2.75	no	no
IAC-322	2.75	no	no
MABS families (BC_3F_4)[b]			
14D	7	no	no
107B	7.25	no	no
214B	7.75	no	no
216C	7	yes	no[c]
226C	1	yes	yes
93B	1.75	yes	-[d]
93C	3.25	yes	-[d]

CHAPTER 3

CONCLUSIONS

Peanut diseases like *Puccinia arachidis* cause economic damage to farms around the world. In countries where peanut is an essential staple of day-to day life, loss of the crop can be devastating. Yet the same places that rely on peanut in this way often don't have access to the most effective chemical controls. Creating lines of pathogen-resistant plants can circumvent these problems and allow farmers to apply fewer fungicides or other methods of control. Using wild peanut species to introgress this resistance can be more difficult than breeding cultivated lines together, but the results are worth it. We decided to screen lines from the marker-assisted backcrossing scheme (MABS) because the wild parents harbor resistance to *P. arachidis*, and because the population already has confirmed resistance to root knot nematode (RKN). The two BC_3 lines with resistance to rust that also have wild-derived RKN resistance will be even more useful in future breeding programs. In order to continue forward with a breeding program to introgress rust and RKN resistance into a new line, more work has to be done to characterize the Seed7 and Seed8 lines. Along with phenotypic characteristics such as seed size and pod morphology, the specific wild alleles that each line contains will contribute to their usefulness in a breeding program. Lines with introgressions on the A-subgenome that are related to RKN, and late leaf spot resistance will be important as well as the new segment we have identified on LG B02. It will also be possible to use the primer assays designed for this project to screen other lines from the MABS population to find more options for breeding.