EXPLORATION OF *ARACHIS STENOSPERMA* AS A SOURCE OF NEMATODE RESISTANCE FOR PEANUT

by

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(Under the Direction of Soraya Bertioli)

ABSTRACT

Arachis hypogaea (peanut) is a very important crop cultivated worldwide. It is an allotetraploid species with low genetic diversity and high susceptibility to root-knot nematode (RKN) *Meloidogyne arenaria*. Most commercial cultivars harbor a single RKN resistance locus derived from the wild species *A. cardenasii*. There is, however, a risk that this resistance could breakdown and could lead to devastating consequences for peanut production, therefore, additional sources of resistance are urgently needed.

Strong resistant to RKN is present in the peanut wild relative *A. stenosperma*. Previously, three QTLs were mapped in the *A. stenosperma* genome (A02, A04 and A09). Here, to validate these chromosome segments within the genetic background of tetraploid peanut, an F_2 population was developed from a cross between *A. hypogaea* with the induced allotetraploid BatSten1 ([*A. batizocoi* x *A. stenosperma*]^{4x}). This population was genotyped using a SNP array and phenotyped for nematode resistance. QTL analysis allowed us to verify the major-effect QTL on chromosome A02, where an

R-gene cluster is co-located, and a secondary QTL on A09, the two QTL providing a disease reduction up to 98.2%.

To incorporate RKN resistance from *A. stenosperma* into peanut cultivars, selected $F_{2:3}$ lines were crossed and backcrossed with advanced peanut breeding lines. Later, phenotypic screening for resistance and genotypic characterization of BC_2F_1 lines allowed us to validate and refine the genomic regions that confer resistance. Furthermore, we performed genome-wide genotyping of advanced backcrossed lines (BC_3F_1s); and lines harboring the resistance alleles and that had a high recurrent genome recovery were selected. In the future, further selection and advancement will be needed for eventual germplasm release. These genotypes that incorporate strong RKN resistance and the markers linked to this trait, represent a valuable tool for introgression of a new nematode resistance into agronomically adapted peanuts and can significantly impact peanut production in RKN-affected areas.

Finally, due to the synthetic origin of the allotetraploid BatSten1, there was the possibility of inter-genomic interactions at the transcriptome level ('genomics shock') between the A and K subgenomes genomes of the synthetic BatSten1. Therefore, we started the exploration of changes in expression of homeologous genes pairs.

INDEX WORDS: Peanut, *Arachis hypogaea*, Root-knot nematode (RKN), *Meloidogyne arenaria*, *Arachis stenosperma*, genome-wide genotyping, QTL mapping, Marker-assisted backcrossing

(MABC), introgression, RNA sequencing (RNA-seq), homoeologous expression bias (HEB).

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B.S., Pontificia Universidad Javeriana, Colombia, 2009

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2019

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December 2019

DEDICATION

This dissertation is dedicated to God and Mary. To my beloved family in Colombia, my greatest blessing from heaven. This could not be possible without your unconditional love and support during my whole life. To my parents that always have believe in me, Pedro Ballén and Miriam Taborda; your hard work, love, dedication, sacrifice, honesty, perseverance and strength are inspirations for my life. To my sisters, best friends and the strongest people I know, Diana Ballén and Soley Ballén. To my beloved grandmothers, Lola Herrera, Maruja Ballén and Teresa Herrera. To my beautiful niece and god-daughter Isabella Gonzales Ballén.

ACKNOWLEDGEMENTS

Firstly, I would to express my admiration and gratitude to Scott Jackson for the opportunity to work under his guidance, support and encouragement. Thank you for being such a kind person and great mentor, and for the music on Fridays!!!

I would like to express all my gratitude to Soraya Bertioli and David Bertioli for their guidance, support, patience and for believing in my work and the person I am. Thank you for being such a kind people and great mentors. To Soraya Bertioli all my admiration; She is inspiration of strength and kindness, and example of woman's strong voice in science.

I would also like to acknowledge the members of my advisory committee, Dr. Jason Wallace, Dr. Peggy Ozias-Akins and Dr. Albert Culbreath for their guidance, technical support, suggestions and expertise. As well to Drs. Patricia Timper, Corrinne Grover, Jonathan Wendel and Chunming Xu for advice in different experiments and analysis.

I want to thanks to all member of the Jackson Lab, Bertioli Lab, Ozias-Akins lab, Corley's group and other IPBGG students/staff for their support in different ways, especially to Larissa Arrais, Ye Chu, Jenny Leverett, Brian Abernathy, Kurk Lance, Deborah Franco, Carolina Chavarro, Maricel Gonzales, Wendy Tsai, Stephanie Botton, Kathleen Monfero, Davis Gimode, Kate Costello, Richard Lu, Mark Hopkins and Deborah Santo.

I would like to thank all the undergrad student workers for their hard work: Emma Matthews, Rida Osman, Victoria Morris, Blake Chen, Casey Miller and Eric Antepenko. It would have never been able to accomplish all of this without your help.

To Joe Tohme, German Plata and Jorge Duitama my former mentors at CIAT, all my gratitude and admiration. Without your support and encouragement, I would have not started this journey. Off course to all my lab mates at CIAT.

Last but not least, I am so grateful with all my true friends in the U.S, Colombia and other parts of the world, specially to Lina Correa (my best friend), Maru Castellamos, Laura Paris, Carla Aranibar, Tito Montenegro, Leynar Leyton, Lisa Wegscheider, Maria Terraza, Megan Lepere-Schloop, Sebastian Samoluk, Maricel Podio, Ana Maria Villegas, Ana Maria Bossa, Mariam Chaib, Daniel Cruz, Yamid Sanabria, Andres Gutierrez, Alexander Aguirre, Harold Suarez, Christian Olaya, Jesus Beltran and German Plata for their friendship.

I sincerely apologize to anyone who I may have omitted.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Peanut (*Arachis hypogaea*)

Peanut, or groundnut (*Arachis hypogaea* L.) is an important oilseed, food and fodder crop cultivated worldwide in an area of 27.9 million ha and with an annual production of 47.1 million tons. There are several major producers globally; in 2017, Asia accounted for about 62.5% of the global production lead by China (36.3%) and India (19.5%), followed by Africa that contributed to 26.1% lead by Nigeria (5.1%), and finally the Americas with 11.3% with the United States (7%) being the major producer in the region. The U.S. has the highest yield with 4.57 tons/ha, whereas Africa has the lowest yield with 0.84 tons/ha in 2017 (FAOSTAT 2019). In the U.S., peanut is grown on more than a half million ha (0.72 Mha), primarily in the Southeast region. Georgia produces nearly 50% of the nation's peanut followed by Texas, Alabama and Florida. Other producing states include North Carolina, Arkansas, South Carolina, Virginia, Mississippi, Oklahoma and New Mexico (https://quickstats.nass.usda.gov, data for 2019) (Fig. 1.1).

This crop is commercially important for the oil industry, for edible products manufacturing and many other processed foods. In addition, peanut is an important source of dietary calories, nutrients and proteins, particularly in developing countries (e.g. sub-Saharan Africa), where peanut products are used as weaning food for children

and to produce milk, flour, soup and porridge, highly nutritious foods for these vulnerable populations (Arya et al. 2016; Mupunga et al. 2017; Settaluri et al. 2012; Singh and Singh 1991).

Peanut origin and genetics

Peanut is an allotetraploid species (AABB, 2n = 4x =40) that evolved from the hybridization of two wild diploid species, *Arachis duranensis* (A genome donor) and *Arachis ipaensis* (B genome donor), followed by a spontaneous chromosome doubling (Bertioli et al. 2016; Bertioli et al. 2019; Halward et al. 1992; Husted 1936; Kochert et al. 1996a; Moretzsohn et al. 2013; Seijo et al. 2004). Peanut has a genome size of ~2.7 Gb, which is approximately the sum of the *A. duranensis* and *A. ipaensis* genome sizes (1.25 Gb and 1.56 Gb, respectively) (Bertioli et al. 2016; Bertioli et al. 2019).

Taxonomically, the genus *Arachis* belongs to the family Fabaceae, subfamily Faboideae, like other economically important legumes (e.g. common bean, soybean, chickpea). The genus is endemic to South America and is composed of approximately 80 described species classified in 9 sections based on geographic distribution, reproductive compatibility and morphology (Krapovickas and Gregory 1994; Krapovickas et al. 2007; Penaloza and Valls 2005; Valls and Simpson 2005). Peanut is found in the section *Arachis* along with 25 wild diploid species (2n = 2x =20), including its progenitors and the wild allotetraploid species *A. monticola* (Krapovickas and Gregory 1994; Krapovickas et al. 2007). Several pieces of evidence including cytogenetic, phylogeographic and molecular studies, have confirmed that *A. duranensis* and *A. ipaensis* are the most likely donors of the A and B peanut subgenomes, respectively

(Grabiele et al. 2012; Kochert et al. 1996a; Moretzsohn et al. 2013; Ramos et al. 2006; Robledo et al. 2009; Robledo and Seijo 2010; Seijo et al. 2007). It has been shown that progeny derived from a cross between the induced allotetraploid IpaDur ([*A. ipaensis* K30076 x *A. duranensis* V14167]$^{(2n=4x=40)}$) and cultivated peanut (Fávero et al. 2006) result in highly fertile, phenotypically normal and vigorous progeny, which also support the close relationship of the A and B genomes of these two wild species with the sub genomes in peanut (Shirasawa et al. 2013).

More recently, it was described that the hybridization that resulted in *A. hypogaea* was likely due to human transport of *A. ipaensis* into the geographic range of *A. duranensis* around 9,400 year ago (Bertioli et al. 2016). According to the percentage of DNA identity*, A. ipaensis* K30076, the only accession in germplasm collections, is likely a descendant of the same population that contributed the *A. hypogaea* B subgenome (99.96% DNA identity). It is still unclear which specific *A. duranensis* population donated the peanut A subgenome. However, four candidates that were the most similar to the A genome sequence of Tifrunner cultivar, include two accessions from Rio Seco, Argentina (30065 and 30067) (99.76% of DNA identity), the accession V14167 (recently sequenced with 99.61% of DNA identity) and the PI 475845 (98.23% of DNA identity) (Bertioli et al. 2016; Bertioli et al. 2019).

Root-knot nematode (RKN) of peanut

Pathogens and pests are an important threat for food crops globally, reducing quality and productivity by 10% to 16% (Oerke 2006; Savary et al. 2012; Strange and Scott 2005). Nematodes, a plant-parasitic pathogen, can result in severe damage to food

crops worldwide, and cause an estimated loss of about $US80 billion annually (Jones et al. 2013; Nicol et al. 2011). In particular, root-knot nematodes (RKN) (*Meloidogyne* spp.) represent a very important limitation for food production and sustainable agriculture in several countries (Jones et al. 2013; Onkendi et al. 2014; Perry et al. 2009; Wesemael et al. 2011).

Meloidogyne spp. affect several major crops worldwide (Jones et al. 2013). These nematodes are the primarily parasites of peanut in all production areas around the world (Timper et al. 2018) and it has been estimated that ~12% of annual losses are caused by nematodes species, which translates to US$1.03 billion (Sasser and Freckman 1987). In particular, *Meloidogyne arenaria* is the most destructive among all nematode species and causes the most peanut yield losses, reduces pod and grain quality, affects plant growth and increases production cost (Holbrook and Stalker 2003; Starr et al. 2002). Of the two races reported for *M. arenaria,* race 1 has the ability to infect peanut whereas race 2 does not (Sasser and Freckman 1987).

In the southern region of the US (Alabama, Florida, Georgia, Texas and sometimes in N. Carolina, S. Carolina and Virginia), *M. arenaria* (race 1) reduces production by 50% or more under dense nematode populations (Dickson and De Waele 2005; Minton and Baujard 1990). At times, 100% losses have been reported in areas of heavily infested fields (Timper et al. 2018). In 2016 alone, $18.7M were calculated in peanut yield losses and $7.2M spent on disease control (Little 2016). In other parts of the world (Ghana, Zimbabwe, India and China) there have also been reports of *M. arenaria* damaging peanut. In African countries (e.g. Uganda), despite the lack of record of nematology research on groundnuts, these pathogens represent a threat for peanut

cultivation due to their presence across this region and their ability to infect most crops (Onkendi et al. 2014; Perry et al. 2009; Sikora 2018; Timper et al. 2018).

Root-knot nematode life cycle

In peanut, nematode infection is characterized by the formation of galls or "knots" in the peanut roots and lesions in the pods and pegs. Above-ground symptoms may be unnoticeable, however as the plants mature, stunting, chlorosis and wilting can be observed. Symptoms are visible both above and below ground ~45-75 days after planting (DAP) and start to become more frequent and severe around 90-120 days. Although symptoms may be visible above ground, galling and egg masses do not appear on roots until 55-90 DAP (Timper et al. 2018).

The nematode life cycle begins when infectious second-stage juveniles (J2) (that are present in the soil) penetrate the peanut root tips and start migrating along the vascular cylinder. Later, the nematodes establish feeding sites by remodeling the parenchymatic and surrounding cells into enlarged specialized multinucleated 'giant' cells. These changes are particularly detrimental because it causes deformation of the xylem and phloem tissues, which involves reduced nutrient and water uptake of infected roots, and affects growth (Timper et al. 2018). Once established, the nematode undergoes three molts to become a reproductive adult female with an enlarged size and globose shape; the three molts include J2 to a third-stage juvenile (J3), to a fourth-stage juvenile (J4) to a female adult stage. Males are usually found when the conditions are not favorable for female development (food limitation or high population density). Later, the female lay hundreds of eggs (300-500 eggs) into gelatinous masses, to keep the eggs

together and to protect them from the environment. The egg masses are usually found on the surface of the galled root tissue. Inside the eggs, embryogenesis occurs for the formation of J2 nematodes. When environmental condition are favorable, the nematodes hatch into the soil, reach new roots and invade the host to start the infectious cycle again (Eisenback 1981; Moens et al. 2009; Williamson and Hussey 1996).

Root-knot nematode management

RKN management is very difficult and costly once the nematode has established in the field. However, there are several management strategies for RKN control and for reducing the disease spread (Timper et al. 2018). Crop rotation and nematicides are the most common methods for nematode management.

Crop rotation is an effective method to reduce nematode population by using non-host or resistant plants. In peanut, rotations with other crops including cotton, corn, bahiagrass, bermudagrass and castorbean is typical (Bailey 1988; Dickson and Melouk 1995; Dunn and Dickson 1995; Hagan 1988; Johnson et al. 1999; Rodríguez-Kábana et al. 1991; Timper et al. 2018). Rotation with bahiagrass is considered one of the best crops preceding peanut, and 3-year rotations or more years is highly recommended (Norden et al. 1977; Rodriguez-Kabana et al. 1994; Timper et al. 2018). Crop rotation is useful to maintain low nematode densities or to progressively reduce high densities over time, rather than 100% elimination of nematodes from the field. This because some nematodes will survive in the field, and most crops and weeds present in the field can support nematode reproduction (Dunn and Dickson 1995). Nonetheless, due to the wide *M. arenaria* host range, implementing control strategies through crop rotation is difficult

(Dong et al. 2007; Johnson et al. 1999; Perry et al. 2009; Rodríguez-Kábana et al. 1991; Sikora 2018; Wesemael et al. 2011). Nematicides are frequently used to prevent pod damage later in the season, but the long-term elimination of nematode population is difficult to achieve with chemical control (Sikora et al. 2005). Moreover, it is costly and has adverse environmental effects (Danchin et al. 2013; Onkendi et al. 2014).

Host plant resistance is the ultimate nematode management strategy (Timper et al. 2018). Development of nematode-resistant cultivars is the most efficient and effective strategy to control nematode populations and to decrease yield losses while reducing the use of nematicides (Dong et al. 2007).

Arachis genetic resources

A. hypogaea has a very narrow genetic diversity as consequence of its recent and unique polypoid origin (Bertioli et al. 2016; Bertioli et al. 2019; Halward et al. 1992; Husted 1936; Kochert et al. 1996a; Moretzsohn et al. 2013). Spontaneous chromosome doubling in the hybrid (*A. ipaensis* x *A. duranensis*) restored the fertility but made the resulting tetraploid reproductively isolated from its diploid wild relatives (Bertioli et al. 2011; Holbrook and Stalker 2003). This, in combination with the effects of the subsequent domestication, has decreased tremendously its ability to withstand biotic and abiotic stresses (Bertioli et al. 2011; Jung et al. 2003; Kochert et al. 1991; Kochert et al. 1996a; Krapovickas et al. 2007; Seijo et al. 2004). Peanut is extremely susceptibility to severe pests and diseases, including root-knot nematode (RKN) (*Meloidogyne arenaria* (Neal)) (described above), rust (*Puccinia arachidis*), early leaf spot (ELS) (*Passalora*

arachidicola), late leaf spot (LLS) (*Nothopassalora personata*), Tomato spotted wilt virus (TSWV) and others (Holbrook and Stalker 2003).

Strong resistance to pest and diseases is not available in the *A. hypogaea* gene pool (Stalker 2017), which imposes certain constraints for crop improvement using cultivated germplasm (Moretzsohn et al. 2004; Pandey et al. 2012). Yet, the wild species of peanut are found in diverse environments (from swamps to grasslands, to rocky ground in semi-arid areas) and are adapted to a wide range of conditions (Bertioli et al. 2011; Krapovickas et al. 2007). The greater genetic diversity in the wild relatives has the potential to broaden peanut's genetic base and improve its performance under disease/pest pressure (Holbrook and Stalker 2003; Simpson 2001a; Stalker 1997; Stalker 2017).

Transferring wild alleles into peanut

Introgression of alleles from wild germplasm into peanut cultivars is believed to be mostly restricted to the species in the section *Arachis* (where peanut is taxonomically classified), but again ploidy differences between wild *Arachis* species and cultivated peanut make the incorporation of wild desired alleles very challenging (Bertioli et al. 2011; Dwivedi et al. 2007; Garcia et al. 1995; Stalker 2017). Nevertheless, overcoming this genetic barrier for broadening the peanut genetic base using wild species is possible, through the development of peanut compatible, wild-derived synthetic allotetraploids (Burow et al. 2001; Fonceka et al. 2009; Fonceka et al. 2012a; Kumari et al. 2014; Leal-Bertioli et al. 2017; Leal-Bertioli et al. 2015c; Simpson 1991; Simpson et al. 1993; Simpson et al. 2003; Simpson and Starr 2001).

Different pathways to develop synthetic allotetraploid have been previously described (Simpson 2001a): 1) in the hexaploid method, a triploid hybrid between *A. hypogaea* and a wild diploid species is developed and later treated with colchicine to double the chromosome number; 2) in another pathway, two diploid wild species are treated independently with colchicine to create the autotetraploid plants (AAAA and BBBB genome composition), and then crossed together to create an allotetraploid, which is partially sterile; 3) an alternative three-way path, is the cross between two A-genome wild diploid species to produce a diploid hybrid, which then is crossed with a third diploid species with B-genome. This tri-species hybrid is later chromosome-doubled with colchicine; and 4) the final route consists in the hybridization of two wild species (usually combining A and B genomes, or closely related genomes) followed by doubling of the AB hybrid to develop the allotetraploid (Simpson 2001a). The third and fourth approaches are especially important because they ensure that both the A and B genomes (or related) are included in the initial crosses, resulting in allotetraploids that are genetically compatible with peanut (Simpson 2001a; Stalker 2017). The last pathway was used to recreate the events that likely resulted in the allotetraploid peanut (Fávero et al. 2006). Also, it is the most preferred method and is currently used in several breeding programs for incorporation of useful wild alleles into elite peanut cultivars (Fonceka et al. 2009; Kumari et al. 2014; Leal-Bertioli et al. 2015c; Mallikarjuna et al. 2012; Mallikarjuna et al. 2011; Stalker 2017).

Disease and pest resistance in wild species

A diverse set of wild diploid species is available for introgression of disease and pest resistance alleles into allotetraploid peanut (Stalker 2017). The first step of incorporation of wild alleles is the hybridization of two diploid species followed by genome duplication (as described above); however, is important to carefully consider the combination of wild species (genomes) to assure successful introgression of wild alleles into the crop (Hajjar and Hodgkin 2007; Leal-Bertioli et al. 2012). Stalker (2017) provided a detailed review list of wild species, their genome composition and their resistances to diseases and pests. The pathogens commonly affecting the peanut crop include root-knot nematode (RKN), rust, early leaf spot (ELS), late leaf spot (LLS), Tomato spotted wilt virus (TSWV) (Holbrook and Stalker 2003), to which several wild species harbor resistance including in *A. stenosperma, A. batizocoi, A. cardenasii*, *A. magna, A. diogoi, A. valida,* and *A. duranensis* (Bera et al. 2018; Guimaraes et al. 2010; Holbrook and Noe 1990a; Holbrook and Stalker 2003; Kumar and Kirti 2015; Leal-Bertioli et al. 2015b; Leal-Bertioli et al. 2009; Leal-Bertioli et al. 2016; Morgante et al. 2013; Mota et al. 2018; Nelson et al. 1989; Nelson et al. 1990; Pande and Rao 2001; Simpson et al. 1993; Simpson et al. 2003; Stalker 1984; Stalker 2017).

RKN resistance

Several efforts to find sources of resistance to root-knot nematode in *Arachis* germplasm have been done. Initially, a systematic search for sources of resistance in *A. hypogaea* germplasm collections was completed (Holbrook and Noe 1990a) and further studies showed that some *A. hypogaea* plant introductions (PI) had moderate level of

resistance with 40-60% reduction of *M. arenaria* population densities (Noe et al. 1992). In 1989 and later in 1990 important breakthrough occurred when strong resistance to *M. arenaria* was found in several wild *Arachis* spp. (*A. chacoensis, A. cardenasii, A. batizocoi, A. stenosperma,* between others) and two complex hybrids; the allotetraploid TP-129 ([*A. batizocoi* K9484 x *A. chacoensis* GKP10602]$^{(2n=4x=40)}$) and the backcrossed line TP-135 (*A. hypogaea* cv. Florunner x [*A. batizocoi* K9484 x *A. chacoensis* GKP10602]$^{(2n=4x=40)}$) (Nelson et al. 1989; Nelson et al. 1990).

The use of wild relatives to introgress RKN resistance into elite peanut began in 1993 when the hybrid TxAG-6 was developed through a complex three-way interspecific cross (described above) (Simpson et al. 1993). This synthetic allotetraploid combined three *Arachis* species*, A. batizocoi, A. cardenasii* and *A. diogoi* (also called *A. chacoensis*), all resistant to RKN (Nelson et al. 1989; Nelson et al. 1990). The first released resistant cultivars, COAN (Simpson and Starr 2001) and NemaTAM (Simpson et al. 2003) were developed from TxAG-6 through a backcrossing schemes involving *A. hypogaea* cv. Florunner as the recurrent parent (Burow et al. 2001). Both cultivars reduced nematode reproduction by more that 90% and had higher yields than susceptible cultivars in nematode infested fields (Starr et al. 2002). Since then, peanut breeding programs have successfully released other cultivars in the U.S. using the same source of resistance from *A. cardenasii* via TxAG-6, e.g. Tifguard, Webb, Georgia-14N and TifNV-High O/L (Branch and Brenneman 2015; Holbrook et al. 2017; Holbrook et al. 2008; Simpson et al. 2013).

To date all RKN resistant cultivars contain an introgression segment from *A. cardenasii*. The resistance is located on a large segment on chromosome A09 and is

dominantly inherited (Burow et al. 1996; Choi et al. 1999). Previously, it was reported that a single dominant resistance gene (*Rma*) was responsible for resistance (Nagy et al. 2010), but now we know that there may be two segments providing different levels of RKN resistance (high and moderate) (Chu et al. 2016a; Clevenger et al. 2017a).

Another source of strong resistance to RKN has been reported in the peanut wild relative *A. stenosperma* (Guimaraes et al. 2010; Proite et al. 2008; Proite et al. 2007) and three chromosome locations (A02, A04 and A09) conferring resistance were identified in the diploid context (Leal-Bertioli et al. 2016). Introgression of this new RKN resistance was initiated with the creation of a diploid hybrid between these two species, followed by chromosome doubling with colchicine to develop the a synthetic allotetraploid BatSten1 ([*Arachis batizocoi* x *A. stenosperma*]$^{(2n=4x=40)}$) (Leal-Bertioli et al. 2015c). In this case, the resistance to *M. arenaria* is conferred by *A. stenosperma* and the location of the QTLs are different from than the resistance derived from *A. cardenasii*, indicating that different R-genes are likely involved (Leal-Bertioli et al. 2016). This resistance is manifest at multiple stages of the infection cycle: much lower rates of juvenile nematode penetration into *A. stenosperma* roots, and those that do penetrate induce the expression of genes associated with hypersensitive response and secondary metabolite production for defense against the infection (Guimaraes et al. 2010; Guimaraes et al. 2015; Morgante et al. 2013; Proite et al. 2008; Proite et al. 2007).

As part of this project, a set of F_2 progeny were derived from the cross of *A. hypogaea* cv. Runner-886 with BatSten1 and QTLs controlling RKN resistance were validated in the tetraploid. Major A02-QTL and secondary A09-QTL were confirmed (Ballén-Taborda et al. Submitted). This framework of knowledge allowed us to

incorporate this new source of resistance into elite peanut cultivars through marker-assisted backcrossing schemes (MABC), work that is being completed and is the main focus of this dissertation. So far, we have developed BC_3F_2s that will be subjected to further selection and advancement for the eventual release of improved germplasm.

Changes due to polyploidization

Polyploidy is an important process in plant evolution (Doyle et al. 2008; Jiao et al. 2011). In autopolyploid species, additional set of chromosomes come from the same species, whereas in allopolyploids additional sets of chromosomes are derived from different species (Doyle et al. 2008; Yoo et al. 2014). In allopolyploidy, the coexistence of divergent genomes within a single nucleus can be accompanied by non-mendelian genetic and epigenetic changes, and with effects on genome structure, gene expression and phenotype (Comai et al. 2003; Comai et al. 2000; Doyle et al. 2008; Jackson and Chen 2010; Yoo et al. 2014).

At a genetic level, the phenomenon known as "genomic/transcriptomic shock" (Buggs et al. 2011; Hegarty et al. 2006; McClintock 1984; Wu et al. 2016; Zhang et al. 2016) have been studied in a variety of plant species using both natural and newly formed allotetraploids, such *Coffea arabica* (Bardil et al. 2011) *Brassica napus* (Wu et al. 2018), *Mimulus* species (Edger et al. 2017), *Arabidopsis* (Chen 2007; Wang et al. 2006), *Triticum* species (Chague et al. 2010; Li et al. 2014; Qi et al. 2012; Zhang et al. 2016), *Oryza sativa* (Xu et al. 2014), *Gossypium* (Flagel et al. 2008; Flagel and Wendel 2010; Rapp et al. 2009; Yoo et al. 2013b), *Tragopogon* (Buggs et al. 2011) *Spartina* (Chelaifa et al. 2010) and *Senecio* (Hegarty et al. 2008; Hegarty et al. 2006).

In allopolyploids, the integration of divergent genomes can involve a variety of genetic/genomics changes including: 1) modification in epigenetic marks (Chen 2007; Comai et al. 2003; Doyle et al. 2008; Kovarik et al. 2008; Liu et al. 2009; Madlung et al. 2002; Rapp and Wendel 2005; Shaked et al. 2001; Soltis et al. 2010; Soltis and Soltis 2009; Wang et al. 2014); 2) homoeologous genome interactions and exchanges (Gaeta et al. 2007; Kovarik et al. 2008; Salmon et al. 2010; Szadkowski et al. 2010); 3) sequence elimination (Han et al. 2005; Jackson and Chen 2010; Shaked et al. 2001; Skalicka et al. 2005; Tate et al. 2009); 4) chromosomal rearrangements (Madlung et al. 2005; Pontes et al. 2004); 5) activation of transposable elements (TE) (Agren et al. 2016; Kashkush et al. 2003; Kraitshtein et al. 2010; Madlung et al. 2005; Mhiri et al. 2019; O'Neill et al. 1998; Wendel et al. 2016); 6) variations in small RNA populations (Fu et al. 2016; Ha et al. 2009; Shen et al. 2014); and 7) alterations in gene expression (gene loss, silencing and activation) (Comai et al. 2003; Comai et al. 2000; Hegarty et al. 2008; Liu et al. 2009).

At a phenotypic level, polyploidy has been shown to affect phenotypes, including plant anatomy, morphology and physiology traits (Adams and Wendel 2005; Masterson 1994; Otto and Whitton 2000). For peanut improvement, transference of valuable alleles from wild species involves hybridization and genome duplication to overcome the ploidy barrier with peanut (Burow et al. 2001; Fonceka et al. 2009; Fonceka et al. 2012a; Kumari et al. 2014; Leal-Bertioli et al. 2017; Leal-Bertioli et al. 2015c; Simpson 1991; Simpson et al. 1993; Simpson et al. 2003; Simpson and Starr 2001), therefore phenotypic changes may occur. Drought-related traits and leaf morphology were studied in *Arachis duranensis*, *Arachis ipaensis* and its derived allotetraploid. It was found that traits such as chlorophyll content was maintained upon tetraploidization, whereas leaf area, stomata

size, trichome density and transpiration profile were modified (Leal-Bertioli et al. 2012). Later, it was observed that after colchicine treatment for tetraploidization of *Arachis* interspecific hybrids, plants were highly fertile with increased vigor, and higher overall plant size, leaf area, leaf thickness, canopy area, root and aerial dry biomass. Additionally, at early stages plants did display some morphological abnormalities *(e.g.* curled leaf lamina, necrotic spots and chlorosis) that disappeared later in the season (Leal-Bertioli et al. 2015c). *Several* anatomical, morphological, and physiological traits were studied in wild diploid species of *Arachis* and synthetic allotetraploids. Most of the traits displayed transgressive behavior (larger leaves, larger stomata and epidermal cells, improved photosynthesis capacity) but other were not modified significantly (e.g. seed dimensions) (Leal-Bertioli et al. 2017). In summary, phenotypic changes after polyploidization are commonly observed and synthetic polyploids may have a similar phenotype to one of the parents, intermediate, absent or displaying transgressive phenotypes (Liu et al. 2007; Xu et al. 2007).

Objectives

The main first objective of this project was the genetic characterization and validation in a tetraploid genetic background of the genomic regions controlling root-knot nematode resistance previously identified in the diploid genome of *A. stenosperma*. For this purpose, a cultivated peanut x wild species F_2 population was developed, phenotyped for RKN resistance and genotyped with a high-density SNP chip. The second main objective was the incorporation of the A02-QTL and A09-QTL from *A. stenosperma* into susceptible cultivated peanut. This dissertation is organized in 3 main topics:

1. Validation of the QTLs associated with root-knot nematode resistance from *A. stenosperma* in the peanut tetraploid scenario (chapter 2).
2. Incorporation of QTL controlling RKN resistance into peanut genome using a marker-assisted backcrossing scheme (MABC). Phenotypic and genotypic validation was performed in the second generation of backcrossed lines (BC_2F_1s). Later, 271 advanced backcrossed lines (BC_3F_1s) were genotyped to track target and non-target introgressions (chapter 3 and 4).
3. Characterization of gene expression of homoeologous gene pairs from A (*A. stenosperma*) and K (*A. batizocoi*) genomes coexisting in the nucleus of the synthetic allotetraploid BatSten1 (chapter 5).

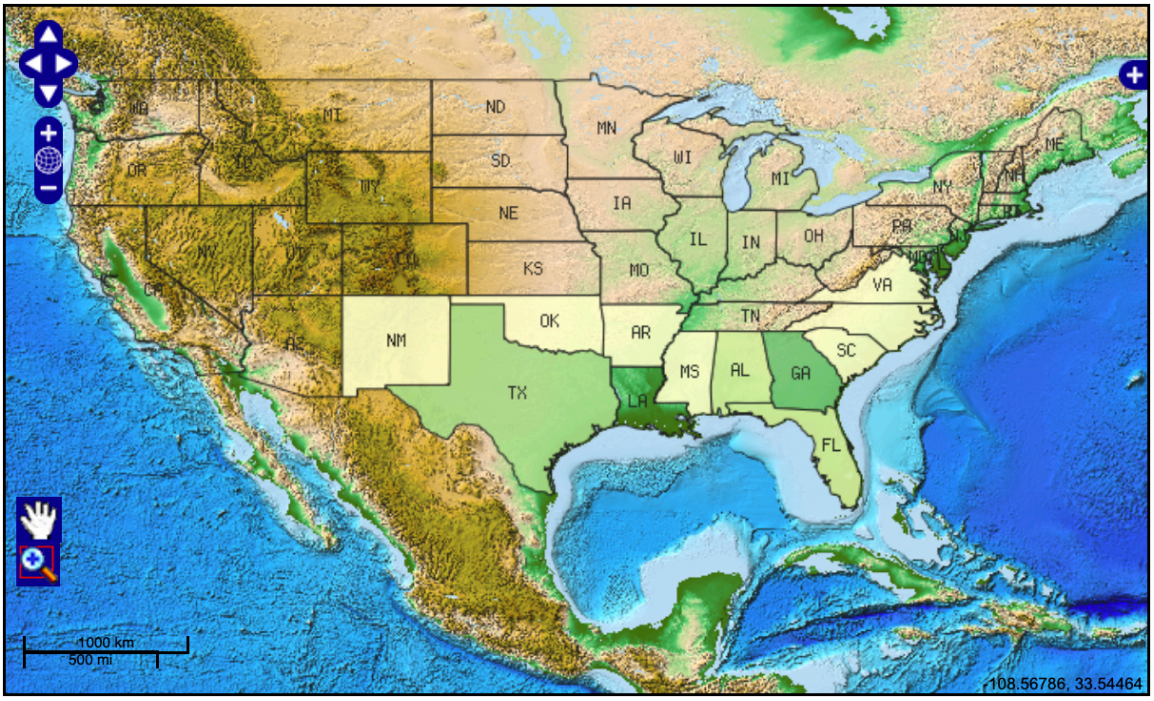

Figure 1.1. U.S. peanut-producing areas (2019 data) (https://quickstats.nass.usda.gov). Major producing states in dark green and minor producing states in yellow color. GA-Georgia; TX-Texas; AL-Alabama; FL- Florida; NC-North Carolina; AR-Arkansas; SC-South Carolina; VA-Virginia; MA-Mississippi; OK-Oklahoma; NM-New Mexico.

References

Adams KL, Wendel JF (2005) Polyploidy and genome evolution in plants. Current Opinion in Plant Biology 8:135-141

Agren JA, Huang HR, Wright SI (2016) Transposable element evolution in the allotetraploid Capsella bursa-pastoris. American journal of botany 103:1197-1202

Arya SS, Salve AR, Chauhan S (2016) Peanuts as functional food: a review. J Food Sci Technol 53:31-41

Bailey JE (1988) Peanut disease control. North Carolina Agricultural extension service. North Carolina State University, pp 51–64

Ballén-Taborda C, Chu C, Jackson S, Ozias-Akins P, Timper P, Holbrook C, Bertioli D, Leal-Bertioli S (Submitted) A new source of root-knot nematode resistance from Arachis stenosperma incorporated into allotetraploid peanut (Arachis hypogaea). Sci Rep

Bardil A, de Almeida JD, Combes MC, Lashermes P, Bertrand B (2011) Genomic expression dominance in the natural allopolyploid Coffea arabica is massively affected by growth temperature. New Phytol 192:760-774

Bera SK, Manohar SS, Variath MT, Chaudhari S, Yaduru S, Thankappan R, Narayana M, Kurapati S, Pandey MK, Sudini HK, Shanmugavel S, Kulandaivelu G, Varshney RK, Pasupuleti J (2018) Assessing variability for disease resistance and nutritional quality traits in an interspecific collection of groundnut (Arachis hypogaea). Plant Breed 137:883-894

Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD, Cannon EK, Liu X, Gao D, Clevenger J, Dash S, Ren L, Moretzsohn MC, Shirasawa K, Huang W, Vidigal B, Abernathy B, Chu Y, Niederhuth CE, Umale P, Araujo AC, Kozik A, Kim KD, Burow MD, Varshney RK, Wang X, Zhang X, Barkley N, Guimaraes PM, Isobe S, Guo B, Liao B, Stalker HT, Schmitz RJ, Scheffler BE, Leal-Bertioli SC, Xun X, Jackson SA, Michelmore R, Ozias-Akins P (2016) The genome sequences of Arachis duranensis and Arachis ipaensis, the diploid ancestors of cultivated peanut. Nat Genet 48:438-446

Bertioli DJ, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, Leal-Bertioli SCM, Ren L, Farmer AD, Pandey MK, Samoluk SS, 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 KD, Korani W, Lanciano S, Lui CG, Mirouze M, Moretzsohn MC, Pham M, Shin JH, Shirasawa K, Sinharoy S, Sreedasyam A, Weeks NT, Zhang X, Zheng Z, Sun Z, Froenicke L, Aiden EL, Michelmore R, Varshney RK, Holbrook CC, Cannon EKS, Scheffler BE, Grimwood J, Ozias-Akins P, Cannon SB, Jackson SA, Schmutz J (2019) The

genome sequence of peanut (Arachis hypogaea), a segmental allotetraploid. Nat Genet 51:877-884

Bertioli DJ, Seijo G, Freitas FO, Valls JFM, Leal-Bertioli SCM, Moretzsohn MC (2011) An overview of peanut and its wild relatives. Plant Gene Resour 9:134-149

Branch WD, Brenneman TB (2015) Registration of 'Georgia-14N' peanut. J Plant Regist 9:159-161

Buggs RJ, Zhang L, Miles N, Tate JA, Gao L, Wei W, Schnable PS, Barbazuk WB, Soltis PS, Soltis DE (2011) Transcriptomic shock generates evolutionary novelty in a newly formed, natural allopolyploid plant. Curr Biol 21:551-556

Burow MD, Simpson CE, Paterson AH, Starr JL (1996) Identification of peanut (Arachis hypogaea L.) RAPD markers diagnostic of root-knot nematode (Meloidogyne arenaria (Neal) Chitwood) resistance. Molecular Breeding 2:369-379

Burow MD, Simpson CE, Starr JL, Paterson AH (2001) Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (Arachis hypogaea L.). broadening the gene pool of a monophyletic polyploid species. Genetics 159:823-837

Chague V, Just J, Mestiri I, Balzergue S, Tanguy AM, Huneau C, Huteau V, Belcram H, Coriton O, Jahier J, Chalhoub B (2010) Genome-wide gene expression changes in genetically stable synthetic and natural wheat allohexaploids. New Phytol 187:1181-1194

Chelaifa H, Monnier A, Ainouche M (2010) Transcriptomic changes following recent natural hybridization and allopolyploidy in the salt marsh species Spartina x townsendii and Spartina anglica (Poaceae). New Phytol 186:161-174

Chen ZJ (2007) Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annu Rev Plant Biol 58:377-406

Choi K, Burow MD, Church G, Burow G, Paterson AH, Simpson CE, Starr JL (1999) Genetics and Mechanism of Resistance to Meloidogyne arenaria in Peanut Germplasm. J Nematol 31:283-290

Chu Y, Gill R, Clevenger J, Timper P, Corley Holbrook C, Ozias-Akins P (2016) Identification of rare recombinants leads to tightly linked markers for nematode resistance in peanut. Peanut Sci 43:88-93

Clevenger J, Chu Y, Arrais Guimaraes L, Maia T, Bertioli D, Leal-Bertioli S, Timper P, Holbrook CC, Ozias-Akins P (2017) Gene expression profiling describes the genetic regulation of Meloidogyne arenaria resistance in Arachis hypogaea and reveals a candidate gene for resistance. Sci Rep 7:1317

Comai L, Madlung A, Josefsson C, Tyagi A (2003) Do the different parental 'heteromes' cause genomic shock in newly formed allopolyploids? Philos Trans R Soc Lond B Biol Sci 358:1149-1155

Comai L, Tyagi AP, Winter K, Holmes-Davis R, Reynolds SH, Stevens Y, Byers B (2000) Phenotypic instability and rapid gene silencing in newly formed arabidopsis allotetraploids. Plant Cell 12:1551-1568

Danchin EG, Arguel MJ, Campan-Fournier A, Perfus-Barbeoch L, Magliano M, Rosso MN, Da Rocha M, Da Silva C, Nottet N, Labadie K, Guy J, Artiguenave F, Abad P (2013) Identification of novel target genes for safer and more specific control of root-knot nematodes from a pan-genome mining. PLoS Pathog 9:e1003745

Dickson DW, De Waele D (2005) Nematode parasites of peanut. In: Sikora RA, Bridge J (eds) Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI Publishing, Wallingford, UK, pp 393-436

Dickson DW, Melouk HA (1995) Management of nematode pests. In: Melouk HA, Shokes FM (eds) Peanut Health Management. APS Press, St. Paul, MN, pp 83-86

Dong W, Holbrook CC, Timper P, Brenneman TB, Mullinix BG (2007) Comparison of methods for assessing resistance to meloidogyne arenaria in peanut. J Nematol 39:169-175

Doyle JJ, Flagel LE, Paterson AH, Rapp RA, Soltis DE, Soltis PS, Wendel JF (2008) Evolutionary genetics of genome merger and doubling in plants. Annu Rev Genet 42:443-461

Dunn RA, Dickson DW (1995) Peanut nematode management. Document RF-NG016 Florida Cooperative Extension Service. University of Florida, Gainsville, Florida

Dwivedi SL, Bertioli DJ, Crouch JH, Valls JF, Upadhyaya HD, Fávero A, Moretzsohn M, Paterson AH (2007) Peanut. In: Kole C (ed) Oilseeds. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 115-151

Edger PP, Smith R, McKain MR, Cooley AM, Vallejo-Marin M, Yuan Y, Bewick AJ, Ji L, Platts AE, Bowman MJ, Childs KL, Washburn JD, Schmitz RJ, Smith GD, Pires JC, Puzey JR (2017) Subgenome Dominance in an Interspecific Hybrid, Synthetic Allopolyploid, and a 140-Year-Old Naturally Established Neo-Allopolyploid Monkeyflower. Plant Cell 29:2150-2167

Eisenback J (1981) Life Cycle of the Root-Knot Nematodes. N.C. State University Graphics, Raleigh, NC

Fávero AP, Simpson CE, Valls JFM, Vello NA (2006) Study of the Evolution of Cultivated Peanut through Crossability Studies among A. ipaensis, A. duranensis and A. hypogaea Crop Sci 46:1546–1552

Flagel L, Udall J, Nettleton D, Wendel J (2008) Duplicate gene expression in allopolyploid Gossypium reveals two temporally distinct phases of expression evolution. BMC Biol 6:16

Flagel LE, Wendel JF (2010) Evolutionary rate variation, genomic dominance and duplicate gene expression evolution during allotetraploid cotton speciation. New Phytol 186:184-193

Fonceka D, Hodo-Abalo T, Rivallan R, Faye I, Sall MN, Ndoye O, Favero AP, Bertioli DJ, Glaszmann JC, Courtois B, Rami JF (2009) Genetic mapping of wild introgressions into cultivated peanut: a way toward enlarging the genetic basis of a recent allotetraploid. BMC Plant Biol 9:103

Fonceka D, Tossim H-A, Rivallan R, Vignes H, Lacut E, de Bellis F, Faye I, Ndoye O, Leal-Bertioli SCM, Valls JFM, Bertioli DJ, Glaszmann J-C, Courtois B, Rami J-F (2012) Construction of Chromosome Segment Substitution Lines in Peanut (Arachis hypogaea L.) Using a Wild Synthetic and QTL Mapping for Plant Morphology. PLoS One 7:e48642

Fu Y, Xiao M, Yu H, Mason AS, Yin J, Li J, Zhang D, Fu D (2016) Small RNA changes in synthetic Brassica napus. Planta 244:607-622

Gaeta RT, Pires JC, Iniguez-Luy F, Leon E, Osborn TC (2007) Genomic changes in resynthesized Brassica napus and their effect on gene expression and phenotype. Plant Cell 19:3403-3417

Garcia GM, Stalker HT, Kochert G (1995) Introgression analysis of an interspecific hybrid population in peanuts (Arachis hypogaea L.) using RFLP and RAPD markers. Genome 38:166-176

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 Syst Evol 298:1151-1165

Guimaraes P, Brasileiro ACM, Proite K, Araujo A, Leal-Bertioli S, Pic-Taylor A, da Silva FR, Morgante C, Ribeiro S, Bertioli D (2010) A study of gene expression in the nematode resistant wild peanut relative, Arachis stenosperma, in response to challenge with Meloidogyne arenaria. Tropical Plant Biology 3:183-192

Guimaraes PM, Guimaraes LA, Morgante CV, Silva OB, Jr., Araujo AC, Martins AC, Saraiva MA, Oliveira TN, Togawa RC, Leal-Bertioli SC, Bertioli DJ, Brasileiro

AC (2015) Root Transcriptome Analysis of Wild Peanut Reveals Candidate Genes for Nematode Resistance. PLoS One 10:e0140937

Ha M, Lu J, Tian L, Ramachandran V, Kasschau KD, Chapman EJ, Carrington JC, Chen X, Wang XJ, Chen ZJ (2009) Small RNAs serve as a genetic buffer against genomic shock in Arabidopsis interspecific hybrids and allopolyploids. Proc Natl Acad Sci U S A 106:17835-17840

Hagan A (1988) Disease and nematode control. In: University A (ed) Alabama cooperative extension servicePeanut Peast Management, Weed, Insect, Disease and Nematode Control Recomendations. Circular ANR-360, Auburn, pp 5-6

Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156:1-13

Halward T, Stalker T, LaRue E, Kochert G (1992) Use of single-primer DNA amplifications in genetic studies of peanut (Arachis hypogaea L.). Plant Mol Biol 18:315-325

Han F, Fedak G, Guo W, Liu B (2005) Rapid and repeatable elimination of a parental genome-specific DNA repeat (pGc1R-1a) in newly synthesized wheat allopolyploids. Genetics 170:1239-1245

Hegarty MJ, Barker GL, Brennan AC, Edwards KJ, Abbott RJ, Hiscock SJ (2008) Changes to gene expression associated with hybrid speciation in plants: further insights from transcriptomic studies in Senecio. Philos Trans R Soc Lond B Biol Sci 363:3055-3069

Hegarty MJ, Barker GL, Wilson ID, Abbott RJ, Edwards KJ, Hiscock SJ (2006) Transcriptome shock after interspecific hybridization in senecio is ameliorated by genome duplication. Curr Biol 16:1652-1659

Holbrook C, Noe PJ (1990) Resistance to Meloidogyne arenaria in Arachis spp. and the implications on development of resistant peanut cultivars. Peanut Sci 17:35-38

Holbrook C, Ozias-Akins P, Chu Y, K. Culbreath A, Kvien C, Brenneman T (2017) Registration of ‘TifNV-high O/L’ peanut. J Plant Regist 11:228-230

Holbrook C, Stalker H (2003) Peanut breeding and genetic resources. Plant Breed Rev 22:297-356

Holbrook C, Timper P, Culbreath A, Kvien C (2008) Registration of 'Tifguard' peanut. J Plant Regist 2:391-410

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 7:396-423

Jackson S, Chen ZJ (2010) Genomic and expression plasticity of polyploidy. Curr Opin Plant Biol 13:153-159

Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS, Soltis DE, Clifton SW, Schlarbaum SE, Schuster SC, Ma H, Leebens-Mack J, dePamphilis CW (2011) Ancestral polyploidy in seed plants and angiosperms. Nature 473:97-100

Johnson AW, Minton NA, Brenneman TB, Burton GW, Culbreath AK, Gascho GJ, Baker SH (1999) Bahiagrass, corn, cotton rotations, and pesticides for managing nematodes, diseases, and insects on peanut. J Nematol 31:191-200

Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, Kikuchi T, Manzanilla-Lopez R, Palomares-Rius JE, Wesemael WM, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14:946-961

Jung S, Tate PL, Horn R, Kochert G, Moore K, Abbott AG (2003) The phylogenetic relationship of possible progenitors of the cultivated peanut. J Hered 94:334-340

Kashkush K, Feldman M, Levy AA (2003) Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nature Genetics 33:102-106

Kochert G, Halward T, Branch WD, Simpson CE (1991) RFLP variability in peanut (Arachis hypogaea L.) cultivars and wild species. Theor Appl Genet 81:565-570

Kochert G, Stalker H, Gimenes M, Galgaro L, C. RL, Moore K (1996) RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, Arachis hypogaea (Leguminosae). American Journal of Botany 83:1281-1291

Kovarik A, Dadejova M, Lim YK, Chase MW, Clarkson JJ, Knapp S, Leitch AR (2008) Evolution of rDNA in Nicotiana allopolyploids: a potential link between rDNA homogenization and epigenetics. Annals of botany 101:815-823

Kraitshtein Z, Yaakov B, Khasdan V, Kashkush K (2010) Genetic and epigenetic dynamics of a retrotransposon after allopolyploidization of wheat. Genetics 186:801-812

Krapovickas A, Gregory WC (1994) Taxonomia del genero Arachis (Leguminosae). Bonplandia 8:1-186

Krapovickas A, Gregory WC, Williams DE, Simpson CE (2007) Taxonomy of the genus Arachis (Leguminosae). Bonplandia 16:7-205

Kumar D, Kirti PB (2015) Transcriptomic and Proteomic Analyses of Resistant Host Responses in Arachis diogoi Challenged with Late Leaf Spot Pathogen, Phaeoisariopsis personata. PLoS One 10:e0117559

Kumari V, Gowda MVC, Tasiwal V, Pandey MK, Bhat RS, Mallikarjuna N, Upadhyaya HD, Varshney RK (2014) Diversification of primary gene pool through introgression of resistance to foliar diseases from synthetic amphidiploids to cultivated groundnut (Arachis hypogaea L.). Crop J 2:110-119

Leal-Bertioli SC, Cavalcante U, Gouvea EG, Ballen-Taborda C, Shirasawa K, Guimaraes PM, Jackson SA, Bertioli DJ, Moretzsohn MC (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 5:1403-1413

Leal-Bertioli SC, Jose AC, Alves-Freitas DM, Moretzsohn MC, Guimaraes PM, Nielen S, Vidigal BS, Pereira RW, Pike J, Favero AP, Parniske M, Varshney RK, Bertioli DJ (2009) Identification of candidate genome regions controlling disease resistance in Arachis. BMC Plant Biol 9:112

Leal-Bertioli SC, Moretzsohn MC, Santos SP, Brasileiro AC, Guimaraes PM, Bertioli DJ, Araujo AC (2017) Phenotypic effects of allotetraploidization of wild Arachis and their implications for peanut domestication. Am J Bot 104:379-388

Leal-Bertioli SC, Santos SP, Dantas KM, Inglis PW, Nielen S, Araujo AC, Silva JP, Cavalcante U, Guimaraes PM, Brasileiro AC, Carrasquilla-Garcia N, Penmetsa RV, Cook D, Moretzsohn MC, Bertioli DJ (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. Ann Bot 115:237-249

Leal-Bertioli SCM, Bertioli DJ, Guimarães PM, Pereira TD, Galhardo I, Silva JP, Brasileiro ACM, Oliveira RS, Silva PÍT, Vadez V, Araujo ACG (2012) The effect of tetraploidization of wild Arachis on leaf morphology and other drought-related traits. Environ Exper Bot 84:17-24

Leal-Bertioli SCM, Moretzsohn MC, Roberts PA, Ballén-Taborda C, Borba TCO, Valdisser PA, Vianello RP, Araújo ACG, Guimarães PM, Bertioli DJ (2016) Genetic mapping of resistance to Meloidogyne arenaria in Arachis stenosperma: A new source of nematode resistance for peanut. G3: Genes, Genomes, Genetics 6:377-390

Li A, Liu D, Wu J, Zhao X, Hao M, Geng S, Yan J, Jiang X, Zhang L, Wu J, Yin L, Zhang R, Wu L, Zheng Y, Mao L (2014) mRNA and Small RNA transcriptomes reveal insights into dynamic homoeolog regulation of allopolyploid heterosis in nascent hexaploid wheat. Plant Cell 26:1878-1900

Little EL (2016) 2016 Georgia Plant Disease Loss Estimate

Liu B, Xu C, Zhao N, Qi B, Kimatu JN, Pang J, Han F (2009) Rapid genomic changes in polyploid wheat and related species: implications for genome evolution and genetic improvement. J Genet Genomics 36:519-528

Liu G, Li Z, Bao M (2007) Colchicine-induced chromosome doubling in Platanus acerifolia and its effect on plant morphology. Euphytica 157:145-154

Madlung A, Masuelli RW, Watson B, Reynolds SH, Davison J, Comai L (2002) Remodeling of DNA methylation and phenotypic and transcriptional changes in synthetic Arabidopsis allotetraploids. Plant Physiol 129:733-746

Madlung A, Tyagi AP, Watson B, Jiang H, Kagochi T, Doerge RW, Martienssen R, Comai L (2005) Genomic changes in synthetic Arabidopsis polyploids. Plant J 41:221-230

Mallikarjuna N, Jadhav D, Reddy K, Husain F, Das K (2012) Screening new Arachis amphidiploids, and autotetraploids for resistance to late leaf spot by detached leaf technique. Eur J Plant Pathol 132:17-21

Mallikarjuna N, Senthilvel S, Hoisington D (2011) Development of new sources of tetraploid Arachis to broaden the genetic base of cultivated groundnut (Arachis hypogaea L.). Genet Resour Crop Evol 58:889-907

Masterson J (1994) Stomatal Size in Fossil Plants: Evidence for Polyploidy in Majority of Angiosperms. Sci 264:421-424

McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792-801

Mhiri C, Parisod C, Daniel J, Petit M, Lim KY, Dorlhac de Borne F, Kovarik A, Leitch AR, Grandbastien MA (2019) Parental transposable element loads influence their dynamics in young Nicotiana hybrids and allotetraploids. New Phytol 221:1619-1633

Minton NA, Baujard P (1990) Nematode parasites of peanut. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, UK, pp 285-320

Moens M, Perry RN, Starr JL (2009) Meloidogyne species - a diverse group of novel and important plant parasites. In: Perry RN, Moens M, Starr JL (eds) Root-knot nematodes. CAB International, Wallingford, UK, pp 1-17

Moretzsohn MC, Gouvea EG, Inglis PW, Leal-Bertioli SC, Valls JF, Bertioli DJ (2013) A study of the relationships of cultivated peanut (Arachis hypogaea) and its most closely related wild species using intron sequences and microsatellite markers. Ann Bot 111:113-126

Moretzsohn MdC, Hopkins MS, Mitchell SE, Kresovich S, Valls JFM, Ferreira ME (2004) Genetic diversity of peanut (Arachis hypogaea L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biol 4:11-11

Morgante C, Brasileiro ACM, Roberts PA, Guimaraes La, Aaujo A, Fonseca LN, Leal-Bertioli S, Bertioli D, Guimarães P (2013) A survey of genes involved in Arachis stenosperma resistance to Meloidogyne arenaria race 1. Functional Plant Biology 40:1298

Mota APZ, Vidigal B, Danchin EGJ, Togawa RC, Leal-Bertioli SCM, Bertioli DJ, Araujo ACG, Brasileiro ACM, Guimaraes PM (2018) Comparative root transcriptome of wild Arachis reveals NBS-LRR genes related to nematode resistance. BMC Plant Biol 18:159

Mupunga I, Mngqawa P, Katerere DR (2017) Peanuts, Aflatoxins and Undernutrition in Children in Sub-Saharan Africa. Nutrients 9

Nagy ED, Chu Y, Guo Y, Khanal S, Tang S, Li Y, Dong WB, Timper P, Taylor CG, Ozias-Akins P, Corley H. C, Beilinson V, Nielsen NC, H. TS, Knapp SJ (2010) Recombination is suppressed in an alien introgression in peanut harboring Rma, a dominant root-knot nematode resistance gene. Molecular Breeding 26:357-370

Nelson SC, Simpson CE, Starr JL (1989) Resistance to Meloidogyne arenaria in Arachis spp. germplasm. J Nematol 21:654-660

Nelson SC, Starr JL, Simpson CE (1990) Expression of resistance to Meloidogyne arenaria in Arachis batizocoi and A. cardenasii. J Nematol 22:242-244

Nicol JM, Turner SJ, Coyne DL, Nijs Ld, Hockland S, Maafi ZT (2011) Current Nematode Threats to World Agriculture. In: Jones J, Gheysen G, Fenoll C (eds) Genomics and Molecular Genetics of Plant-Nematode Interactions. Springer Netherlands, Dordrecht, pp 21-43

Noe JP, Holbrook CC, Minton NA (1992) Field Evaluation of Susceptibility to Meloidogyne arenaria in Arachis hypogaea Plant Introductions. J Nematol 24:712-716

Norden AJ, Perry VG, Martin FG, NeSmith J (1977) Effect of Age of Bahiagrass Sod on Succeeding Peanut Crops. Peanut Science 4:71-74

O'Neill RJ, O'Neill MJ, Graves JA (1998) Undermethylation associated with retroelement activation and chromosome remodelling in an interspecific mammalian hybrid. Nature 393:68-72

Oerke EC (2006) Crop Losses to Pests. The Journal of Agricultural Science 144:31-43

Onkendi EM, Kariuki GM, Marais M, Moleleki LN (2014) The threat of root-knot nematodes (Meloidogyne spp.) in Africa: a review. Plant Pathology 63:727-737

Otto SP, Whitton J (2000) Polyploid incidence and evolution. Ann Rev Gen 34:401-437

Pande S, Rao JN (2001) Resistance of Wild Arachis Species to Late Leaf Spot and Rust in Greenhouse Trials. Plant Dis Report 85:851-855

Pandey MK, Monyo E, Ozias-Akins P, Liang X, Guimarães P, Nigam SN, Upadhyaya HD, Janila P, Zhang X, Guo B, Cook DR, Bertioli DJ, Michelmore R, Varshney RK (2012) Advances in Arachis genomics for peanut improvement. Biotechnol Adv 30:639-651

Penaloza APS, Valls JFM (2005) Chromosome number and satellite chromosome morphology of eleven species of Arachis (Leguminosae). Bonplandia 14:65-72

Perry RN, Moens M, Starr JL (2009) Root-knot nematodes. CAB International, Wallingford, UK

Pontes O, Neves N, Silva M, Lewis MS, Madlung A, Comai L, Viegas W, Pikaard CS (2004) Chromosomal locus rearrangements are a rapid response to formation of the allotetraploid Arabidopsis suecica genome. Proc Natl Acad Sci U S A 101:18240-18245

Proite K, Carneiro R, Falcão R, Gomes A, Leal-Bertioli S, Guimarães P, Bertioli D (2008) Post-infection development and histopathology of Meloidogyne arenaria race 1 on Arachis spp. Plant Pathology 57:974-980

Proite K, Leal-Bertioli SC, Bertioli DJ, Moretzsohn MC, da Silva FR, Martins NF, Guimaraes PM (2007) ESTs from a wild Arachis species for gene discovery and marker development. BMC Plant Biol 7

Qi B, Huang W, Zhu B, Zhong X, Guo J, Zhao N, Xu C, Zhang H, Pang J, Han F, Liu B (2012) Global transgenerational gene expression dynamics in two newly synthesized allohexaploid wheat (Triticum aestivum) lines. BMC Biol 10:3

Ramos ML, 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. Mol Genet Genomics 275:578-592

Rapp RA, Udall JA, Wendel JF (2009) Genomic expression dominance in allopolyploids. BMC Biol 7:18

Rapp RA, Wendel JF (2005) Epigenetics and plant evolution. New Phytol 168:81-91

Robledo G, Lavia GI, Seijo G (2009) Species relations among wild Arachis species with the A genome as revealed by FISH mapping of rDNA loci and heterochromatin detection. Theor Appl Genet 118:1295-1307

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. Theor Appl Genet 121:1033-1046

Rodriguez-Kabana R, Kokalis-Burelle N, Robertson DG, King PS, Wells LW (1994) Rotations with Coastal Bermudagrass, Cotton, and Bahiagrass for Management of Meloidogyne arenaria and Southern Blight in Peanut. J Nematol 26:665-668

Rodríguez-Kábana R, Robertson DG, Weaver CF, Wells L (1991) Rotations of bahiagrass and castorbean with peanut for the management of Meloidogyne arenaria. J Nematol 23:658-661

Salmon A, Flagel L, Ying B, Udall JA, Wendel JF (2010) Homoeologous nonreciprocal recombination in polyploid cotton. New Phytol 186:123-134

Sasser JN, Freckman DW (1987) A World Perspective on Nematology: The Role of the Society. In: Veech JA, Dickson DW (eds) Vistas on Nematology. Society of Nematologist, Inc., Hyattsville, Maryland, pp 7-14

Savary S, Ficke A, Aubertot J-N, Hollier C (2012) Crop losses due to diseases and their implications for global food production losses and food security. Food Security 4

Seijo G, Lavia GI, Fernández A, Krapovickas A, Ducasse DA, Bertioli DJ, Moscone EA (2007) Genomic relationships between the cultivated peanut (Arachis hypogaea, Leguminosae) and its close relatives revealed by double GISH. Am J Bot 94:1963-1971

Seijo JG, Lavia GI, Fernandez A, Krapovickas A, Ducasse D, Moscone EA (2004) Physical mapping of the 5S and 18S-25S rRNA genes by FISH as evidence that Arachis duranensis and A. ipaensis are the wild diploid progenitors of A. hypogaea (Leguminosae). Am J Bot 91:1294-1303

Settaluri VS, Kandala CVK, Puppala N, Sundaram J (2012) Peanuts and Their Nutritional Aspects¡ A Review. Food and Nutrition Sciences 3:1644-1650

Shaked H, Kashkush K, Ozkan H, Feldman M, Levy AA (2001) Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell 13:1749-1759

Shen Y, Zhao Q, Zou J, Wang W, Gao Y, Meng J, Wang J (2014) Characterization and expression patterns of small RNAs in synthesized Brassica hexaploids. Plant molecular biology 85:287-299

Shirasawa K, Bertioli DJ, Varshney RK, Moretzsohn MC, Leal-Bertioli SCM, Thudi M, Pandey MK, Rami J-F, Foncéka D, Gowda MVC, 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 research : an international journal for rapid publication of reports on genes and genomes 20:173-184

Sikora RA (2018) Plant parasitic nematodes in subtropical and tropical agriculture, 3rd edition. edn. CABI, Boston, MA

Sikora RA, Bridge J, Starr JL (2005) Management practices: an overview of integrated nematode management technologies. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB Internationa, Wallingford, pp 793–825

Simpson C (1991) Pathways for introgression of pest resistance into Arachis hypogaea L. Peanut Sci 18:22-26

Simpson C (2001) Use of Wild Arachis Species/Introgression of Genes into A. hypogaea L. Peanut Sci 28:114-116

Simpson C, C. Nelson S, L. Starr J, E. Woodard K, D. Smith O (1993) Registration of TxAG-6 and TxAG-7 peanut germplasm. Crop Sci 33:1418

Simpson C, L. Starr J, Church G, D. Burow M, H. Paterson A (2003) Registration of NemaTAM peanut. Crop Sci 43:1561

Simpson C, L. Starr J, R. Baring M, D. Burow M, M. Cason J, N. Wilson J (2013) Registration of ‘Webb’ peanut. J Plant Regist 7:265

Simpson C, Starr J (2001) Registration of ‘COAN’ peanut. Crop Sci 41:918

Singh B, Singh U (1991) Peanut as a source of protein for human foods. Plant Food Hum Nutr 41:165-177

Skalicka K, Lim KY, Matyasek R, Matzke M, Leitch AR, Kovarik A (2005) Preferential elimination of repeated DNA sequences from the paternal, Nicotiana tomentosiformis genome donor of a synthetic, allotetraploid tobacco. New Phytol 166:291-303

Soltis DE, Buggs RJA, Doyle JfJ, Soltis PS (2010) What we still don't know about polyploidy. Taxon 59:1387-1403

Soltis PS, Soltis DE (2009) The role of hybridization in plant speciation. Annu Rev Plant Biol 60:561-588

Stalker H (1997) Peanut (Arachis hypogaea L.). Field Crops Research 53:205–217

Stalker HT (1984) Utilizing Arachis cardenasii as a source of Cercospora leafspot resistance for peanut improvement. Euphytica 33:529-538

Stalker TH (2017) Utilizing Wild Species for Peanut Improvement. Crop Sci 57:1102

Starr J, R. Morgan E, E. Simpson C (2002) Management of the peanut root-knot nematode, Meloidogyne arenaria, with host resistance. Plant Health Prog

Strange RN, Scott PR (2005) Plant disease: A threat to global food security. Annu Rev Phytopathol 43:83-116

Szadkowski E, Eber F, Huteau V, Lode M, Huneau C, Belcram H, Coriton O, Manzanares-Dauleux MJ, Delourme R, King GJ, Chalhoub B, Jenczewski E, Chevre AM (2010) The first meiosis of resynthesized Brassica napus, a genome blender. New Phytol 186:102-112

Tate JA, Joshi P, Soltis KA, Soltis PS, Soltis DE (2009) On the road to diploidization? Homoeolog loss in independently formed populations of the allopolyploid Tragopogon miscellus (Asteraceae). BMC Plant Biol 9:80

Timper P, Dickson DW, Steenkamp S (2018) Nematode parasites of groundnut. In: Sikora RA, Coyne DL (eds) Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, pp 411-445

Valls JOS, Simpson CE (2005) New species of Arachis (leguminosae) from Brazil, Paraguay and Bolivia. Bonplandia 14:35-63

Wang H, Jiang J, Chen S, Qi X, Fang W, Guan Z, Teng N, Liao Y, Chen F (2014) Rapid genetic and epigenetic alterations under intergeneric genomic shock in newly synthesized Chrysanthemum morifolium x Leucanthemum paludosum hybrids (Asteraceae). Genome Biol Evol 6:247-259

Wang J, Tian L, Lee HS, Wei NE, Jiang H, Watson B, Madlung A, Osborn TC, Doerge RW, Comai L, Chen ZJ (2006) Genomewide nonadditive gene regulation in Arabidopsis allotetraploids. Genetics 172:507-517

Wendel JF, Jackson SA, Meyers BC, Wing RA (2016) Evolution of plant genome architecture. Genome Biol 17:37

Wesemael W, Viaene N, Moens M (2011) Root-knot nematodes (Meloidogyne spp.) in Europe. Nematology 13:3-16

Williamson VM, Hussey RS (1996) Nematode pathogenesis and resistance in plants. The Plant cell 8:1735-1745

Wu J, Lin L, Xu M, Chen P, Liu D, Sun Q, Ran L, Wang Y (2018) Homoeolog expression bias and expression level dominance in resynthesized allopolyploid Brassica napus. BMC Genomics 19:586

Wu Y, Sun Y, Wang X, Lin X, Sun S, Shen K, Wang J, Jiang T, Zhong S, Xu C, Liu B (2016) Transcriptome shock in an interspecific F1 triploid hybrid of Oryza revealed by RNA sequencing. J Integr Plant Biol 58:150-164

Xu C, Bai Y, Lin X, Zhao N, Hu L, Gong Z, Wendel JF, Liu B (2014) Genome-wide disruption of gene expression in allopolyploids but not hybrids of rice subspecies. Mol Biol Evol 31:1066-1076

Xu W, Fang HY, Xu D, Xu WL, Liu XS, Yang JG (2007) Controlling welding hot cracking based on electromagnetic force. Sci Technol Weld Joi 12:659-663

Yoo MJ, Liu X, Pires JC, Soltis PS, Soltis DE (2014) Nonadditive gene expression in polyploids. Annu Rev Genet 48:485-517

Yoo MJ, Szadkowski E, Wendel JF (2013) Homoeolog expression bias and expression level dominance in allopolyploid cotton. Heredity 110:171-180

Zhang H, Gou X, Zhang A, Wang X, Zhao N, Dong Y, Li L, Liu B (2016) Transcriptome shock invokes disruption of parental expression-conserved genes in tetraploid wheat. Sci Rep 6:26363

CHAPTER 2

A NEW SOURCE OF ROOT-KNOT NEMATODE RESISTANCE FROM *ARACHIS STENOSPERMA* INCORPORATED INTO ALLOTETRAPLOID PEANUT (*ARACHIS HYPOGAEA*) [1]

[1] Ballén-Taborda C, Chu C, Jackson S, Ozias-Akins P, Timper P, Holbrook C, Bertioli D, Leal-Bertioli S. Accepted and soon to be published by *Scientific reports*.

Abstract

Root-knot nematode is a very destructive pathogen, to which most peanut cultivars are highly susceptible. Strong resistance is present in the wild diploid peanut relatives. Previously, QTLs controlling nematode resistance were identified on chromosomes A02, A04 and A09 of *Arachis stenosperma*. Here, to study the inheritance of these resistance alleles within the genetic background of tetraploid peanut, an F_2 population was developed from a cross between peanut and an induced allotetraploid that incorporated *A. stenosperma*, [*Arachis batizocoi* x *A. stenosperma*]^{4x}. This population was genotyped using a SNP array and phenotyped for nematode resistance. QTL analysis allowed us to verify the major-effect QTL on chromosome A02 and a secondary QTL on A09, each contributing to a percentage reduction in nematode multiplication up to 98.2%. These were validated in selected $F_{2:3}$ lines. The genome location of the large-effect QTL on A02 is rich in genes encoding TIR-NBS-LRR protein domains that are involved in plant defenses. We conclude that the strong resistance to RKN, derived from the diploid *A. stenosperma*, is transferrable and expressed in tetraploid peanut. Currently it is being used in breeding programs for introgressing a new source of nematode resistance and to widen the genetic basis of agronomically adapted peanut lines.

Introduction

Arachis hypogaea L. (peanut or groundnut) is an important oilseed, food and forage crop, cultivated worldwide in tropical and subtropical regions with annual production of 64.2 million tons. In the USA, peanut was grown on more than a half million ha (0.72 ha) with an average yield of 4.57 tonnes/ha in 2016 (FAOSTAT 2018).

Peanut is an allotetraploid species with very low genetic diversity due to its recent polyploid origin (Bertioli et al. 2016; Bertioli et al. 2019; Halward et al. 1992; Husted 1936; Kochert et al. 1996b; Moretzsohn et al. 2013). It is highly susceptible to several pests and diseases, including root-knot nematode (RKN) *Meloidogyne arenaria* (Neal). This is in part due to the absence of gene flow with diploid wild relatives with resistant alleles (Holbrook and Stalker 2003). Root-knot nematode causes substantial yield losses, reduces pod and grain quality, affects plant growth and increases production cost (Holbrook and Stalker 2003; Starr et al. 2002). Crop rotation and nematicides are commonly used for nematode management. Due to the ability of *M. arenaria* to infect most crops, few non-host crops are available to reduce populations of *M. arenaria* and crop damage (Johnson et al. 1999; Rodríguez-Kábana et al. 1991). Additionally, chemical control for nematode management is not only costly, but also presents concerns for effects on human health and the environment that have led to the loss of registration of many of the commonly used nematicides (Danchin et al. 2013). Development of high-yielding and nematode-resistant cultivars is an efficient and effective way to control nematode populations and decrease yield losses while reducing the use of nematicides (Dong et al. 2007).

Natural sources of resistance to RKN are not present in cultivated peanut, but found in wild relatives that can be utilized to enhance peanut performance under disease pressure (Holbrook and Noe 1990a; Nelson et al. 1989; Stalker 1997). The first use of a wild relative to introgress RKN resistance into peanut dates from 2001, with the release of the cultivar COAN (Simpson and Starr 2001). The resistance was based on a chromosome A09 segment from the wild species *Arachis cardenasii* Krapov. & W.C.

Greg. (Chu et al. 2016a). The segment was introgressed through a backcrossing scheme involving interspecific hybrids (Simpson 1991; Simpson et al. 1993). Since then, several other cultivars have been released in the U.S. using the same source of resistance: NemaTAM, Tifguard, Webb, Georgia-14N and TifNV-High O/L (Branch and Brenneman 2015; Holbrook et al. 2017; Holbrook et al. 2008; Simpson et al. 2003; Simpson et al. 2013).

Additional sources of resistance are important for the development of new high-yielding and nematode-resistant peanut cultivars, and to reduce the risk of resistance breakdown in the varieties currently used (Starr et al. 2002). Previously, we studied the peanut wild relative *Arachis stenosperma* Krapov. & W. C. Greg., which harbors resistance to a number of pests, including the RKN *Meloidogyne* spp. and foliar diseases, such as late leaf spot and rust (Leal-Bertioli et al. 2010; Leal-Bertioli et al. 2009; Proite et al. 2008; Proite et al. 2007). Subsequently, using diploid mapping populations, we identified a large-effect QTL controlling RKN resistance on chromosome A02 together with minor effect QTLs on A04 and A09 of *A. stenosperma* (accession V10309) (Leal-Bertioli et al. 2016). Strong resistance to RKN also has been reported in the wild diploid species *A. batizocoi* Krapov. & W. C. Greg. (accession K9484) (Nelson et al. 1990).

In this study, an F_2 population derived from the cross of *A. hypogaea* with an induced allotetraploid (*A. batizocoi* x *A. stenosperma*)^{4x} (Leal-Bertioli et al. 2015c) was used to identify genome regions that confer RKN resistance within a tetraploid genetic background; to develop reliable molecular markers tightly linked to the resistance loci for selection in breeding programs; and finally, to characterize the genetic behavior of wild-cultivated crosses. This research will contribute to the production of advanced peanut

lines that incorporate wild-derived chromosome segments that confer a new source of resistance to RKN.

Materials and Methods

Plant materials

Wild *Arachis* accessions were obtained from the USDA-GRIN system (https://www.ars-grin.gov/). To introgress the diploid nematode resistance into tetraploid peanut, a synthetic allotetraploid (BatSten1) was created using the peanut wild relatives *A. stenosperma* PI666100 (original collection voucher V10309) and *A. batizocoi* PI298639 (original collection voucher K9484), as described in Leal-Bertioli et al., (2015) (Leal-Bertioli et al. 2015a). This wild-derived allotetraploid combines the A genome of *A. stenosperma* (Moretzsohn et al. 2013; Tallury et al. 2005) and a K genome (B genome *sensu lato*) of *A. batizocoi* (Moretzsohn et al. 2013; Robledo and Seijo 2010). An F_2 segregating population was created by selfing an F_1 derived from a cross between *A. hypogaea* cv. Runner IAC-886 (herein called Runner-886) and BatSten1. This population was named RBS-F_2 and had initially, 196 individuals. To be able to perform the bioassays in different years, the RBS-F_2 population was maintained in the greenhouse by vegetative propagation. The majority of the individuals, but not all, were maintained for the duration of this work.

Root-knot nematode resistance evaluation

The RBS-F_2 population was evaluated for resistance to root-knot nematode (RKN) *M. arenaria* Chitwood race 1 under greenhouse conditions (Chu et al. 2007; Leal-

Bertioli et al. 2016) in a randomized complete block design with five replicates per genotype. The tetraploid parents (BatSten1 and Runner-886) and the diploid wild species *A. batizocoi* and *A. stenosperma* were used as controls. RKN populations were maintained and extracted from eggplant (*Solanum melongena* cv. Black Beauty). *Arachis* seeds were planted in nursery pots (15 cm in diameter 10 cm in height) filled with Promix (Premier Horticulture, Quakertown, PA) and maintained in a greenhouse. Two-month-old cuttings from each F_2 line were established in steam-sterilized sandy soil and inoculated with 10,000 second-stage RKN juveniles (J2) by distributing the inoculum in two 2-cm deep holes at the base of the plant. Eight weeks later, plants were uprooted, rinsed, and the roots weighed after removing excess water with a paper towel. Roots were stained with 0.05% phloxin B solution for 3 to 5 min. Nematode eggs were extracted from roots using 0.5% NaOCl (Holbrook et al. 2003; Hussey and Barker 1973). Assays were conducted over three years, with 155, 105 and 99 segregating F_2s, respectively, as a few individuals died with time. Resistance was assessed using three different traits: 1) Numbers of eggs per gram of root (EGR); 2) Nematode reproductive factor (RF = Pf/Pi; where Pf is the final egg population and Pi the initial J2 population (Oostenbrink 1966); and 3) Root-galling index or egg masses: 0 (no galling or no egg masses), 1 (1-2 galls), 2 (3-10 galls), 3 (11-30 galls), 4 (31-100 galls) and 5 (more than 100 galls or egg masses per root system) (Holbrook et al. 2003).

Statistical analysis

The Shapiro-Wilk test was used to test normality of phenotypic data. Non-parametric Kruskal-Wallis one-way analysis of variance (Kruskal and Wallis 1952) was

used to assess the global differences in phenotypic traits at a 5% level of significance ($P<0.05$) among RBS-F_2 lines and controls for each year using the Statistical package R. Non-normal phenotype data were transformed to Log_{10} and $Log_{10}(x+1)$ for QTL identification. Additionally, the Best Linear Unbiased Predictors (BLUPs) of random effects were calculated for each trait using the *ranef* function in R. BLUPs were calculated in order to control for missing phenotypic data and transformed to Log_{10} and $Log_{10}(x+1)$.

SNP genotyping, analysis and data filtering

Genomic DNAs of 196 individuals from the RBS-F_2 population and controls (BatSten1, Runner-886, *A. stenosperma* and *A. batizocoi*) were extracted from leaves using the DNeasy *Plant* Mini *Kit* (*QIAGEN*) according to manufacturer's instructions. DNAs were quantified with PicoGreen and samples were submitted for genotyping with the 'Axiom_Arachis v01' 58K high-density SNP array (Clevenger et al. 2017b; Pandey et al. 2017). The genotypic data were extracted, processed and analyzed using the Axiom Analysis Suite 2.0 software (http://www.affymetrix.com). Output was analyzed using Unix scripts (Script 2.S1) and data were visualized as a color map in Microsoft Excel. The strategy to identify polymorphic SNP markers included three different steps:

Firstly, informative SNP assay results were extracted from SNP calling using a panel of diploid species plus a single tetraploid genotype (*A. hypogaea* Runner-886). This set of markers was filtered to reveal SNP markers specific to each of the three parental species in the pedigree of the F_2 population as follows:

- *A. stenosperma*-characteristic markers: *A. stenosperma* ≠ (*A. batizocoi* = *A. hypogaea*)
- *A. batizocoi*-characteristic markers: *A. batizocoi* ≠ (*A. stenosperma* = *A. hypogaea*)
- Runner-886 characteristic markers: *A. hypogaea* ≠ (*A. stenosperma* = *A. batizocoi*)

Secondly, SNP assay results were extracted from SNP calling of tetraploid genotypes only (Runner-886, BatSten1 and RBS-F_2 population). Finally, the three sets of informative SNP markers identified in the first step were retrieved using the panel of tetraploid genotypes, followed by merging and filtering as BatSten1 ≠ *A. hypogaea*.

Genetic Mapping and QTL discovery

Genetic maps for A and B subgenomes were constructed using Kosambi's genetic map function (Kosambi 1943) and maximum likelihood algorithm in JoinMap v.4.0 (Stam 1993; Van Ooijen 2006). The goodness of fit Chi-square test was performed to evaluate the expected 1:2:1 segregation ratio for the F_2 population for each locus ($P<0.01$). The genetic map was visualized by calculating pairwise logarithm of the odds (LOD) scores and recombination fractions using the *plot.rf* function in R/QTL (Broman and Sen 2009; Broman et al. 2003).

The genetic map in combination with transformed RKN measurements of resistance for three years were used for QTL identification using R/QTL software following the procedure described in "A guide to QTL mapping with R/qtl" (Broman and Sen 2009). Due to the spike observed in the phenotype data distribution at zero (*null* phenotype, Fig. 2.1), we employed a two-part binary plus normal analysis method using

the *scanone* function (Broman and Sen 2009; Broman 2003; Broman et al. 2003). The two-part model is suitable for data with non-normal distribution prior and after transformations, as we observed in our case (Fig. 2.1). This model performs two different analyses. First, the phenotype is treated as a binary trait (0 or >0), and then as a quantitative trait, for those individuals with phenotypic values above zero (Broman 2003; Holland and Coles 2011). The two-part model calculates the LOD scores for each tested genome position to assess the significant association with the trait of interest (Broman and Sen 2009; Broman 2003; Broman et al. 2003). 1000 permutations were used to identify genome-wide LOD significance thresholds for QTL identification at 1% and 5% level of significance (Churchill and Doerge 1994; Doerge and Churchill 1996; Zhou et al. 2016). 95% Bayesian credible interval was calculated with the *bayesint* function and LOD support interval with *lodint function* in R/qtl. The percentage of phenotypic variability explained by a QTL (R^2) and the estimated effect was assessed using the *fitqtl* function in R/qtl (Broman and Sen 2009). Physical positions for each marker on the A and B subgenomes were determined, respectively, based on the *A. duranensis* and *A. ipaensis* pseudomolecules (https://www.peanutbase.org/) (Bertioli et al. 2016).

Meiotic behavior analysis and tetrasomic inheritance

Given that the genome of tetraploid peanut harbors regions where homeologous recombination can occur (Bertioli et al. 2016; Bertioli et al. 2019; Clevenger et al. 2017b; Leal-Bertioli et al. 2015a; Nguepjop et al. 2016), we examined our data for evidence of such chromosomal behavior. When assuming recombination only between homologous chromosomes, the expected segregation ratio in the F_2 population should be

$1(A_1A_1):2(A_1A_2):1(A_2A_2)$ for A-subgenome and 1(BB):2(BK):1(KK) for the B/K-subgenome, but when homeologous chromosomes recombine during the parental and/or F_1 meiosis, the expected segregation ratio in the F_2 progeny changes, as described by Nguepjop et al. (2016) (Nguepjop et al. 2016).

In order to determine lines exhibiting homeologous recombination and the markers associated with these regions, all 1499 polymorphic markers were visually and manually inspected. The criterion was to analyze data points with "No Call" data or forming unexpected genetic clusters, similar to the rationale described in Leal-Bertioli et al., (2015) (Leal-Bertioli et al. 2015a) but applied to an F_2 population. Genotypes and markers were scored as "tetrasomic" on the color map. Additionally, segregation distortion from 1:2:1 ratio ($P<0.01$) was analyzed for markers segregating in a disomic manner.

Results

Nematode screening

Resistance to RKN was evaluated over three years by measuring three traits: EGR (eggs/gram of root), RF (reproductive factor), and GI (galling index). *Arachis stenosperma* V10309*, A. batizocoi* K9484 and the induced allotetraploid BatSten1 ([*A. batizocoi* x *A. stenosperma*]$^{(2n=4x=40)}$) were found to be resistant to RKN, with no or small galls and low egg production. Cultivar Runner-886 was susceptible in all the assays and presented the highest values for all traits (Fig. 2.1 and Table 2.S1). Individuals from the RBS-F_2 population (derived from a cross between *A. hypogaea* cv. Runner IAC-886 and the synthetic allotetraploid BatSten1) showed varying degrees of resistance to RKN. The

frequency distributions of EGR, RF and GI traits were distinctly non-normal, being skewed towards resistance (Fig. 2.1). EGR, RF and GI values for the RBS-F_2 were significantly different at $P<0.05$ for 2014 and 2016 assays, but not for 2015. Values for the two parents (Runner-886 and BatSten1) were significantly different ($P<0.05$) for 2014 and 2016 assays (in 2015 data for BatSten1 was not available). Transgressive segregation was also observed: across years, on average 49.3 %, 48.0 % and 59.0 % segregating individuals were as, or more, resistant than *A. stenosperma* for EGR, RF and GI, respectively. For the three measured traits, few lines were more susceptible than Runner-886; on average 0.7 %, 0.3 % and 0.3 % individuals were as, or more, susceptible than Runner-886 for EGR, RF and GI, respectively. Several individuals that produced galls did not support the production of nematode eggs. These traits were highly correlated: Pearson correlation was calculated in Minitab v.15.1.0.0 between EGR, RF and GI and significant values ($P \leq 0.01$) ranged between 0.281 and 0.887.

Genotyping and genetic mapping

Using the Affymetrix 'Axiom_Arachis' SNP array (Clevenger et al. 2017b; Pandey et al. 2017) 196 F_2 progeny and controls were genotyped. Two individuals were eliminated since they had too many missing data points. For the rest of the analyses, data of 194 progenies were used. A total of 1587 polymorphic SNP markers were identified from our filtering strategy, 911 assigned to A-subgenome (*A. stenosperma*-specific markers) and 676 to B/K-subgenome (*A. batizocoi*-specific markers); additionally, 9696 *A. hypogaea*-specific markers were identified. Only markers derived from *A. stenosperma* and *A. batizocoi* were utilized for genetic mapping and QTL identification.

The physical positions of the A-genome markers were determined according to the position of their homologues in the *A. duranensis* pseudomolecules and the B-genome markers based on the *A. ipaensis* pseudomolecules (https://www.peanutbase.org/) (Bertioli et al. 2016). After removing low-quality and unlinked markers, 1499 SNP markers were ordered into 20 linkage groups (LGs) that ranged in size from 100.7 cM (LG A05) to 359.5 cM (LG A02), spanning a total map distance of 3984.9. The average distance between adjacent markers ranged from 1.35 cM (LG A01) to 3.8 cM (LG A06) and the largest distance was 29.48 cM (LG A06). The number of markers in each linkage group varied from 36 (LG B04) to 217 (LG A01) (Fig. 2.S1 and Table 2.S2). There was a strong relationship between genetic and physical positions of their homologues. As expected, higher recombination frequencies were observed in the distal parts of the chromosomes. LG A07 presents an inversion on the lower end relative to its diploid ancestor, but consistent with the genome sequence of *A. hypogaea* (Fig. 2.S2) (Bertioli et al. 2019). The heatmap of the marker-pairwise estimated recombination fractions versus LOD scores indicated that there is only linkage within LGs, with the exception of LGs A05 and B05, where markers seem to be tightly associated with each other, especially at the beginning and end of the linkage groups (Fig. 2.S3). This is consistent with tetrasomic genome composition and recombination between alleles between these homeologous chromosomes (Bertioli et al. 2016; Bertioli et al. 2019).

QTL identification

A large-effect QTL for all three measurements of resistance to RKN was detected at the bottom of LG A02 with LOD scores above 5.9 and with a peak at marker

A02_89159922_Sten (320.7 cM) at genome-wise α = 0.05 and 0.01 thresholds (Fig. 2.2, Table 2.1 and Fig. 2.S4.A-C). Using EGR and RF data, the QTL was identified at 1% level of significance (Fig. 2.S4.A-B) and for GI data at 5% (Fig. 2.S4.C). A second QTL for EGR and RF was observed in LG A04 below the α = 0.05 genome-wise threshold. This QTL seems to comprise two separate loci with LOD scores above 3.2. The highest peak was near A04_111013470_Sten SNP marker (160.6 cM), while the lower peak was near A04_110684871_Sten (139.6 cM) (Fig. 2.2, Table 2.1 and Fig. 2.S5.A-B). A third QTL, for RF, was present in LG A09 with LOD score of 3.7 at marker A09_116335836_Sten (139.6) (Fig. 2.2, Table 2.1 and Fig. 2.S5.C).

All QTLs derived from *A. stenosperma* are colocalized with the ones found in a previous study on a *A. stenosperma*-derived diploid population (Fig. 2.2) (Leal-Bertioli et al. 2016). The QTL on LG A02 contributed to a percentage reduction up to 98.2% in nematode multiplication. Although putative QTL on A09 did not pass the estimated threshold, it reduced the nematode development up to 97.8%. Putative QTL on A04 did not contribute to improve nematode resistance (Table 2.1). Although *A. batizocoi* was also found to be resistant to RKN (Nelson et al. 1990), *A. batizocoi*-derived QTLs associated with nematode resistance were not identified.

The analysis of phenotypic effects of markers tightly linked to QTLs contributing to nematode resistance reveals that most F_2 lines with *A. stenosperma* alleles at QTLs on LG A02 and A09 showed the lowest phenotypic mean scores for all the measurements of resistance (EGR, RF and GI); whereas, F_2 plants carrying *A. hypogaea* alleles had a higher incidence of the disease (Fig. 2.3.A,C), Graphs for RF and GI are not shown, but have the same trend. This was also supported with the positive additive effect values

(Table 2.1), suggesting that these genome segments contribute to reduced egg production, nematode reproduction and gall formation, as previously described (Leal-Bertioli et al. 2016). This tendency was also observed when analyzing combined effect of two QTLs. F_2 lines that were homozygous for *A. stenosperma* alleles on A02 and A09 together had low disease scores (Fig. 2.3.D), on average presenting reduction of EGR, RP and GI, of 87.3%, 95.2% and 67.5%, respectively. Conversely, the putative QTL on LG A04 does not produce an improved resistance when the F_2 lines were homozygous or heterozygous for the *A. stenosperma* alleles (Fig. 2.3.B,E-F), which is supported with the negative additive effect we found for this segment (Table 2.1). It is possible that this trend on the A04 putative QTL reveals underdominance, where heterozygous F_2 lines have an inferior performance for nematode infection than either the cultivated or wild homozygous genotypes (Fig. 2.3.B). Alternatively, the lower resistance could have arisen from unfavorable gene interactions (Schierup 1996).

Validation of resistance

Progeny of F_2 lines produced on average 10 seeds per plant (median = 4). Six lines that produced between 12 and 36 seeds were selected to be planted in the field for yield and disease resistance evaluation. Two lines that carried markers associated to QTLs in LG02 and LG09 were selected and advanced to the $F_{2:3}$ generation. To confirm the RKN resistance on the $F_{2:3}$ lines, cuttings where phenotyped for RKN resistance using the same screening approach described in Methods but using eight replicates per genotype and inoculation with 5.1K second-stage juveniles (J2) per pot (Table 2.2). According to Kruskal-Wallis test ($P<0.05$), there was a significant difference in RKN

resistance between genotypes. Further analysis included the Wilcoxon signed rank test for pairwise comparisons using FDR (false discovery rate) correction, in order to group the samples by significant similarity (P>0.05). According to the grouping, for EGR, RF and GI all the F_2-derived F_3 lines were significantly different from the TifGp-2 susceptible genotype (Table 2.2). These lines were also genotyped using the 'Axiom_Arachis v01' 58K high-density SNP array (Clevenger et al. 2017b; Pandey et al. 2017) and data processed similarly to the F_2 genotypic data. Only genotyping calls for the *A. stenosperma* and *A. batizocoi*-derived markers were taken into account, the data was then filtered as BatSten1 ≠ *A. hypogaea* (Script 2.S2) and then visually inspected for the *A. stenosperma* introgressions along the three chromosome segments associated with nematode resistance in LG A02, A04 and A09. $F_{2:3}$-7 and $F_{2:3}$-34 were found to carry the resistance segments in heterozygous or homozygous states for *A. stenosperma* alleles (Table 2.2).

Inheritance patterns and segregation distortion

In an F_2 population with disomic recombination, three main genotypic classes are expected: the two parental and the hybrid types (Fig. 2.4.A). A set of 1156 SNPs (77.1%) were inherited as expected under disomic inheritance: 694 (46.3%) markers followed the Mendelian segregation ratio expected for an F_2 population (1:2:1) and 462 (30.8%) showed significant deviation (P<0.01) (Table 2.3). From the 234 (15.6%) distorted loci located in the A-genome, distorted blocks were skewed toward the cultivated genotype specially on LGs A04 and A05. Among the 228 (15.2%) loci in B/K genome, we found a

biased segregation in favor of wild in B06 and an excess of cultivated alleles in B07 (Fig. 2.S6).

During marker analyses, it was also noted that several markers exhibited unexpected genotypic classes, in other words additional clusters to the three genotypic classes expected with disomic recombination. To investigate the nature of these markers, the clustering of 1499 polymorphic SNPs for the 194 F_2 lines were visually inspected and confirmed. The percentage of markers in each LG that showed homeologous recombination in at least one genotype, ranged from 4.3% (LG B03) to 75% (LG A04) with an average of 22.9% (343 SNPs) (Table 2.S2). These informative SNPs showed unexpected clustering patterns (Fig. 2.4.B-D), which was explained by recombination between homeologous chromosomes (Table 2.3). A total of 565 individual data points across the whole data set (1499 SNPs x 194 individuals) (0.19%) were informative to identify homeologous recombination. Lines that are triplex (single allele replaced by its homeolog) or quadriplex (both alleles replaced by homeolog) are expected only when individuals have undergone polysomic recombination; Fig. 2.4.C-D shows examples of assays that can distinguish two products of homeologous recombination: triplex (green) and quadriplex (gray). In the example in Fig. 2.4.B. it is unclear whether or not the individual that has undergone homeologous recombination for this locus is triplex or quadriplex, since there is no information about the allele dosage in the B-subgenome. Segregation ratio and distortion was not possible to describe for the markers with tetrasomic behavior, since the information about the F_1 hybrid and its gametic allelic constitution is unknown. Additionally, we observed blocks of genome substitution especially on LG A02/B02 (Fig. 2.5), A03, A04, A08 and A10/B10. A04 was the LG

with highest number of markers exhibiting homeologous recombination. All of above is consistent with previous reports for F_2 progeny (Nguepjop et al. 2016), RIL populations (Clevenger et al. 2017b; Leal-Bertioli et al. 2015a) and when mapping *A. hypogaea* RIL sequences against *A. duranensis* and *A. ipaensis* genomes (Bertioli et al. 2016).

Discussion

The peanut wild relative *A. stenosperma* has been shown to be resistant to multiple pests and pathogens including *M. arenaria* Race 1. This resistance is manifest at multiple stages of the infection cycle: both much lower rates of penetration of nematodes, and hypersensitive response to the few nematodes that do penetrate are observed (Proite et al. 2008). Genes involved in hypersensitive response and secondary metabolite production for defense against nematode infection have been identified in differential analyses of gene expression and histology (Guimaraes et al. 2010; Proite et al. 2008; Proite et al. 2007). Prior to the present work, RKN resistance segments on A02, A04 and A09 of the wild *A. stenosperma* were discovered using the simplified genetic context of a diploid mapping population derived from a cross of *A. duranensis* x *A. stenosperma* (Leal-Bertioli et al. 2016). Here, we used an F_2 population from the cross of the cultivar Runner-886 and the wild-derived allotetraploid BatSten1 (Leal-Bertioli et al. 2015c) to analyze the genome regions conferring resistance in a tetraploid background. The aim of this work was to provide a framework of knowledge to incorporate this new source of resistance into elite peanut cultivars through marker-assisted backcrossing schemes (MABC) (Bernardo 2008), work that is now in progress. Using the high-density genetic map and RKN phenotypic data measured on the F_2 progeny, allowed us to validate the

previously described diploid QTLs, in the tetraploid context of cultivated peanut. We identified three *A. stenosperma* QTLs associated with nematode resistance: on the bottom of LG 02, middle of LG 04 and bottom of LG 09.

The QTL on LG A02 was consistently associated with lower EGR, RF and GI (measures of resistance) (Fig. 2.2, Table 2.1 and Fig. 2.S4). The QTL located on LG A02 was found in the same region as the previously described diploid QTL (Fig. 2.2). The diploid QTL peak was mapped at 87.4Mbp with an interval from 83.6Mbp to 92.5Mbp (Bertioli et al. 2016; Leal-Bertioli et al. 2016). Here in the tetraploid context we located this resistance segment at 89.2Mbp with an interval between 84.3Mbp and 92.1Mbp. The second tetraploid QTL on LG A04 was near the diploid QTL at 111.0 Mbp. The QTL we identified in A09 was located at 116.3 Mbp, a little further from the previously reported diploid position (112.8 Mbp) (Fig. 2.2) (Bertioli et al. 2016). With this information, we could demonstrate that RKN resistance from *A. stenosperma* is transferable and stable in tetraploid genotypes.

The chromosome segment conferring resistance on LG A02 is homologous to the region of the reference genome of *A. duranensis*, that harbors multiple resistance-genes (R-genes) (Bertioli et al. 2016). A gene encoding a toll/interleukin-1 receptor (TIR)-like-nucleotide binding (NBS)-leucine-rich repeat (LRR), associated with plant immune defenses (Chisholm et al. 2006; Feys and Parker 2000; Tameling et al. 2002) is close to the A02-QTL peak (89.2Mbp) (Fig. 2.2). Recently, at a different genome location, a different TIR-NBS-LRR gene was observed to be constitutively expressed in the cultivar Tifguard, which carries RKN resistance from the wild species *A. cardenasii* and absent in the susceptible cultivar Gregory (Clevenger et al. 2017a).

The effects of the A02 and A09-QTL on nematode resistance was confirmed in selected F_3 lines ($F_{2:3}$-7 and $F_{2:3}$-34) carrying resistance loci in a heterozygous (Table 2.2, "-/+") or homozygous (Table 2.2, "+/+") state. The presence of *A. stenosperma* alleles at the QTL positions significantly reduced egg production (EGR), inhibited the nematode reproduction (RF) and decreased gall formation in comparison with susceptible genotype TifGP-2. We can infer that the presence of resistance segments is important to halt the completion of different steps of nematode life cycle. In the future we intend to carry out histological work on advanced backcross lines carrying different combinations of wild species chromosome segments, to provide insights into the timing and nature of the resistances conferred by these chromosome segments as in Proite et al., (2008) (Proite et al. 2008). Currently lines $F_{2:3}$-7 and $F_{2:3}$-34 are being crossed and backcrossed with several agronomically elite peanut lines.

Although the main focus of this study was to discover, introgress and validate QTLs associated with nematode resistance, interesting non-disomic inheritance of markers was also detected on the F_2 genotyping data. In a tetraploid hybrid context with cultivated peanut, the *A. stenosperma* genome is expected to recombine predominantly with *A. hypogaea* A-subgenome and the K genome (B genome *sensu lato*) of *A. batizocoi* with the *A. hypogaea* B-subgenome. Previously, genetic mapping and QTL identification studies assumed disomic inheritance in *Arachis* tetraploid species (Fonceka et al. 2012b; Hong et al. 2010; Shirasawa et al. 2013; Varshney et al. 2009; Zhou et al. 2014). However, Leal-Bertioli et al., (2015) (Leal-Bertioli et al. 2015a) provided the first molecular evidence of non-homologous alleles recombination in peanut, when unexpected genotyping patterns where detected for some loci in cultivated x artificially

induced allotetraploid RIL lines as well as the parent. Later, this phenomenon was reported in an F_2 progeny, also derived from a peanut by synthetic tetraploid cross (Nguepjop et al. 2016). More recently homeologous recombination has been reported in pure *A. hypogaea* crosses (Bertioli et al. 2016; Bertioli et al. 2019; Clevenger et al. 2017b).

In this study, using Affymetrix genotyping, most SNP markers were inherited as expected for disomic segregation, but others appeared to have undergone homeologous recombination. The percentage of markers showing homeologous recombination found in this study (22.9%) was higher than previously found by other groups (Nguepjop et al. 2016) for an F_2 progeny (11.05%). LG A04 had the highest number of markers showing tetrasomic behavior (Table 2.S2); in one F_2 plant we observed almost a complete substitution of the B/K04 and B/K10 alleles by their homeologs on A04 and A10. This recombination between homeologous chromosomes supports the idea that cultivated peanut has segmental genetic inheritance, where behavior is mostly disomic but partially polysomic. Since homeologous recombination occurs mostly in gene-rich regions (distal parts of chromosomes), understanding this phenomenon is not only important to avoid disregarding these genomic segments during genetic mapping (Clevenger et al. 2017b), but also to address peanut breeding strategies to transfer genes between species and to accelerate the accumulation of favorable alleles through marker-assisted introgression (Nguepjop et al. 2016).

From the analysis of segregation distortion (Chi-square test, $P<0.01$), we observed that markers with significant deviation from expected Mendelian segregation ratio (1:2:1) were distributed as clusters, possibly located within segregation distortion regions

(SDRs) (Wang et al. 2012). The SDRs have been shown to be present in interspecific or wide crosses in plants (Avni et al. 2014; Harushima et al. 1996; Ting et al. 2014; Wang et al. 2012) and also described in *A. hypogaea* intra and interspecific mapping populations (Gautami et al. 2012; Hong et al. 2010; Nguepjop et al. 2016; Zhou et al. 2014). Here, distorted markers on LG A04, A05 and B07 showed a bias toward *A. hypogaea*, indicating strong selection against the *A. stenosperma* alleles. In chromosome B06, we found a biased segregation in favor of wild *A. batizocoi* (Fig. 2.S6). In general, more distorted loci were found in favor of the *A. hypogaea* parent.

Conclusions

This research allowed us to transfer to and validate QTLs for RKN resistance derived from the diploid *A. stenosperma* into a tetraploid background. We observed that the chromosome segments carrying RKN resistance behaved normally in an induced tetraploid and in crosses with cultivated peanut. Currently, diagnostic markers are being used for the selection of backcrossed lines with resistance to RKN. Additionally, we were able to confirm the segmental genetic behavior with predominantly disomic, but partly tetrasomic genetic inheritance. This research will contribute to the production of peanut varieties that incorporate a new source of resistance to RKN from the wild species *A. stenosperma*. Since all the current RKN resistant cultivars have alleles derived *A. cardenasii*, expanding the gene pool will help ensure continued protection of the peanut crop from losses due to this pest. It will also enable lower inputs of agrochemicals and fuel, reducing environmental impact, higher profitability and more stable peanut yields.

Acknowledgements

The authors would like to acknowledge the Peanut Research Foundation, the Georgia Peanut Commission, the National Peanut Board, the National Science Foundation (grant # MCB-1543922) and NIFA project (award # 2018-67013-28139) for providing funds for this work. To the Glenn and Helen Burton Feeding the Hungry Scholarship Award for the recognition of the impact of this work (C.B.T). We are also indebted to Jenny Leverett, Stephanie Botton, Larissa Arrais, Shannon Atkinson, Jason Golden, and student workers for laboratory and greenhouse assistance.

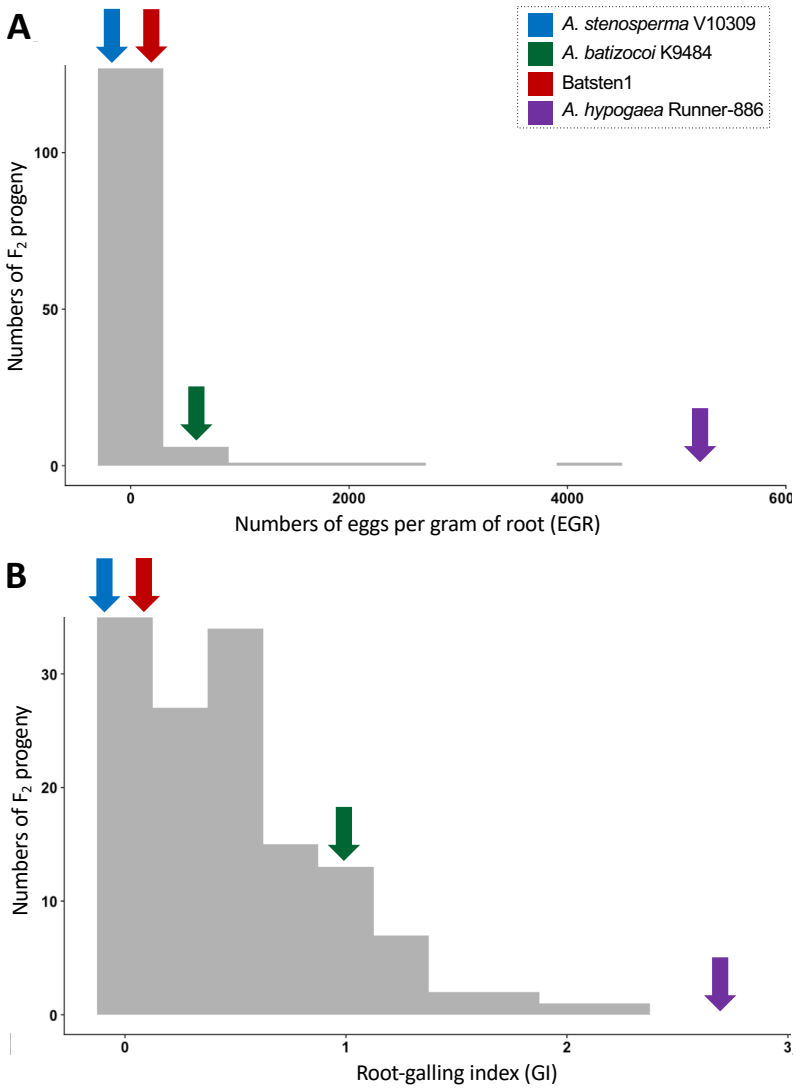

Figure 2.1: Frequency distribution of disease resistance to *Meloidogyne arenaria* Race 1 among the RBS-F_2 population for 2014 assay. Eggs per gram of root (EGR) **(A)** and galling index (GI) **(B)**. As expected, *A. stenosperma*, BatSten1 and *A. batizocoi* were resistant and *A. hypogaea* Runner IAC-886 was susceptible. The RBS-F_2 progeny showed a distinctly non-normal distribution, with genotypes having a skewed phenotypic frequency distribution towards resistance (zero value). Number of F_2 individuals in y-axis and phenotypic values in x-axis. The means of the parents are significantly different ($P<0.05$).

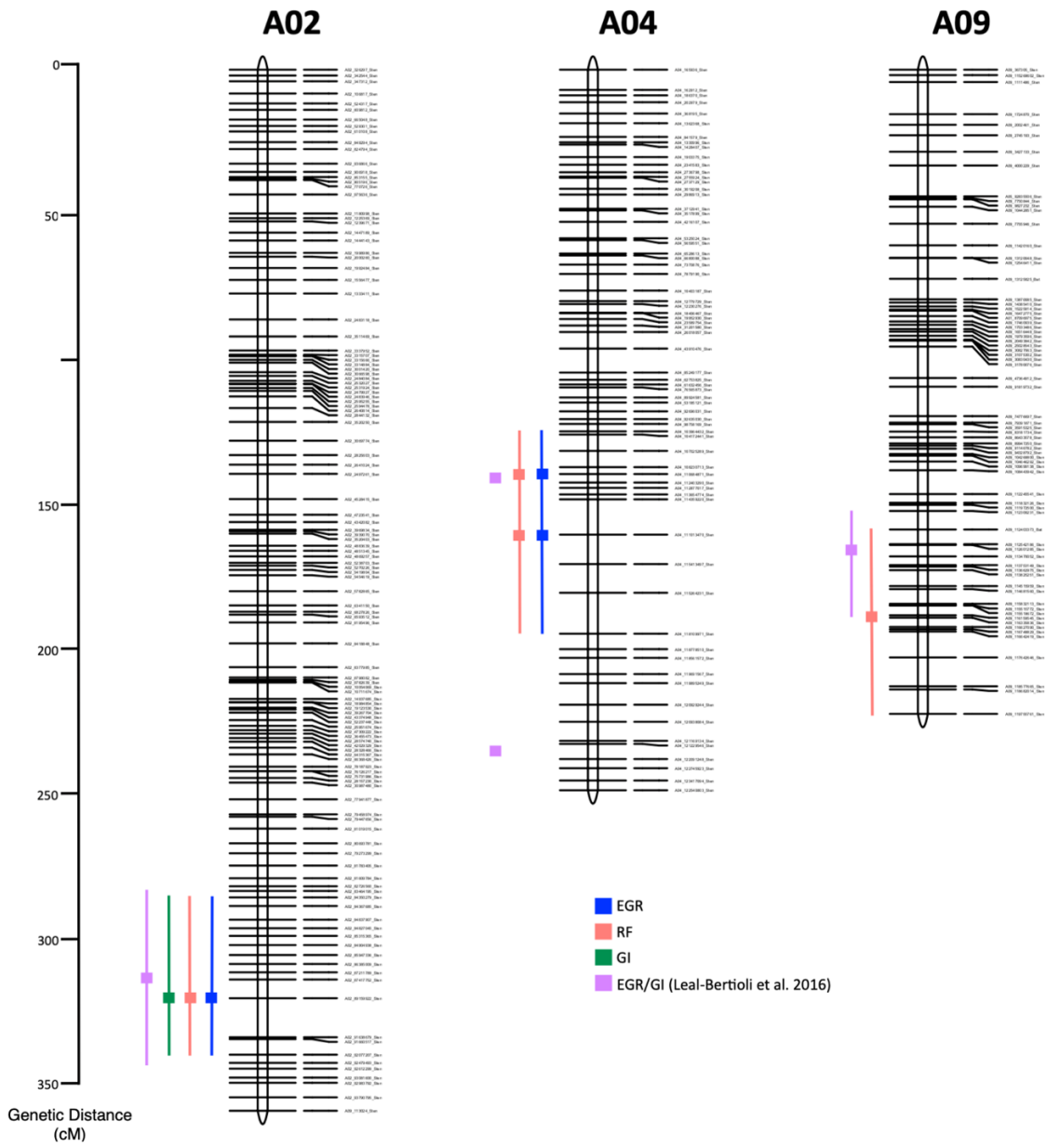

Figure 2.2: Linkage groups A02, A04 and A09 of the high-density linkage map obtained by the analysis of the RBS-F2 population using JoinMap v.4.0. These linkage groups harbored QTL identified in this study (in blue, orange and green) and previously report (purple) (Leal-Bertioli et al. 2016). QTL peaks are indicated as colored boxes alongside the linkage groups and QTL intervals as lines.

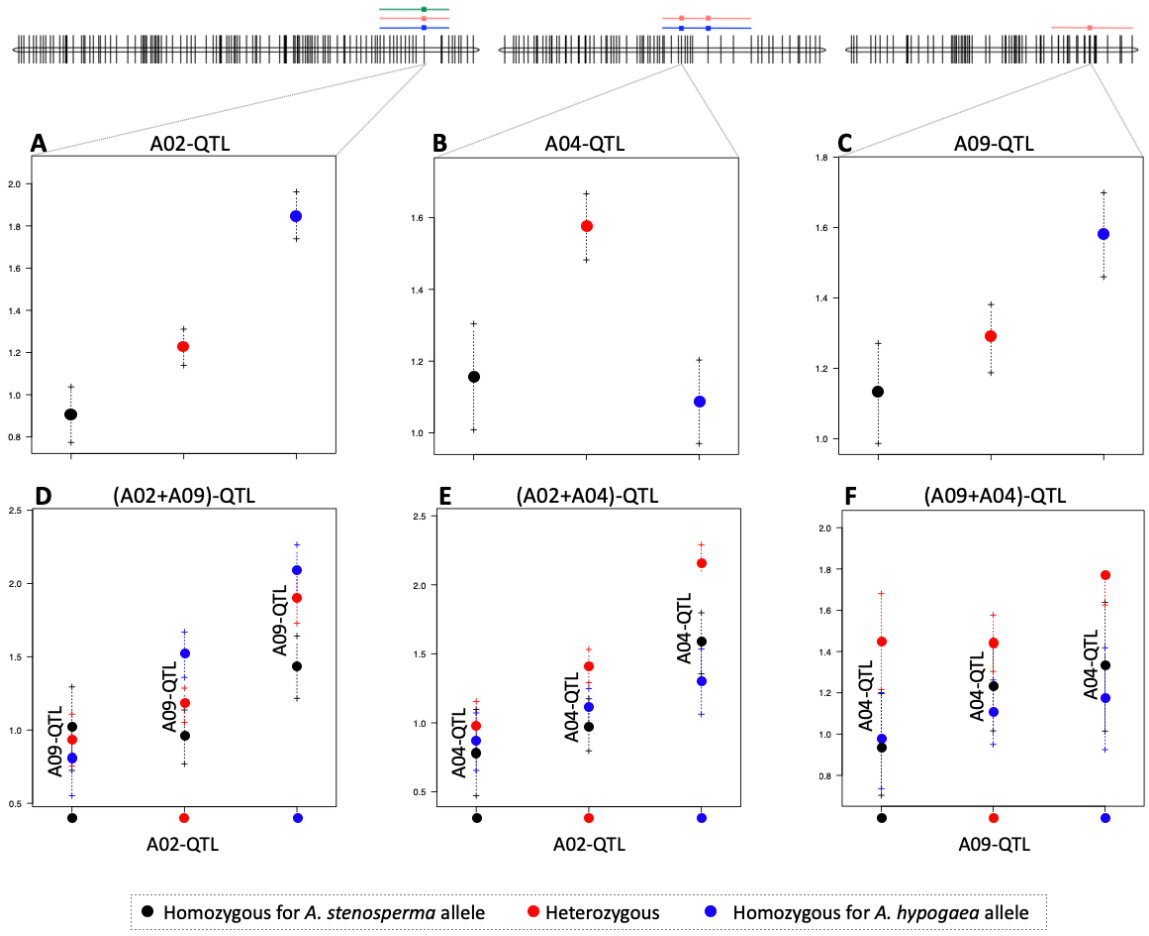

Figure 2.3: QTL effect plot of Log transformed data number of eggs per gram of root (EGR) at QTL on A02 (A02_89159922_Sten) **(A)**, A04 (A04_111013470_Sten or A04_110684871_Sten, both have similar behavior) **(B)** and A09 (A09_116335836_Sten) **(C)**; Effect of combination of QTL on A02 and A09 **(D)**, A02 and A04 **(E)** and A09 and A04 **(F)**; Phenotype values on logarithmic scale (Y-axis) as a function of genotypic class (X-axis). Bars at each genotypic class represent standard error of mean.

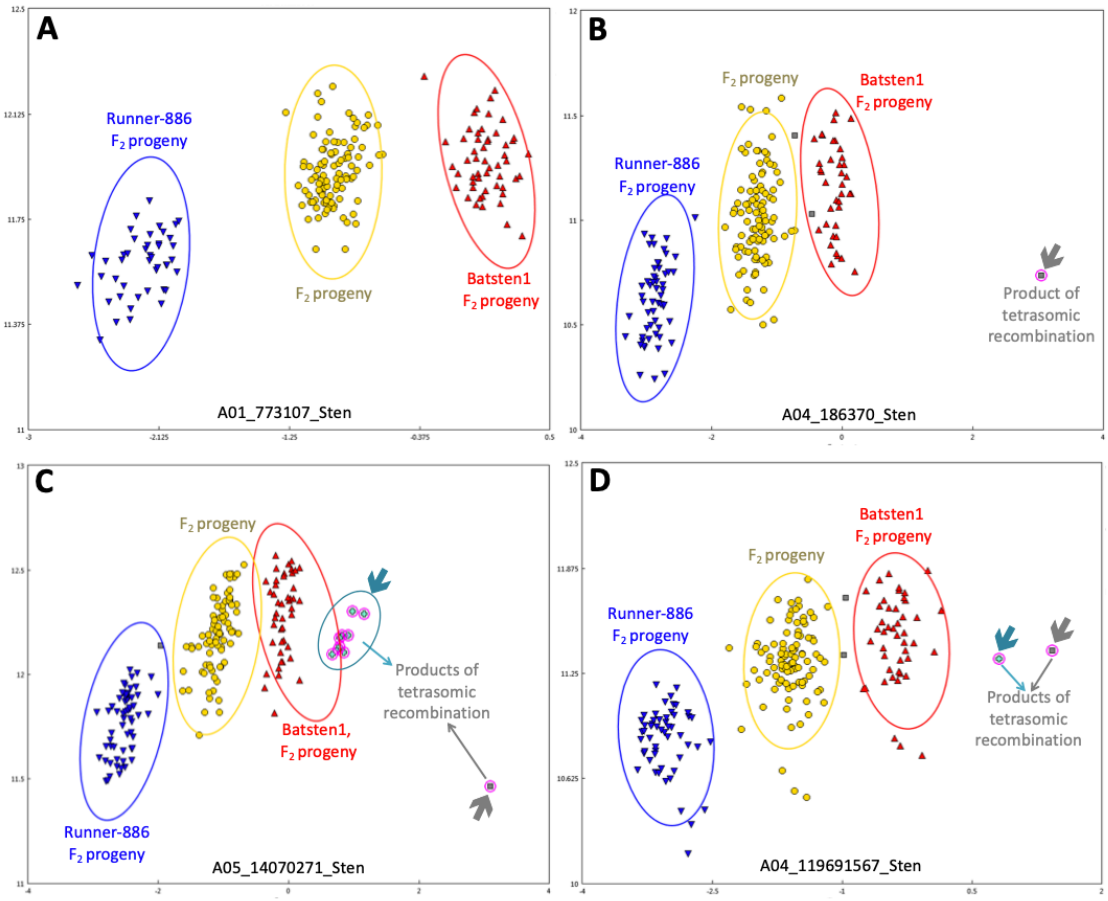

Figure 2.4: Examples of SNP markers segregation in the F_2 population under disomic **(A)** and tetrasomic inheritance **(B-D).** One product of homeologous recombination is detected and indicated by a gray arrow (triplex/quadriplex) **(B)** and two products of homeologous recombination are detected and indicated by a aquamarine (triplex) and gray arrows (quadriplex) **(C-D).**

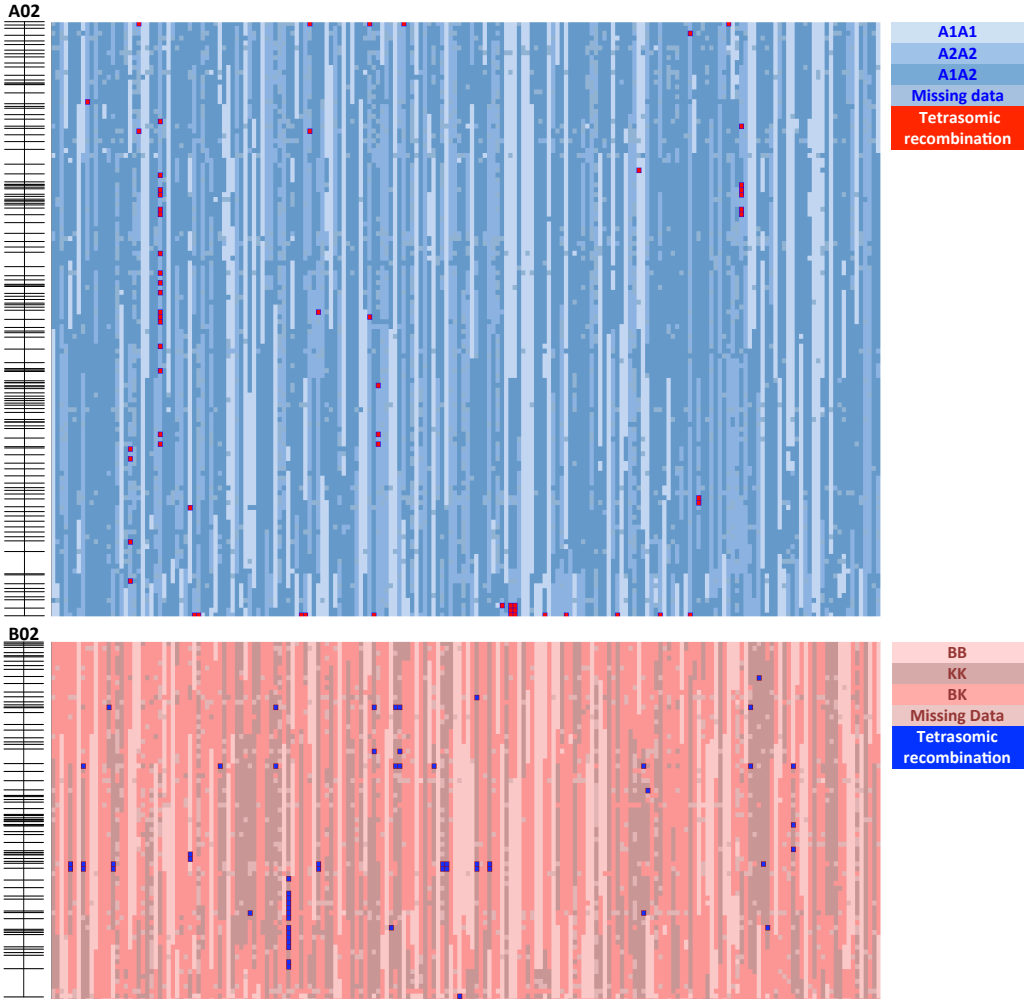

Figure 2.5: Genotyping color map of 194 F_2 progeny for linkage groups A02 and B02. Each column represents an F_2 line and rows represent markers. Blue and red colors denote the A and B/K-subgenomes, respectively. Cultivated (A1A1), wild (A2A2) and heterozygous (A1A2) genotypes for the A-genome are represented by light blue, blue and dark blue, respectively. Cultivated (BB), wild (KK) heterozygous (BK) genotypes for the B-genome are represented by light red, dark red and red, respectively. Red in the A-genome and blue in the B-genome color indicate homeologous recombination events. Linkage maps on the side are included for illustration purposes.

Table 2.1: Identified QTL for resistance to RKN on the RBS-F_2 population.

Trait Symbol	LG[a]	Genetic position[b]	Physical Position[c]	Nearest Marker	LOD[d]	LOD threshold[e]	95% Bayes / LOD interval[f]	Additive Effect[g]	R^2 (%)[h]	%[i]	QTL Name(s)
EGR2014LOG	A02	320.7	89.2	A02_89159922_Sten	8.1	5.60 (1%), 4.73 (5%)	(320.7) / (314.2-334.1)	0.473	22.2	96.3	A02
RF2014LOG	A02	320.7	89. 2	A02_89159922_Sten	8.3	5.67 (1%), 4.71 (5%)	(320.7) / (314.2-334.1)	0.499	23.0	98.2	A02
GI2014LOG1	A02	320.7	89. 2	A02_89159922_Sten	5.9	6.63 (1%), 5.36 (5%)	(320.7) / (314.2-334.1)	0.065	15.4	61.3	A02
EGR2014LOG	A04	139.6 160.6	110.7 111.0	A04_110684871_Sten A04_111013470_Sten	3.2 3.6	5.60 (1%), 4.73 (5%)	(131.6-160.6)/ (125.9- 170.7)	-0.005	Do not reduce nematode multiplication		A04a A04b
RF2014LOG	A04	139.6 160.6	110.7 111.0	A04_110684871_Sten A04_111013470_Sten	3.3 3.4	5.67 (1%), 4.71 (5%)	(125.9-160.6)/ (124.8- 170.7)	-0.068			A04a A04b
RFBLUPS1416LOG	A09	189.3	116.3	A09_116335836_Sten	3.7	5.6 (1%), 4.66 (5%)	(44.7-214.0)/ (158.8-222.5)	0.134	9.97	97.8	A09

LOD, logarithm of the odds; EGR2014LOG, Eggs per gram of root Log_{10} transformation for 2014; RF2014LOG, Reproduction factor Log_{10} transformation for 2014; GI2014LOG1, galling index Log_{10} (x+1) transformation for 2014; BLUPs for Reproduction factor Log_{10} transformation for 2014+2016;

[a] Linkage group

[b] Map position in Kosambi cM

[c] Physical position is based on *A. duranensis* pseudomolecules (https://www.peanutbase.org/) (Bertioli et al. 2016).

[d] LOD score at QTL peak

[e] LOD threshold based on 1000 permutations at 1% and 5% level of significance.

[f] 95% Bayes credible intervals/LOD support interval

[g] Positive values indicate that alleles come from *A. stenosperma* V10309 and negative values indicate that alleles come from *A. hypogaea* Runner-886.

[h] Proportion of the phenotypic variance explained by the QTL

[i] Percentage (%) decrease in nematode multiplication

Table 2.2: Summary of presence/absence of markers linked RKN resistance segments from *A. stenosperma* in chromosomes A02, A09 and A04 (associated with susceptibility), and EGR, RF and GI disease average values and grouping by Wilcoxon signed rank (P>0.05) for selected RBS-$F_{2:3}$ lines and susceptible control. Homozygous for *A. hypogaea* alleles as "-/-"; homozygous for resistance segments as "+/+"; and heterozygous as "-/+".

LG	Marker	F_2-derived F_3 lines		*A. hypogaea* TifGP-2
		$F_{2:3}$-7	$F_{2:3}$-34	
A02	A02_83464195_Sten	+/+	+/+	-/-
	A02_84827045_Sten	+/+	-/+	-/-
	A02_85315365_Sten	-/+	-/+	-/-
	A02_86385009_Sten	+/+	-/+	-/-
	A02_89159922_Sten	+/+	-/+	-/-
	A02_91638679_Sten	+/+	-/+	-/-
	A02_92077207_Sten	+/+	-/+	-/-
A04	A04_104172441_Sten	-/+	-/+	-/-
	A04_108230713_Sten	-/+	-/+	-/-
	A04_110684871_Sten	-/+	-/+	-/-
	A04_112403290_Sten	-/+	-/+	-/-
	A04_113654774_Sten	-/+	-/+	-/-
	A04_118561572_Sten	-/+	-/+	-/-
	A04_118778510_Sten	+/+	+/+	-/-
	A04_119895249_Sten	+/+	+/+	-/-
	A04_120929244_Sten	+/+	+/+	-/-
	A04_120938084_Sten	+/+	+/+	-/-
	A04_121169134_Sten	+/+	-/+	-/-
	A04_121229546_Sten	+/+	-/+	-/-
	A04_122540803_Sten	+/+	-/+	-/-
A09	A09_112245541_Sten	-/+	+/+	-/-
	A09_112309231_Sten	-/+	+/+	-/-
	A09_112542186_Sten	-/+	+/+	-/-
	A09_112601285_Sten	-/+	+/+	-/-
	A09_113470052_Sten	-/+	+/+	-/-
	A09_113662975_Sten	-/+	+/+	-/-
	A09_114515959_Sten	-/+	+/+	-/-
	A09_114681560_Sten	-/+	+/+	-/-
	A09_115268602_Sten	-/-	-/-	-/-
	A09_115832113_Sten	-/+	+/+	-/-
	A09_116627090_Sten	-/+	+/+	-/-
	A09_118577665_Sten	-/-	+/+	-/-
	A09_118682014_Sten	-/-	+/+	-/-
Eggs per gram of root - EGR		30.22 ± 28.83 (a)	231.82 ± 264.30 (a)	2163.78 ± 1688.27 (b)
Reproduction factor - RF		0.12 ± 0.08 (a)	0.21 ± 0.23 (a)	5.49 ± 2.96 (b)
Galling index - GI		0.17 ± 0.41 (a)	0.00 ± 0.00 (a)	2.57 ± 0.79 (b)

Table 2.3: Segregation of SNP markers in the RBS-F_2 population.

Type of inheritance						
Disomic				Tetrasomic		Total
A-genome		B/K-genome		A-genome	B/K-genome	
Mendelian	Distorted	Mendelian	Distorted	238 (15.9%)	105 (7.0%)	1499
394 (26.3%)	234 (15.6%)	300 (20.0%)	228 (15.2%)			

*Percentage of marker in each category is indicated in parenthesis

References

Avni R, Nave M, Eilam T, Sela H, Alekperov C, Peleg Z, Dvorak J, Korol A, Distelfeld A (2014) Ultra-dense genetic map of durum wheat × wild emmer wheat developed using the 90K iSelect SNP genotyping assay. Molecular Breeding 34:1549-1562

Bernardo R (2008) Molecular markers and selection for complex traits in plants: learning from the last 20 Years. Crop Sci 48:1649-1664

Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD, Cannon EK, Liu X, Gao D, Clevenger J, Dash S, Ren L, Moretzsohn MC, Shirasawa K, Huang W, Vidigal B, Abernathy B, Chu Y, Niederhuth CE, Umale P, Araujo AC, Kozik A, Kim KD, Burow MD, Varshney RK, Wang X, Zhang X, Barkley N, Guimaraes PM, Isobe S, Guo B, Liao B, Stalker HT, Schmitz RJ, Scheffler BE, Leal-Bertioli SC, Xun X, Jackson SA, Michelmore R, Ozias-Akins P (2016) The genome sequences of Arachis duranensis and Arachis ipaensis, the diploid ancestors of cultivated peanut. Nat Genet 48:438-446

Bertioli DJ, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, Leal-Bertioli SCM, Ren L, Farmer AD, Pandey MK, Samoluk SS, 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 KD, Korani W, Lanciano S, Lui CG, Mirouze M, Moretzsohn MC, Pham M, Shin JH, Shirasawa K, Sinharoy S, Sreedasyam A, Weeks NT, Zhang X, Zheng Z, Sun Z, Froenicke L, Aiden EL, Michelmore R, Varshney RK, Holbrook CC, Cannon EKS, Scheffler BE, Grimwood J, Ozias-Akins P, Cannon SB, Jackson SA, Schmutz J (2019) The genome sequence of peanut (Arachis hypogaea), a segmental allotetraploid. Nat Genet 51:877-884

Branch WD, Brenneman TB (2015) Registration of 'Georgia-14N' peanut. J Plant Regist 9:159-161

Broman K, Sen Ś (2009) A guide to QTL mapping with R/qtl. Springer

Broman KW (2003) Mapping quantitative trait loci in the case of a spike in the phenotype distribution. Genetics 163:1169-1175

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889-890

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803-814

Chu Y, C. Holbrook C, Timper P, Ozias-Akins P (2007) Development of a PCR-based molecular marker to select for nematode resistance in peanut. Crop Sci 47:841-847

Chu Y, Gill R, Clevenger J, Timper P, Corley Holbrook C, Ozias-Akins P (2016) Identification of rare recombinants leads to tightly linked markers for nematode resistance in peanut. Peanut Sci 43:88-93

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963-971

Clevenger J, Chu Y, Arrais Guimaraes L, Maia T, Bertioli D, Leal-Bertioli S, Timper P, Holbrook CC, Ozias-Akins P (2017a) Gene expression profiling describes the genetic regulation of Meloidogyne arenaria resistance in Arachis hypogaea and reveals a candidate gene for resistance. Sci Rep 7:1317

Clevenger J, Chu Y, Chavarro C, Agarwal G, Bertioli DJ, Leal-Bertioli SCM, Pandey MK, Vaughn J, Abernathy B, Barkley NA, Hovav R, Burow M, Nayak SN, Chitikineni A, Isleib TG, Holbrook CC, Jackson SA, Varshney RK, Ozias-Akins P (2017b) Genome-wide SNP genotyping resolves signatures of selection and tetrasomic tecombination in peanut. Mol Plant 10:309-322

Danchin EG, Arguel MJ, Campan-Fournier A, Perfus-Barbeoch L, Magliano M, Rosso MN, Da Rocha M, Da Silva C, Nottet N, Labadie K, Guy J, Artiguenave F, Abad P (2013) Identification of novel target genes for safer and more specific control of root-knot nematodes from a pan-genome mining. PLoS Pathog 9:e1003745

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285-294

Dong W, Holbrook CC, Timper P, Brenneman TB, Mullinix BG (2007) Comparison of methods for assessing resistance to meloidogyne arenaria in peanut. J Nematol 39:169-175

Feys BJ, Parker JE (2000) Interplay of signaling pathways in plant disease resistance. Trends Genet 16:449-455

Fonceka D, Tossim HA, Rivallan R, Vignes H, Faye I, Ndoye O, Moretzsohn MC, Bertioli DJ, Glaszmann JC, Courtois B, Rami JF (2012) Fostered and left behind alleles in peanut: interspecific QTL mapping reveals footprints of domestication and useful natural variation for breeding. BMC Plant Biol 12:26

Gautami B, Fonceka D, Pandey MK, Moretzsohn MC, Sujay V, Qin H, Hong Y, Faye I, Chen X, BhanuPrakash A, Shah TM, Gowda MV, Nigam SN, Liang X, Hoisington DA, Guo B, Bertioli DJ, Rami JF, Varshney RK (2012) An international reference consensus genetic map with 897 marker loci based on 11

mapping populations for tetraploid groundnut (Arachis hypogaea L.). PLoS One 7:e41213

Guimarães P, Brasileiro ACM, Proite K, Araujo A, Leal-Bertioli S, Pic-Taylor A, da Silva FR, Morgante C, Ribeiro S, Bertioli D (2010) A study of gene expression in the nematode resistant wild peanut relative, Arachis stenosperma, in response to challenge with Meloidogyne arenaria. Tropical Plant Biology 3:183-192

Halward T, Stalker T, LaRue E, Kochert G (1992) Use of single-primer DNA amplifications in genetic studies of peanut (Arachis hypogaea L.). Plant Mol Biol 18:315-325

Harushima Y, Kurata N, Yano M, Nagamura Y, Sasaki T, Minobe Y, Nakagahra M (1996) Detection of segregation distortions in an indica-japonica rice cross using a high-resolution molecular map. Theor Appl Genet 92:145-150

Holbrook C, Noe PJ (1990) Resistance to Meloidogyne arenaria in Arachis spp. and the implications on development of resistant peanut cultivars. Peanut Sci 17:35-38

Holbrook C, Ozias-Akins P, Chu Y, K. Culbreath A, Kvien C, Brenneman T (2017) Registration of 'TifNV-high O/L' peanut. J Plant Regist 11:228-230

Holbrook C, Stalker H (2003) Peanut breeding and genetic resources. Plant Breed Rev 22:297-356

Holbrook C, Timper P, Culbreath A (2003) Resistance to and root-knot nematode in peanut interspecific breeding lines. Crop Sci 43:1109-1113

Holbrook C, Timper P, Culbreath A, Kvien C (2008) Registration of 'Tifguard' peanut. J Plant Regist 2:391-410

Holland JB, Coles ND (2011) QTL controlling masculinization of ear tips in a maize (Zea mays L.) intraspecific cross. G3: Genes, Genomes, Genetics 1:337-341

Hong Y, Chen X, Liang X, Liu H, Zhou G, Li S, Wen S, Holbrook CC, Guo B (2010) A SSR-based composite genetic linkage map for the cultivated peanut (Arachis hypogaea L.) genome. BMC Plant Biol 10:17

Hussey RS, Barker KR (1973) A comparison of methods of collecting inocula of Meloidogyne species, including a new technique. Plant Disease Reporter 57:1025–1028

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 7:396-423

Johnson AW, Minton NA, Brenneman TB, Burton GW, Culbreath AK, Gascho GJ, Baker SH (1999) Bahiagrass, corn, cotton rotations, and pesticides for managing nematodes, diseases, and insects on peanut. J Nematol 31:191-200

Kochert G, Stalker H, Gimenes M, Galgaro L, Romero Lopes C, Moore K (1996) RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, Arachis hypogaea (Leguminosae). American Journal of Botany 83:1281-1291

Kosambi DD (1943) The estimation of map distances from recombination values. Annals of human genetics 12:172-175

Kruskal W, Wallis W (1952) Use of ranks in one-criterion variance analysis. J Amer Statist Assn 47:583-621

Leal-Bertioli S, Farias M, Tanno P, Guimarães P, Brasileiro AC, Bertioli D, Araujo AC (2010) Ultrastructure of the initial interaction of puccinia arachidis and Cercosporidium personatum with leaves of Arachis hypogaea and Arachis stenosperma. Journal of Phytopathology 158:792-796

Leal-Bertioli S, Shirasawa K, Abernathy B, Moretzsohn M, Chavarro C, Clevenger J, Ozias-Akins P, Jackson S, Bertioli D (2015a) Tetrasomic recombination is surprisingly frequent in allotetraploid Arachis. Genetics 199:1093-1105

Leal-Bertioli SC, Jose AC, Alves-Freitas DM, Moretzsohn MC, Guimaraes PM, Nielen S, Vidigal BS, Pereira RW, Pike J, Favero AP, Parniske M, Varshney RK, Bertioli DJ (2009) Identification of candidate genome regions controlling disease resistance in Arachis. BMC Plant Biol 9:112

Leal-Bertioli SC, Santos SP, Dantas KM, Inglis PW, Nielen S, Araujo AC, Silva JP, Cavalcante U, Guimaraes PM, Brasileiro AC, Carrasquilla-Garcia N, Penmetsa RV, Cook D, Moretzsohn MC, Bertioli DJ (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. Ann Bot 115:237-249

Leal-Bertioli SCM, Moretzsohn MC, Roberts PA, Ballén-Taborda C, Borba TCO, Valdisser PA, Vianello RP, Araújo ACG, Guimarães PM, Bertioli DJ (2016) Genetic mapping of resistance to Meloidogyne arenaria in Arachis stenosperma: A new source of nematode resistance for peanut. G3: Genes, Genomes, Genetics 6:377-390

Moretzsohn MC, Gouvea EG, Inglis PW, Leal-Bertioli SC, Valls JF, Bertioli DJ (2013) A study of the relationships of cultivated peanut (Arachis hypogaea) and its most closely related wild species using intron sequences and microsatellite markers. Ann Bot 111:113-126

Nelson SC, Simpson CE, Starr JL (1989) Resistance to Meloidogyne arenaria in Arachis spp. germplasm. J Nematol 21:654-660

Nelson SC, Starr JL, Simpson CE (1990) Expression of resistance to Meloidogyne arenaria in Arachis batizocoi and A. cardenasii. J Nematol 22:242-244

Nguepjop JR, Tossim H-A, Bell JM, 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. Frontiers in plant science 7:1635-1635

Oostenbrink M (1966) Major characteristics of the relation between nematodes and plants. Mededelingen Landbouwhogeschool Wageningen 66:1-46

Pandey MK, Agarwal G, Kale SM, Clevenger J, Nayak SN, Sriswathi M, Chitikineni A, Chavarro C, Chen X, Upadhyaya HD, Vishwakarma MK, Leal-Bertioli S, Liang X, Bertioli DJ, Guo B, Jackson SA, Ozias-Akins P, Varshney RK (2017) Development and evaluation of a high density genotyping 'Axiom_Arachis' array with 58 K SNPs for accelerating genetics and breeding in groundnut. Sci Rep 7:40577-40577

Proite K, Carneiro R, Falcão R, Gomes A, Leal-Bertioli S, Guimarães P, Bertioli D (2008) Post-infection development and histopathology of Meloidogyne arenaria race 1 on Arachis spp. Plant Pathology 57:974-980

Proite K, Leal-Bertioli SC, Bertioli DJ, Moretzsohn MC, da Silva FR, Martins NF, Guimaraes PM (2007) ESTs from a wild Arachis species for gene discovery and marker development. BMC Plant Biol 7

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. Theor Appl Genet 121:1033-1046

Rodríguez-Kábana R, Robertson DG, Weaver CF, Wells L (1991) Rotations of bahiagrass and castorbean with peanut for the management of Meloidogyne arenaria. J Nematol 23:658-661

Schierup M (1996) Inbreeding depression and outbreeding depression in plants. Heredity 77:461

Shirasawa K, Bertioli DJ, Varshney RK, Moretzsohn MC, Leal-Bertioli SCM, Thudi M, Pandey MK, Rami J-F, Foncéka D, Gowda MVC, 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 research : an international journal for rapid publication of reports on genes and genomes 20:173-184

Simpson C (1991) Pathways for introgression of pest resistance into Arachis hypogaea L. Peanut Sci 18:22-26

Simpson C, C. Nelson S, L. Starr J, E. Woodard K, D. Smith O (1993) Registration of TxAG-6 and TxAG-7 peanut germplasm. Crop Sci 33:1418

Simpson C, L. Starr J, Church G, D. Burow M, H. Paterson A (2003) Registration of NemaTAM peanut. Crop Sci 43:1561

Simpson C, L. Starr J, R. Baring M, D. Burow M, M. Cason J, N. Wilson J (2013) Registration of 'Webb' peanut. J Plant Regist 7:265

Simpson C, Starr J (2001) Registration of 'COAN' peanut. Crop Sci 41:918

Stalker H (1997) Peanut (Arachis hypogaea L.). Field Crops Research 53:205–217

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JOINMAP. The plant journal 3:739-744

Starr J, R. Morgan E, E. Simpson C (2002) Management of the peanut root-knot nematode, Meloidogyne arenaria, with host resistance. Plant Health Prog

Tallury SP, Hilu KW, Milla-Lewis SR, Friend S, Alsaghir M, Stalker H, Quandt D (2005) Genomic affinities in Arachis section Arachis (Fabaceae): Molecular and cytogenetic evidence. Theor Appl Genet 111:1229-1237

Tameling WIL, Elzinga SDJ, Darmin PS, Vossen JH, Takken FLW, Haring MA, Cornelissen BJC (2002) The tomato R gene products I-2 and MI-1 are functional ATP binding proteins with ATPase activity. The Plant cell 14:2929-2939

Ting N-C, Jansen J, Mayes S, Massawe F, Sambanthamurthi R, Ooi LC-L, Chin CW, Arulandoo X, Seng T-Y, Alwee SSRS, Ithnin M, Singh R (2014) High density SNP and SSR-based genetic maps of two independent oil palm hybrids. BMC genomics 15:309-309

Van Ooijen JW (2006) JoinMap® 4.0: software for the calculation of genetic linkage maps in experimental population. Wageningen: Kyazma BV

Varshney R, Bertioli D, C Moretzsohn M, Vadez V, L K, Aruna R, Nigam S, J Moss B, Seetha K, Koppolu R, He G, J Knapp S, Hoisington D (2009) The first SSR-based genetic linkage map for cultivated groundnut (Arachis hypogaea L.). Theor Appl Genet 118:729-739

Wang G, He QQ, Xu ZK, Song R (2012) High segregation distortion in maize B73 x teosinte crosses. Genetics and molecular research : GMR 11:693-706

Zhou H, Liu S, Liu Y, Liu Y, You J, Deng M, Ma J, Chen G, Wei Y, Liu C, Zheng Y (2016) Mapping and validation of major quantitative trait loci for kernel length in wild barley (Hordeum vulgare ssp. spontaneum). BMC genetics 17:130

Zhou X, Xia Y, Ren X, Chen Y, Huang L, Huang S, Liao B, Lei Y, Yan L, Jiang H (2014) Construction of a SNP-based genetic linkage map in cultivated peanut based on large scale marker development using next-generation double-digest restriction-site-associated DNA sequencing (ddRADseq). BMC genomics 15:351-351

Appendix 2.A

Supplementary Figures

Figure 2.S1. High-density linkage map obtained through the analysis of the RBS-F_2 population using JoinMap. 20 linkage groups for A subgenome and B subgenome. Linkage group names are shown at the bottom of the figure; distance (cM) is shown to the left of the figure and marker names are shown to the right of each LG

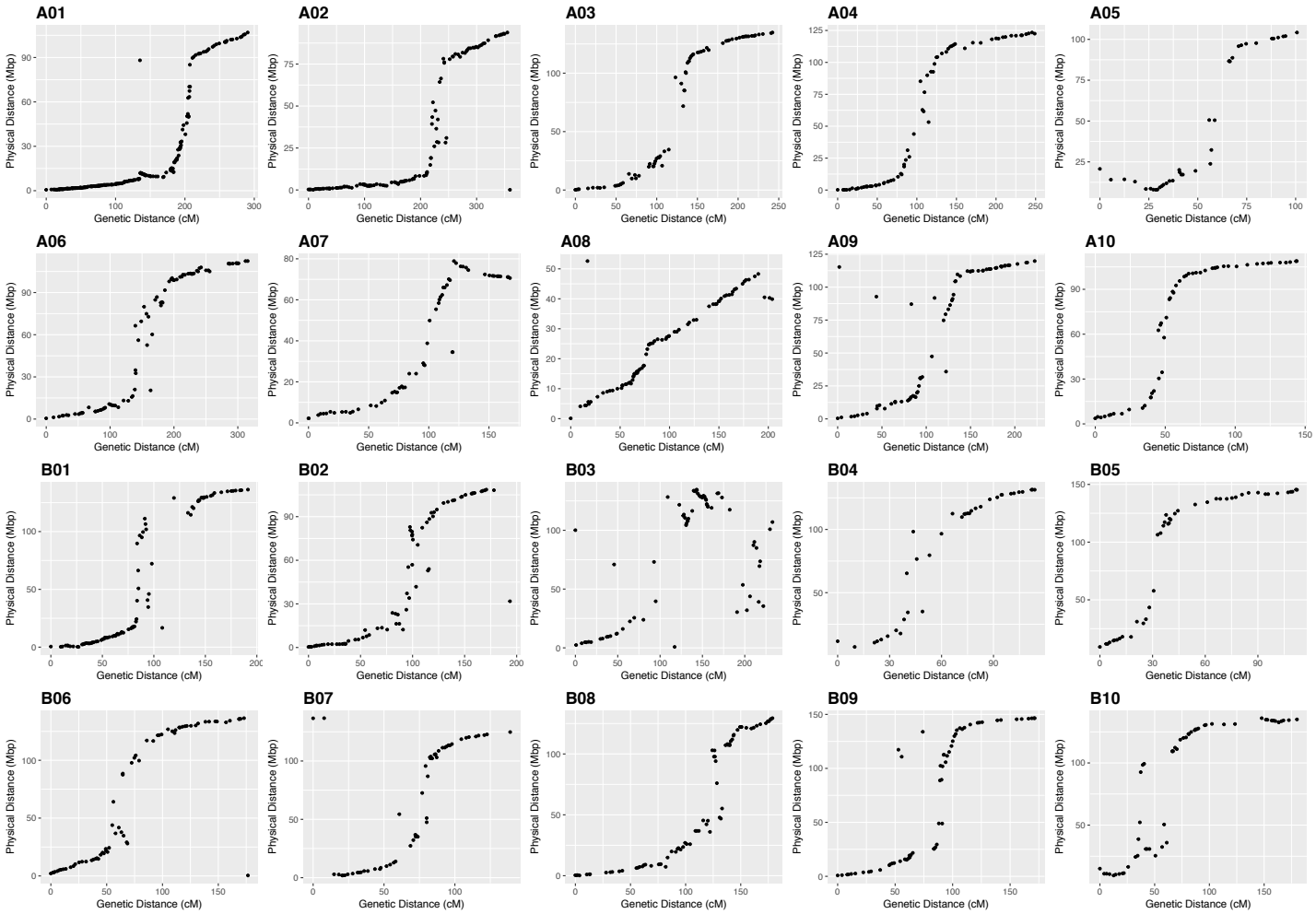

Figure 2.S2. Relationship plot between genetic distance (x-axis) and physical distance (y-axis) for 10 A-subgenome (markers derived from *A. stenosperma*) and 10 B/K-subgenome (markers derived from *A. batizocoi*) linkage groups.

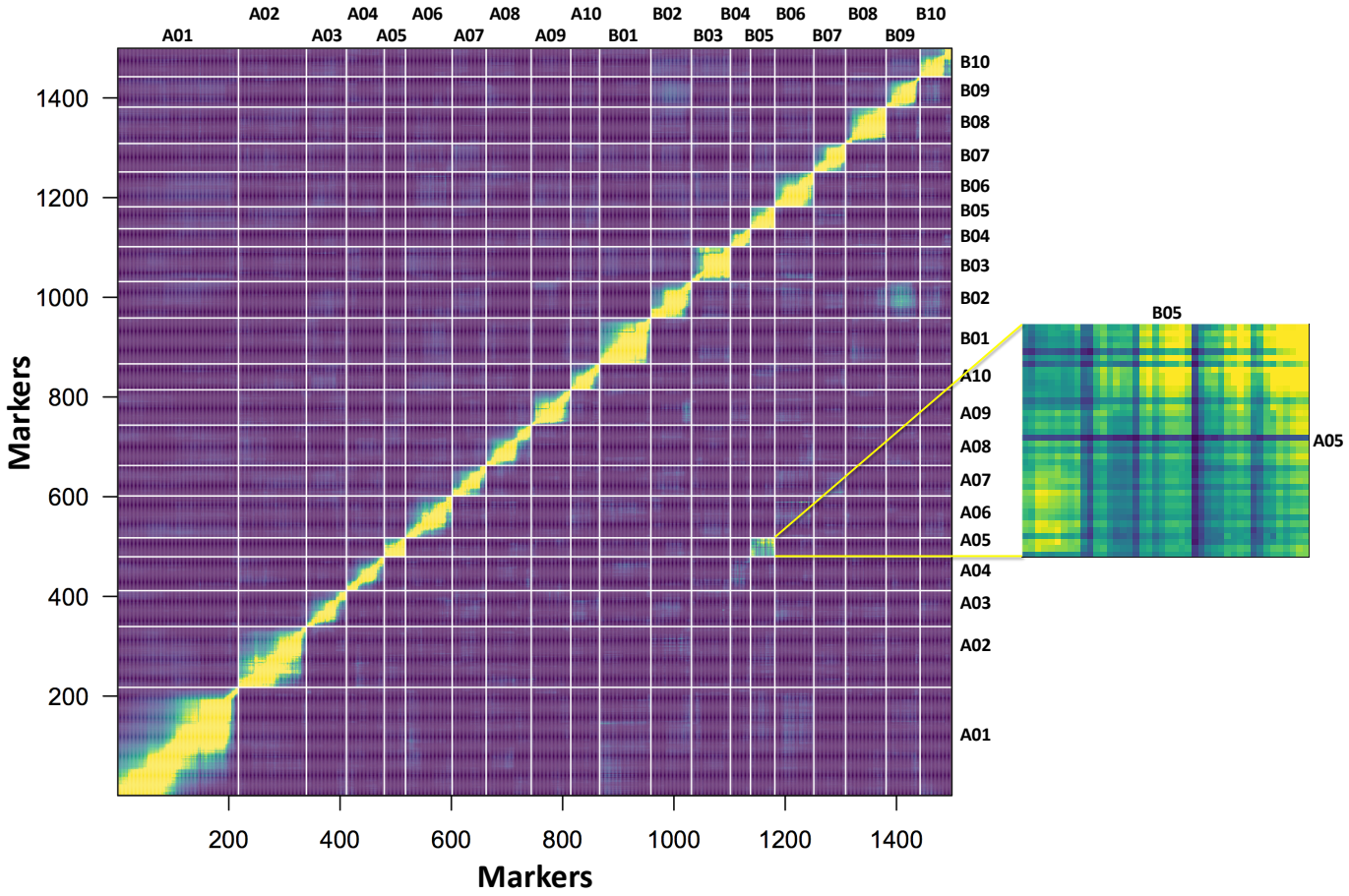

Figure 2.S3. Plot of estimated recombination fractions (above diagonal) and LOD scores for tests of r = 1/2 (below diagonal) for all pairs of markers in the linkage map. Yellow indicates linkage, while blue indicates pairs that are not linked. Magnified look of the relationship between homeologous chromosomes A05 and B05 is shown in the right panel.

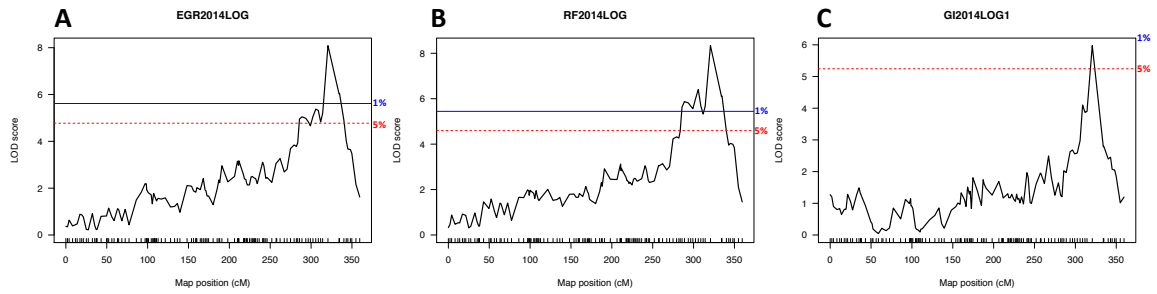

Figure 2.S4. Identified QTL on chromosomes A02 using two-part model for RKN resistance for EGR2014LOG, Eggs per gram of root Log10 transformation for 2014 **(A)**; RF2014LOG, Reproduction factor Log10 transformation for 2014 **(B)**; and GI2014LOG1, galling index Log10 (x+1) transformation for 2014 **(C)**. Genome-wise LOD threshold at 1% (P<0.01, horizontal blue solid lines) and 5% (P<0.05, horizontal red dashed lines) level of significance based on 1000 permutations. Genetic distance (cM) in x-axis and LOD scores in y-axis.

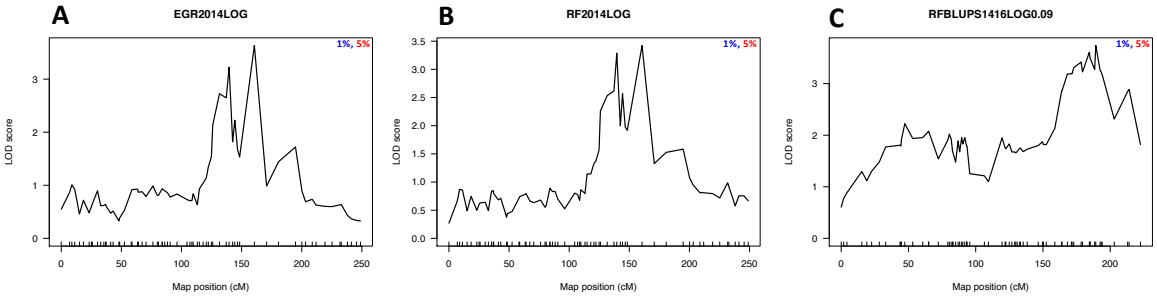

Figure 2.S5. Identified QTL on chromosomes A04 (A-B) and A09 **(C)** using two-part model for RKN resistance for EGR2014LOG, Eggs per gram of root Log10 transformation for 2014 (A), RF2014LOG, Reproduction factor Log10 transformation for 2014 **(B)** and RFBLUPS1416LOG0.09, Reproduction factor Log10 transformation for 2014 and 2016 BLUPs **(C)**. Genome-wise LOD threshold at 1% (P<0.01) and 5% (P<0.05) level of significance based on 1000 permutations above curves. Genetic distance (cM) in x-axis and LOD scores in y-axis.

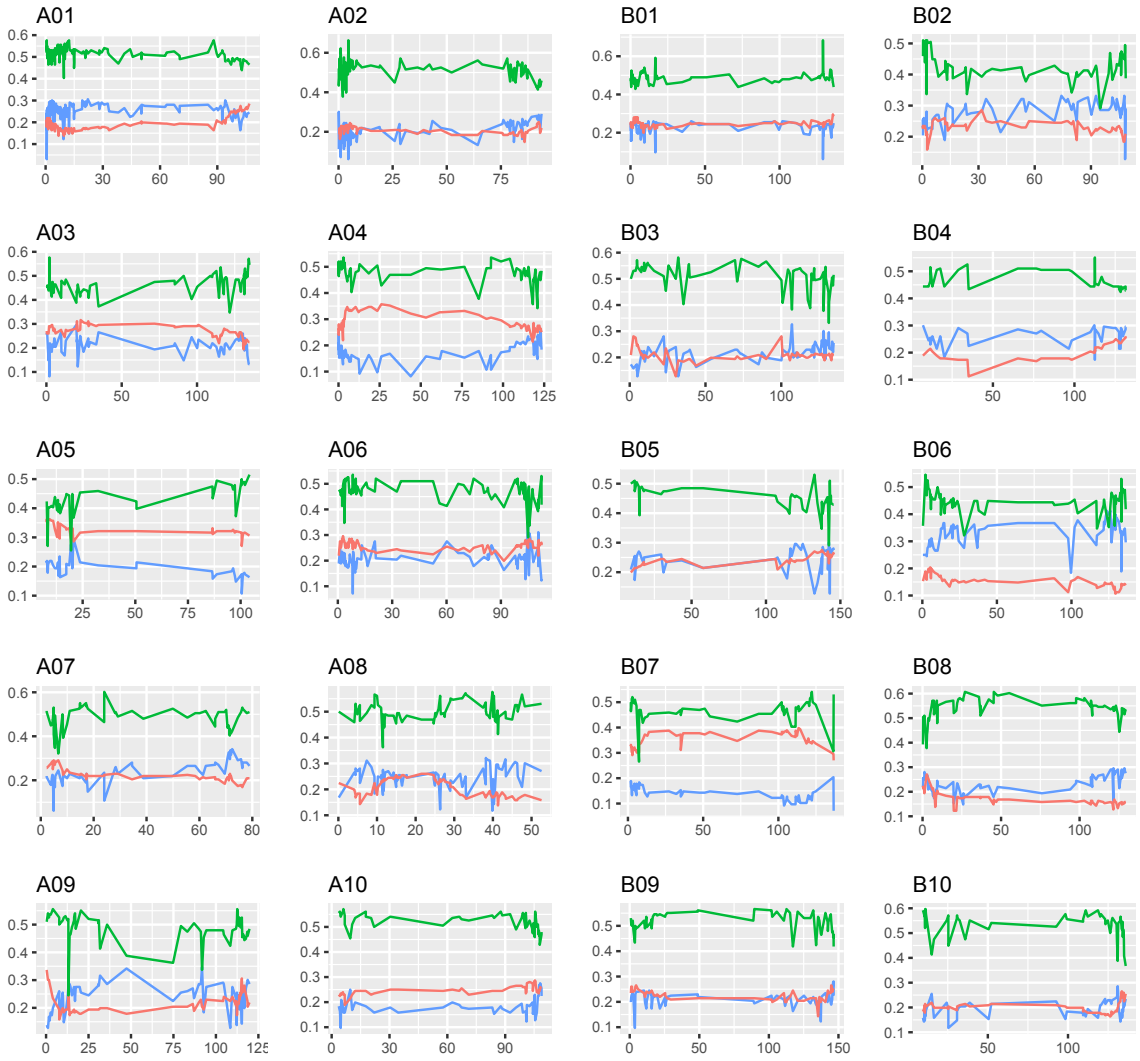

Figure 2.S6. Distribution of segregation distortion across the 20 linkage groups in the RBS-F2 population genetic map. Y-axis represents proportion of genotype (%) and X-axis represents physical position (Mbp). Wild alleles in blue, cultivated alleles in red and heterozygous alleles in green.

Appendix 2.B

Supplementary Tables

Table 2.S1: Root-knot nematode phenotyping values for control genotypes for 2014, 2015 and 2016 and number of phenotyped individuals in comparison with midparent, *A. stenosperma* and *A. hypogaea* Runner-886. Number of eggs per gram of root (EGR), Reproduction factor (RF) and galling index (GI).

	EGR-2014	**EGR-2015**	**EGR-2016**	**RF--2014**	**RF2015**	**RF-2016**	**GI-2014**	**GI-2015**	**GI-2016**
A. stenosperma V10309	0	250 (±433.01)	13.75 (±27.5)	0	0.06 (±0.09)	0.015 (±0.03)	0	0.5 (±0.58)	0
A. batizocoi K9484	621.09 (±768.27)	26.09 (±58.33)	88.33 (±152.99)	0.04 (±0.042)	0.033 (±0.074)	0.06 (±0.104)	1 (±1.41)	0.2 (±0.45)	1 (±1.73)
BatSten1	2.70 (±6.04)	-	55.67 (±96.42)	0.001 (±0.002)	-	0.1 (± 0.17)	0	-	0.33 (±0.58)
Runner-886	5320.67 (±5686.27)	3613.48 (±2518.27)	1971.2 (±2046.31)	1.24 (±1.35)	5.85 (±4.85)	4.49 (±5.89)	2.75 (±2.22)	2 (±1.15)	2.4 (±0.55)
Total phenotyped individuals	155	99	99	155	98	99	155	105	99
MidParent	2661.69	-	1013.43	0.617	-	2.294	1.375	-	1.37
More resistant than midparent	154	-	92	152	-	99	149	-	98
Less resistant than midparent	1	-	7	3	-	0	6	-	1
Equal or more resistant than *A. stenosperma* V10309	24	94	30	24	78	42	40	97	40
Less resistant than *A. stenosperma* V10309	130	6	71	130	21	59	115	9	61
Equal or more susceptible than *A. hypogaea* IAC886	0	0	2	1	0	0	0	1	0

Table 2.S2: Description of 10 linkage groups in the calculated genetic map. Genetic length in cM, number of SNPs in each LG, average and largest distance between SNP markers and number of SNP markers that undergone homeologous recombination. Lowest and highest values in each category are highlighted in red.

LG[a]	Length (cM)	SNP number	Average distance (cM) [b]	Largest distance (cM) [c]	Tetrasomic markers (%)[d]
A01	290.56	**217**	**1.35**	8.26	12.0
A02	**359.52**	122	2.97	13.48	28.7
A03	242.26	72	3.41	17.46	31.9
A04	248.87	68	3.71	14.12	**75.0**
A05	**100.69**	38	2.72	**8.04**	10.5
A06	315.52	84	**3.80**	**29.48**	20.2
A07	167.50	61	2.79	13.74	23.0
A08	203.99	81	2.55	12.80	32.1
A09	222.49	71	3.18	11.11	15.5
A10	143.99	52	2.82	9.55	59.6
B01	191.36	92	2.10	13.58	12.0
B02	193.57	73	2.69	15.41	37.0
B03	232.70	70	3.37	14.19	4.3
B04	113.80	**36**	3.25	11.41	13.9
B05	112.27	44	2.61	9.81	22.7
B06	176.56	70	2.56	8.27	12.9
B07	139.03	57	2.48	16.36	5.3
B08	178.78	73	2.48	14.55	16.4
B09	171.40	61	2.86	13.71	16.4
B10	180.02	57	3.21	24.37	26.3
Total/average	Total = 3984.89	Total = 1944	Average = 2.85	-	Average = 22.9

[a] Linkage group
[b] Average distance between SNP markers (cM)
[c] Largest distance between SNP markers (cM)
[d] Percentage (%) of markers showing homeologous recombination (%)

Appendix 2.C

Supplementary Scripts

Script 2.S1. Commands used for marker filtering and processing to identify polymorphic SNPs for the F_2 population.

```
#Input file:
        TetraploisControlsRBSF2s.txt

# Identification of A. batizocoi K9484 characteristic markers:
        egrep "^\S+\tAA\tAA\tBB\tBB\tAA\tAA" DiploidControls+Runner.txt >> Bat-markers-1.txt
        egrep "^\S+\tBB\tBB\tAA\tAA\tBB\tBB" DiploidControls+Runner.txt >> Bat-markers-2.txt

        head -7 TetraploisControlsRBSF2s.txt > RBS-Bat-markers.txt
        for i in `cut -f 1 Bat-markers-1.txt` ; do grep "$i" TetraploisControlsRBSF2s.txt | perl -lpe
        "s/AB/ab/g" | perl -lpe "s/BB/bb/g" | perl -lpe "s/AA/aa/g" >> RBS-Bat-markers.txt ; done
        for i in `cut -f 1 Bat-markers-2.txt` ; do grep "$i" TetraploisControlsRBSF2s.txt | perl -lpe
        "s/AB/ab/g" | perl -lpe "s/AA/bb/g" | perl -lpe "s/BB/aa/g" >> RBS-Bat-markers.txt ; done

        head -7 RBS-Bat-markers.txt > RBS-Bat-markers-filter.txt
        egrep "^\S+\tbb\tbb\taa\taa" RBS-Bat-markers.txt >> RBS-Bat-markers-filter.txt

#A. stenosperma V10309 characteristic markers:
        egrep "^\S+\tBB\tBB\tBB\tBB\tAA\tAA" DiploidControls+Runner.txt >> Sten-markers-1.txt
        egrep "^\S+\tAA\tAA\tAA\tAA\tBB\tBB" DiploidControls+Runner.txt >> Sten-markers-2.txt

        head -7 TetraploisControlsRBSF2s.txt > RBS-Sten-markers.txt
        for i in `cut -f 1 Sten-markers-1.txt` ; do grep "$i" TetraploisControlsRBSF2s.txt | perl -lpe
        "s/AB/ab/g" | perl -lpe "s/AA/bb/g" | perl -lpe "s/BB/aa/g" >> RBS-Sten-markers.txt ; done
        for i in `cut -f 1 Sten-markers-2.txt` ; do grep "$i" TetraploisControlsRBSF2s.txt | perl -lpe
        "s/AB/ab/g" | perl -lpe "s/BB/bb/g" | perl -lpe "s/AA/aa/g" >> RBS-Sten-markers.txt ; done

        head -7 RBS-Sten-markers.txt > RBS-Sten-markers-filter.txt
        egrep "^\S+\tbb\tbb\taa\taa" RBS-Sten-markers.txt >> RBS-Sten-markers-filter.txt

#A. hypogaea Runner-IAC-886 characteristic markers:
        egrep "^\S+\t.B\t.B\tAA\tAA\tAA\tAA" DiploidControls+Runner.txt >> Hyp-markers-1.txt
        egrep "^\S+\tA.\tA.\tBB\tBB\tBB\tBB" DiploidControls+Runner.txt >> Hyp-markers-2.txt

        head -7 TetraploisControlsRBSF2s.txt > RBS-Hyp-markers.txt
        for i in `cut -f 1 Hyp-markers-1.txt` ; do grep "$i" TetraploisControlsRBSF2s.txt | perl -lpe
        "s/AB/ab/g" | perl -lpe "s/AA/bb/g" | perl -lpe "s/BB/aa/g" >> RBS-Hyp-markers.txt ; done
        for i in `cut -f 1 Hyp-markers-2.txt` ; do grep "$i" TetraploisControlsRBSF2s.txt | perl -lpe
        "s/AB/ab/g" | perl -lpe "s/BB/bb/g" | perl -lpe "s/AA/aa/g" >> RBS-Hyp-markers.txt ; done

        head -7 RBS-Hyp-markers.txt > RBS-Hyp-markers-filter.txt
        egrep "^\S+\tbb\tbb\taa\taa" RBS-Hyp-markers.txt >> RBS-Hyp-markers-filter.txt
```

Script 2.S2. Commands used for marker filtering and processing to analyse genotyping data of the controls $F_{2:3}$ lines and to identify introgressions.

```
#Input file:
        RBS_Genotyping_tetra.txt

#Filtering:
        head -7 RBS_Genotyping_tetra.txt > RBS_Genotyping_Tetra_BatStenMap.txt
        for i in `cut -f 1 BatStenMap_MarkersList.txt` ; do grep "$i" RBS_Genotyping_tetra.txt >>
        RBS_Genotyping_Tetra_BatStenMap.txt ; done
        egrep "^\S+\tAA\tAB" RBS_Genotyping_Tetra_BatStenMap.txt | perl -lpe "s/AA/1/g" | perl -lpe
        "s/AB/2/g" | perl -lpe "s/BB/3/g" | perl -lpe "s/NoCall/-/g" > RBS_GenotypingTetraF2-3_1.txt
        egrep "^\S+\tAA\tBB" RBS_Genotyping_Tetra_BatStenMap.txt | perl -lpe "s/AA/1/g" | perl -lpe
        "s/BB/2/g" | perl -lpe "s/AB/3/g" | perl -lpe "s/NoCall/-/g" > RBS_GenotypingTetraF2-3_2.txt
        egrep "^\S+\tAB\tAA" RBS_Genotyping_Tetra_BatStenMap.txt | perl -lpe "s/AB/1/g" | perl -lpe
        "s/AA/2/g" | perl -lpe "s/BB/3/g" | perl -lpe "s/NoCall/-/g" > RBS_GenotypingTetraF2-3_3.txt
        egrep "^\S+\tAB\tBB" RBS_Genotyping_Tetra_BatStenMap.txt | perl -lpe "s/AB/1/g" | perl -lpe
        "s/BB/2/g" | perl -lpe "s/AA/3/g" | perl -lpe "s/NoCall/-/g" > RBS_GenotypingTetraF2-3_4.txt
        egrep "^\S+\tBB\tAA" RBS_Genotyping_Tetra_BatStenMap.txt | perl -lpe "s/BB/1/g" | perl -lpe
        "s/AA/2/g" | perl -lpe "s/AB/3/g" | perl -lpe "s/NoCall/-/g" > RBS_GenotypingTetraF2-3_5.txt
        egrep "^\S+\tBB\tAB" RBS_Genotyping_Tetra_BatStenMap.txt | perl -lpe "s/BB/1/g" | perl -lpe
        "s/AB/2/g" | perl -lpe "s/AA/3/g" | perl -lpe "s/NoCall/-/g" > RBS_GenotypingTetraF2-3_6.txt

        head -7 RBS_Genotyping_tetra.txt > RBS_GenotypingTetraF2-3_All.txt
        cat  RBS_GenotypingTetraF2-3_1.txt  RBS_GenotypingTetraF2-3_2.txt
        RBS_GenotypingTetraF2-3_3.txt  RBS_GenotypingTetraF2-3_4.txt  RBS_GenotypingTetraF2-
        3_5.txt RBS_GenotypingTetraF2-3_6.txt >> RBS_GenotypingTetraF2-3_All.txt
```

CHAPTER 3

DEVELOPMENT AND GENETIC CHARACTERIZATION OF PEANUT ADVANCED BACKCROSSED LINES THAT INCORPORATE ROOT-KNOT NEMATODE RESISTANCE FROM *ARACHIS STENOSPERMA*

Introduction

The peanut (*Arachis hypogaea*) is an important oil, food and fodder crop cultivated worldwide with an annual production of 64.2 million tons and grown in more than a half million ha (0.72 Mha) (FAOSTAT 2019). Is an allotetraploid species (AABB, 2n = 4x =40) with a narrow genetic base as result of a recent and likely unique polypoid origin (Bertioli et al. 2016; Bertioli et al. 2019; Halward et al. 1992; Husted 1936; Kochert et al. 1996a; Moretzsohn et al. 2013). Genetic improvement using low level of polymorphism present in cultivated genotypes imposes certain constraints for improvement (Moretzsohn et al. 2004; Pandey et al. 2012), and limited gene flow with its diploid wild relatives due to sexual barriers (Bertioli et al. 2011) has resulted in susceptibility to severe pests and diseases. Among the pathogens commonly affecting this crop, root-knot nematode (RKN) (*Meloidogyne arenaria* (Neal)) (Holbrook and Stalker 2003) causes significant yield losses for about 50% or more under dense nematode populations (Dickson and De Waele 2005; Minton and Baujard 1990). It also reduced pod and grain quality, negatively impacts plant growth and increases production costs (Holbrook and Stalker 2003; Starr et al. 2002).

Resistance to many pests/diseases is not found in cultivated peanut, but its wild relatives comprise a diverse genetic pool that can be utilized to enhance peanut performance under disease/pest pressure (Holbrook and Noe 1990a; Nelson et al. 1989; Stalker 1997). Resistance to RKN that is present in modern commercial varieties (Branch and Brenneman 2015; Holbrook et al. 2017; Holbrook et al. 2008; Simpson et al. 2003; Simpson et al. 2013) is derived from a single chromosome segment on A09 from the wild species *Arachis cardenasii* (Chu et al. 2016a). Even though this resistance has been durable thus far, it is important to find additional sources of resistance for the development of new high-yielding and nematode-resistant peanut cultivars and to reduce the risk of resistance breakdown in the varieties currently used (Starr et al. 2002) in order to ensure continued protection of the peanut crop from losses due to RKN.

Strong resistance to RKN has been reported in the diploid wild *Arachis* species *A. stenosperma* V10309 (Guimaraes et al. 2010; Proite et al. 2008; Proite et al. 2007). Three chromosome locations (A02, A04 and A09) conferring resistance were identified (Leal-Bertioli et al. 2016). Later, validation of major QTL in A02 and secondary QTL in A09 was completed by using a tetraploid F_2 population between Runner-886 and BatSten1 ([*Arachis batizocoi* x *A. stenosperma*]$^{(2n=4x=40)}$) where each of the QTL contributing to a percentage reduction in nematode multiplication of up to 98.2% (Refer to Chapter 2 for more details) (Ballén-Taborda et al. Submitted).

Transfer of resistance alleles from wild species can be accomplished through the development of peanut compatible wild-derived synthetic allotetraploids (Kumari et al. 2014; Leal-Bertioli et al. 2017; Leal-Bertioli et al. 2015c; Simpson 1991), and segregating progeny harboring R-genes can be used as donor parents in marker-assisted

backcrossing (MABC) schemes. This breeding method is labor intensive, but a very efficient way to incorporate of simply-inherited traits (e.g. resistance to pest/diseases) from a donor parent with inferior agronomically performance (wild relative or unadapted genotype) into elite/superior cultivars or adapted varieties that lack the desired attributes (Collard and Mackill 2008; Jiang 2013a, b, 2015). This method is particularly useful for selecting lines either without phenotypic screening or during early developmental stages (e.g. seed or seedling) (Collard et al. 2005; Collard and Mackill 2008; Frisch et al. 1999a; Hospital and Charcosset 1997). Molecular markers for marker-assisted backcrossing (MABC) associated with a trait can be used for foreground selection and unlinked markers for background selection (Collard et al. 2005; Frisch et al. 1999a; Hospital 2003; Hospital and Charcosset 1997). It has been suggested that at least three cycles of MABC are required to recover a plant that has the more or less same genetic composition of a particular elite cultivar, except for the target trait (Frisch et al. 1999b, 2000; Hospital and Charcosset 1997; Jiang 2015; Lecomte et al. 2004).

In peanut, MABC breeding has been used to transfer and pyramid desired traits into adapted elite cultivars (Pandey et al. 2016), for example: 1) high oleic/linoleic acid content incorporated into the nematode-resistant cultivar Tifguard (Chu et al. 2011); 2) chromosome segment substitution lines (CSSLs) developed for QTL mapping and introgression of different wild-derived traits of interest (Fonceka et al. 2012a); 3) QTL controlling rust resistance was incorporated into three cultivars grown in India (Varshney et al. 2014a); 4) late leaf spot (LLS) and rust resistance were bred into the Indian variety TMV2 (Kolekar et al. 2017); and 5) improved oleic acid in four Chinese cultivars (Huang et al. 2019).

In this project, we used a MABC with the aim to incorporate RKN resistance from *A. stenosperma* into cultivated peanut. Three breeding elite lines from Tifton were used as recurrent parents (RP): 5-646-10, 13-1014 and TifGp-2. $F_{2:3}$ lines ($F_{2:3}$-7 and $F_{2:3}$-34) derived from a cross between *A. hypogaea* cv. Runner-886 and the synthetic allotetraploid BatSten1) harboring resistance RKN segments in chromosomes A02 and A09 were used as donor parents (DP). Three cycles of backcrossing were completed in three consecutive years (2016, 2017 and 2018) in two locations (Athens and Tifton, GA). To accelerate the breeding process, 22 SNP markers were used in each crossing step for foreground selection of lines with introgressed resistance. In addition, phenotypic screening for RKN resistance and genotypic characterization of BC_2F_1 lines allowed us to validate and refine the resistance chromosome segments. Lastly, high-throughput genotyping of 271 advanced backcrossed lines (BC_3F_1) was done to aid in the selection of superior lines harboring RKN resistance alleles while having recovered most of the recurrent genome. Future experiments will include phenotypic screening of advanced backcrossed lines (BC_3F_2) for nematode resistance with the aim to make additional selections. The present work represents an important step for developing new high-yielding nematode-resistant peanut cultivars by releasing advanced breeding lines that can be used directly in breeding programs. Additionally, SNP markers tightly linked to the QTL will be useful to facilitate the introgression of *A. stenosperma* resistance into elite peanut cultivars.

Materials and Methods

Plant materials

Wild *Arachis* accessions were obtained from the USDA-GRIN system (https://www.ars-grin.gov/). To introgress the diploid nematode resistance into tetraploid peanut, a synthetic allotetraploid (BatSten1, ([*A. batizocoi* x *A. stenosperma*]$^{(2n=4x=40)}$)) was created using the peanut wild relatives *A. stenosperma* PI666100 (original collection voucher V10309) and *A. batizocoi* PI298639 (original collection voucher K9484), as described in Leal-Bertioli et al., (2015). This wild-derived allotetraploid combines the A genome of *A. stenosperma* (Moretzsohn et al. 2013; Tallury et al. 2005) and a K genome of *A. batizocoi* (Moretzsohn et al. 2013; Robledo and Seijo 2010). An F_2 segregating population was created by selfing an F_1 derived from a cross between *A. hypogaea* cv. Runner IAC-886 (herein called Runner-886) and BatSten1 (described above). From this population, F_2-derived F_3 ($F_{2:3}$) homozygous progeny from four selected superior lines (F_2-7, F_2-13, F_2-34 and F_2-73) were used as donors for nematode resistance alleles from *A. stenosperma* in our backcrossing scheme (Fig. 3.1) (see "Marker-assisted breeding" section below for more details). Male parents in each cycle of crossing comprised backcrossed lines (BC_nF_{1s}) developed and selected the previous year.

The female recipient lines from Tifton (GA) included: 1) TifGP-2 is a RKN-susceptible runner-type peanut and a near isogenic sister line of the RKN-resistant 'Tifguard' cultivar. TifGP-2 has good yield and grade, and normal oleic content (Holbrook et al. 2012); 2) 5-646-10, a Florida-07 x Tifguard derived breeding line susceptible to root knot nematode, with good yield and grade, and high oleic oil content (Holbrook, unpublished data); and 3) 13-1014, a RKN susceptible and high oleic oil

breeding line derived from [C1805-617-1 (Florida-07 x Tifguard) x GA-06G] (Holbrook, unpublished data).

Peanut hybridization

Artificial hybridization took place in the greenhouse to backcross the recurrent parents (RP) with the F_2-derived F_3 ($F_{2:3}$) lines harboring RKN resistance. Since peanut has hermaphrodite flowers, emasculation is required before crossing to prevent self-pollination and to more easily cross-pollinate with desired pollen. Briefly, emasculation of flowers to be used as female progenitor was performed, usually after 4 pm. To emasculate a flower from recipient plant (female), we looked for flower buds, and with a thin tweezer carefully removed the lower lip of the calyx to expose the closed flower, then gently the standard and wings (keel) were moved aside (still attached) and all eight anthers were removed. After, we placed the keel back to protect the stigma. A small colored hair clip was attached to mark the emasculated flower (Fig. 3.2.A,B). The following day, pollination occurred between 7 and 10 am. For this, a flower to be used as male progenitor was picked, the keel was pulled, and with flat tweezers the anthers were taken and squeezed to obtain pollen. The pollen was gently rubbed onto the stigma of the emasculated flower (from female progenitor) and marked with a dated paper tag adjacent to the flower (Fig. 3.2.C). After pollination, water was sprinkled on the plant and floor to increase humidity to improve success of pollination. Female flowers that were not emasculated were removed every morning to prevent self-pollination. Between five and 10 days after pollination peg tips were observed emerging from the bottom of the pollinated flowers. Using a wire folded at the top to form a circle, we allow the peg to

grow through it, to be easily identified later during harvest (Fig. 3.2.D,E) (http://plantbreeding.coe.uga.edu/index.php?title=20.3_Peanut) (Chu et al. 2016b; Fonceka et al. 2012a; Nigam et al. 1990).

Marker-assisted breeding

A marker-assisted backcrossing (MABC) approach was used to incorporate RKN-resistance QTL derived from *A. stenosperma* into cultivated peanut. The scheme presented in Fig. 3.1, was done at two locations, Athens, GA and Tifton, GA. Progeny from 10 superior F_2 lines, derived from the cross between Runner-886 and BatSten1 ([*Arachis batizocoi* x *A. stenosperma*]$^{(2n=4x=40)}$) (refer to chapter 2 and to Ballén-Taborda et al., submitted, for description of F_2 development) that produced a higher number of seeds were planted in the field in 2015 for yield and disease resistance evaluation. Four F_2 lines (F_2-7, F_2-13, F_2-34 and F_2-73) were selected based on the following criteria: (1) better vigor, as visually more leaf biomass 2) late leaf spot (LLS) resistance and no root-knot nematode (RKN) multiplication; 3) good agronomic traits and; 4) harboring QTLs for nematode resistance per molecular genotyping (Table 3.1).

Selected F_2 lines were advanced to $F_{2:3}$ generation. For two F_2-derived F_3 lines ($F_{2:3}$-7 and $F_{2:3}$-34) resistance to RKN was validated, for the other two lines (F_2-13 and F_2-73) resistance was not possible to confirm due to missing data (Chapter 2, Ballén-Taborda et al. Submitted). These lines were used as donor parents in our backcrossing process (Fig. 3.1, purple box). The recurrent parents (RP) were TifGP-2, 5-646-10 and 13-1014 (see "Plant material" section above for more details) (Fig. 3.1, orange boxes).

The first cycle of backcrossing occurred in the summer of 2016 in Athens and Tifton under greenhouse-controlled conditions. Ten and 28 cross combinations were used in Athens and Tifton (GA), respectively. Nineteen $F_{2:3}$ plants of $F_{2:3}$-7 and $F_{2:3}$-34 lines were used as donor parents (Table 3.2). Breeding line 13-1014 was not included in this crossing cycle. A second cycle of backcrossing was performed in the summer of 2017, where 14 cross combinations were used in Athens and Tifton (7 in each location). Eleven BC_1F_1 lines (developed the previous year) were selected as they harbored RKN resistance chromosome segments based on KASP genotyping and were used as male parents. Twenty-one BC_2F_1 were selected as they had RKN resistance chromosome segments based on KASP genotyping and were used as male parents (Table 3.3 and Fig. 3.1, red box). A third cycle of backcrossing was performed in the summer of 2018 under greenhouse conditions. A total of 10 and 11 cross combinations were used in Athens and Tifton, respectively (Table 3.4 and Fig. 3.1 red box).

In each cycle of backcrossing, pods from hybridization were harvested during the fall of each year, dried, cleaned and shelled for photo documentation. Then, seeds were organized, weighed and measured. KASP genotyping was performed using 22 SNP markers using cotyledon tissue (Fig. 3.3 and Table 3.S1, gray shaded markers). Refer to the "Foreground selection" section for a detailed description of this process. BC_1F_2, BC_2F_2 and BC_3F_2 seeds from male parents were harvested each year and stored at 4ºC (Fig. 3.1, green boxes).

Parallel to the backcrossing experiment in 2018, phenotypic screening for RKN resistance and genotypic characterization of 10 selected BC_2F_1 lines located in Athens was performed (Table 3.4 and Fig. 3.1, light blue box). From the 283 BC_2F_1 seeds

developed in Athens during the third backcrossing cycle, 81 were randomly selected for seed DNA KASP assays. As we obtained a good number of lines (52/81) harboring the alleles of interest, high-throughput genotyping from 271 advanced backcrossed lines (BC_3F_1) was completed in 2019. The objective was to select the lines harboring RKN resistance alleles in chromosomes A02 and A09 while having also recovered most of the recurrent genome (Fig. 3.1, dark blue box).

SNP primer design

For selection of backcrossed lines, three SNP primers per SNP position were designed to detect the regions associated with resistance to RKN. Two allele-specific primers (one labelled with FAM dye and the other with VIC dye) and a common flanking primer were designed for use in KASP endpoint genotyping (Kompetitive Allele-Specific PCR) (LGC Biosearch technologies). The primers were designed using the web-based program BatchPrimer3 (https://wheat.pw.usda.gov/demos/BatchPrimer3/) (You et al. 2008) using the "Allele-specific primers and allele flanking primers" option. The parameters used were 60−120 bp in size, GC content of 30-80% and Tm between 58 and 60°. Twenty-two KASP assays were designed (Table 3.S1, gray shaded markers). KASP primer assay mix per SNP comprised of 12 ul (100 uM) of each allele-specific primer, 30 ul (100 uM) of flanking marker and 46 ul of H_2O.

Foreground selection

In order to make marker-assisted selections (MAS) prior to seed germination (to save time and space), we used DNAs from seed chips for genotyping (Fig. 3.3). High

quality DNA was extracted without disturbing the embryo and without reducing either DNA quality or quantity (Gao et al. 2008). Once the seed were harvested, dried, and cleaned, they were organized into 24-well plates linked to a detailed spreadsheet (Fig. 3.3.A). Seed measurements were taken for weight (g), length (mm) and width (mm) and photo documented (see below).

A small section (~50-100 mg) of the peanut cotyledon (opposite to the embryo) was cut carefully with a razor blade, chopped into small pieces (Fig. 3.3.B) and transferred to a 2 ml tube to be macerated with a pellet pestle (Fig. 3.3.C). A bead was placed inside the tube, rapidly frozen in liquid nitrogen, and ground into fine powder for 30 sec (twice) using a grinder (RETSCH MM301). Tissue was store at -80°C until DNA extraction. Genomic DNA was extracted from seed tissue using the DNeasy *Plant* Mini *Kit (QIAGEN)* according to manufacturer's instructions (Fig. 3.3.D). Extracted DNAs were quantified with spectrophotometer NanoDrop 2000 (v.1.4.2) (Thermo Scientific) for quality and fluorometer QUBIT 2.0 (Invitrogen) for quantity. DNAs were diluted to 10 ng/ul for KASP assays. DNAs were extracted from all potential backcrossed lines in each backcrossing cycle and controls (BatSten1, Runner-886, 5-646-10, 13-1014, TifGp-2, *A. stenosperma* and *A. batizocoi*)

KASP genotyping was performed using a modified manufacture's protocol. Twenty-two SNP markers located in the vicinity of the QTL at bottom and top of chr A02 and top of chr A09 were used for selection and SNP markers in A04 were used to select against *A. stenosperma* alleles (Table 3.S1, gray shaded markers). Single KASP reactions of 5 ul consisted in 2.5 ul of KASP 2x Master Mix (Low Rox 5000 V4.0), 0.07 ul of primer assay mix (12 uM of each allele-specific primer and 30 uM of common primer),

1.93 ul of water and 0.5 ul of DNA (10 ng/ul, 5 ng). Two replicates per primer per sample were included in each run, as well as no-template controls (NTCs) (water). A C100 touch Thermal Cycler (BIO-RAD) was used with the following modified KASP thermal cycling conditions: 94°C for 15 min; 8 cycles of 94°C for 20 sec and touchdown starting at 61°C for 1 min (dropping 0.6° per cycle); 31 cycles of 94°C for 10 sec and 55°C for 1 min; 9 cycles of 94°C for 20 sec, and 57°C for 1 min: 4°C forever (https://biosearch-cdn.azureedge.net/assetsv6/KASP-genotyping-chemistry-User-guide.pdf). Fluorescence was read with a LightCycler® 480 Instrument II and analyzed using the LightCycler® 480 Software (v.1.5.1.62) (Roche Life Science) (Fig. 3.3.E).

Seeds of the backcrossed genotypes that presented the desired regions (segments associated with RKN resistance in A02 and A09) were selected and germinated (Fig. 3.3.G,H).

High-throughput genotyping, analysis and data filtering for BC_3F_1s

Genomic DNAs from lyophilized young folded leaflets from 288 samples (271 BC_3F_1 lines, diploid controls *A. stenosperma* and *A. batizocoi* and tetraploid controls BatSten1, *A. hypogaea* genotypes Runner-886, 5-646-10, 13-1014, TifGp-2, Tifguard and Tifrunner were extracted using the DNeasy 96 Plant Kit (*QIAGEN*) according to manufacturer's instructions. DNAs were checked for quality with spectrophotometer NanoDrop 2000 (v.1.4.2) (Thermo Fisher Scientific) for quantified with fluorometer Qubit 2.0 (Invitrogen). DNAs were submitted for genotyping with the 'Axiom_Arachis' 47K high-density SNP array v.02 (Clevenger et al. 2018; Korani et al. 2019). The genotypic data was extracted and processed using the Axiom™ Analysis Suite software

(v.4.0.3.3, Applied Biosystems) (http://www.affymetrix.com). Output was analyzed using custom Unix scripts (Script 3.S1) and resulting data were visualized as a color map in Microsoft Excel.

The strategy to identify polymorphic SNP markers included two main steps. Firstly, SNP assay results were extracted from SNP calling using tetraploid controls and BC_3F_1s. This set of markers was filtered to reveal polymorphic SNP markers between parental genotypes (BatSten1 ≠ *A. hypogaea* Runner-886) and genotypes were replaced by numbers (1 for BatSten, 2 for Runner-886 and 3 for a different genotype). Secondly, SNP assays from markers present in the genetic map previously developed here (*A. stenosperma*-specific and *A. batizocoi*-specific markers) (Chapter 2, Fig. 2.S1, Ballén-Taborda et al. Submitted) were recovered. Using the Statistical package R and the 'dist' function, genotypic data was used to perform the principal component analysis (PCA) to spot any errors and to evaluate the population structure.

Seed size evaluation

In each cycle of backcrossing, developed seeds (BC_1F_1, BC_2F_1 and BC_3F_1) were dried, pods were cleaned, and shelled seed were prepared for photo documentation. Seeds were weighed using a precision balance XS403S (Mettler Toledo), and measured a digital caliper (Ted Pella, Inc) at the widest and longest point. Since each individual BC_nF_1 line were unique and replicated measurements were not possible to take, statistical analysis was not performed. For controls, weight, length and width from 10 individual seeds per genotype were measured. Shapiro-Wilk test was used to verify normality. Non-parametric Kruskal-Wallis test (Kruskal and Wallis 1952) was use to estimate differences

in seed dimensions ($P<0.05$) among controls. Wilcoxon signed rank test using FDR (false discovery rate) correction was performed to group the samples by significant similarity ($P>0.05$).

Results

Marker-assisted breeding and phenotyping

Three generations of marker-assisted backcrossing (MABC) for introgression of *A. stenosperma* RKN resistance were produced in two locations, Athens and Tifton, GA. The first cycle of backcrosses was finished in 2016. From the 38 cross combinations, 1008 potential BC_1F_1s seeds were collected (Table 3.2). After running the KASP genotyping assays using 22 markers associated with the resistance (Table 3.S1, gray shaded markers), 17 seeds were selected as carrying all three nematode resistance segments in A02 and A09 (Fig. 3.4.A-C). From these, 11 seeds that successfully germinated were used as male parents for next cycle of backcrossing (Table 3.2 and Fig. 3.5.A, introgressions in red). The second cycle of introgression took place in 2017, where 14 cross combinations were made (Table 3.3). Here, 61 potential BC_2F_1 seeds were obtained and genotyped with KASP markers to again identify the carriers of nematode resistance segments (Fig. 3.4.D-F). A total of 21 seeds were selected and successfully germinated for next cycle of crossing (Table 3.3 and Fig. 3.5.B, introgressions in red).

The third cycle of introgression occurred in 2018. In total, 21 cross combinations were made in Athens and Tifton resulting in 397 potential BC_3F_1 seeds. From these, 283 seeds came from 10 cross combinations done in Athens and 114 seeds came from 11 cross combinations done in Tifton (Table 3.4). From the set of BC_3F_1 seeds developed in

Athens, 81 were randomly selected and evaluated for the presence of resistance segments (Fig. 3.4.G-I) using the KASP assays (Table 3.S1, gray shaded markers) and seed tissue. From this group, 52 BC_3F_1s harbored the segments from *A. stenosperma* (Table 3.4 and Fig. 3.5.C, introgressions in red). As we obtained a good number of lines (52/81, 64.2%) harboring the segments of interest, we decided to genotype all the population with the SNP array (Clevenger et al. 2018; Korani et al. 2019).

Out of the 397 BC_3F_1, a total of 271 seeds were successfully germinated, grown in the greenhouse and leaf tissue was genotyped with the SNP array; 126 seeds were not advanced as some did not germinate (47 seeds) and progeny from susceptible BC_2F_1 male parent (BC_2F_1_BRD_C0055_Seed17) (50 seeds) (Chapter 4) and lines not carrying resistance segments based on KASP genotyping (29 seeds) were not included (Table 3.4).

In parallel to the backcrossing experiment in 2018, phenotypic screening for RKN resistance and genotypic characterization of 10 selected BC_2F_1 lines were performed (Fig. 3.1, light blue box). Detailed information of these experiments is found in Chapter 4.

<u>High-throughput genotyping of BC_3F_1s and selection</u>

The 271 BC_3F_1s lines that were germinated and grown in greenhouse and controls were genotyped with the Affymetrix 'Axiom_Arachis' SNP array (Clevenger et al. 2018; Korani et al. 2019). A total of 930 polymorphic SNP markers from the genetic map (Chapter 2, Fig. 2.S1, Ballén-Taborda et al. Submitted) were recovered using the filtering strategy described in Script 3.S1. Of these, 527 markers were assigned to A-subgenome (*A. stenosperma*-specific markers) and 403 to B/K-subgenome (*A. batizocoi*- specific markers). The physical positions of the A-genome markers were determined by the

position of their homologues in the *A. duranensis* pseudomolecules and the B/K-genome markers based on the *A. ipaensis* pseudomolecules (https://www.peanutbase.org/) (Bertioli et al. 2016).

Affymetrix clustering plots of SNP markers linked to QTL controlling RKN resistance at the bottom of chromosomes A02 (Fig. 3.6.A,B) and A09 (Fig. 3.6.C-E) show the distribution of BC_3F_1 lines and controls. Red clusters for SNPs located in A02 and blue groups in A09 comprise genotypes without the *A. stenosperma*-derived alleles (Runner-886, 5-646-10, 13-1014, TifGp-2, Tifguard, Tifrunner, *A. batizocoi* and BC_3F_1s without those regions). Red/Blue cluster comprises of *A. stenosperma* and the derived allotetraploid BatSten1. Yellow clusters include the lines with incorporated RKN resistance from *A. stenosperma*. When analyzing all genome-wide markers, BC_3F_1 backcrossed lines showed a percent of identity to the recurrent parents (5-646-10, 13-1014 and TifGp-2) and other cultivated accessions between 80.2% and 98.8% while still carrying between 1.1% and 19.1% of the wild donor genome (Fig. 3.8.A, Fig. 3.S1 and Fig. 3.S2). We obtained between 0.1% to 0.7% missing data. Of the 271 genotyped lines, 253 (93.4%) were true progeny from hybridization and 18 (6.6%) were products of self-pollination.

In order to visualize the distribution of the backcrossed population according to the proportion of wild introgression (%) in each chromosome, we displayed the data in a violin plot (Fig. 3.7). These plots allowed us to observe that more individuals (dots) with high percentage of wild genome were observed more often in the A-subgenome, especially in chromosomes A02 and A09 where foreground selection for *A. stenosperma* alleles was applied during the backcrossing process (Fig. 3.7, Fig. 3.S1 and Fig. 3.S2).

Then, we performed the principal component analysis (PCA) on the genotyping data to evaluate the grouping behavior of the BC_3F_1s. Each line was labeled with the percentage of wild genome in A-subgenome and in chromosomes A02 and A09, as well as A08 as control as no introgressions were observed (Fig. 3.8). Here, each dot indicates an individual BC_3F_1 and the color meaning the percentage of wild genome (blue close to 100% and red close to *A. hypogaea* genome). In A02, the percentage of wild alleles was dividing the data into two horizontal clusters, one closer to *A. hypogaea* (bottom cluster in red, 0 - 55.2% of wild genome) and a second group (top cluster in blue, 43.1 - 98.3% of wild genome) (Fig. 3.8.B). Percentage of wild genome in A09 seems to be affecting the clustering as well, with data points closer to *A. hypogaea* in red and others less related in blue (Fig. 3.8.C). In contrast, A08 (an example) the only chromosome in A-subgenome did not have effect on the clustering (Fig. 3.8.D).

Seed size evaluation

Seed weight, length and width measurements were taken on 11 BC_1F_1, 30 BC_2F_1 and 253 BC_3F_1 lines, along with wild and cultivated controls. Significant differences were observed between the control genotypes according to Kruskal-Wallis test and Wilcoxon Test ($P>0.05$), where wild genotypes were significantly different to the cultivated genotypes for seed measurement data (Table 3.5). Each backcrossed line (BC_nF_1) was considered genetically unique so replicates were nonexistent and therefore statistical analyses were not possible to estimate. However, it was possible to observe that data for seed measurements of the backcrossed lines were increasing each generation.

For the 11 BC_1F_1s, seed weight ranged between 0.29 to 1.46 g (data for length and width was not collected). Seed weight for the 30 BC_2F_1s was similar, ranging from 0.25 to 1.47 g, and length and width varied from 11.01 to 21.46 mm and 7.48 to 13.91 mm, respectively. The seed weight of the 253 BC_3F_1s varied more than the other BC generations, between 0.09 to 1.31 g, and length and width were between 9.98 to 20.83 mm and 3.71 to 13.42 mm, respectively (Fig. 3.9.A-C and Table 3.5). Despite the wider range of seed dimensions and weight, we see clear size recovery in the BC generations, e.g. BC_3F_1 seeds and pods derived from 5-646-10 x BC_2F_1:C2633-2_3(16) from the 2018 backcross cycle (Table 3.4 and Fig. 3.9 D,E), where pod and seed size are similar to the cultivated genotypes and clearly larger than wild species and the BatSten1 allotetraploid.

Discussion

Incorporation of RKN resistance from *A. stenosperma*

Root-knot nematode (RKN) (*Meloidogyne* spp.) is a serious constraint for crop production (Jones et al. 2013). Peanut is a particularly susceptible crop, where RKN produces root galls and pods/seed damage that results in significant economic losses (Holbrook and Stalker 2003; Starr et al. 2002). Although resistance from *Arachis cardenasii* (Chu et al. 2016a) is present in several commercial peanut cultivars (Branch and Brenneman 2015; Holbrook et al. 2017; Holbrook et al. 2008; Simpson et al. 2003; Simpson et al. 2013), it is imperative find other sources of resistance to RKN (Stuthman et al. 2007) to ensure protection in the event that the pathogen is able to overcome the current resistance source (Starr et al. 2002). Among the potential new sources of resistance to RKN infection, the peanut wild relative *A. stenosperma* has numerous

resistance genes (R-genes) that will help expand the peanut gene pool (Burow et al. 2001). Previously, resistance has been identified and validated in chromosomes A02 and A09 (Ballén-Taborda et al. Submitted; Leal-Bertioli et al. 2016).

In peanut, transferring genes/alleles from the wild species is not a straightforward process due to ploidy differences (Bertioli et al. 2011; Dwivedi et al. 2007; Garcia et al. 1995; Stalker 2017); however this genetic incompatibility can be addressed through the development of peanut compatible wild-derived synthetic allotetraploids (Leal-Bertioli et al. 2017; Leal-Bertioli et al. 2015c; Simpson 1991). It was not until the development of the synthetic allotetraploid BatSten1 ([*Arachis batizocoi* x *A. stenosperma*]$^{(2n=4x=40)}$) (Glover et al. 2016; Leal-Bertioli et al. 2015c) that transfer of RKN resistance from *A. stenosperma* was possible. MABC is a useful and efficient breeding strategy to incorporate major genes or few QTL (e.g. resistance to pest/diseases) into elite cultivars (Collard and Mackill 2008; Jiang 2013b; Sousa et al. 2019). This breeding strategy has been used to introgress/pyramid several traits into peanut, including RKN, rust, late leaf spot and oleic to linoleic (O/L) acid ratio (Chu et al. 2011; Fonceka et al. 2012a; Huang et al. 2019; Kolekar et al. 2017; Simpson and Starr 2001; Varshney et al. 2014a).

Here, we employed MABC with the objective to incorporate RKN resistance from *A. stenosperma* into high-yielding peanut breeding lines. We selected $F_{2:3}$ resistant homozygous progeny (derived from Runner-886 x BatSten1) as donors in our MABC scheme. Three cycles of backcrossing were undertaken and foreground selection was key for accelerating the breeding process (Collard et al. 2005; Frisch et al. 1999a; Hospital 2003; Hospital and Charcosset 1997; Jiang 2013a, 2015), as well as the use of seed tissue to increase efficiency of selection and to reduce time, space and resources required for

seed germination and seedling growth (e.g. growth chamber, greenhouse space, soil, pots) (Collard et al. 2005; Collard and Mackill 2008; Frisch et al. 1999a; Hospital and Charcosset 1997). In parallel, phenotyping and genotyping of BC_2F_1 lines allowed us to validate and delineate RKN resistance in A02 and A09 to a ~8Mbp and ~6.5Mbp regions on the bottom of each chromosome, respectively (Chapter 2 for more details).

Finally, we genotyped the 271 BC_3F_1 lines using the Axion *Arachis* SNP array, where 93.4% (253) of the progeny are a result of hybridization, not selfing, which is a good success rate of pollination. When performing the PCA analysis of the genotypic data, it was possible to observe that chromosomes harboring high percentage of wild alleles were driving the distribution pattern between the BC_3F_1 lines (Fig. 3.8, Fig. 3.S1 and Fig. 3.S2). High percentage of wild alleles in A02 was the major factor for the grouping (Fig. 3.8.B). In contrast, A08 (Fig. 3.8.D), as well as other chromosomes in B-subgenome with few or no introgressions from the wild species, were not important factors for the data distribution (Fig. 3.7 Fig. 3.8, Fig. 3.S1 and Fig. 3.S2). The position of the wild controls (*A. stenosperma* and *A. batizocoi*) in the PCA plot, could be due ascertainment bias, that make them appear distantly related to BatSten1 (Fig. 3.8).

Genome-wide genotyping of these backcrossed lines was performed at the 3rd cycle of crossing, until then only selection for resistance segments was performed. Thus, there was variation for wild segments across lines and chromosomes (Fig. 3.7, Fig. 3.S1 and Fig. 3.S2) resulting in an observed 18-point range of recurrent parent genome recovery (RPG) between the backcrossed lines (80.2% and 98.8%). This observed RPG was consistent with the expected value for a third generation of backcrossing (~95.5%) (Fonceka et al. 2012; Hasan et al. 2015; Hospital 2003). The observed wide range of

recurrent parent genome recovery is expected when the genetic background is not screened at early generations for elimination and this had been seen in other studies (Arbelaez et al. 2015; Gramazio et al. 2017). It is also common to observe deviation from the expected average of genome recovery within backcrossed lines (Babu et al. 2004; Collard et al. 2005; Hasan et al. 2015; Hospital 2003).

Having a wide range of elite genome recovery (some individuals carrying more or less recurrent parent genome) indicates that additional cycles of backcrossing to assure maximum RP genome representation and discard non-target introgressions would be useful (Babu et al. 2004; Collard et al. 2005; Fonceka et al. 2012a; Frisch et al. 1999b, 2000; Hasan et al. 2015; Hospital 2003; Hospital and Charcosset 1997; Jiang 2015; Lecomte et al. 2004). However, we can also use these introgressions in our favor. First, lines that contain the highest RP genome and carry target resistance in chromosomes A02-bottom and A09-bottom can be used to continue nematode resistance screening and advancement of materials. We can also study the effect of different introgression sizes in these chromosomes. Second, wild introgressions in non-target chromosomes in A-subgenome from *A. stenosperma* (mostly observed) and in B-subgenome from *A. batizocoi* (Fig. 3.7, Fig. 3.S1 and Fig. 3.S2), could be used to study and characterize genomic regions that underlie other agronomic traits of interest (Fonceka et al. 2012a; Yano 2001; Zamir 2001), including other diseases. We have selected 29 advanced backcrossed lines (BC_3F_1s) for this purpose.

Lastly, large blocks of introgressions were observed, rather than small segments or single genes, especially in the target chromosomes A02 and A09 (Fig. 3.S1 and Fig. 3.S2). This appears to be common in *A. hypogaea* when genes from wild species have

been introgressed (Garcia et al. 1995; Nagy et al. 2010) and can be explained by suppressed recombination. Large introgression blocks result in linkage drag, which brings along undesired traits that can reduce the performance of cultivated × wild progeny, which limits the use of progenies until linkage between good and undesired characteristics is broken (Leal-Bertioli et al. 2012; Stalker 2017). Suppressed crossing-over between homeologous chromosomes is common in interspecific populations segregating for alien introgressions (Chetelat et al. 2000; Nagy et al. 2010). The reduced meiotic recombination is possible due to the absence of microcolinearity and diverging haplotypes (Schweiger et al. 2016). As chromosome pairing and recombination between different genomes of different species is likely be preferential (Garcia et al. 1995), it is important to carefully select the wild species (genomes) to be used to assure successful introgression of only the target wild alleles into *A. hypogaea* (Hajjar and Hodgkin 2007; Leal-Bertioli et al. 2012).

Genetic background

The expression of introgressed traits can be affected by genetic background, epistasis, environment and unusual recombination on the phenotypic expression of genes (Leal-Bertioli et al. 2015c; Leal-Bertioli et al. 2016; Lecomte et al. 2004; Liao et al. 2001). Efficacy and durability of QTL can be greatly influenced by the genetic background in which a segment is introgressed. Superior durability and enhanced efficiency of disease resistance is observed when resistance genes are deployed in a partially resistant background. In other words, not just the introgressed resistance gene alone may be entirely responsible for a desired or observed phenotype; the resistance may

be enhanced due to the favorable genetic interactions between genes in the recurrent parent (quantitative resistance) and the introgressed major resistance gene (qualitative resistance) (Barbary et al. 2014; Brun et al. 2010; Fournet et al. 2013; Gallois et al. 2018; Jacquet et al. 2005; Palloix et al. 2009; Quenouille et al. 2012; Quenouille et al. 2014). Therefore, in order to breed efficiently, increase durability and successfully incorporate resistance from wild species into peanut cultivars (Barbary et al. 2014; de Paula et al. 2017; Lecomte et al. 2004), it is essential to test whether the genetic background into which the QTL will be incorporated affects their expression (Bernardo 2008). Here, RKN resistance segments in this specific tetraploid scenario have been introgressed in two recurrent parents (5-646-10 and 13-1014); however, further analysis in other different recipient lines needs to be completed to fully understand the effect of genetic background on expression of RKN resistance.

Seed size

Wild species have a great genetic diversity for broadening the peanut genetic base but at the same time they have poor agronomic characteristics (Stalker 2017). When using wild species as donor of traits of interest, other undesired traits are also introgressed due to linkage drag (Varshney et al. 2014b). However, it is possible to eliminate undesired traits through marker-assisted selection, while restoring superior agronomic attributes from the elite genome (Babu et al. 2004; Hospital 2005; Jiang 2013a, 2015). An example is the case of TxAG-6, a wild-derived synthetic allotetraploid with good resistance to RKN from *A. cardenasii* (Simpson et al. 1993), but due to its wild composition and significant linkage drag, it had very low yields, and inferior pod and

seed characteristics (Stalker 2017). Later, using MABC, these characteristics were recovered from cultivated germplasm to release commercial cultivars such as NemaTAM, Tifguard, Webb, Georgia-14N and TifNV-High O/L (Branch and Brenneman 2015; Holbrook et al. 2017; Holbrook et al. 2008; Simpson et al. 2003; Simpson et al. 2013). In addition, unexpectedly, wild alleles can contribute to larger seed size (Fonceka et al. 2012b; Leal-Bertioli et al. 2017; Suassuna et al. 2019).

Although the main focus of this study was to incorporate RKN from *A. stenosperma*, we also examined seed weight, length and width in each generation of introgression. We did this with the aim to observe the seed size recovery as we progressed through our MABC scheme, as seed size is an important trait associated with germination, vigor and yield, and is important for the peanut industry and market (Singh et al. 1998). Although we could not use statistical analysis as each BC seed was unique, our measurements showed that by the BC_3 generation, we had mostly recovered the elite peanut's large seeds (Fig. 3.9.A-E and Table 3.5).

Implications for breeding for disease resistance

Genetic maps and a quantitative trait loci (QTL) have been reported for numerous crops and traits; consequently, marker-assisted breeding is possible as these DNA marker-phenotype associations have been established (Collard and Mackill 2008). Despite the availability of numerous QTL studies and marker information, examples of routine implementation in plant breeding programs for the release of breeding lines or cultivars are lower than one would expect. This is not to say that there have not been successful examples of using QTL information to breed crops (Babu et al. 2004;

Bernardo 2008, 2016; Collard et al. 2005; Young 1999). In particular, this work represents a successful example of QTL introgression from a wild relative species despite the differences in ploidy with peanut cultivated genotypes. Also, this is the second report of incorporation of root-knot nematode resistance using interspecific synthetic allotetraploids through MABC (two-way hybrid); after the release of COAN cultivar (three-way hybrid) (Simpson and Starr 2001) widely used in modern varieties.

Future work

After self-pollination of advanced backcrossed lines (BC_3F_1s) in the greenhouse during the summer of 2019, we observed segregation for other traits, such us foliar disease resistance, flower color and plant architecture (growth habit). This present additional opportunities for the future:

1. Screen for nematode resistance in homozygous progeny of selected BC_3F_2s with the aim to make additional selections focusing on individuals harboring RKN resistance segments in A02-bottom and A09-bottom, or both, and recombinants within those chromosome segments (Fig. 3.S1 and Fig. 3.S2).

2. These selfed BC_3s will allow us to study the molecular basis of other traits from the wild species *A. stenosperma* and A. *batizocoi*. These plants displayed different levels of resistance to foliar diseases, thus, we intend to carry out studies on the effect of wild introgression in non-target chromosomes (Fig. 3.S1 and Fig. 3.S2) on resistance to early leaf spot (ELS) and late leaf spot (LLS). It is possible to stablish an advance backcross QTL mapping (AB-QTL) to study these regions (Bhanu et al. 2017; Tanksley and Nelson 1996).

Summary and Conclusions

Wild diploid peanut species have been and are increasing in importance to improve cultigen performance under biotic and abiotic pressures. The wild relative *A. stenosperma* has become a source of alleles for root-knot nematode resistance. Transferring valuable alleles into elite peanut cultivars is possible through the development of induced allotetraploids. Here, we crossed the synthetic tetraploid BatSten1 ([*A. batizocoi* x *A. stenosperma*]$^{(2n=4x=40)}$) with the cultivated genotype Runner-886. A selected homozygous segregating progeny ($F_{2:3}$) obtained from this cross was used as donor to incorporate RKN resistance from *A. stenosperma* through three cycles of marker-assisted backcrossing. These advanced peanut lines that incorporate RKN resistance will be used for further analysis, selection and advancement and will be valuable germplasm resources for other peanut breeding programs. Additionally, SNP markers linked to the introgressed regions will be useful to facilitate the introgression of RKN resistance into elite peanuts. Ultimately, these will lead to the developing of new high-yielding cultivars with strong and durable resistance to RKN, and possibly to foliar diseases. This work has the potential to positively impact peanut cultivation, which consequently, will contribute to food security in the developing world where peanut plays an important nutritional role, and to sustainably increase production in industrialized countries.

Acknowledgements

I would like to acknowledge the contribution of Dr. Ye (Juliet) Chu, Dr. Larissa Arrais, Stephanie Botton, Kathleen Monfero, Jenny Leverett and Maricel Gonzales for

performing peanut crossings and phenotyping. To all members of Jackson, Bertioli and Ozias-Akins labs for laboratory and greenhouse assistance. To the Glenn and Helen Burton Feeding the Hungry Scholarship Award for the recognition of the contribution and impact of this work.

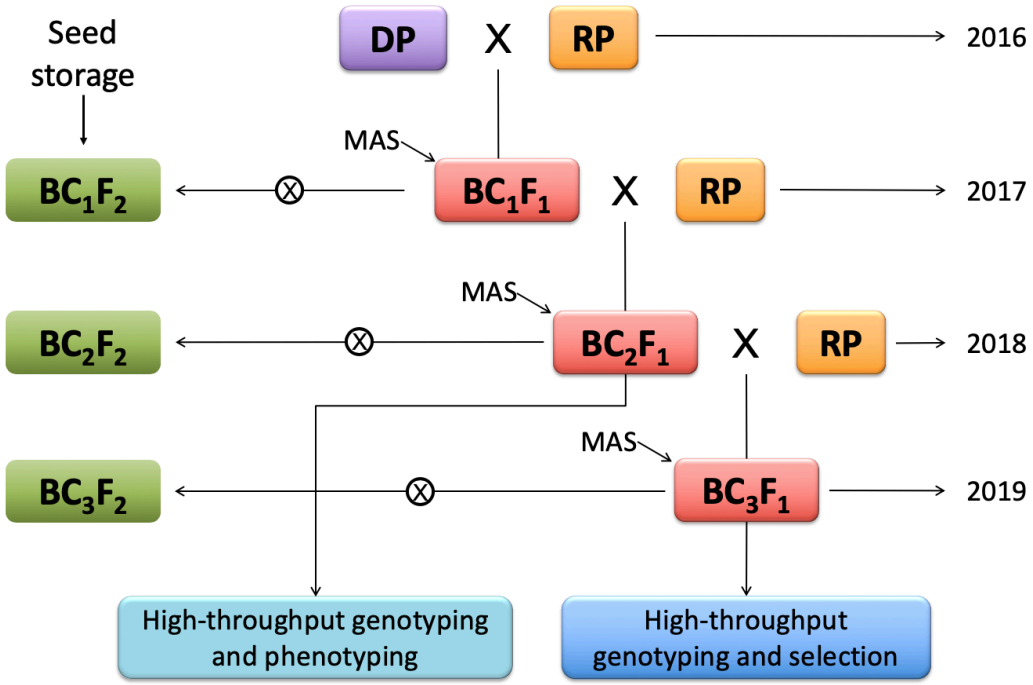

Figure 3.1. Schematic of the marker-assisted backcrossing scheme for incorporation of root-knot nematode resistance from the wild species *A. stenosperma* V10309 into peanut breeding lines from Tifton, GA. Superior F_2-derived F_3 lines (Chapter 2, Ballén-Taborda et al. Submitted) were selected as donor parents (DP, purple box). Recurrent parents (RP, orange boxes) included TifGp-2, 5-646-10 and 13-1014; First, second and third generation of backcrossing cycles were performed in Athens and Tifton in 2016, 2017 and 2018. In each cycle genotyping was performed to identify lines carrying RKN resistance segments (Marker-assisted selection - MAS); BC_nF_1 (BC_1F_1 and BC_2F_1) progeny from each cycle were used as male parents for the next backcrossing cycle (red boxes). BC_nF_2 progeny (BC_1F_2, BC_2F_2 and BC_3F_2) were stored in a cold room (Green boxes). Self-pollination represented by ⊗. BC_2F_1s were genotyped and phenotyped for RKN resistance as a validation step (light blue box). BC_3F_1s were subjected to genome-wide genotyping for selection (dark blue box).

Figure 3.2. Overview of the crossing process in peanut. Closed flowers (buds) ready to emasculate (pink arrow) **(A)**; emasculated flower (pink arrow) marked with a hair clip **(B)**; pollination **(C)**; peg tip emergence (pink arrows) during 5-10 days after pollination **(D)**; peg enlarging and growing down to the soil (pink arrow), marked with a folded wire **(E)**.

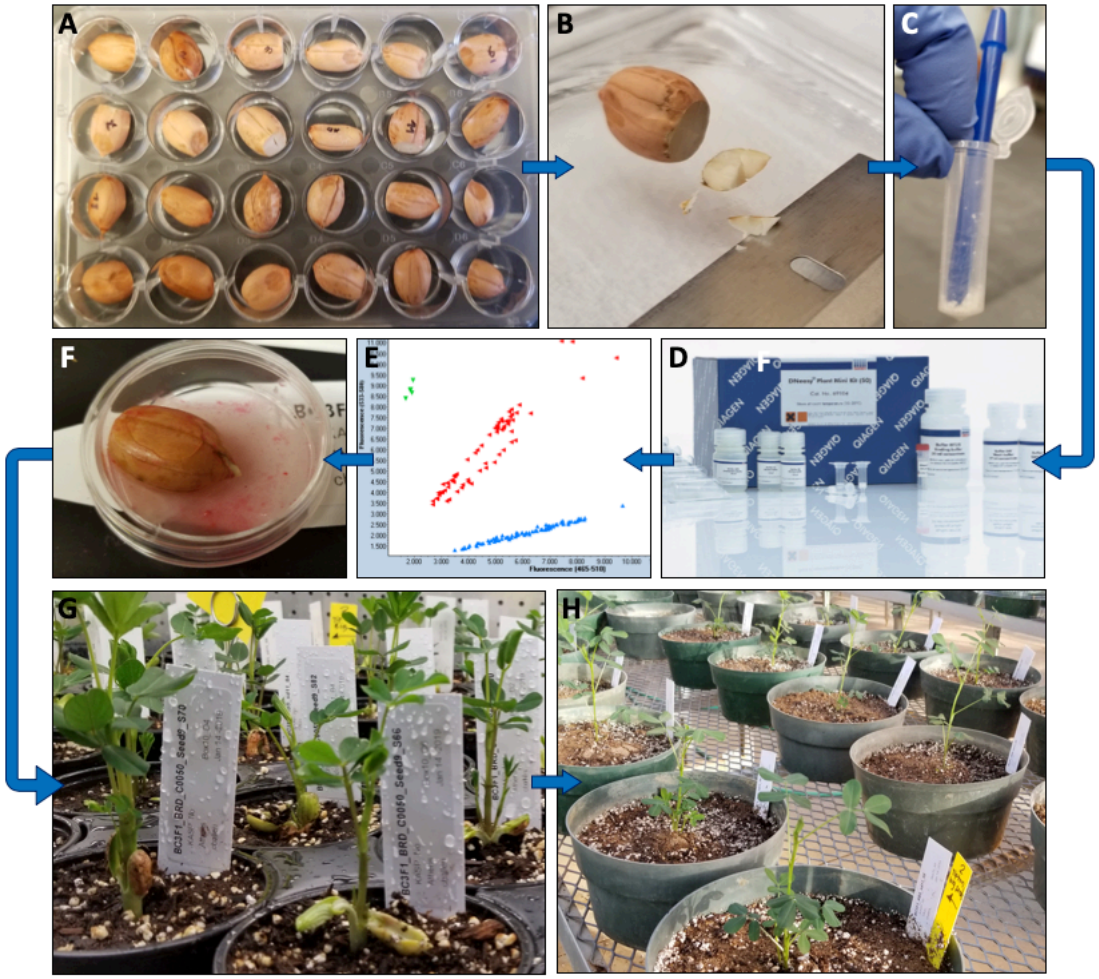

Figure 3.3. Overview of the steps for seed chip for genotyping and germination. Potential backcrossed lines organized in 24 well-plates **(A)**; Seed piece collected from seeds **(B)**; maceration of seed tissue inside 2 ml tubes **(C)**; DNA isolation using the DNeasy *Plant* Mini *Kit (Q*iagen) and quantification **(D)**; KASP genotyping and selection **(E)**; selected seed treated with fungicide to prevent contamination and Ethylene solution (0.5 %) to induce germination (emergent embryonic root) **(F)**; two-week old seedlings growing in growth chamber **(G)**; three-week-old seedlings transferred to the greenhouse **(H)**.

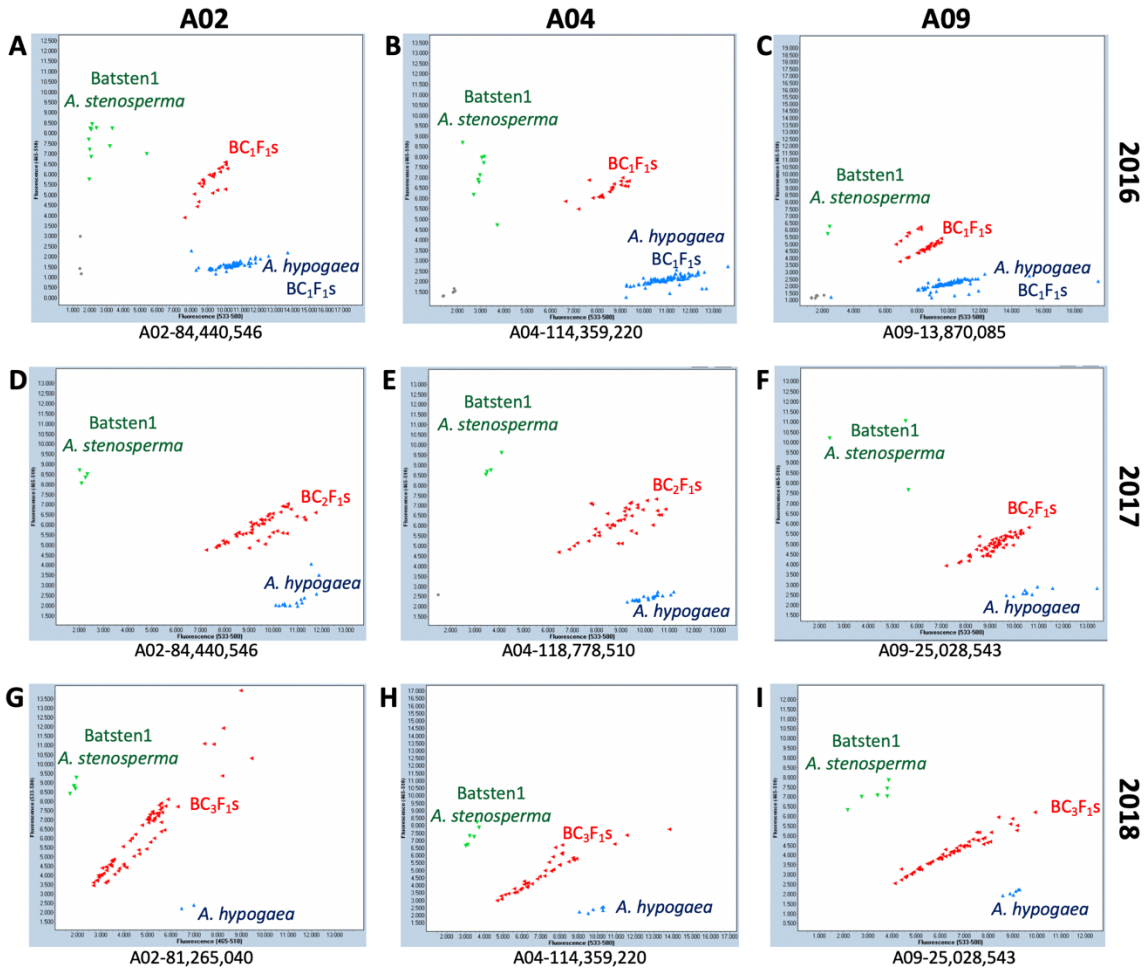

Figure 3.4. Screenshots of genotyping of Kompetitive Allele-Specific PCR assays (KASP) clustering for single-nucleotide polymorphism (SNP) associated with nematode resistance from *A. stenosperma*. Examples of SNPs localized in chromosome A02 **(A,D,G)**, A04 **(B,E,H)** and A09 **(C,F,I)**. KASP experiments run in 2016 for BC_1F_1s **(A-C)**; KASP assays run in 2017 for BC_2F_1s **(D-F)**; and KASP experiments run in 2018 for BC_3F_1s **(G-I)**. Three clusters are present: *A. stenosperma* V10309 -derived alleles (green), blue *A. hypogaea* alleles (blue) and true backcrossed lines (red) for each cycle of backcrossing.

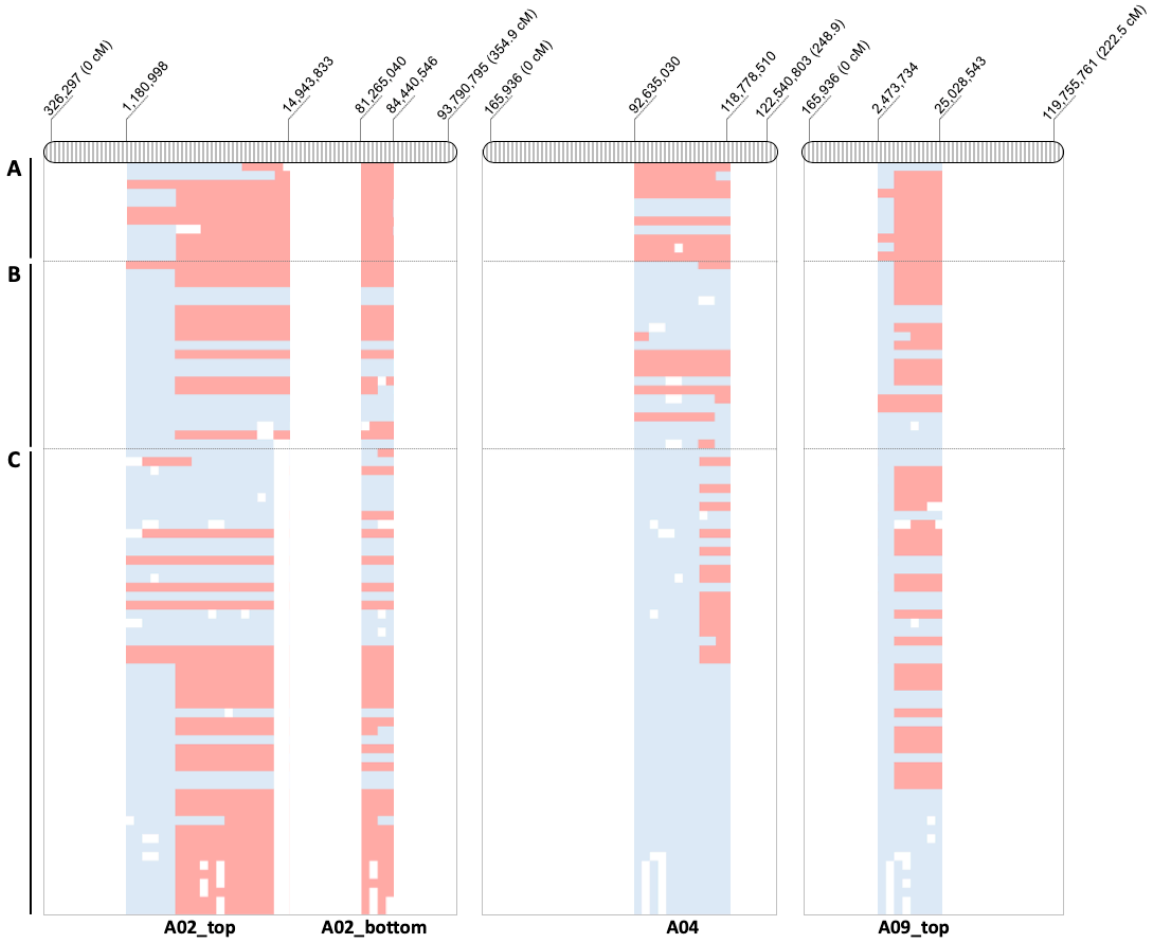

Figure 3.5. Graphical representation of *A. stenosperma*-derived segments correlated with RKN resistance. Horizontal lines represent genotypes, and columns represent positions on chromosomes A02, A09 and A04 (associated with susceptibility), revealed using KASP genotyping for 22 SNP markers (Table 3.S1, gray shaded markers). Backcrossed lines (11 BC_1F_1s **(A)** 21 BC_2F_1s **(B)** 52 BC_3F_1s **(C)**) were tested and selected based on the presence of the *A. stenosperma* derived alleles. KASP Physical positions in base pairs (bp) at top of the figure and genetic distance at start and end of the linkage groups (cM). Introgressions from *A. stenosperma* in heterozygous state are indicated in red; *A. hypogaea* alleles in light blue; white blocks indicate chromosome segments not tested or missing data.

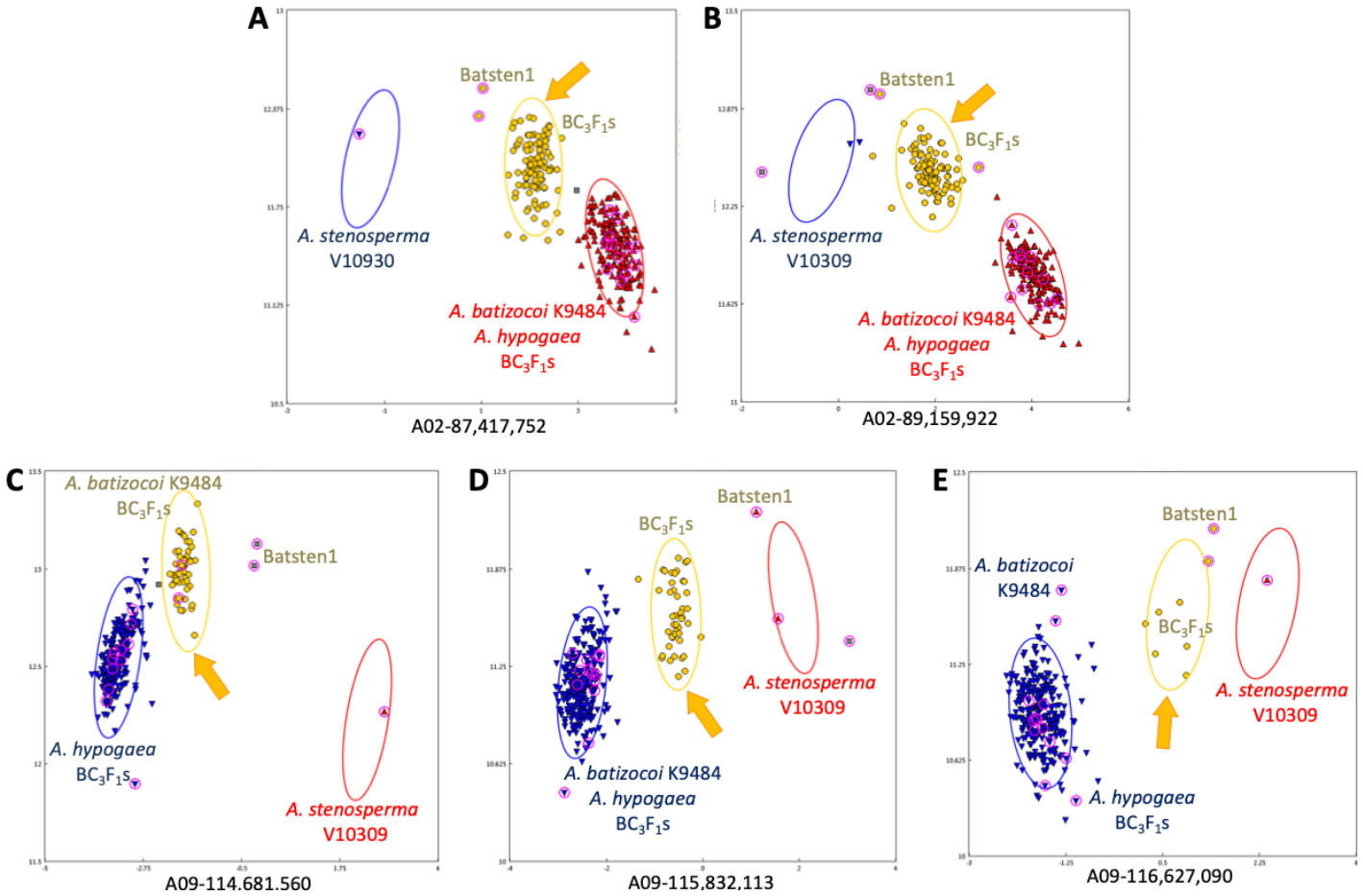

Figure 3.6. Screenshots of genotyping with the second version of the 'Axiom_Arachis' 47K high-density SNP array (Clevenger et al. 2018; Korani et al. 2019). SNPs associated with nematode resistance localized in chromosome A02 **(A,B)** and A09 **(C,D,E)** from *A. stenosperma* visualized in the BC_3F_1s and controls. True backcrossed lines inside yellow clusters (indicated by arrows).

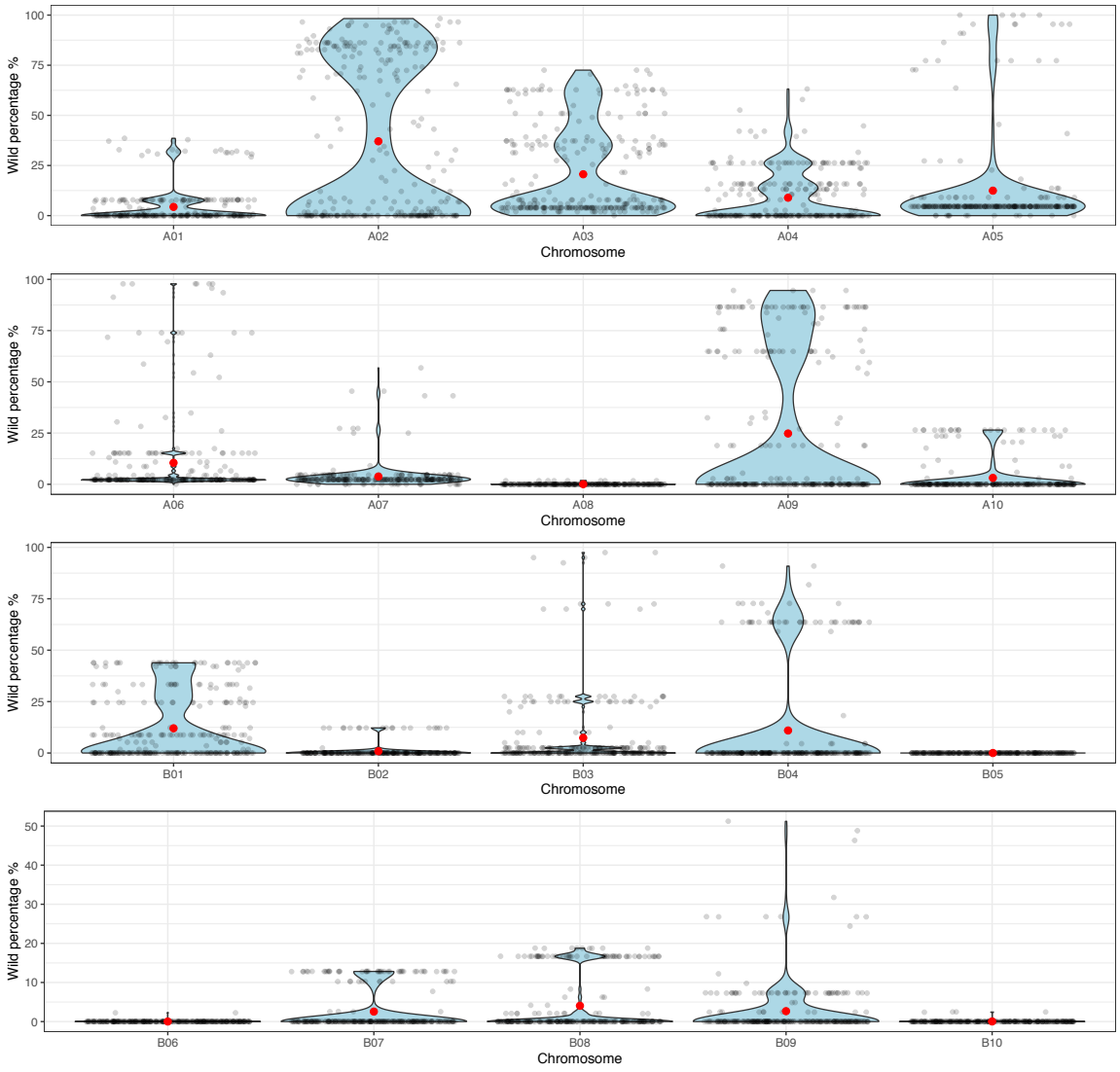

Figure 3.7. Violin plot for proportion of wild genome (%) (y-axis) in each of the 10 A- and 10 B-subgenome chromosomes (x-axis) for the 271 BC_3F_1 lines. Black dots indicate each individual BC_3F_1 and red dot, the mean.

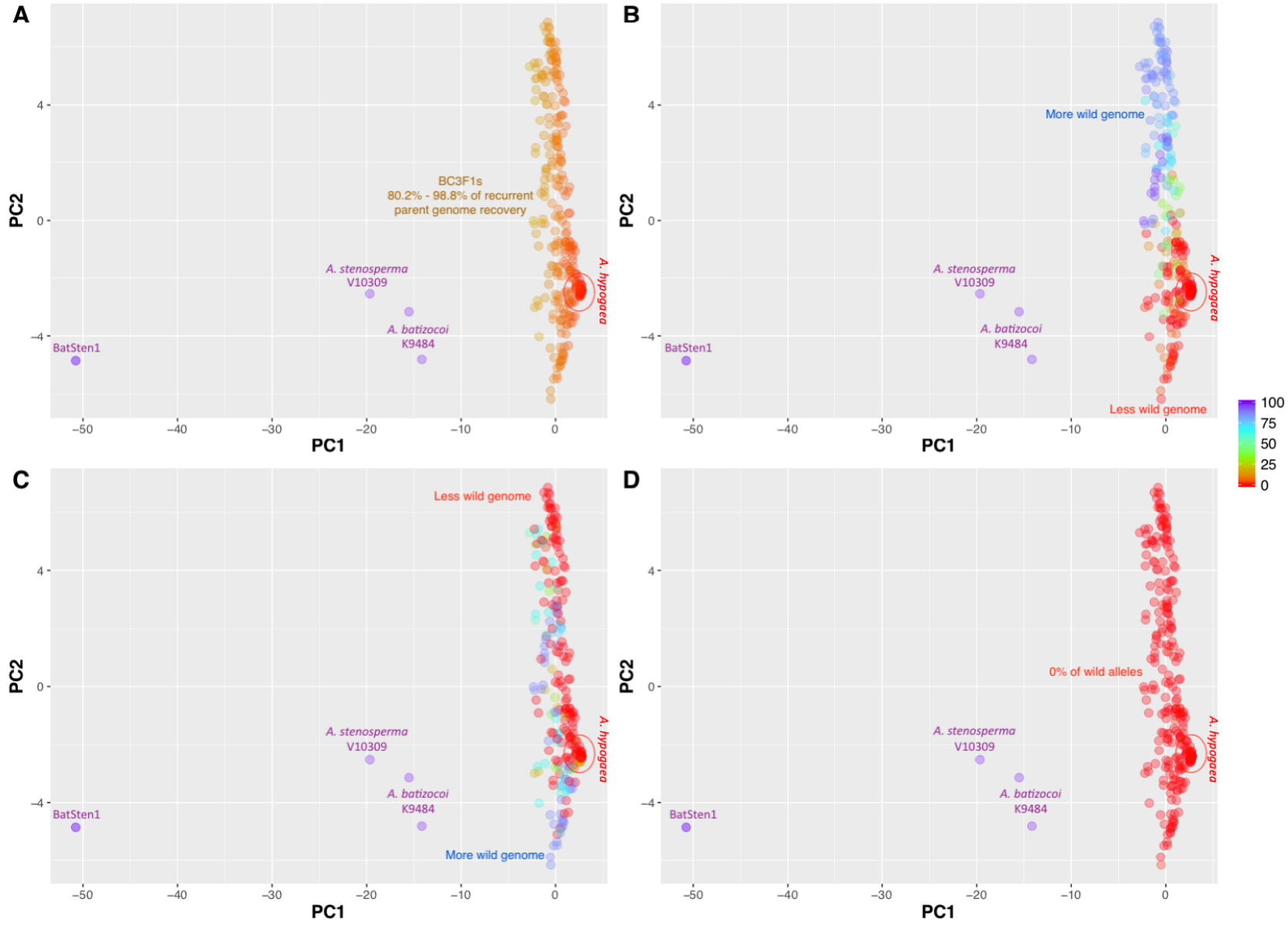
A
B
C
D
PC1
PC2
BC3F1s
80.2% - 98.8% of recurrent
parent genome recovery
More wild genome
Less wild genome
0% of wild alleles
A. stenosperma
V10309
A. batizocoi
K9484
BatSten1
A. hypogaea
100
75
50
25
0

Figure 3.8. Principal components analysis (PCA) for high-throughput genotyping data of BC_3F_1 lines, diploid controls *A. stenosperma*, *A. batizocoi*, the synthetic allotetraploid BatSten1 (purple clusters) and *A. hypogaea* genotypes (red cluster) using the Affymetrix SNP array v.02 (Clevenger et al. 2018; Korani et al. 2019). Proportion of *A. stenosperma* alleles in A-subgenome (**A**), in chr A02 **(B)**, A09 **(C)** and A08 **(D)** are indicated by color for the BC_3F_1s cluster. The legend on the right side indicates the percentage of wild genome. Alleles were coded to maximize the difference between *A. hypogaea* and BatSten1 (see Methods for details).

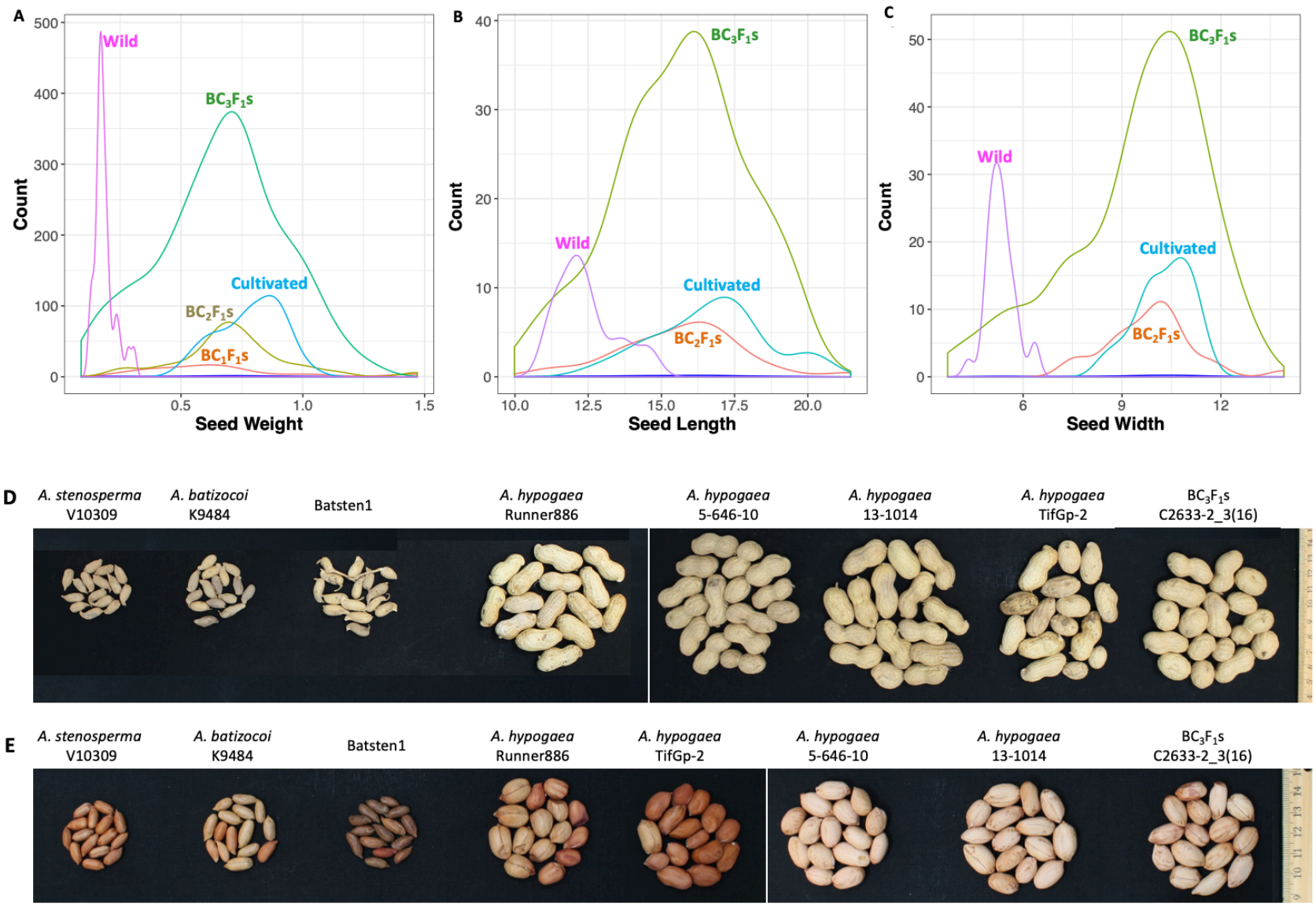
A
Count
Seed Weight
Wild
BC3F1s
Cultivated
BC2F1s
BC1F1s
B
Count
Seed Length
Wild
BC3F1s
Cultivated
BC2F1s
C
Count
Seed Width
Wild
BC3F1s
Cultivated
BC2F1s
D
A. stenosperma V10309
A. batizocoi K9484
Batsten1
A. hypogaea Runner886
A. hypogaea 5-646-10
A. hypogaea 13-1014
A. hypogaea TifGp-2
BC3F1s C2633-2_3(16)
E
A. stenosperma V10309
A. batizocoi K9484
Batsten1
A. hypogaea Runner886
A. hypogaea TifGp-2
A. hypogaea 5-646-10
A. hypogaea 13-1014
BC3F1s C2633-2_3(16)

Figure 3.9. Distribution of seed weight (g) **(A)**, length (mm) **(B)** and width (mm) **(C)** for BC_1F_1s (orange), BC_2F_1s (dark yellow) and BC_3F_1s (green), wild controls (magenta) and cultivated genotypes (blue); count/frequency in y-axis and seed measurements in x-axis **(A-C);** Data for length and width of BC_1F_1 is not available. Examples of pods **(D)** and seeds **(E)** of diploid species (*A. stenosperma* and *A. batizocoi*), synthetic allotetraploid BatSten1, cultivated genotypes (Runner-886, 5-646-10, 13-1014, TifGp-2) and from BC_3F_1 progeny from 5-646-10 x BC_2F_1:C2633-2_3(16) cross combination from 2018 backcross cycle (Table 3.4). Ruler on the side of each image in centimeters (cm). Groups of 15 pods and seeds are presented.

Table 3.1. Phenotypic and genotypic characterization of selected F_2 Lines.

F_2 Line	F_3 seeds	LLS	RKN		Genotype				
		DLA	Galls/g	RF	A02_76,738,828	A02_84,440,546	A04_106,874,754	A09_112,901,114	A09_114,001,128
F_2-7	24	0.02 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	+/+	+/+	+/+	+/-	+/-
F_2-13	50	0.00 ± 0.00	24.60 ± 28.51	0.03 ± 0.03	+/-	-/-	+/-	-/-	-/-
F_2-34	48	0.10 ± 0.24	0.00 ± 0.00	0.00 ± 0.00	+/-	+/-	+/-	+/+	+/+
F_2-73	15	0.02 ± 0.03	160.10 ± 231.08	0.12 ± 0.20	-/-	+/-	+/-	+/+	+/+
V10309	-	0.00 ± 0.00	0.00 ± 0.00	-	+/+	+/+	+/+	+/+	+/+
K9484	-	0.00 ± 0.00	621.09 ± 768.27	-	+/+	+/-	-/-	-/-	+/+
BatSten1	-	0.00 ± 0.00	2.7 ± 6.04	-	+/+	+/+	+/-	+/-	+/+
Runner-886	-	0.12 ± 0.24	5320.67 ± 5686.27	-	-/-	-/-	-/-	-/-	-/-

Notes: LLS - late leaf spot screening; RKN - root-knot nematode screening; DLA - percentage of disease leaf area; Galls/g - total number of galls per gram of root; RF - Reproduction factor. V10309 - *A. stenosperma* V10309; K9484 - *A. batizocoi* K9484; Runner-886 - *A. hypogaea* cv. Runner-886. Homozygous for *A. hypogaea* alleles in light blue "-/-"; homozygous for resistance segments in light green "+/+"; and heterozygous in light red "-/+"; Missing data indicated by "-".

Table 3.2. Cross combinations for the first cycle of backcrossing completed in 2016 and results. Athens and Tifton crossing experiments are detailed.

Year	Location	RP	$F_{2:3}$	Reps	No. of potential BC_1F_1s	Selected
2016	Athens	TifGP-2	F_2:7_Athens_F_3:1	2	165	0
2016	Athens	TifGP-2	F_2:13_Athens_F_3:1	2	54	0
2016	Athens	TifGP-2	F_2:34_Athens_F_3:6	2	43	1
2016	Athens	TifGP-2	F_2:73_Athens_F_3:1	2	104	0
2016	Athens	TifGP-2	F_2:167_Athens_F_3:1	2	0	0
2016	Athens	5-646-10	F_2:7_Athens_F_3:1	2	88	1
2016	Athens	5-646-10	F_2:13_Athens_F_3:1	2	34	0
2016	Athens	5-646-10	F_2:34_Athens_F_3:6	2	90	0
2016	Athens	5-646-10	F_2:73_Athens_F_3:1	2	166	0
2016	Athens	5-646-10	F_2:167_Athens_F_3:1	2	0	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:13 F_3:1	2	18	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:13 F_3:1	2	17	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:13 F_3:2	1	7	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:13 F_3:2	1	10	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:13 F_3:3	2	12	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:13 F_3:3	2	4	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:13 F_3:4	1	5	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:13 F_3:4	2	9	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:13 F_3:5	1	1	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:13 F_3:5	1	2	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:34 F_3:6	1	9	3
2016	Tifton	5-646-10	Tifton_RBS_F_2:34 F_3:6	1	5	3
2016	Tifton	TifGP-2	Tifton_RBS_F_2:34 F_3:7	1	9	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:34 F_3:7	1	8	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:7 F_3:2	2	39	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:7 F_3:2	1	18	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:7 F_3:5	1	11	3
2016	Tifton	5-646-10	Tifton_RBS_F_2:7 F_3:5	1	8	6
2016	Tifton	5-646-10	Tifton_RBS_F_2:73 F_3:10	2	9	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:73 F_3:6	1	10	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:73 F_3:6	1	3	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:73 F_3:7	2	9	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:73 F_3:7	2	13	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:73 F_3:8	1	8	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:73 F_3:8	1	10	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:73 F_3:9	1	1	0
2016	Tifton	5-646-10	Tifton_RBS_F_2:73 F_3:9	1	6	0
2016	Tifton	TifGP-2	Tifton_RBS_F_2:73 F_3:10	2	3	0
				Total = 58	Total = 1008	**Total = 17**

Notes: RP - female recurrent parent; 19 $F_{2:3}$ - male parents; Reps - number of reps per cross combination; No. of potential BC_1F_1s - number of potential BC_1F_1 seeds harvested and seed chip genotyped with KASP using 22 SNPs (Table 3.S1, gray shaded markers); Selected - Selected BC_1F_1 lines base on KASP genotyping and germinated for next cycle of crossing.

Table 3.3. Cross combinations for the second cycle of backcrossing completed in 2017 and results. Athens and Tifton crossing experiments are detailed.

Year	Location	RP	BC_1F_1	RKN introgression	Reps	No. of potential BC_2F_1s	Selected
2017	Athens	5-646-10	BC_1F_1:49	A02_top-sml; **A02_bottom**; A04	2	7	0
2017	Athens	5-646-10	BC_1F_1:C2633-2_3	A02_top-L; **A02_bottom**; A04; A09_top	2	9	1
2017	Athens	TifGp-2	BC_1F_1:C2641-2_10	A02_top; **A02_bottom**; A04; A09_top	1	0	0
2017	Athens	TifGp-2	BC_1F_1:C2641-2_11	A02_top; **A02_bottom**; A09_top	1	1	0
2017	Athens	TifGp-2	BC_1F_1:C2632-2_1	A02_top-L; **A02_bottom**; A09_top	1	2	0
2017	Athens	TifGp-2	BC_1F_1:C2632-2_2	A02_top-L; **A02_bottom**; A04; A09_top	1	4	0
2017	Athens	TifGp-2	BC_1F_1:03	**A02_bottom**; A04; A09_top	1	1	0
2017	Tifton	5-646-10	C2642-2_3	A02_top; **A02_bottom**; A09_top	1	8	5
2017	Tifton	13-1014	C2642-2_3	A02_top; **A02_bottom**; A09_top	1	3	2
2017	Tifton	13-2113	C2642-2_4	A02_top; **A02_bottom**; A04; A09_top	1	2	1
2017	Tifton	5-646-10	C2642-2_6	A02_top; **A02_bottom**; A04; A09_top	1	11	4
2017	Tifton	13-1014	C2642-2_6	A02_top; **A02_bottom**; A04; A09_top	1	3	2
2017	Tifton	5-646-10	C2642-2_7	A02_top; **A02_bottom**; A04; A09_top	1	4	2
2017	Tifton	13-2113	C2642-2_7	A02_top; **A02_bottom**; A04; A09_top	1	6	4
						Total = 61	**Total = 21**

Notes: RP - female recurrent parent; 11 BC_1F_1 - male parents; RKN introgression - RKN resistance introgressed present in the BC_2F_1 male parents base on KASP genotyping. Segments in bold highlighting the most important segments for nematode resistance according to Ballén-Taborda et al. Submitted; Reps - number of reps per cross combination; No. of potential BC_2F_1s - number of potential BC_2F_1 seeds harvested and seed chip genotyped with KASP using 22 SNPs (Table 3.S1, gray shaded markers); Selected - Selected BC_2F_1 lines base on KASP genotyping and germinated for next cycle of crossing.

Table 3.4. Cross combinations for the third cycle of backcrossing completed in 2018 and results. Athens and Tifton crossing experiments are detailed.

Year	Location	RP	BC_2F_1	RKN introgression	Reps	No. of potential BC_3F_1s	KASP Genotyping	Affymetrix Genotyping
2018	Athens	5-646-10	BC_2F_1:C2633-2_3(16)	A02 top-L; **A02 Bottom**; A04_bottom; A09 top	4	71	26 (24)	67
2018	Athens	5-646-10	BC_2F_1_BRD_C0049_Seed1	A02 top; **A02 Bottom**; A09 top	2	26	12 (8)	20
2018	Athens	5-646-10	BC_2F_1_BRD_C0049_Seed2	A02 top; **A02 Bottom**; A09 top	2	8	5 (4)	6
2018	Athens	5-646-10	BC_2F_1_BRD_C0049_Seed7	A09 top	2	18	9 (2)	5
2018	Athens	5-646-10	BC_2F_1_BRD_C0049_Seed8	A02 top; **A02 bottom**	3	20	9 (3)	11
2018	Athens	13-1014	BC_2F_1_BRD_C0050_Seed9	A02 top; **A02 bottom**	4	85	17 (11)	75
2018	Athens	5-646-10	BC_2F_1_BRD_C0055_Seed15	A02 top; **A02 Bottom**; A04	1	5	3 (0)	0
2018	Athens	5-646-10	BC_2F_1_BRD_C0055_Seed17	A04; A09 top	2	50	0	0
2018	Athens	5-646-10	BC_2F_1_BRD_C0057_Seed28	A04_bottom; A09_top	1	0	0	0
2018	Athens	5-646-10	BC_2F_1_BRD_C0058_Seed33	A04	1	0	0	0
2018	Tifton	13-1014	RBS_ BC_2F_1_sd4	A09_top	1	5	0	5
2018	Tifton	5-646-10	RBS_ BC_2F_1_sd11	A02_top; **A02 bottom**; A09_top	1	15	0	15
2018	Tifton	5-646-10	RBS_ BC_2F_1_sd13	A02_top; **A02 bottom**; A09_top	1	16	0	15
2018	Tifton	5-646-10	RBS_ BC_2F_1_sd14	A09_top	1	13	0	9
2018	Tifton	13-1014	RBS_ BC_2F_1_sd20	A04; A09_top	1	7	0	1
2018	Tifton	5-646-10	RBS_ BC_2F_1_sd26	A02_top; **A02 bottom**; A09_top	1	26	0	21
2018	Tifton	13-1014	RBS_ BC_2F_1_sd27	A02_top; **A02 bottom**; A04	1	7	0	4
2018	Tifton	5-646-10	RBS_ BC_2F_1_sd30	A09_top	1	4	0	2
2018	Tifton	5-646-10	RBS_ BC_2F_1_sd34	**A02 bottom**	1	9	0	9
2018	Tifton	13-1014	RBS_ BC_2F_1_sd35	A02_top; **A02_bottom**	1	1	0	1
2018	Tifton	5-646-10	RBS_ BC_2F_1_sd36	None	1	11	0	5
						Total = 397	Total = 81 (52)	**Total = 271**

Notes: RP - female recurrent parent; 21 BC_2F_1 - male parents; RKN introgression - RKN resistance introgressed present in the BC_2F_1 male parents base on KASP genotyping. Segments in bold highlighting the most important segments for nematode resistance (Ballén-Taborda et al. Submitted); Reps - number of reps per cross combination; No. of potential BC_3F_1s - number of potential BC_3F_1 seeds harvested; KASP genotyping - BC_3F_1 seed chip genotyped with KASP using 22 SNPs (Table 3.S1, gray shaded markers), first number represent total number of genotyped seeds and in parenthesis the total of materials harboring resistance alleles; Affymetrix genotyping – number of BC_3F_1 leaf tissue genotyped with the second version of the 'Axiom_Arachis' 47K high-density SNP array (Clevenger et al. 2018; Korani et al. 2019).

Table 3.5. Average and standard deviation for seed weight, length and width measured in diploid wild species, induced allotetraploid BatSten1 and cultivated controls. Minimum and maximum values for weight, length and width dimensions for controls and BC_nF_1 developed seeds (BC_1F_1, BC_2F_1 and BC_3F_1).

Control genotype	Type	Weight (g)	Length (mm)	Width (mm)	Weight min-max (g)	Length min-max (mm)	Width min-max (mm)
A. stenosperma V10309	Diploid wild species	0.17 ± 0.01 (b)	11.86 ± 0.64 (d)	5.21 ± 0.21 (c)	0.16-0.19	11.10-13.39	4.93-5.57
A. batizocoi K9484	Diploid wild species	0.22 ± 0.05 (c)	12.95 ± 1.21 (e)	5.7 ± 0.43 (d)	0.14-0.31	11.26-14.70	5.01-6.37
BatSten1	Induced allotetraploid	0.16 ± 0.03 (b)	12.52 ± 0.92 (e)	5.05 ± 0.33 (c)	0.13-0.20	11.18-14.45	4.34-5.41
A. hypogaea Runner-886	Cultivated control	0.74 ± 0.17 (a)	15.08 ± 1.7 (c)	10.23 ± 1.01 (ab)	0.51-0.94	12.61-17.77	8.4-11.26
A. hypogaea 5-646-10	Cultivated (Recurrent parent)	0.81 ± 0.1 (a)	17.21 ± 1.87 (ab)	10.43 ± 0.51 (ab)	0.67-1.03	14.30-20.22	9.71-11.2
A. hypogaea 13-1014	Cultivated (Recurrent parent)	0.84 ± 0.1 (a)	18.04 ± 1.62 (a)	10.65 ± 0.89 (a)	0.58-0.97	15.30-20.19	8.50-11.54
A. hypogaea TifGp-2	Cultivated (Recurrent parent)	0.75 ± 0.12 (a)	16.49 ± 1.22 (bc)	9.81 ± 0.63 (b)	0.59-0.91	14.23-17.66	9.06-10.89
BC_1F_1s	Backcrossed line	-	-	-	0.29-1.46	-	-
BC_2F_1s	Backcrossed line	-	-	-	0.25-1.47	11.01-21.46	7.48-13.91
BC_3F_1s	Backcrossed line	-	-	-	0.09-1.31	9.98-20.83	3.71-13.42

Notes: Weight (g), length (mm) and width (mm) columns with the same letter do not differ significantly ($p<0.05$). Min-max - minimum and maximum values for weight, length and width.

References

Arbelaez JD, Moreno LT, Singh N, Tung C-W, Maron LG, Ospina Y, Martinez CP, Grenier C, Lorieux M, McCouch SR (2015) Development and GBS-genotyping of introgression lines (ILs) using two wild species of rice, O. meridionalis and O. rufipogon, in a common recurrent parent, O. sativa cv. Curinga. Molecular Breeding 35:81

Babu R, Nair SK, Prasanna BM, Gupta HS (2004) Integrating marker-assisted selection in crop breeding - Prospects and challenges. Current Science 87:607-619

Ballén-Taborda C, Chu C, Jackson S, Ozias-Akins P, Timper P, Holbrook C, Bertioli D, Leal-Bertioli S (Submitted) A new source of root-knot nematode resistance from Arachis stenosperma incorporated into allotetraploid peanut (Arachis hypogaea). Sci Rep

Barbary A, Palloix A, Fazari A, Marteu N, Castagnone-Sereno P, Djian-Caporalino C (2014) The plant genetic background affects the efficiency of the pepper major nematode resistance genes Me1 and Me3. Theor Appl Genet 127:499-507

Bernardo R (2008) Molecular markers and selection for complex traits in plants: learning from the last 20 Years. Crop Sci 48:1649-1664

Bernardo R (2016) Bandwagons I, too, have known. Theor Appl Genet 129:2323-2332

Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD, Cannon EK, Liu X, Gao D, Clevenger J, Dash S, Ren L, Moretzsohn MC, Shirasawa K, Huang W, Vidigal B, Abernathy B, Chu Y, Niederhuth CE, Umale P, Araujo AC, Kozik A, Kim KD, Burow MD, Varshney RK, Wang X, Zhang X, Barkley N, Guimaraes PM, Isobe S, Guo B, Liao B, Stalker HT, Schmitz RJ, Scheffler BE, Leal-Bertioli SC, Xun X, Jackson SA, Michelmore R, Ozias-Akins P (2016) The genome sequences of Arachis duranensis and Arachis ipaensis, the diploid ancestors of cultivated peanut. Nat Genet 48:438-446

Bertioli DJ, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, Leal-Bertioli SCM, Ren L, Farmer AD, Pandey MK, Samoluk SS, 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 KD, Korani W, Lanciano S, Lui CG, Mirouze M, Moretzsohn MC, Pham M, Shin JH, Shirasawa K, Sinharoy S, Sreedasyam A, Weeks NT, Zhang X, Zheng Z, Sun Z, Froenicke L, Aiden EL, Michelmore R, Varshney RK, Holbrook CC, Cannon EKS, Scheffler BE, Grimwood J, Ozias-Akins P, Cannon SB, Jackson SA, Schmutz J (2019) The genome sequence of peanut (Arachis hypogaea), a segmental allotetraploid. Nat Genet 51:877-884

Bertioli DJ, Seijo G, Freitas FO, Valls JFM, Leal-Bertioli SCM, Moretzsohn MC (2011) An overview of peanut and its wild relatives. Plant Gene Resour 9:134-149

Bhanu A, Y G, Mn S (2017) Advanced Backcross QTL Method: A Brief Overview. Trends in Biosciences 10:20-25

Branch WD, Brenneman TB (2015) Registration of 'Georgia-14N' peanut. J Plant Regist 9:159-161

Brun H, Chevre AM, Fitt BD, Powers S, Besnard AL, Ermel M, Huteau V, Marquer B, Eber F, Renard M, Andrivon D (2010) Quantitative resistance increases the durability of qualitative resistance to Leptosphaeria maculans in Brassica napus. The New phytologist 185:285-299

Burow MD, Simpson CE, Starr JL, Paterson AH (2001) Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (Arachis hypogaea L.). broadening the gene pool of a monophyletic polyploid species. Genetics 159:823-837

Chetelat RT, Meglic V, Cisneros P (2000) A Genetic Map of Tomato Based on BC1 Lycopersicon esculentum × Solanum lycopersicoides Reveals Overall Synteny but Suppressed Recombination Between These Homeologous Genomes. Genetics 154:857-867

Chu Y, Gill R, Clevenger J, Timper P, Corley Holbrook C, Ozias-Akins P (2016a) Identification of rare recombinants leads to tightly linked markers for nematode resistance in peanut. Peanut Sci 43:88-93

Chu Y, Wu CL, Holbrook CC, Ozias-Akins P (2016b) Conditions that Impact Artificial Hybridization of Arachis hypogaea L. Peanut Sci 43:106-115

Chu Y, Wu CL, Holbrook CC, Tillman BL, Person G, Ozias-Akins P (2011) Marker-Assisted Selection to Pyramid Nematode Resistance and the High Oleic Trait in Peanut. The Plant Genome 4:110-117

Clevenger JP, Korani W, Ozias-Akins P, Jackson S (2018) Haplotype-Based Genotyping in Polyploids. Frontiers in plant science 9:564

Collard B, Jahufer Z, Brouwer JB, Pang E (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142:169-196

Collard BC, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans R Soc Lond B Biol Sci 363:557-572

de Paula AF, Dinato NB, Vigna BBZ, Favero AP (2017) Recombinants from the crosses between amphidiploid and cultivated peanut (Arachis hypogaea) for pest-resistance breeding programs. PLoS One 12:e0175940

Dickson DW, De Waele D (2005) Nematode parasites of peanut. In: Sikora RA, Bridge J (eds) Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI Publishing, Wallingford, UK, pp 393-436

Dwivedi SL, Bertioli DJ, Crouch JH, Valls JF, Upadhyaya HD, Fávero A, Moretzsohn M, Paterson AH (2007) Peanut. In: Kole C (ed) Oilseeds. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 115-151

Fonceka D, Tossim H-A, Rivallan R, Vignes H, Lacut E, de Bellis F, Faye I, Ndoye O, Leal-Bertioli SCM, Valls JFM, Bertioli DJ, Glaszmann J-C, Courtois B, Rami J-F (2012a) Construction of Chromosome Segment Substitution Lines in Peanut (Arachis hypogaea L.) Using a Wild Synthetic and QTL Mapping for Plant Morphology. PLoS One 7:e48642

Fonceka D, Tossim HA, Rivallan R, Vignes H, Faye I, Ndoye O, Moretzsohn MC, Bertioli DJ, Glaszmann JC, Courtois B, Rami JF (2012b) Fostered and left behind alleles in peanut: interspecific QTL mapping reveals footprints of domestication and useful natural variation for breeding. BMC Plant Biol 12:26

Fournet S, C. Kerlan M, Renault L, P. Dantec J, Rouaux C, Montarry J (2013) Selection of nematodes by resistant plants has implications for local adaptation and cross-virulence. Plant Pathology 62:184-193

Frisch M, Bohn M, Melchinger AA (1999a) Minimum Sample Size and Optimal Positioning of Flanking Markers in Marker-Assisted Backcrossing for Transfer of a Target Gene. Crop Sci 39:967-975

Frisch M, Bohn M, Melchinger AE (1999b) Comparison of Selection Strategies for Marker-Assisted Backcrossing of a Gene. Crop Sci 39:1295-1301

Frisch M, Bohn M, Melchinger AE (2000) PLABSIM: software for simulation of marker-assisted backcrossing. J Hered 91:86-87

Gallois JL, Moury B, German-Retana S (2018) Role of the Genetic Background in Resistance to Plant Viruses. Int J Mol Sci 19

Gao S, Martinez C, Skinner JD, Krivanek FA, Crouch HJ, Xu Y (2008) Development of a seed DNA-based genotyping system for marker-assisted selection in maize. Molecular Breeding 22:477-494

Garcia GM, Stalker HT, Kochert G (1995) Introgression analysis of an interspecific hybrid population in peanuts (Arachis hypogaea L.) using RFLP and RAPD markers. Genome 38:166-176

Glover NM, Redestig H, Dessimoz C (2016) Homoeologs: What Are They and How Do We Infer Them? Trends Plant Sci 21:609-621

Gramazio P, Prohens J, Plazas M, Mangino G, Herraiz FJ, Vilanova S (2017) Development and Genetic Characterization of Advanced Backcross Materials and An Introgression Line Population of Solanum incanum in a S. melongena Background. Frontiers in plant science 8

Guimaraes P, Brasileiro ACM, Proite K, Araujo A, Leal-Bertioli S, Pic-Taylor A, da Silva FR, Morgante C, Ribeiro S, Bertioli D (2010) A study of gene expression in the nematode resistant wild peanut relative, Arachis stenosperma, in response to challenge with Meloidogyne arenaria. Tropical Plant Biology 3:183-192

Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156:1-13

Halward T, Stalker T, LaRue E, Kochert G (1992) Use of single-primer DNA amplifications in genetic studies of peanut (Arachis hypogaea L.). Plant Mol Biol 18:315-325

Hasan MM, Rafii MY, Ismail MR, Mahmood M, Rahim HA, Alam MA, Ashkani S, Malek MA, Latif MA (2015) Marker-assisted backcrossing: a useful method for rice improvement. Biotechnol Biotechnol Equip 29:237-254

Holbrook C, Noe PJ (1990) Resistance to Meloidogyne arenaria in Arachis spp. and the implications on development of resistant peanut cultivars. Peanut Sci 17:35-38

Holbrook C, Ozias-Akins P, Chu Y, K. Culbreath A, Kvien C, Brenneman T (2017) Registration of ‘TifNV-high O/L’ peanut. J Plant Regist 11:228-230

Holbrook C, Stalker H (2003) Peanut breeding and genetic resources. Plant Breed Rev 22:297-356

Holbrook C, Timper P, Culbreath A, Kvien C (2008) Registration of 'Tifguard' peanut. J Plant Regist 2:391-410

Hospital F (2003) Marker-assisted breeding. In: HJ N (ed) Plant molecular breeding. Blackwell Publishing and CRC Press, Oxford, Boca Raton, pp 30–59

Hospital F (2005) Selection in backcross programmes. Philosophical transactions of the Royal Society of London Series B, Biological sciences 360:1503-1511

Hospital F, Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. Genetics 147:1469-1485

Huang B, Qi F, Sun Z, Miao L, Zhang Z, Liu H, Fang Y, Dong W, Tang F, Zheng Z, Zhang X (2019) Marker-assisted backcrossing to improve seed oleic acid content in four elite and popular peanut (Arachis hypogaea L.) cultivars with high oil content. Breeding Science 69:234-243

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 7:396-423

Jacquet M, Bongiovanni M, Martinez M, Verschave P, Wajnberg E, Castagnone-Sereno P (2005) Variation in resistance to the root-knot nematode Meloidogyne incognita in tomato genotypes bearing the Mi gene. Plant path 54:93-99

Jiang G-L (2013a) Molecular Markers and Marker-Assisted Breeding in Plants. In: Andersen S (ed) Plant Breeding from Laboratories to Fields. InTech, Croatia, pp 45–83

Jiang G-L (2013b) Plant Marker-Assisted Breeding and Conventional Breeding: Challenges and Perspectives. Adv Crop Sci Tech

Jiang G-L (2015) Molecular Marker-Assisted Breeding: A Plant Breeder's Review. In: al. JMA-Ke (ed) Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools. Springer International Publishing Switzerland, Switzerland, pp 431-472

Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, Kikuchi T, Manzanilla-Lopez R, Palomares-Rius JE, Wesemael WM, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14:946-961

Kochert G, Stalker H, Gimenes M, Galgaro L, C. RL, Moore K (1996) RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, Arachis hypogaea (Leguminosae). American Journal of Botany 83:1281-1291

Kolekar RM, Sukruth M, Shirasawa K, Nadaf HL, Motagi BN, Lingaraju S, Patil PV, Bhat RS (2017) Marker-assisted backcrossing to develop foliar disease-resistant genotypes in TMV 2 variety of peanut (Arachis hypogaea L.). Plant Breeding 136:948-953

Korani W, Clevenger JP, Chu Y, Ozias-Akins P (2019) Machine Learning as an Effective Method for Identifying True Single Nucleotide Polymorphisms in Polyploid Plants. Plant Genome 12

Kruskal W, Wallis W (1952) Use of ranks in one-criterion variance analysis. J Amer Statist Assn 47:583-621

Kumari V, Gowda MVC, Tasiwal V, Pandey MK, Bhat RS, Mallikarjuna N, Upadhyaya HD, Varshney RK (2014) Diversification of primary gene pool through introgression of resistance to foliar diseases from synthetic amphidiploids to cultivated groundnut (Arachis hypogaea L.). Crop J 2:110-119

Leal-Bertioli SC, Moretzsohn MC, Santos SP, Brasileiro AC, Guimaraes PM, Bertioli DJ, Araujo AC (2017) Phenotypic effects of allotetraploidization of wild Arachis and their implications for peanut domestication. Am J Bot 104:379-388

Leal-Bertioli SC, Santos SP, Dantas KM, Inglis PW, Nielen S, Araujo AC, Silva JP, Cavalcante U, Guimaraes PM, Brasileiro AC, Carrasquilla-Garcia N, Penmetsa RV, Cook D, Moretzsohn MC, Bertioli DJ (2015) 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. Ann Bot 115:237-249

Leal-Bertioli SCM, Bertioli DJ, Guimarães PM, Pereira TD, Galhardo I, Silva JP, Brasileiro ACM, Oliveira RS, Silva PÍT, Vadez V, Araujo ACG (2012) The effect of tetraploidization of wild Arachis on leaf morphology and other drought-related traits. Environ Exper Bot 84:17-24

Leal-Bertioli SCM, Moretzsohn MC, Roberts PA, Ballén-Taborda C, Borba TCO, Valdisser PA, Vianello RP, Araújo ACG, Guimarães PM, Bertioli DJ (2016) Genetic mapping of resistance to Meloidogyne arenaria in Arachis stenosperma: A new source of nematode resistance for peanut. G3: Genes, Genomes, Genetics 6:377-390

Lecomte L, Duffe P, Buret M, Servin B, Hospital F, Causse M (2004) Marker-assisted introgression of five QTLs controlling fruit quality traits into three tomato lines revealed interactions between QTLs and genetic backgrounds. Theor Appl Genet 109:658-668

Liao CY, Ph W, Hu B, Yi K (2001) Effects of genetic background and environment on QTLs and epistasis for rice (Oryza sativa L.) panicle number. Theor Appl Genet 103:104-111

Minton NA, Baujard P (1990) Nematode parasites of peanut. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, UK, pp 285-320

Moretzsohn MC, Gouvea EG, Inglis PW, Leal-Bertioli SC, Valls JF, Bertioli DJ (2013) A study of the relationships of cultivated peanut (Arachis hypogaea) and its most

closely related wild species using intron sequences and microsatellite markers. Ann Bot 111:113-126

Moretzsohn MdC, Hopkins MS, Mitchell SE, Kresovich S, Valls JFM, Ferreira ME (2004) Genetic diversity of peanut (Arachis hypogaea L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biol 4:11-11

Nagy ED, Chu Y, Guo Y, Khanal S, Tang S, Li Y, Dong WB, Timper P, Taylor CG, Ozias-Akins P, Corley H. C, Beilinson V, Nielsen NC, H. TS, Knapp SJ (2010) Recombination is suppressed in an alien introgression in peanut harboring Rma, a dominant root-knot nematode resistance gene. Molecular Breeding 26:357-370

Nelson SC, Simpson CE, Starr JL (1989) Resistance to Meloidogyne arenaria in Arachis spp. germplasm. J Nematol 21:654-660

Nigam SN, Vasudeva MJ, Gibbons RW (1990) Artificial Hybridization in Groundnut. Information Bulletin no 29 Patancheru, AP 502 324, India: International Crops Research Institute for the Semi-Arid Tropics 26:159-162

Palloix A, Ayme V, Moury B (2009) Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. The New phytologist 183:190-199

Pandey MK, Monyo E, Ozias-Akins P, Liang X, Guimarães P, Nigam SN, Upadhyaya HD, Janila P, Zhang X, Guo B, Cook DR, Bertioli DJ, Michelmore R, Varshney RK (2012) Advances in Arachis genomics for peanut improvement. Biotechnol Adv 30:639-651

Pandey MK, Roorkiwal M, Singh VK, Ramalingam A, Kudapa H, Thudi M, Chitikineni A, Rathore A, Varshney RK (2016) Emerging Genomic Tools for Legume Breeding: Current Status and Future Prospects. Frontiers in plant science 7:455-455

Proite K, Carneiro R, Falcão R, Gomes A, Leal-Bertioli S, Guimarães P, Bertioli D (2008) Post-infection development and histopathology of Meloidogyne arenaria race 1 on Arachis spp. Plant Pathology 57:974-980

Proite K, Leal-Bertioli SC, Bertioli DJ, Moretzsohn MC, da Silva FR, Martins NF, Guimaraes PM (2007) ESTs from a wild Arachis species for gene discovery and marker development. BMC Plant Biol 7

Quenouille J, Montarry J, Palloix A, Moury B (2012) Farther, slower, stronger: how the plant genetic background protects a major resistance gene from breakdown

Quenouille J, Paulhiac E, Moury B, Palloix A (2014) Quantitative trait loci from the host genetic background modulate the durability of a resistance gene: a rational basis for sustainable resistance breeding in plants. Heredity 112:579-587

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. Theor Appl Genet 121:1033-1046

Schweiger W, Steiner B, Vautrin S, Nussbaumer T, Siegwart G, Zamini M, Jungreithmeier F, Gratl V, Lemmens M, Mayer KFX, Bérgès H, Adam G, Buerstmayr H (2016) Suppressed recombination and unique candidate genes in the divergent haplotype encoding Fhb1, a major Fusarium head blight resistance locus in wheat. Theor Appl Genet 129:1607-1623

Simpson C (1991) Pathways for introgression of pest resistance into Arachis hypogaea L. Peanut Sci 18:22-26

Simpson C, C. Nelson S, L. Starr J, E. Woodard K, D. Smith O (1993) Registration of TxAG-6 and TxAG-7 peanut germplasm. Crop Sci 33:1418

Simpson C, L. Starr J, Church G, D. Burow M, H. Paterson A (2003) Registration of NemaTAM peanut. Crop Sci 43:1561

Simpson C, L. Starr J, R. Baring M, D. Burow M, M. Cason J, N. Wilson J (2013) Registration of 'Webb' peanut. J Plant Regist 7:265

Simpson C, Starr J (2001) Registration of 'COAN' peanut. Crop Sci 41:918

Singh A, Nautiyal P, Zala PV (1998) Growth and yield of groundnut varieties as influenced by seed size. Tropical Science 38:48-56

Sousa F, Neiva J, Martins N, Jacinto R, Anderson L, Raimondi PT, Serrao EA, Pearson GA (2019) Increased evolutionary rates and conserved transcriptional response following allopolyploidization in brown algae. Evolution 73:59-72

Stalker H (1997) Peanut (Arachis hypogaea L.). Field Crops Research 53:205–217

Stalker TH (2017) Utilizing Wild Species for Peanut Improvement. Crop Sci 57:1102

Starr J, R. Morgan E, E. Simpson C (2002) Management of the peanut root-knot nematode, Meloidogyne arenaria, with host resistance. Plant Health Prog

Stuthman DD, Leonard KJ, Miller-Garvin J (2007) Breeding Crops for Durable Resistance to Disease. Advances in Agronomy 95:319-367

Suassuna T, Suassuna N, Bogiani J, Perina F, Fragoso D, Sofiatti V, Medeiros E, Moretzsohn M, Leal-Bertioli S, Bertioli D, Heuert J, Assunção H, Luiz A, Colnago L, Gondim T, Vasconcellos R, Schwengber J, Bezerra J (2019) BRS 425: the first runner peanut cultivar related to wild ancestral species CULTIVAR RELEASE. CBAB 19:373-377

Tallury SP, Hilu KW, Milla-Lewis SR, Friend S, Alsaghir M, Stalker H, Quandt D (2005) Genomic affinities in Arachis section Arachis (Fabaceae): Molecular and cytogenetic evidence. Theor Appl Genet 111:1229-1237

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191-203

Varshney RK, Pandey MK, Janila P, Nigam SN, Sudini H, Gowda MV, Sriswathi M, Radhakrishnan T, Manohar SS, Nagesh P (2014a) Marker-assisted introgression of a QTL region to improve rust resistance in three elite and popular varieties of peanut (Arachis hypogaea L.). Theor Appl Genet 127:1771-1781

Varshney RK, Terauchi R, McCouch SR (2014b) Harvesting the Promising Fruits of Genomics: Applying Genome Sequencing Technologies to Crop Breeding. PLOS Biology 12:e1001883

Yano M (2001) Genetic and molecular dissection of naturally occurring variation. Current Opinion in Plant Biology 4:130-135

You FM, Huo N, Gu YQ, Luo MC, Ma Y, Hane D, Lazo GR, Dvorak J, Anderson OD (2008) BatchPrimer3: a high throughput web application for PCR and sequencing primer design. BMC Bioinformatics 9:253

Young ND (1999) A cautiously optimistic vision for marker-assisted breeding. Molecular Breeding 5:505-510

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nat Rev Genet 2:983-989

Appendix 3.A

Supplementary Figures

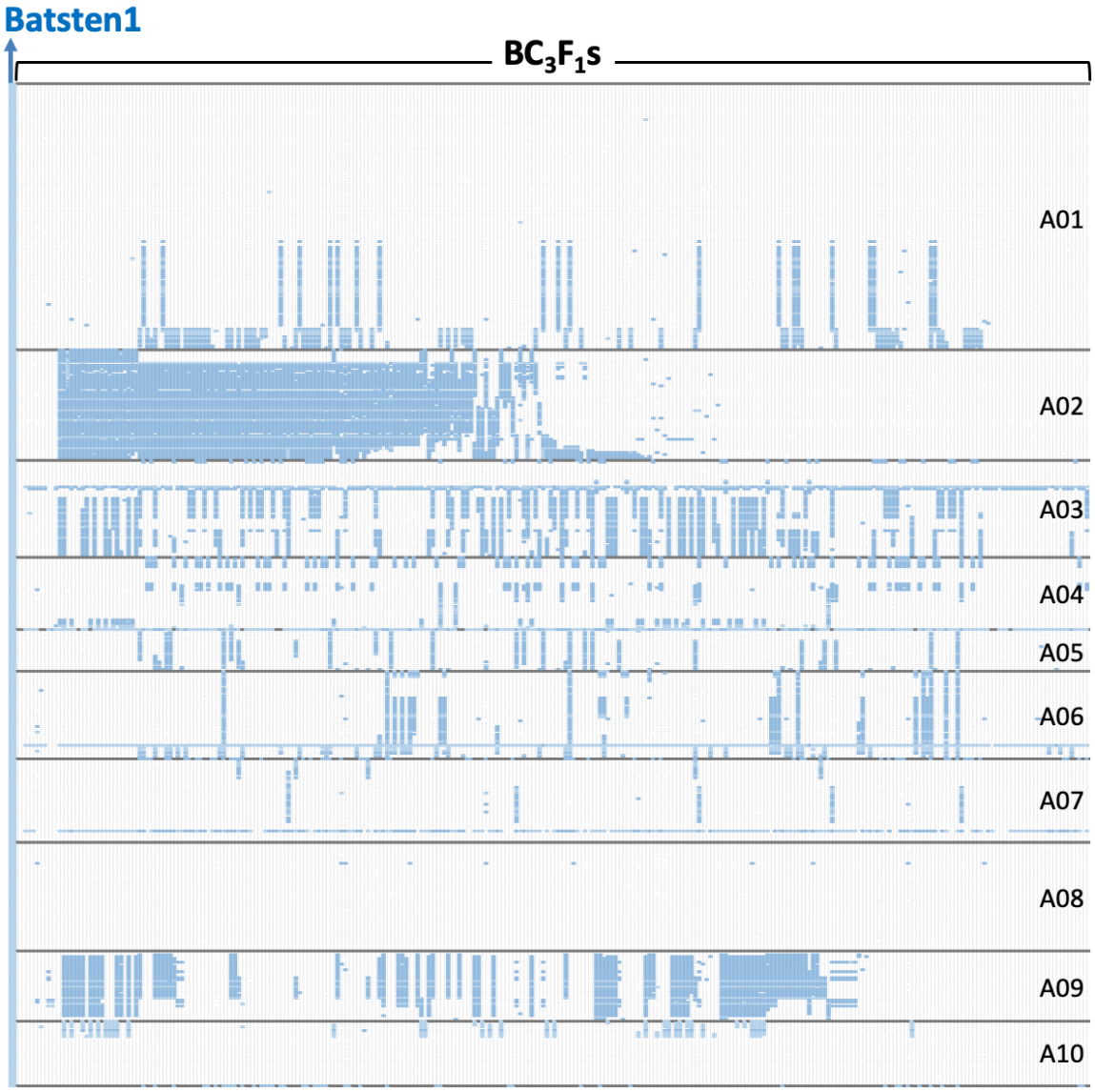

Figure 3.S1. Introgressions in the BC_3F_1 lines in A-subgenome from *A. stenosperma* (in blue). SNP markers in rows and BC_3F_1 lines in columns. Blue solid line indicates the synthetic allotetraploid BatSten1. Chromosome names to the right side of the figure.

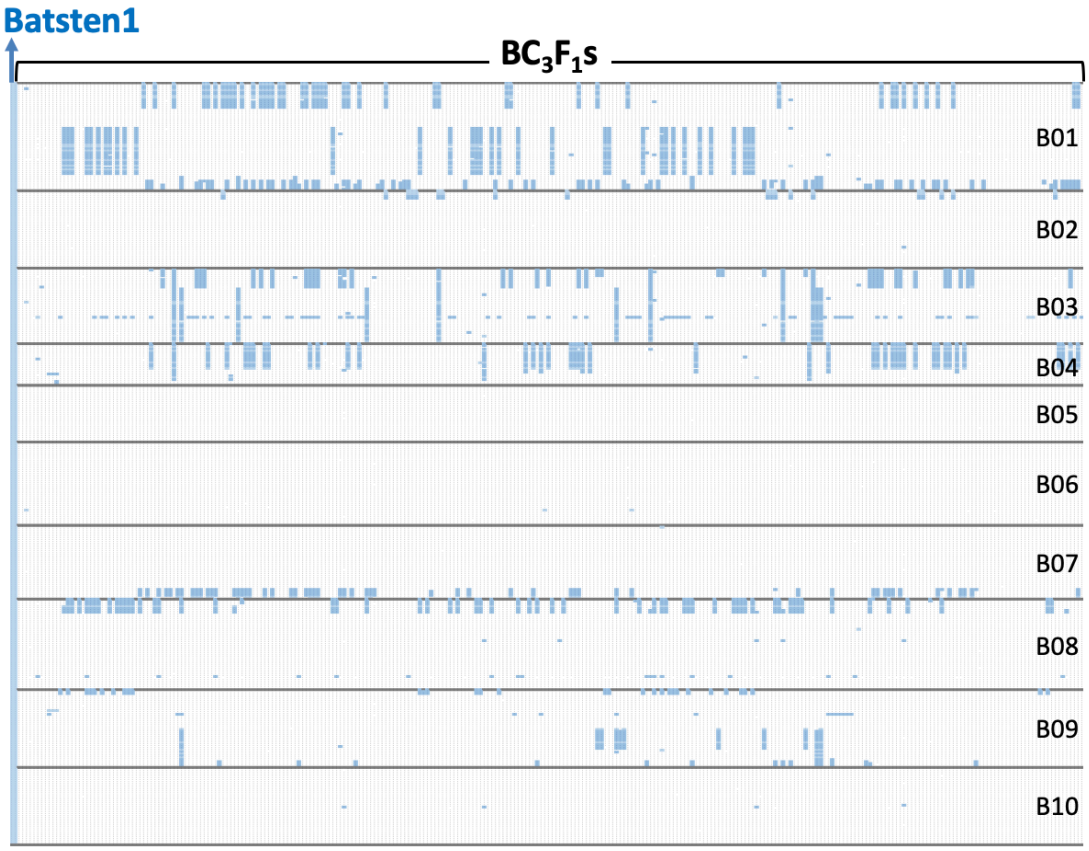

Figure 3.S2. Introgressions in the BC_3F_1 lines in A-subgenome from *A. batizocoi* (in blue). SNP markers in rows and BC_3F_1 lines in columns. Blue solid line indicates the synthetic allotetraploid BatSten1. Chromosome names to the right side of the figure.

Appendix 3.B

Supplementary Table

Table 3.S1. Information about KASP assays distributed across the resistance segments and R-gene clusters. Primer name (with linkage group, position on *A. duranensis* pseudomolecule, orientation and dye), primer sequence, primer type, SNP, SNP amplification pattern and reference. Gray shaded SNP assay markers used for foreground selection of true hybrids in each cycle of backcrossing.

Primer name	Sequence	Type	SNP	Amplification pattern	Reference
1_Aradu.A02_222671_Rev	TCAGTAACCTCTAAATCTTATCATCCA	AF		ND	Unpublished work
1_Aradu.A02_222671_FwdFAM	GAAGGTGACCAAGTTCATGCTAACTTGGTGAGGTAAAACAGAACAG	AS	G	ND	Unpublished work
1_Aradu.A02_222671_FwdVIC	GAAGGTCGGAGTCAACGGATTAACTTGGTGAGGTAAAACAGAACAA	AS	A	ND	Unpublished work
7_Aradu.A02_1180998_Fwd	TCCGACTTCTGTTGACTGGA	AF		As ≠ Ah	Unpublished work
7_Aradu.A02_1180998_RevVIC	GAAGGTCGGAGTCAACGGATTAATACCACACTAGCTCCCATATCC	AS	G	As ≠ Ah	Unpublished work
7_Aradu.A02_1180998_RevFAM	GAAGGTGACCAAGTTCATGCTCAATACCACACTAGCTCCCATATCT	AS	A	As ≠ Ah	Unpublished work
9_Aradu.A02_1556477_Fwd	AAGGGAGTTCAATCATGGAAAA	AF		As ≠ Ah	Unpublished work
9_Aradu.A02_1556477_RevVIC	GAAGGTCGGAGTCAACGGATTCCATGTTTCACCACTAATTCTCC	AS	G	As ≠ Ah	Unpublished work
9_Aradu.A02_1556477_RevFAM	GAAGGTGACCAAGTTCATGCTAACCATGTTTCACCACTAATTCTCT	AS	A	As ≠ Ah	Unpublished work
13_Aradu.A02_1993223_Rev	CAAGAATCAAATGGGAGTCCA	AF		As ≠ Ah	Unpublished work
13_Aradu.A02_1993223_FwdFAM	GAAGGTGACCAAGTTCATGCTACTTCTTGACAATCTTTCTTCCG	AS	G	As ≠ Ah	Unpublished work
13_Aradu.A02_1993223_FwdVIC	GAAGGTCGGAGTCAACGGATTCACTTCTTGACAATCTTTCTTCCA	AS	A	As ≠ Ah	Unpublished work
14_Aradu.A02_2340663_Rev	TCCATGGTACTCTGCATATCACT	AF		N	Unpublished work
14_Aradu.A02_2340663_FwdVIC	GAAGGTCGGAGTCAACGGATTCAACCGTTCAACAAGAGGG	AS	G	N	Unpublished work
14_Aradu.A02_2340663_FwdFAM	GAAGGTGACCAAGTTCATGCTCCAACCGTTCAACAAGAGGA	AS	A	N	Unpublished work
21_Aradu.A02_2844132_Fwd	CCTCCTAAATAGTACTGGTTCATTCC	AF		As ≠ Ah	Unpublished work
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Arachis_spp_rep_c2472p187vGT-AF_Fwd	CACATTAATCCAGGCTAACACAA	AF		N	Unpublished work
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Arachis_spp_rep_c2472p187vGT-AS-RevFAM	GAAGGTGACCAAGTTCATGCTTTGGATACTTTTTGGAGACTTGG	AS	C	N	Unpublished work
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35_Aradu.A02_6633078_FwdFAM	GAAGGTGACCAAGTTCATGCTGTTCAGAAGGAGAAGAAGTTAGGAGA	AS	A	As ≠ Ah	Unpublished work
38_Aradu.A02_8065316_Rev	TTCTCTTCACTGCGGCTTCT	AF		As ≠ Ah	Unpublished work
38_Aradu.A02_8065316_FwdFAM	GAAGGTGACCAAGTTCATGCTGCAATAGCGACGACGGG	AS	G	As ≠ Ah	Unpublished work
38_Aradu.A02_8065316_FwdVIC	GAAGGTCGGAGTCAACGGATTTAGCAATAGCGACGACGGA	AS	A	As ≠ Ah	Unpublished work
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12_Aradu.A02_83464195_Rev VIC	GAAGGTCGGAGTCAACGGATTGTTAAAAGAGCTTGTGACTTGTGACT	AS	A	ND	Unpublished work
Nem_Aradu.A02_83608917_Fwd_	TTTGTGGCTGCAATAACTTCA	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A02_83608917_Rev_VIC	GAAGGTCGGAGTCAACGGATTCATGACATTGTAAGTGGCAAAAAC	AS	G	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A02_83608917_Rev_FAM	GAAGGTGACCAAGTTCATGCTCATGACATTGTAAGTGGCAAAAAT	AS	A	ND	Leal-Bertioli et al. 2016
13_Aradu.A02_84350279_Rev	TACCTTAACCACGCCATCG	AF		ND	Unpublished work
13_Aradu.A02_84350279_Fwd FAM	GAAGGTGACCAAGTTCATGCT GGGAGTTTGGAAGCGAGG	AS	G	ND	Unpublished work
13_Aradu.A02_84350279_Fwd VIC	GAAGGTCGGAGTCAACGGATTGGGAGTTTGGAAGCGAGA	AS	A	ND	Unpublished work
Nem_Aradu.A02_84440546_Rev_	GCGATTAATACATTCAACAACCA	AF		As ≠ Ah	Leal-Bertioli et al. 2016
Nem_Aradu.A02_84440546_Fwd_FAM	GAAGGTGACCAAGTTCATGCTGCTCTCCTTCTTGGTGGTTTG	AS	G	As ≠ Ah	Leal-Bertioli et al. 2016
Nem_Aradu.A02_84440546_Fwd_VIC	GAAGGTCGGAGTCAACGGATTGCTCTCCTTCTTGGTGGTTTA	AS	A	As ≠ Ah	Leal-Bertioli et al. 2016
Nem_Aradu.A02_84440594_Rev_	GGAAGCGGATTCCACTCA	AF		N	Leal-Bertioli et al. 2016
Nem_Aradu.A02_84440594_Fwd_FAM	GAAGGTGACCAAGTTCATGCTGAAGTGTGTCATAATCTCCAAAGTG	AS	G	N	Leal-Bertioli et al. 2016
Nem_Aradu.A02_84440594_Fwd_VIC	GAAGGTCGGAGTCAACGGATTCTGAAGTGTGTCATAATCTCCAAAGTA	AS	A	N	Leal-Bertioli et al. 2016
14_Aradu.A02_84895487_Rev	AACTGATCTTTACCCGATTGCT	AF		ND	Unpublished work
14_Aradu.A02_84895487_Fwd VIC	GAAGGTCGGAGTCAACGGATTGCTATCCTAAAATGACAGAAAAAGTCAG	AS	G	ND	Unpublished work
14_Aradu.A02_84895487_Fwd FAM	GAAGGTGACCAAGTTCATGCT GCTATCCTAAAATGACAGAAAAAGTCAA	AS	A	ND	Unpublished work
15_Aradu.A02_85315365_Fwd	TCGGTAATGTGTTGCCTTTG	AF		ND	Unpublished work
15_Aradu.A02_85315365_Rev FAM	GAAGGTGACCAAGTTCATGCT AACATTGCATATAACAGCACCTTCT	AS	A	ND	Unpublished work
15_Aradu.A02_85315365_Rev VIC	GAAGGTCGGAGTCAACGGATTATTGCATATAACAGCACCTTCG	AS	C	ND	Unpublished work
16_Aradu.A02_86218781_Fwd	GACCTGTATTTGCATACTCAGCA	AF		ND	Unpublished work
16_Aradu.A02_86218781_Rev VIC	GAAGGTCGGAGTCAACGGATTGGAGTGGTAACTTTGCATTGAA	AS	T	ND	Unpublished work
16_Aradu.A02_86218781_Rev FAM	GAAGGTGACCAAGTTCATGCT GGAGTGGTAACTTTGCATTGAG	AS	C	ND	Unpublished work
17_Aradu.A02_86870675_Rev	GAGCAGGTCTAAAGAACAAGAAAGA	AF		ND	Unpublished work
17_Aradu.A02_86870675_Fwd VIC	GAAGGTCGGAGTCAACGGATTCGTTAGAAAACCACAATTCCAAG	AS	G	ND	Unpublished work
17_Aradu.A02_86870675_Fwd FAM	GAAGGTGACCAAGTTCATGCT CGTTAGAAAACCACAATTCCAAA	AS	A	ND	Unpublished work
DS_c1614_886_A02_88903581_Rev_	AGCTGAGGAGAACCCCTTTT	AF		ND	Leal-Bertioli et al. 2016
DS_c1614_886_A02_88903581_Fwd_FAM	GAAGGTGACCAAGTTCATGCTCAGATACAGTGACAGATATGAATGGTG	AS	G	ND	Leal-Bertioli et al. 2016
DS_c1614_886_A02_88903581_Fwd_VIC	GAAGGTCGGAGTCAACGGATTTCAGATACAGTGACAGATATGAATGGTA	AS	A	ND	Leal-Bertioli et al. 2016
18_Aradu.A02_91550268_Fwd	AATATAGGAGAAAGAAGTGAGGCAAT	AF		ND	Unpublished work
18_Aradu.A02_91550268_Rev FAM	GAAGGTGACCAAGTTCATGCT GCCAACAAGTAAGCACCCAA	AS	T	ND	Unpublished work
18_Aradu.A02_91550268_Rev VIC	GAAGGTCGGAGTCAACGGATTGCCAACAAGTAAGCACCCAG	AS	C	ND	Unpublished work
19_Aradu.A02_91638679_Rev	TCAGCAACATCGGATCCTC	AF		ND	Unpublished work
19_Aradu.A02_91638679_Fwd VIC	GAAGGTCGGAGTCAACGGATTGCATTGAACCAAGCATCG	AS	G	ND	Unpublished work
19_Aradu.A02_91638679_Fwd FAM	GAAGGTGACCAAGTTCATGCT GGCATTGAACCAAGCATCA	AS	A	ND	Unpublished work
20_Aradu.A02_92077207_Fwd	CTATGACATTGTCGTCCATCG	AF		ND	Unpublished work
20_Aradu.A02_92077207_Rev VIC	GAAGGTCGGAGTCAACGGATTAGCCAAATCCATTTTCAAGC	AS	G	ND	Unpublished work

20_Aradu.A02_92077207_Rev FAM	GAAGGTGACCAAGTTCATGCT CAGCCAAATCCATTTTCAAGA	AS	T	ND	Unpublished work
TOG894171_695_A02_92486807_Rev_	CTTCTGTTGGGGTGTTGGAT	AF		ND	Leal-Bertioli et al. 2016
TOG894171_695_A02_92486807_Fwd_VIC	GAAGGTCGGAGTCAACGGATTYATTAATCAGGCAATAGCAACG	AS	G	ND	Leal-Bertioli et al. 2016
TOG894171_695_A02_92486807_Fwd_FAM	GAAGGTGACCAAGTTCATGCTCTAYATTAATCAGGCAATAGCAACA	AS	A	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A02_92631394_Fwd_	AAGAAATTGGGCGTTTTCAG	AF		N	Leal-Bertioli et al. 2016
Nem_Aradu.A02_92631394_Rev_FAM	GAAGGTGACCAAGTTCATGCTATCCCCATATCTAGTGTCTTCTGC	AS	G	N	Leal-Bertioli et al. 2016
Nem_Aradu.A02_92631394_Rev_VIC	GAAGGTCGGAGTCAACGGATTCAATCCCCATATCTAGTGTCTTCTGT	AS	A	N	Leal-Bertioli et al. 2016
21_Aradu.A02_92983792_Fwd	AGTTTATGATCTCATTATGGAGGAAGA	AF		ND	Unpublished work
21_Aradu.A02_92983792_Rev VIC	GAAGGTCGGAGTCAACGGATTCTCTTGGGCAATCAAATTCC	AS	G	ND	Unpublished work
21_Aradu.A02_92983792_Rev FAM	GAAGGTGACCAAGTTCATGCT TTATCTCTTGGGCAATCAAATTCT	AS	A	ND	Unpublished work
22_Aradu.A02_93061221_Fwd	TTTTCAGATCTTCGTGCGAGT	AF		ND	Unpublished work
22_Aradu.A02_93061221_Rev FAM	GAAGGTGACCAAGTTCATGCT GAAGTAGAAGAAACGAGAATTAACGAAC	AS	G	ND	Unpublished work
22_Aradu.A02_93061221_Rev VIC	GAAGGTCGGAGTCAACGGATTGAAGTAGAAGAAACGAGAATTAACGAAA	AS	T	ND	Unpublished work
23_Aradu.A02_93081600_Fwd	CGATTCAGGAAGAGCTGCAC	AF		ND	Unpublished work
23_Aradu.A02_93081600_Rev FAM	GAAGGTGACCAAGTTCATGCT CCTTTCTGTTGAATTGCTTCTCAC	AS	G	ND	Unpublished work
23_Aradu.A02_93081600_Rev VIC	GAAGGTCGGAGTCAACGGATTCCTTTCTGTTGAATTGCTTCTCAT	AS	A	ND	Unpublished work
24_Aradu.A02_93293318_Fwd	TACTCTTACTACAGAATCCCGATTAAA	AF		ND	Unpublished work
24_Aradu.A02_93293318_Rev VIC	GAAGGTCGGAGTCAACGGATTTGGTAATCGGAGCTGAAAAGTATAA	AS	T	ND	Unpublished work
24_Aradu.A02_93293318_Rev FAM	GAAGGTGACCAAGTTCATGCT TGGTAATCGGAGCTGAAAAGTATAG	AS	C	ND	Unpublished work
25_Aradu.A02_93414423_Fwd	TGCTTCGAGTTTTTGGGAGA	AF		ND	Unpublished work
25_Aradu.A02_93414423_Rev FAM	GAAGGTGACCAAGTTCATGCT TCTTATAACACGTTGCAACTAAAGGA	AS	T	ND	Unpublished work
25_Aradu.A02_93414423_Rev VIC	GAAGGTCGGAGTCAACGGATTCTTATAACACGTTGCAACTAAAGGG	AS	C	ND	Unpublished work
26_Aradu.A02_93471506_Fwd	GATTCATGTTCGATGAGTCCTTC	AF		ND	Unpublished work
26_Aradu.A02_93471506_Rev VIC	GAAGGTCGGAGTCAACGGATTAAACGAAGATGACGAAGATAACAAC	AS	G	ND	Unpublished work
26_Aradu.A02_93471506_Rev FAM	GAAGGTGACCAAGTTCATGCT AAACGAAGATGACGAAGATAACAAT	AS	A	ND	Unpublished work
27_Aradu.A02_93790795_Fwd	AGGCTCCACATCCATTCTCA	AF		ND	Unpublished work
27_Aradu.A02_93790795_Rev FAM	GAAGGTGACCAAGTTCATGCT GGTTGTTTGATGATGCCATTT	AS	A	ND	Unpublished work
27_Aradu.A02_93790795_Rev VIC	GAAGGTCGGAGTCAACGGATTGGTTGTTTGATGATGCCATTG	AS	C	ND	Unpublished work
82_Aradu.A04_92635030_Fwd	ATCAAGAGGGTTTGCAATTCTG	AF		As ≠ Ah	Unpublished work
82_Aradu.A04_92635030_RevFAM	GAAGGTGACCAAGTTCATGCTTTCATATAAATATTAAGTCCCACATTCAA	AS	T	As ≠ Ah	Unpublished work
82_Aradu.A04_92635030_RevVIC	GAAGGTCGGAGTCAACGGATTTTCATATAAATATTAAGTCCCACATTCAG	AS	C	As ≠ Ah	Unpublished work
83_Aradu.A04_96553308_Fwd	GGATGTTAAGTTTGACAACTTTGG	AF		ND	Unpublished work
83_Aradu.A04_96553308_RevFAM	GAAGGTGACCAAGTTCATGCTGAAGTGGGCCTTCTAATTGGTTA	AS	T	ND	Unpublished work
83_Aradu.A04_96553308_RevVIC	GAAGGTCGGAGTCAACGGATTAGTGGGCCTTCTAATTGGTTG	AS	C	ND	Unpublished work
85_Aradu.A04_104172441_Fwd	AAATTTGTAGAAATATGAGGCCTAGAA	AF		As ≠ Ah	Unpublished work
85_Aradu.A04_104172441_RevVIC	GAAGGTCGGAGTCAACGGATTTCAAGGCCATCAACACCA	AS	T	As ≠ Ah	Unpublished work
85_Aradu.A04_104172441_RevFAM	GAAGGTGACCAAGTTCATGCTCAAGGCCATCAACACCG	AS	C	As ≠ Ah	Unpublished work
TOG906490_74_A04_106874754_Fwd_	TTCATTCCATAAGCCCAACC	AF		ND	Leal-Bertioli et al. 2016
TOG906490_74_A04_106874754_Rev_VIC	GAAGGTCGGAGTCAACGGATTAACTTTTCGAATCCTCATATTGCT	AS	A	ND	Leal-Bertioli et al. 2016
TOG906490_74_A04_106874754_Rev_FAM	GAAGGTGACCAAGTTCATGCTTTTTCGAATCCTCATATTGCG	AS	C	ND	Leal-Bertioli et al. 2016
87_Aradu.A04_108230713_Fwd	TTGACCATGCGCATAATAAAAG	AF		As ≠ Ah	Unpublished work
87_Aradu.A04_108230713_RevVIC	GAAGGTCGGAGTCAACGGATTGAAAGCATAAGAATGGGGTACAAC	AS	G	As ≠ Ah	Unpublished work
87_Aradu.A04_108230713_RevFAM	GAAGGTGACCAAGTTCATGCTGAAAGCATAAGAATGGGGTACAAT	AS	A	As ≠ Ah	Unpublished work
TOG937303_589_A04_108564975_Rev_	CCATCACAAAAGAACAAAACAAC	AF		ND	Leal-Bertioli et al. 2016
TOG937303_589_A04_108564975_Fwd_FAM	GAAGGTGACCAAGTTCATGCTAATTACTCGTTGGAGTAGTTGATGG	AS	G	ND	Leal-Bertioli et al. 2016
TOG937303_589_A04_108564975_Fwd_VIC	GAAGGTCGGAGTCAACGGATTGAATTACTCGTTGGAGTAGTTGATGA	AS	A	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_109789467_Rev_	CCAAAGCTCTTTTCCAGGTT	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_109789467_Fwd_FAM	GAAGGTGACCAAGTTCATGCTCAATAGAAACAGCAAAGCAATGG	AS	G	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_109789467_Fwd_VIC	GAAGGTCGGAGTCAACGGATTCAATAGAAACAGCAAAGCAATGA	AS	A	ND	Leal-Bertioli et al. 2016
89_Aradu.A04_112224690_Rev	AGGTGGGGATTTGAGTGTTG	AF		As ≠ Ah	Unpublished work
89_Aradu.A04_112224690_FwdVIC	GAAGGTCGGAGTCAACGGATTATGGGGAATGCTATTCTTTGAG	AS	G	As ≠ Ah	Unpublished work

89_Aradu.A04_112224690_FwdFAM	GAAGGTGACCAAGTTCATGCTATGGGGAATGCTATTCTTTGAA	AS	A	As ≠ Ah	Unpublished work
Nem_Aradu.A04_113373632_Rev_	TCCTCATCATCATCTTTCTCCA	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_113373632_Fwd_FAM	GAAGGTGACCAAGTTCATGCTAGGTTGGTCAAGGGTTTCAG	AS	G	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_113373632_Fwd_VIC	GAAGGTCGGAGTCAACGGATTAGGTTGGTCAAGGGTTTCAA	AS	A	ND	Leal-Bertioli et al. 2016
92_Aradu.A04_114359220_Fwd	TTCATTAGTGTCTTTTGTTGCTGA	AF		As ≠ Ah	Unpublished work
92_Aradu.A04_114359220_RevFAM	GAAGGTGACCAAGTTCATGCTCCCGGAACTATTTTTTTTCCC	AS	G	As ≠ Ah	Unpublished work
92_Aradu.A04_114359220_RevVIC	GAAGGTCGGAGTCAACGGATTTCCCGGAACTATTTTTTTTCCT	AS	A	As ≠ Ah	Unpublished work
Nem_Aradu.A04_114769893_Fwd_	TCAAGTCGTGTGTTCTCTACACC	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_114769893_Rev_FAM	GAAGGTGACCAAGTTCATGCTTCTTGTGACATGAGCTACAACTTCT	AS	A	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_114769893_Rev_VIC	GAAGGTCGGAGTCAACGGATTTTGTGACATGAGCTACAACTTCG	AS	C	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_115457181_Rev_	TGTGGACAGATGGAAAACACA	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_115457181_Fwd_VIC	GAAGGTCGGAGTCAACGGATTTTCGGCGTTGGACTGTG	AS	G	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_115457181_Fwd_FAM	GAAGGTGACCAAGTTCATGCTCTTCGGCGTTGGACTGTA	AS	A	ND	Leal-Bertioli et al. 2016
94_Aradu.A04_116396168_Rev	TTTTGATTAGTAAGGAAAGCGTAAC	AF		N	Unpublished work
94_Aradu.A04_116396168_FwdFAM	GAAGGTGACCAAGTTCATGCTCTTCTGACTTTCCATTAACATTCTGT	AS	T	N	Unpublished work
94_Aradu.A04_116396168_FwdVIC	GAAGGTCGGAGTCAACGGATTTCTGACTTTCCATTAACATTCTGC	AS	C	N	Unpublished work
Nem_Aradu.A04_117955004_Fwd_	TCACGGTCCATGTATTCAGC	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_117955004_Rev_VIC	GAAGGTCGGAGTCAACGGATTCGTTAGCAGTTGGACAAACAAAC	AS	G	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_117955004_Rev_FAM	GAAGGTGACCAAGTTCATGCTCGTTAGCAGTTGGACAAACAAAT	AS	A	ND	Leal-Bertioli et al. 2016
96_Aradu.A04_118778510_Rev	CAACATGGCTGCAAAGTTACC	AF		As ≠ Ah	Unpublished work
96_Aradu.A04_118778510_FwdVIC	GAAGGTCGGAGTCAACGGATTATTAAGGACTCTCTTGTGGTTGAAG	AS	G	As ≠ Ah	Unpublished work
96_Aradu.A04_118778510_FwdFAM	GAAGGTGACCAAGTTCATGCTATTAAGGACTCTCTTGTGGTTGAAA	AS	A	As ≠ Ah	Unpublished work
99_Aradu.A04_120888870_Rev	TTATCAACATCGTCAAGAACCA	AF		ND	Unpublished work
99_Aradu.A04_120888870_FwdFAM	GAAGGTGACCAAGTTCATGCTGATATAAAACAAAGTCTCCGCAATAAG	AS	G	ND	Unpublished work
99_Aradu.A04_120888870_FwdVIC	GAAGGTCGGAGTCAACGGATTGATATAAAACAAAGTCTCCGCAATAAA	AS	A	ND	Unpublished work
Nem_Aradu.A04_121132127_Rev_	AGATTTTCTGGGCCCATTTT	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_121132127_Fwd_VIC	GAAGGTCGGAGTCAACGGATTGCCAAGCAAAGTAATGCCG	AS	G	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_121132127_Fwd_FAM	GAAGGTGACCAAGTTCATGCTGCCAAGCAAAGTAATGCCA	AS	A	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_121183243_Rev_	AAGGTTGGGAATGTCAAGGA	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_121183243_Fwd_FAM	GAAGGTGACCAAGTTCATGCTAACTGGTAGGTTTGGAAATAATCG	AS	G	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A04_121183243_Fwd_VIC	GAAGGTCGGAGTCAACGGATTAAACTGGTAGGTTTGGAAATAATCC	AS	C	ND	Leal-Bertioli et al. 2016
101_Aradu.A04_123417094_Fwd	CAGCCTTGGTTGATCTATATGC	AF		N	Unpublished work
101_Aradu.A04_123417094_RevVIC	GAAGGTCGGAGTCAACGGATTAGAACAACCTATACGCAAAATCAAC	AS	G	N	Unpublished work
101_Aradu.A04_123417094_RevFAM	GAAGGTGACCAAGTTCATGCTAGAACAACCTATACGCAAAATCAAT	AS	A	N	Unpublished work
154_Aradu.A09_2473734_Rev	CCAGTACCCCCAAAGATGTA	AF		As ≠ Ah	Unpublished work
154_Aradu.A09_2473734_FwdFAM	GAAGGTGACCAAGTTCATGCTCGATTTAGACCCTTAGTAGTCCCT	AS	T	As ≠ Ah	Unpublished work
154_Aradu.A09_2473734_FwdVIC	GAAGGTCGGAGTCAACGGATTCGATTTAGACCCTTAGTAGTCCCC	AS	C	As ≠ Ah	Unpublished work
158_Aradu.A09_3927064_Rev	CCAAGCATTGAATAGCTACCAG	AF		ND	Unpublished work
158_Aradu.A09_3927064_FwdVIC	GAAGGTCGGAGTCAACGGATTGGGAACTGCTAGCTTGTGTG	AS	G	ND	Unpublished work
158_Aradu.A09_3927064_FwdFAM	GAAGGTGACCAAGTTCATGCTGATGGGAACTGCTAGCTTGTGTA	AS	A	ND	Unpublished work
159_Aradu.A09_8102594_Fwd	TTTGGACTTGTTCGGTTGCT	AF		ND	Unpublished work
159_Aradu.A09_8102594_RevVIC	GAAGGTCGGAGTCAACGGATTGTAGAATTTCTCCTGCACTGTCC	AS	G	ND	Unpublished work
159_Aradu.A09_8102594_RevFAM	GAAGGTGACCAAGTTCATGCTTGTAGAATTTCTCCTGCACTGTCA	AS	T	ND	Unpublished work
161_Aradu.A09_11528416_Rev	TTGGATCACACTCTATTGACACAA	AF		ND	Unpublished work
161_Aradu.A09_11528416_FwdFAM	GAAGGTGACCAAGTTCATGCTTGTGAGACAAAGGAGAAGAACG	AS	G	ND	Unpublished work
161_Aradu.A09_11528416_FwdVIC	GAAGGTCGGAGTCAACGGATTAGTGTGAGACAAAGGAGAAGAACA	AS	A	ND	Unpublished work
163_Aradu.A09_13870085_Rev	TTGTTTGGTTTTGAATCTCCA	AF		As ≠ Ah	Unpublished work
163_Aradu.A09_13870085_FwdVIC	GAAGGTCGGAGTCAACGGATTATTGTTATACATGATTGCCAGTTTG	AS	G	As ≠ Ah	Unpublished work
163_Aradu.A09_13870085_FwdFAM	GAAGGTGACCAAGTTCATGCTGATTATTGTTATACATGATTGCCAGTTTA	AS	A	As ≠ Ah	Unpublished work
164_Aradu.A09_20334783_Rev	TCGTTCATCGTCGGATTCAT	AF		As ≠ Ah	Unpublished work
164_Aradu.A09_20334783_FwdFAM	GAAGGTGACCAAGTTCATGCTAAGAAGACGAACCCCAACAATA	AS	A	As ≠ Ah	Unpublished work

164_Aradu.A09_20334783_FwdVIC	GAAGGTCGGAGTCAACGGATTAGAAGACGAACCCCAACAATC	AS	C	As ≠ Ah	Unpublished work
165_Aradu.A09_25028543_Fwd	GCTACGACACTTGGTAACAAATACTG	AF		As ≠ Ah	Unpublished work
165_Aradu.A09_25028543_RevFAM	GAAGGTGACCAAGTTCATGCTCACAAAATGCAGAGAGAAAAGGTT	AS	A	As ≠ Ah	Unpublished work
165_Aradu.A09_25028543_RevVIC	GAAGGTCGGAGTCAACGGATTACAAAATGCAGAGAGAAAAGGTG	AS	C	As ≠ Ah	Unpublished work
166_Aradu.A09_30666168_Rev	AAACACAAAATCCCAAAATGC	AF		ND	Unpublished work
166_Aradu.A09_30666168_FwdVIC	GAAGGTCGGAGTCAACGGATTGGGAGACGAGAGTTGGTGGT	AS	T	ND	Unpublished work
166_Aradu.A09_30666168_FwdFAM	GAAGGTGACCAAGTTCATGCTGGAGACGAGAGTTGGTGGC	AS	C	ND	Unpublished work
169_Aradu.A09_40832926_Fwd	GATCACCATACACACAGCTTAACA	AF		N	Unpublished work
169_Aradu.A09_40832926_RevVIC	GAAGGTCGGAGTCAACGGATTGCGTGTATGAAGAACCACCTTA	AS	T	N	Unpublished work
169_Aradu.A09_40832926_RevFAM	GAAGGTGACCAAGTTCATGCTCGTGTATGAAGAACCACCTTG	AS	C	N	Unpublished work
170_Aradu.A09_49729729_Rev	TCTTTGTTTAATGTGTCACCTTGG	AF		ND	Unpublished work
170_Aradu.A09_49729729_FwdFAM	GAAGGTGACCAAGTTCATGCTCATGTCAATAGCAAAGAAGATGGA	AS	A	ND	Unpublished work
170_Aradu.A09_49729729_FwdVIC	GAAGGTCGGAGTCAACGGATTATGTCAATAGCAAAGAAGATGGC	AS	C	ND	Unpublished work
Nem_Aradu.A09_112396428_Fwd_	TATGATTGGCCCCCTAAATG	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112396428_Rev_FAM	GAAGGTGACCAAGTTCATGCTAGCCCCCTCTTCTAAAACAAC	AS	G	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112396428_Rev_VIC	GAAGGTCGGAGTCAACGGATTCAGCCCCCTCTTCTAAAACAAT	AS	A	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112396635_Rev_	CCTGGCTTCATGTTTGATGA	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112396635_Fwd_VIC	GAAGGTCGGAGTCAACGGATTAATGTTACAAAAGGATCCCCAG	AS	G	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112396635_Fwd_FAM	GAAGGTGACCAAGTTCATGCTAATGTTACAAAAGGATCCCCAA	AS	A	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112399976_Rev_	TGACGAGAAGGGGAAAGAAA	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112399976_Fwd_FAM	GAAGGTGACCAAGTTCATGCTAATCCTATTACTAAATCGCTGCTTTT	AS	T	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112399976_Fwd_VIC	GAAGGTCGGAGTCAACGGATTAATCCTATTACTAAATCGCTGCTTTC	AS	C	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112901114_Rev_	CTCCCCAATTTCTCAGCAAG	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112901114_Fwd_FAM	GAAGGTGACCAAGTTCATGCTAGGTGTTGACAGAATTACAACCG	AS	G	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_112901114_Fwd_VIC	GAAGGTCGGAGTCAACGGATTGAGGTGTTGACAGAATTACAACCA	AS	A	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_114001128_Rev_	TTAAAGCCCCTGCTTTTTCA	AF		ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_114001128_Fwd_FAM	GAAGGTGACCAAGTTCATGCTATGAGGGAACAACCAGCACTA	AS	A	ND	Leal-Bertioli et al. 2016
Nem_Aradu.A09_114001128_Fwd_VIC	GAAGGTCGGAGTCAACGGATTTGAGGGAACAACCAGCACTC	AS	C	ND	Leal-Bertioli et al. 2016
TOG896942_133_A09_114770700_Fwd_	AAAGAAAGGGCTCCCTAATTTC	AF		ND	Leal-Bertioli et al. 2016
TOG896942_133_A09_114770700_Rev_FAM	GAAGGTGACCAAGTTCATGCTGGGCACAAAAATTCGCTACA	AS	T	ND	Leal-Bertioli et al. 2016
TOG896942_133_A09_114770700_Rev_VIC	GAAGGTCGGAGTCAACGGATTGGCACAAAAATTCGCTACG	AS	C	ND	Leal-Bertioli et al. 2016
DS_c14276_456_A09_115161052_Rev_	AGGAGTCATGGGATGGAATG	AF		ND	Leal-Bertioli et al. 2016
DS_c14276_456_A09_115161052_Fwd_VIC	GAAGGTCGGAGTCAACGGATTTTTGGAAACATCAGCAAAGGA	AS	A	ND	Leal-Bertioli et al. 2016
DS_c14276_456_A09_115161052_Fwd_FAM	GAAGGTGACCAAGTTCATGCTTGGAAACATCAGCAAAGGC	AS	C	ND	Leal-Bertioli et al. 2016
TOG896078_413_A09_116503861_Rev_	GTGGAAGAAATAGCAAAATGGA	AF		ND	Leal-Bertioli et al. 2016
TOG896078_413_A09_116503861_Fwd_VIC	GAAGGTCGGAGTCAACGGATTAAGGAGTTATGGAGATGGTAAGTTTT	AS	T	ND	Leal-Bertioli et al. 2016
TOG896078_413_A09_116503861_Fwd_FAM	GAAGGTGACCAAGTTCATGCTAAGGAGTTATGGAGATGGTAAGTTTC	AS	C	ND	Leal-Bertioli et al. 2016
TOG903757_1119_A09_116533871_Rev_	CCCAAGAAGCAGGGTACTTT	AF		ND	Leal-Bertioli et al. 2016
TOG903757_1119_A09_116533871_Fwd_VIC	GAAGGTCGGAGTCAACGGATTACTTGATTTGATATGAGATTTCCTG	AS	G	ND	Leal-Bertioli et al. 2016
TOG903757_1119_A09_116533871_Fwd_FAM	GAAGGTGACCAAGTTCATGCTCACTTGATTTGATATGAGATTTCCTC	AS	C	ND	Leal-Bertioli et al. 2016

Primer name - LG, Position_Orientation_Dye; LG, Linkage Group; AF, allele flaking marker; AS, allele specific marker; As, *A. stenosperma*; Ah, *A. hypogaea* Runner-886; ND, Pattern not defined; N, assay did not work. Gray shade indicates KASP assays that were tested and worked properly to identify plants carrying the SNP of interest.

Appendix 3.C

Supplementary Script

Script 3.S1. Unix scripts for analysis and filtering of genotyping data for BC_3F_1 lines and controls.

```
#Input file:
        Geno_BC3F1s_tetra1.txt

#Filtering and replace genotype by numbers BatSten1 (1), Runner-886 (2), different (3):
        BatSten1 (Fourth and fifth columns) ≠ Runner-886 (sixth and seventh columns)
        AA ≠ AB
        AA ≠ BB
        AB ≠ AA
        AB ≠ BB
        BB ≠ AA
        BB ≠ AB

        awk '{if($4=="AA" && $5=="AA" && $6== "AB" && $7== "AB") print $0}' Geno_BC3F1s_tetra1.txt |
        perl -lpe "s/AA/1/g" | perl -lpe "s/AB/2/g" | perl -lpe "s/BB/3/g" | perl -lpe "s/NoCall/-/g" >
        Geno_BC3F1s_tetra1_1.txt &

        awk '{if($4=="AA" && $5=="AA" && $6== "BB" && $7== "BB") print $0}' Geno_BC3F1s_tetra1.txt |
        perl -lpe "s/AA/1/g" | perl -lpe "s/BB/2/g" | perl -lpe "s/AB/3/g" | perl -lpe "s/NoCall/-/g" >
        Geno_BC3F1s_tetra1_2.txt &

        awk '{if($4=="AB" && $5=="AB" && $6== "AA" && $7== "AA") print $0}' Geno_BC3F1s_tetra1.txt |
        perl -lpe "s/AB/1/g" | perl -lpe "s/AA/2/g" | perl -lpe "s/BB/3/g" | perl -lpe "s/NoCall/-/g" >
        Geno_BC3F1s_tetra1_3.txt &

        awk '{if($4=="AB" && $5=="AB" && $6== "BB" && $7== "BB") print $0}' Geno_BC3F1s_tetra1.txt |
        perl -lpe "s/AB/1/g" | perl -lpe "s/BB/2/g" | perl -lpe "s/AA/3/g" | perl -lpe "s/NoCall/-/g" >
        Geno_BC3F1s_tetra1_4.txt &

        awk '{if($4=="BB" && $5=="BB" && $6== "AA" && $7== "AA") print $0}' Geno_BC3F1s_tetra1.txt |
        perl -lpe "s/BB/1/g" | perl -lpe "s/AA/2/g" | perl -lpe "s/AB/3/g" | perl -lpe "s/NoCall/-/g" >
        Geno_BC3F1s_tetra1_5.txt &

        awk '{if($4=="BB" && $5=="BB" && $6== "AB" && $7== "AB") print $0}' Geno_BC3F1s_tetra1.txt |
        perl -lpe "s/BB/1/g" | perl -lpe "s/AB/2/g" | perl -lpe "s/AA/3/g" | perl -lpe "s/NoCall/-/g" >
        Geno_BC3F1s_tetra1_6.txt &

#Concatenate files:
        head -7 Geno_BC3F1s_tetra1.txt > Geno_BC3F1s_tetra1_All.txt
        cat Geno_BC3F1s_tetra1_1.txt Geno_BC3F1s_tetra1_2.txt Geno_BC3F1s_tetra1_3.txt
        Geno_BC3F1s_tetra1_4.txt Geno_BC3F1s_tetra1_5.txt Geno_BC3F1s_tetra1_6.txt >>
        Geno_BC3F1s_tetra1_All.txt

#Filter by genetic map (Ballén-Taborda et al. Submitted):
head -7 Geno_BC3F1s_tetra1_All.txt > Geno_BC3F1s_tetra1_All_BatStenMap.txt
        for i in `cut -f 1 BatStenMap_MarkersList.txt` ; do grep "$i" Geno_BC3F1s_tetra1_All.txt >>
        Geno_BC3F1s_tetra1_All_BatStenMap.txt ; done
```

CHAPTER 4

CHARACTERIZATION OF ROOT-KNOT NEMATODE RESISTANCE FROM *ARACHIS STENOSPERMA* INCORPORATED INTO ADVANCED BACKCROSSED LINES OF PEANUT

Introduction

Arachis hypogaea L. (peanut or groundnut) is a grain legume crop cultivated worldwide in tropical and subtropical regions with an annual production of 64.2 million tons. In the USA, peanut is grown on more than a half million ha (0.72 Mha) with an average yield of 4.57 tons/ha in 2017 (FAOSTAT 2019). Peanut is an important source of dietary nutrients and proteins, particularly in developing countries, and has commercially importance for the oil industry for use in edible products and in many processed foods (Arya et al. 2016; Mupunga et al. 2017; Settaluri et al. 2012; Singh and Singh 1991). Peanut is an allotetraploid species (AABB, 2n = 4x =40) with very low genetic diversity due to its recent polyploid origin (Bertioli et al. 2016; Bertioli et al. 2019; Halward et al. 1992; Husted 1936; Kochert et al. 1996; Moretzsohn et al. 2013). Because it is an allotetraploid, it has limited gene flow with its diploid wild relatives due to sexual barriers (Bertioli et al. 2011) which has resulted in susceptibility to severe pests and diseases, including root-knot nematode (RKN) (*Meloidogyne arenaria* (Neal)), rust (*Puccinia arachidis*), early leaf spot (ELS) (*Passalora arachidicola*), late leaf spot (LLS)

(*Nothopassalora personata*), Tomato spotted wilt virus (TSWV) and others (Holbrook and Stalker 2003).

M. arenaria is an economically important nematode that affects several major crops (Jones et al. 2013) including peanut in several production regions. Nematode infection is characterized by the formation of galls or "knots" in the peanut roots. Briefly, the life cycle begins when the infectious second-stage juveniles (J2) (that are present in the soil) penetrate the peanut root tips and start moving along the vascular cylinder. Later, the nematodes establish feeding sites by remodeling the parenchymatic and surrounding cells into enlarged multinucleated giant cells which result in the formation of galls. Once established, the nematode undergoes three molts to become a reproductive adult female that lays hundreds of eggs into gelatinous masses that are later released into the soil. Embryogenesis inside the eggs allow the formation of J2 nematodes that hatch to start the cycle again (Moens et al. 2009; Williamson and Hussey 1996).

Crop rotation and nematicides are commonly used for nematode management. Due to the ability of *M. arenaria* to infect most crops species, only a few non-host crops are available to control nematodes populations and to reduce crop damage (Dong et al. 2007; Perry et al. 2009; Sikora 2018; Wesemael et al. 2011). Additionally, chemical control is not only costly but harmful to human health and environment (Danchin et al. 2013; Onkendi et al. 2014). Therefore, development of nematode-resistant and high yielding cultivars is the most efficacious way to control nematode populations and decrease yield losses while reducing the use of nematicides (Dong et al. 2007).

In peanut, RKN results in yield loss, reduced pod and grain quality, negatively impacts plant growth and increases production costs (Holbrook and Stalker 2003; Starr et

al. 2002). For the southern region of the US it has been estimated that peanut production can be reduced by 50% or more under dense nematode populations (Dickson and De Waele 2005; Minton and Baujard 1990). At times, 100% losses have been reported in areas of heavily infested fields (Timper et al. 2018). In other parts of the world, it is expected that these pathogens are an important threat for peanut cultivation, due to their presence across this region and their wide range of hosts (Dong et al. 2007; Perry et al. 2009; Sikora 2018).

Strong resistance to RKN is not available in peanut. Moderate levels is present in *A. hypogaea* plant introductions (PI) (Noe et al. 1992). However, peanut wild relatives harbor resistance to several pests and pathogens (Holbrook and Noe 1990; Nelson et al. 1989). The diploid wild *Arachis* species can be used to develop synthetic tetraploids that are compatible with peanut to transfer desirable alleles into elite cultivars (Burow et al. 2001). Previously, successful transference of root-knot nematode into cultivated peanut was accomplished through backcrossing schemes involving synthetic allotetraploids (Simpson and Starr 2001). The resistance was located on a chromosome A09 segment from the wild species *Arachis cardenasii* Krapov. & W.C. Greg. (Chu et al. 2016; Simpson 2001; Simpson et al. 1993; Simpson et al. 2003). Since then, nearly all nematode-resistant cultivars grown in the US utilize this single source of resistance, e.g. NemaTAM, Tifguard, Webb, Georgia-14N and TifNV-High O/L (Branch and Brenneman 2015; Holbrook et al. 2017; Holbrook et al. 2008; Simpson et al. 2003; Simpson et al. 2013). Since the resistance to RKN in modern commercial varieties is derived from this single source, there is a risk of resistance breakdown (Kiyosawa 1982; Palloix et al. 2009). Therefore, characterization of new sources of resistance is essential

to provide stronger and more durable resistance (Stuthman et al. 2007), and to ensure continued protection of the peanut crop from losses due to RKN.

The peanut wild relative *A. stenosperma* has been described as very resistant to the root-knot nematode (Guimarães et al. 2010; Proite et al. 2008; Proite et al. 2007). Previously, RKN resistance segments in A02, A04 and A09 of the wild *A. stenosperma* were discovered in a diploid RIL mapping population derived from a cross of *A. duranensis* x *A. stenosperma* (Leal-Bertioli et al. 2016). Later, resistance chromosome segments in bottom A02 and bottom A09 were confirmed in a tetraploid background, each of the fragments contributing to a percentage reduction in nematode multiplication of up to 98.2% and that putative QTL on A04 did not contribute to improve nematode resistance (Ballén-Taborda et al. Submitted) (Chapter 2). To incorporate RKN resistance from the wild species *A. stenosperma* into peanut cultivars, F_2 lines derived from the cross of a induced allotetraploid ((*A. batizocoi* K9484 x *A. stenosperma* V10309)^{4x}) (Leal-Bertioli et al. 2015) and *A. hypogaea* Runner-886 were first selected base on SNP (single nucleotide polymorphism) markers linked to the resistance and for agronomic traits; secondly, they were crossed and backcrossed with peanut elite breeding lines.

At least three cycles of marker-assisted backcrossing for incorporation of resistance from wild species are required to recover a plant that is similar to the recurrent parent and carry the trait of interest (Frisch et al. 1999, 2000; Hospital and Charcosset 1997; Jiang 2015; Lecomte et al. 2004). As a step to accomplish this goal, and to refine the genetic positions of the most important RKN resistance segments, this study focused on the phenotypic screening for RKN resistance and genotypic characterization of BC_2F_1 lines. Here, nine BC_2F_1 (used as male parents for BC_3 generation) were evaluated for

nematode resistance using a rooted-detached leaf bioassay and genotyped using the *Arachis* SNP array (Clevenger et al. 2018; Korani et al. 2019). For six it was possible to confirm phenotypically their ability to stop nematode development and one was susceptible. Additionally, the phenotype and genotype data allowed us to validate the A02-bottom and A09-bottom chromosome segments as key to resistance, spanning regions of ~8Mbp and ~6.5Mbp, respectively. Introgressions in A04 did not show correlation with resistance. With this information, more accurate selections of resistant lines can be made to continue the RKN resistance integration. This study is important for the successful development of advanced germplasm that incorporate *A. stenosperma* chromosome segments for strong and durable resistance to RKN, which will contribute to the improvement of elite peanut lines.

Materials and Methods

Plant materials

Wild *Arachis* accessions (*A. batizocoi* K9484 and *A. stenosperma* V10309), synthetic allotetraploid (BatSten1) (Leal-Bertioli et al. 2015) and cultivated peanut, *A. hypogea*, genotypes (Runner-886, 5-646-10, 13-1014, TifGp-2, Tifguard and Tifrunner) (Table 4.1) were used for phenotyping and genotyping (https://wildpeanutlab.uga.edu/). The BC_2F_1s lines were developed in summer of 2017 during the second cycle of backcrossing then grown in a greenhouse for RKN resistance evaluation and genotyping in 2018.

Ex vitro hairy root induction for detached leaf bioassays

Bioassays were done using the detached leaf technique, which consists of inducing hairy roots in peanut petioles in a moistened cotton layer covered with filter paper in a petri dish. This was done as a non-destructive method of evaluation, and to allow us to have technical replicates from a single plant. To set up the experiment, either the second or third fully expanded healthy leaves were collected. The quadrifoliates were carefully rinsed under running water, dried using paper towels and then with the petiole submerged in water, a fresh cut was made to prevent water logging. Immediately, the petioles were covered with wet cotton after dipping in Clonex®(HDI - Hydrodynamics International, Lansing, MI, USA) rooting hormone gel. Leaves were placed on the petri dishes over a microscope slide with the adaxial side upward. Detached leaves were maintained under controlled conditions at 25±2°C, ~64.9±6.0% of humidity and 16 h photoperiod (Guimaraes et al. 2017). When the first roots appeared after ~20 days, the cotton covering the petiole was replaced with wet vermiculite (Fig. 4.1.A,B). Autoclaved distillated water was used as needed to keep the leaves alive during the experiment timeframe (Fávero et al. 2009; Guimaraes et al. 2017; Leal-Bertioli et al. 2009; Mallikarjuna et al. 2012).

Root-knot nematode resistance evaluation

To study reproduction of root-knot nematode (RKN) *Meloidogyne arenaria* (Neal) Chitwood race 1 (Fig. 4.2), BC_2F_1s lines, susceptible and resistant controls (Table 4.1) were evaluated for resistance using the detached leaf bioassay in a Randomized

Complete Block Design with 15 replicates per genotype and placed in a controlled-environment bioassay room (as described above) (Guimaraes et al. 2017).

Thirty days after root induction (Fig. 4.1.A), roots were inoculated with ~3,000 J2/ml (second-stage juveniles/ml) obtained from Dr. P. Timper (USDA, ARS, Tifton, GA) (Fig. 4.2). Detached leaves were inoculated one block at a time by applying 1ml of inoculum on top the roots and covering with vermiculite (Fig. 4.1.B). The nematode solution was continuously homogenized on a stirring plate during inoculation. To evaluate penetration and development of the nematodes inside the roots after 6-8 days after inoculation (DAI), roots were evaluated by staining with cotton blue solution (Fig. 4.1.C,D) using a modified protocol (Bybd et al. 1983). Briefly, roots were rinsed on water and soaked in 6% hypochlorite for 4 min. Then, washed with running water for 45s and soaked in water for 15 min. Finally, roots were stained in boiling cotton blue solution and rinsed in boiling 50% glycerol. Root were pressed between petri dishes and observed under a stereoscope.

To monitor different stages of the nematode cycle (J2 or swollen J3/J4 (SJ3/SJ4)), the infected roots were evaluated at 13-15 DAI using cotton blue (as described above) (Fig. 4.1.C,D). Later, the roots were processed at 36-48 DAI to quantify galls and egg production. Galls on the roots were quantified under a dissecting stereoscope (Fig. 4.1.E, green arrows). Roots were then were treated with 75 mg/L erioglaucine to distinguish egg masses (Fig. 4.1.E, blue arrows). Briefly, the infected root system was stained with erioglaucine solution for 20 min and rinsed in water (Petrillo et al. 2006). The number of galls (three times and averaged) and eggs masses were recorded and divided by the

weight of the root (Guimaraes et al. 2017). Pictures were taken under dissecting stereoscope and microscope.

Statistical analysis

Data was statistically analyzed using the package R. A Shapiro-Wilk test was used to verify for normal distribution of phenotypic data. Non-parametric Kruskal-Wallis one-way analysis of variance (Kruskal and Wallis 1952) was used to assesses global differences in phenotypic traits at a 5% level of significance ($P<0.05$) among BC_2F_1s lines and controls. Further analysis included the Wilcoxon signed rank test for pairwise comparisons using FDR (false discovery rate) correction to group samples by significant similarity ($P>0.05$).

High-throughput genotyping, analysis and data filtering

Genomic DNA of 23 samples including 10 BC_2F_1 lines, diploid controls (*A. stenosperma* and *A. batizocoi*) and tetraploid controls (BatSten1, Runner-886, 5-646-10, 13-1014, TifGp-2, Tifrunner) (Table 4.2) were extracted from leaves using the DNeasy *Plant* Mini *Kit* (*QIAGEN*) according to manufacturer's instructions. DNAs were quantified with PicoGreen and samples were submitted for genotyping with the second version of the 'Axiom_Arachis' 47K high-density SNP array (Clevenger et al. 2018; Korani et al. 2019). The genotypic data was extracted, processed and analyzed using the Axiom Analysis Suite 2.0 software (http://www.affymetrix.com). Output was analyzed using custom Unix scripts (Script 4.S1) and the resulting data were visualized as a color

map in Microsoft Excel. The strategy to identify polymorphic SNP markers included two main steps:

Firstly, SNP assay results were extracted from SNP calling using a panel of controls and BC_2F_1s. This set of markers was filtered to reveal polymorphic SNP markers for parental genotypes (BatSten1 ≠ Runner-886) and genotypes were replaced by numbers (1 for BatSten, 2 for Runner-886 genotype and 3 for a different genotype). Then, SNP assays from markers present in the genetic map (*A. stenosperma*-specific and *A. batizocoi*-specific markers) (Chapter 2, Ballén-Taborda et al. Submitted) were recovered. Using the Statistical package R and the 'mydist' function, genotypic data was used to perform principal component analysis (PCA) to evaluate the clustering behavior of the backcrossed lines in relation to the wild, synthetic and cultivated genotypes.

Pollen viability evaluation

To evaluate pollen quality (PV) of the BC_2F_1s in comparison with control genotypes (Table 4.3), flowers were collected early morning (between 8 am and 10 am) and dyed with acetocarmine staining solution (Hestop-Harrison 1992). Briefly, keel (fused petals covering the stamen) of individual flowers was removed to expose the anthers. Anthers were collected into 1.5 ml tubes containing acetocarmine solution for examination. Stained pollen grains were observed and counted under microscope at 40X magnification. Viable pollen was identified by having bold red coloring and round shape, and nonviable grains were colorless and misshapen or oblong (Vara Prasad et al. 1999) (Fig. 4.3). Pollen viability from 10 individual flowers (reps) per genotype (% of viable pollen grains) was assessed as the ratio of the number of viable pollen grains to the

number of total pollen grains multiplied by 100 (Gaaliche et al. 2013; Tareq et al. 2015). A Shapiro-Wilk test was used to verify normality. Non-parametric Kruskal-Wallis test (Kruskal and Wallis 1952) was use to estimate differences in pollen viability ($P<0.05$) among BC_2F_1s lines and controls. Wilcoxon signed rank test using FDR (false discovery rate) correction was performed to group the samples by statistical similarity ($P>0.05$).

Results

Root-knot nematode resistance evaluation

9 BC_2F_1s lines harboring QTL from *A. stenosperma*, 5 susceptible and 3 resistant controls were evaluated for resistance using a detached leaf bioassay (Table 4.1). Initially, 15 leaves (Reps) were collected for each of the 17 genotypes to be evaluated. Rooted leaves were inoculated with ~3,000 J2/ml after 30 days of root induction; at this time the roots were on average 5 cm in length. During the assay, leaves were evaluated for nematode penetration and development of nematodes inside the roots at 6-8 DAI and 13-15 DAI. Staining with cotton blue allowed us to observe that *M. arenaria* was able to penetrate and infect the roots at early stages in the life cycle in all genotypes, and the establishment of feeding sites on susceptible genotypes, such us *A. hypogaea* Runner-886 (Fig. 4.4). A total of 8 reps were screened for galls and egg masses production at 36-48 DAI. Number of galls and number of eggs masses in relation to root weight were used as parameters to assess resistance/susceptibility to RKN (Galls/g and Eggs masses/g) (Guimaraes et al. 2017).

As expected, all *A. hypogaea* susceptible cultivars (Tifrunner, Runner-886, 5-646-10 and 13-1014) showed this trend, along with a single BC_2F_1 (BC_2F_1_Seed17); *M.*

arenaria was able to infect the roots (Fig. 4.4), reach maturity and complete the life cycle to produce eggs (Fig. 4.5 and Fig. 4.6) (Guimaraes et al. 2017). We did not observe significant difference between genotypes (Wilcoxon Test P>0.05) (Fig. 4.7). The number of galls per gram of root (galls/g) ranged between 74.49 ± 64.84 and 198.98 ± 56.03 and the number of egg masses per gram of root (eggs masses/g) varied between 4.91 ± 6.38 and 16.95 ± 14.82 (Table 4.1).

In contrast, the resistant wild species *A. stenosperma*, *A. batizocoi*, the induced allotetraploid BatSten1 and six backcrossed (BC) lines had very low numbers of eggs and galls, and did not differ significantly (P>0.05) (BC_2F_1:C2633-2_3(16), BC_2F_1_Seed1, BC_2F_1_Seed8, BC_2F_1_Seed9, BC_2F_1_Seed28 and BC_2F_1_Seed33) (Fig. 4.7). In general, these materials exhibited high levels of resistance to RKN. Although, nematodes were able to penetrate the roots (Fig. 4.4), no or few galls and no egg production was observed in the infected roots (Fig. 4.5 and Fig. 4.8). In summary, galls/g fluctuated between 0.00 ± 0.00 and 18.59 ± 37.89 and eggs masses/g was null in all cases (Table 4.1).

Although, the susceptible genotype TifGp-2 and the BC lines BC_2F_1_Seed2 and BC_2F_1_Seed7 could not be statistically tested due to missing data (Fig. 4.7 and Table 4.1, Indicated by "*"), we did observe some resistance/susceptibility. TifGp-2 was susceptible, as expected, it had an average 196.27 ± 96.67 galls/g and 22.77 ± 21.95 eggs masses/g. BC_2F_1_Seed7 showed moderate resistance with an average of 16.28 ± 23.02 galls/g and no eggs masses/g observed (Fig. 4.4, Fig. 4.5, Fig. 4.6, Fig. 4.7 and Table 4.1). Differently, BC_2F_1_Seed2 showed higher levels of resistance to RKN as no nematodes were observed inside the roots at early stages in the life cycle (Fig. 4.4), a few galls/g (6.09 ± 12.18) and no egg masses were found at later stages (Fig. 4.5, Fig. 4.7,

Fig. 4.8 and Table 4.1). No phenotypic data was collected for BC_2F_1_Seed15 line due to TSWV (tomato spotted wilt virus) infection that prevented the collection of healthy leaves.

High-throughput genotyping

BC_2F_1s lines carry different combination of resistant segments from *A. stenosperma* in Chr A02 and A09. This was assessed based on 22 KASP assays distributed in these chromosome locations (Chapter 3, Table 3.S1, gray shaded markers). In order to fully characterize the chromosome segments and identify to the most important ones, the BC_2F_1s lines and controls were genotyped with the Affymetrix 'Axiom_Arachis' SNP array (Clevenger et al. 2018; Korani et al. 2019) (Table 4.2). A total of 1040 polymorphic SNP markers (present the genetic map, Chapter 2, Ballén-Taborda et al. Submitted) were recovered after filtering (Script 4.S1), 609 assigned to A-subgenome (*A. stenosperma*-specific markers) and 431 to B/K-subgenome (*A. batizocoi*-specific markers); The physical positions of the A-genome markers were determined according to the position of their homologues in the *A. duranensis* pseudomolecules and the B-genome markers based on the *A. ipaensis* pseudomolecules (https://www.peanutbase.org/) (Bertioli et al. 2016).

The principal component analysis (PCA) performed on the genotyping data, allowed us to observe that the BC_2F_1s clustered very close to the *A. hypogaea* genotypes. Backcrossed lines showed a recurrent parent genome (RPG) recovery between 75.7% to 87.2%. That is, they still carry 8.3% to 15.5% of wild donor genome (Fig. 4.9).

Pollen viability evaluation

Pollen viability (PV) of wild accessions *A. stenosperma*, *A. batizocoi*, the induced allotetraploid BatSten1, recurrent parents 5-646-10 and 13-1014 and 9 BC_2F_1 lines was evaluated, as an indication of fertility. Individuals showed varying degrees of pollen viability, ranging from 65.58% to 94.90% (Table 4.3). In control genotypes, PV varied from 71.69% to 94.90% and the BC_2F_1s, it fluctuated from 65.58% and 89.81%, reflecting the genetic distance of the parental genotypes. Although, significant difference was observed between the genotypes according to Kruskal-Wallis test and Wilcoxon Test ($P>0.05$), no grouping trend was observed. In other words, we observed a high pollen viability in the cultivated genotypes (5-646-10 and 13-1014), *A. stenosperma* and some BC_2F_1s, and lower pollen viability for BatSten1, *A. batizocoi* and other BC_2F_1s (Fig. 4.10). No results are presented for BC_2F_1_Seed15 due to the TSWV infection that affected the production of flowers.

Discussion

A. stenosperma, a peanut wild diploid relative, has been shown to be resistant to multiple pests and pathogens including the root-knot nematode, *M. arenaria* (Neal) Race 1. Genes involved in hypersensitive response and secondary metabolite production for defense against nematode infection have been described in gene expression analysis and histology experiments (Guimarães et al. 2010; Morgante et al. 2013; Proite et al. 2008; Proite et al. 2007). Previously, genetic loci involved in resistance were localized on chromosomes A02, A04 and A09 in *A. stenosperma* in a diploid cross (Leal-Bertioli et al. 2016). Later, using a tetraploid F_2 population between Runner-886 and BatSten1

([*Arachis batizocoi* x *A. stenosperma*]$^{(2n=4x=40)}$), resistance in A02 and A09 were validated. Most importantly, QTL in bottoms of A02 and A09 were found to provide strong resistance. Putative QTL on A04 did not contribute to improve nematode resistance (Chapter 2) (Ballén-Taborda et al. Submitted).

Here, we tested RKN resistance in 9 BC_2F_1s that were also used as male parents for a third cycle of introgression (BC_3F_1s). To assess resistance, we used *ex vitro* rooted-detached leaf bioassay taking advantage of the ability in peanut to generate roots spontaneously from petioles of detached leaves (Guimaraes et al. 2017). This is a rapid, efficient, simple and low-cost screening technique that has been widely used to assess the expression of genes in response to external stimuli. This method represents an alternative to whole plant phenotyping for the evaluation of materials under controlled conditions and with an uniform pathogen/pest density (Collier et al. 2005; Guimaraes et al. 2017; Shanks and Morgan 1999; Sharma et al. 2005). In peanut, this method has been used to study root-knot nematode resistance in *Agrobacterium rhizogenes*-transformed cultivars (Chu et al. 2014; Guimaraes et al. 2017), resistance to late leaf spot (Fávero et al. 2009; Michelotto et al. 2015), early leaf spot and rust in cultivated germplasm (Fávero et al. 2009; Leal-Bertioli et al. 2009).

Initially, as part of the backcrossing process, the BC_2F_1s were selected using KASP genotyping assays targeting the location of the QTL mapped in the diploid (Chapter 2 - 3 and Table 3.S1, gray shaded markers). This genotyping approach was used to determine true backcross progeny with targeted genomic regions, but the downside was that it gave a limited image of *A. stenosperma* introgressions across the genome. To obtain a more comprehensive view of the introgressions, particularly the flanking regions

of the QTL, the BC_2F_1s were genotyped using the Affymetrix 'Axiom_Arachis' SNP array (Clevenger et al. 2018; Korani et al. 2019). These BC_2F_1 backcrossed lines showed a recurrent parent genome (RPG) recovery between 75.7% to 87.2%, close to what is expected for second generation of backcrossed lines (Fig. 4.9) (Fonceka et al. 2012; Hasan et al. 2015; Hospital 2003). Combination of phenotypes and high-resolution genotypes allowed us to better refine the targeted QTL and define the most significant genomic segments for RKN resistance.

Visualization of the genotypic data allowed us to track introgressions from *A. stenosperma* (Fig. 4.11.A,B), especially in the location of two QTL associated with nematode resistance in chr A02 and A09 (Ballén-Taborda et al. Submitted; Leal-Bertioli et al. 2016). According to Ballen-Taborda et al. (Submitted) (Chapter 2), the segment located at the bottom of chromosome A02 contributes up to a 98.2% reduction in nematode multiplication. This QTL is homologous to a region in the reference genome of diploid progenitor *A. duranensis*, that harbors multiple resistance-genes (R-genes) (Bertioli et al. 2016), located between 85.3Mbp and 93.8Mbp (8.5Mbp) (Fig. 4.11. A-C, translucent red blocks). Several of the R-genes present in this region encode for putative disease resistance protein families (TIR-NBS-LRR class) associated with plant immune defenses (Chisholm et al. 2006; Feys and Parker 2000; Tameling et al. 2002). In order to illustrate the potential usefulness and importance of introgressed genes from wild species, a TIR-NBS-LRR gene for RKN resistance from *A. cardenasii* is constitutively expressed in the cultivar Tifguard but absent in the susceptible cultivar Gregory (Clevenger et al. 2017).

The A09-QTL confers strong nematode resistance but no R-genes are found in the corresponding region of the *A. duranensis* genome (Bertioli et al. 2016). In the present study, the segment between 113.5Mbp and 120.0 Mbp (6.5Mbp) of A09 appears to play an essential role for peanut defense against RKN infection, especially when the A02-QTL is absent (Fig. 4.11.A-C, translucent red blocks). The QTL in A09 may correspond to the one from *A. cardenasii* (3.4 - 113.7 Mbp) though at a slightly different position on *A. duranensis* pseudomolecules (peak ~116.3 Mbp). The QTL on A04 did not appear to contribute to improved nematode resistance (Ballén-Taborda et al. Submitted; Bertioli et al. 2016).

According to the genotyping data, eight BC_2F_1s lines should be resistant as segments in Chr in A02-bottom and A09-bottom, or both, are present in their genomes (BC_2F_1:C2633-2_3(16), BC_2F_1_Seed1, BC_2F_1_Seed2, BC_2F_1_Seed8, BC_2F_1_Seed9, BC_2F_1_Seed15, BC_2F_1_Seed28 and BC_2F_1_Seed33) (Fig. 4.11.B,C, translucent red blocks and Table 4.2). We confirmed this phenotypically, except for BC_2F_1_Seed2 and BC_2F_1_Seed15 (Fig. 4.7 and Table 4.1, Indicated by "*"). During early life cycle stages nematodes were able to penetrate the roots (Fig. 4.4), but likely the QTL on A02 and A09 or both played a role to halt the completion of the nematode life cycle as no or few galls and no egg production was observed (Fig. 4.5, Fig. 4.7, Fig. 4.8 and Table 4.1). BC_2F_1_Seed28 was resistant but it harbors a fragment at bottom of Chr A09 and the A02 segment is absent, which indicates that the bottom of A09 is key for disease protection (Fig. 4.11.B,C and Table 4.2). Although, the BC_2F_1_Seed33 line was found to be resistant to RKN, we cannot conclude that the *A. stenosperma* resistance segment present in this line is fully responsible for the resistance (Fig. 4.7, Fig. 4.11 and Table 4.1), as the

recurrent parent 13-2113 used to develop this line harbors resistance from *A. cardenasii* (Drs. Ye (Juliet) Chu and Corley Holbrook, personal comm.). The resistance from *A. cardenasii* located in Chr A09 could be contributing to the desired phenotype (Chu et al. 2016). Lines harboring segments only in A04 or/and top of A09 were expected to be susceptible. This was confirmed phenotypically for BC_2F_1_Seed17 and some evidence for it in BC_2F_1_Seed7 (Fig. 4.11.B,C and Table 4.2), where the nematode was able to penetrate (Fig. 4.4), reach maturity and complete the life cycle (Fig. 4.5, Fig. 4.6, Fig. 4.7 and Table 4.1). The high-density SNP array was used to more finely map these QTL and that those markers can be used to facilitate the introgression of RKN resistance from *A. stenosperma* in breeding programs (Fig. 4.11 D).

Although the main focus of this study was to correlate the phenotypic data for RKN resistance and high-throughput SNP information, pollen fertility (PV) of BC_2F_1s and controls was also characterized. For the most part, our results agreed with that of Leal-Bertioli et al (2015) as cultivated peanut genotypes and *A. stenosperma* showed a high pollen fertility (average of 91.94%) and most of the BC_2F_1 lines have lower pollen viability (79.19%) (Fig. 4.10 and Table 4.3), which suggests that the K genome of *A. batizocoi* (Moretzsohn et al. 2013; Robledo and Seijo 2010) might influence fertility due to incompatible K-B genome interactions (Leal-Bertioli et al. 2015). Measuring pollen fertility may be used to identify the lines with a potential to produce large number of seeds.

Conclusions

The characterization of root-knot nematode resistance from *A. stenosperma* incorporated into advanced peanut backcrossed lines (BC_2F_1s) is an important step to validate the association of QTL in A02 and A09 with nematode resistance (Ballén-Taborda et al. Submitted; Leal-Bertioli et al. 2016). This present study allowed us to delineate the chromosomal segments in A02-bottom and A09-bottom to ~8Mbp and ~6.5 Mbp regions, respectively. With this information it was possible to accurately select progeny harboring the most important QTL for further backcrosses and advancement. Although no QTL were detected in other regions of these chromosomes where R-genes were reported (Bertioli et al. 2016), it might be useful to explore these regions for resistance to other peanut diseases and pests. This research will contribute to the production of peanut varieties that incorporate a new source of resistance to RKN from the wild species *A. stenosperma*, which will help to ensure continued protection of the peanut crop from losses due to RKN.

Acknowledgements

I would like to acknowledge the contribution of Drs. Patricia Timper and Larissa Arrais during the nematode phenotyping experiments and to Rida Salim Osman and Victoria Morris for the pollen viability evaluation. To all members of Jackson and Bertioli labs for laboratory and greenhouse assistance.

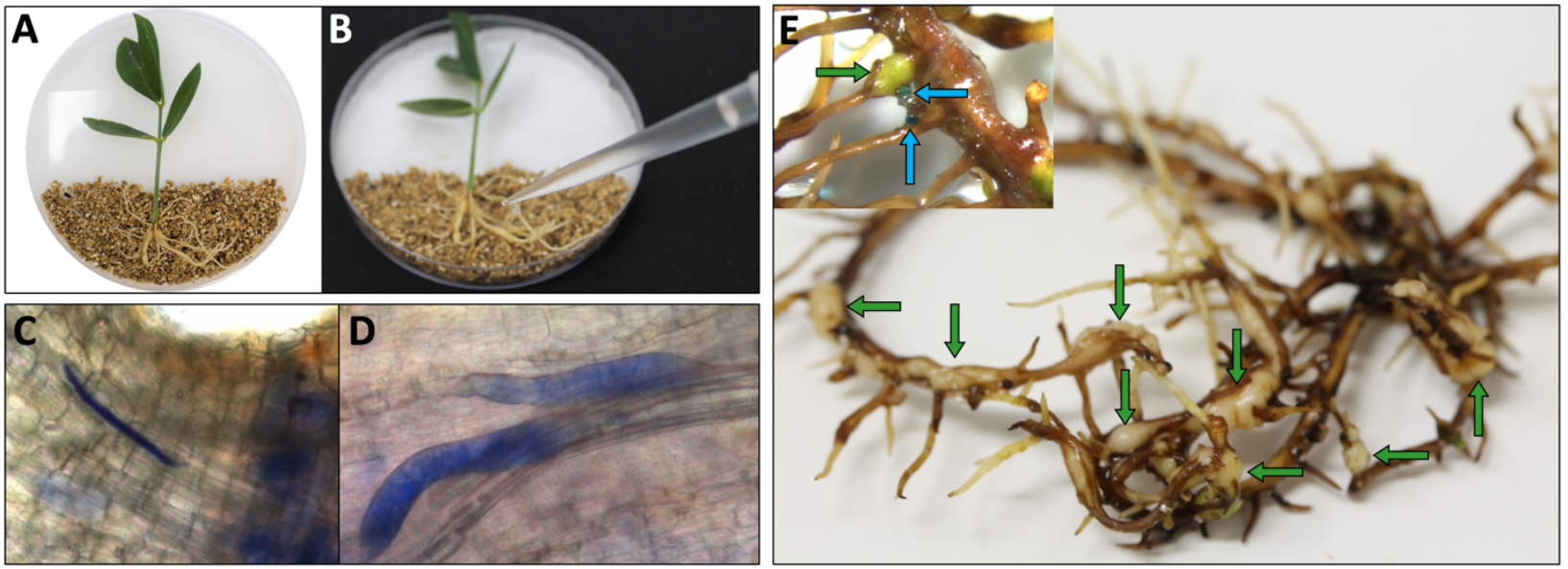

Figure 4.1. Overview of the steps for Root-knot nematode (RKN) *Meloidogyne arenaria* evaluation using detached leaf bioassays. Roots emergence in the detached leaves **(A)**; Inoculation with 1 ml of nematode suspension at ~3,000 J2/ml after 30 days of root induction **(B)**; Second-stage juveniles (J2) **(C)** and swollen J3/J4 (SJ3/SJ4) stained with cotton blue solution **(D)**; Galls and egg masses observed and quantified in the susceptible cultivar *A. hypogaea* Runner-886. Eggs masses stained with Erioglaucine solution. Galls are indicated by green arrows and egg masses by blue arrows **(E)**.

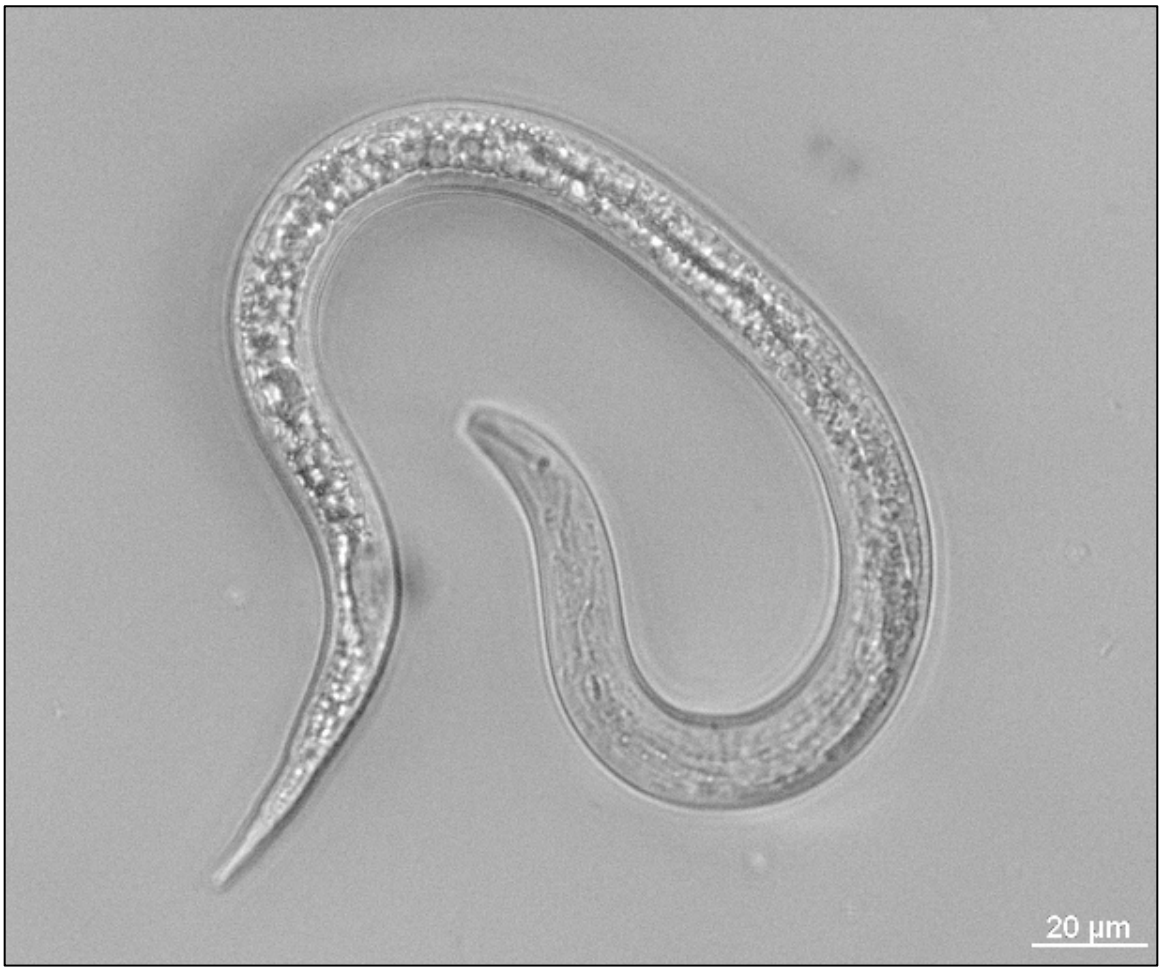

Figure 4.2. *Meloidogyne arenaria* (Neal) Chitwood race 1. Second-stage juvenile (J2). 20X magnification.

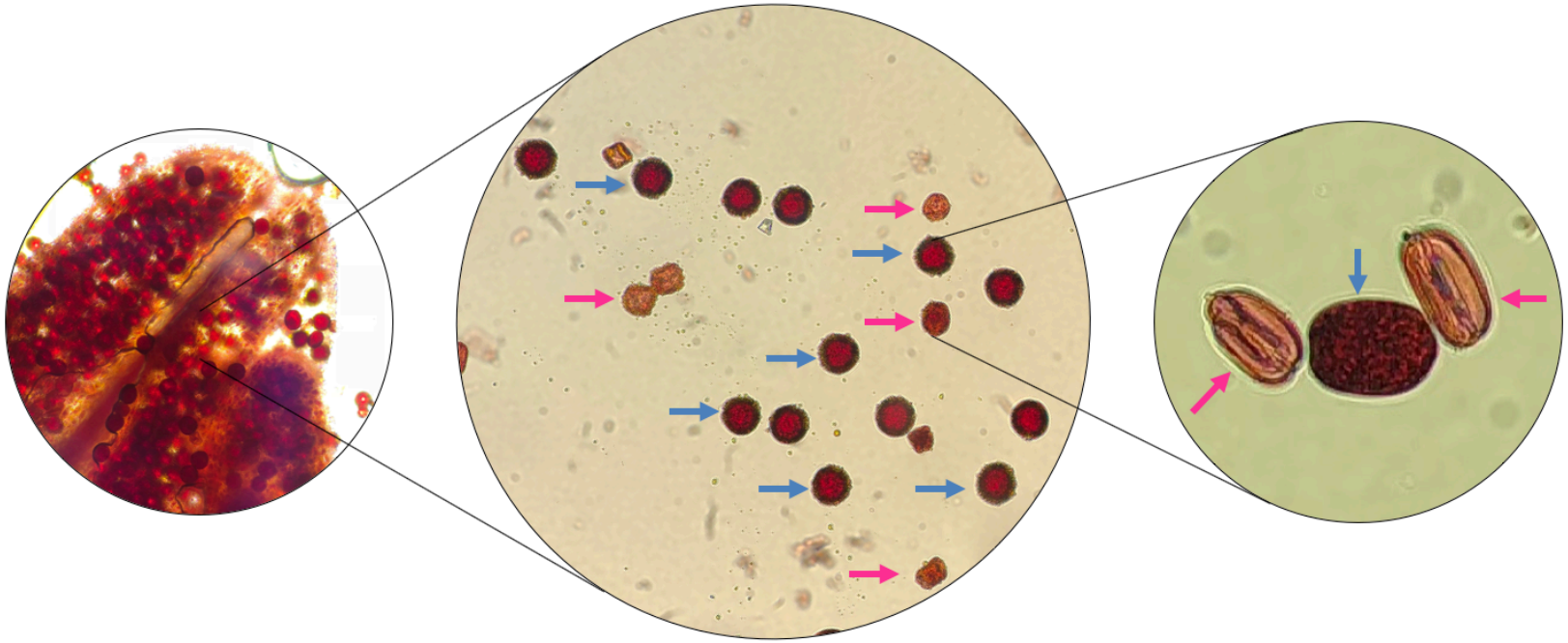

Figure 4.3. Images of pollen grains dyed with an acetocarmine solution and observed under microscope. Pollen grains inside the peanut anthers (left image); floating viable pollen (red color and round shape) and nonviable pollen grains (colorless and oblong shape) indicated by blue and magenta arrows, respectively (middle and right pictures).

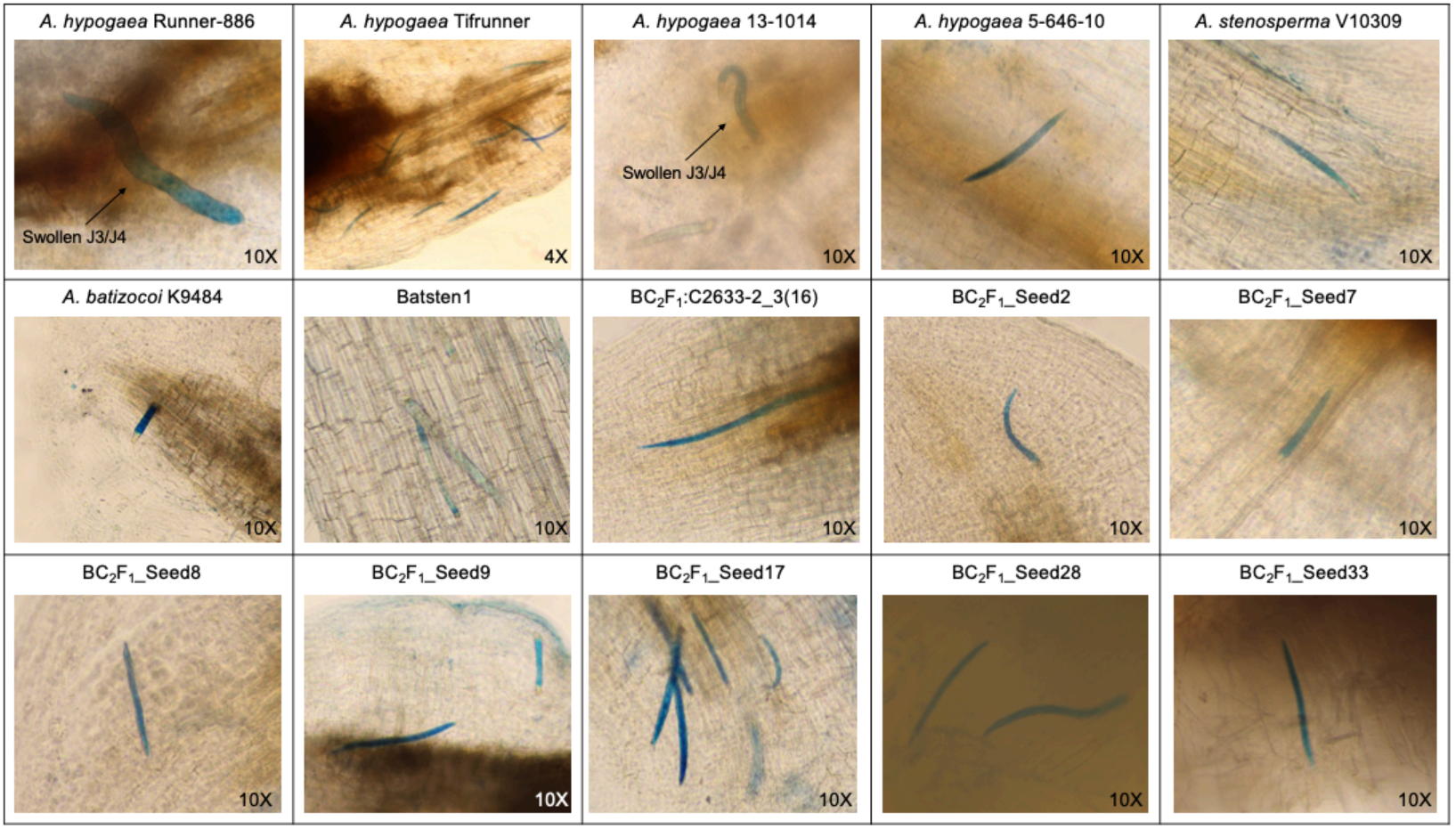

Figure 4.4. Images of *M. arenaria* penetration and infection inside the hairy roots of BC_2F_1s and control genotypes at 13-15 DAI. Roots stained with cotton blue solution were observed under dissecting stereoscope and microscope. Establishment of feeding sites and swollen J3/J4 (SJ3/SJ4) was detected in Runner-886. Photos taken at 4X and 10X magnification.

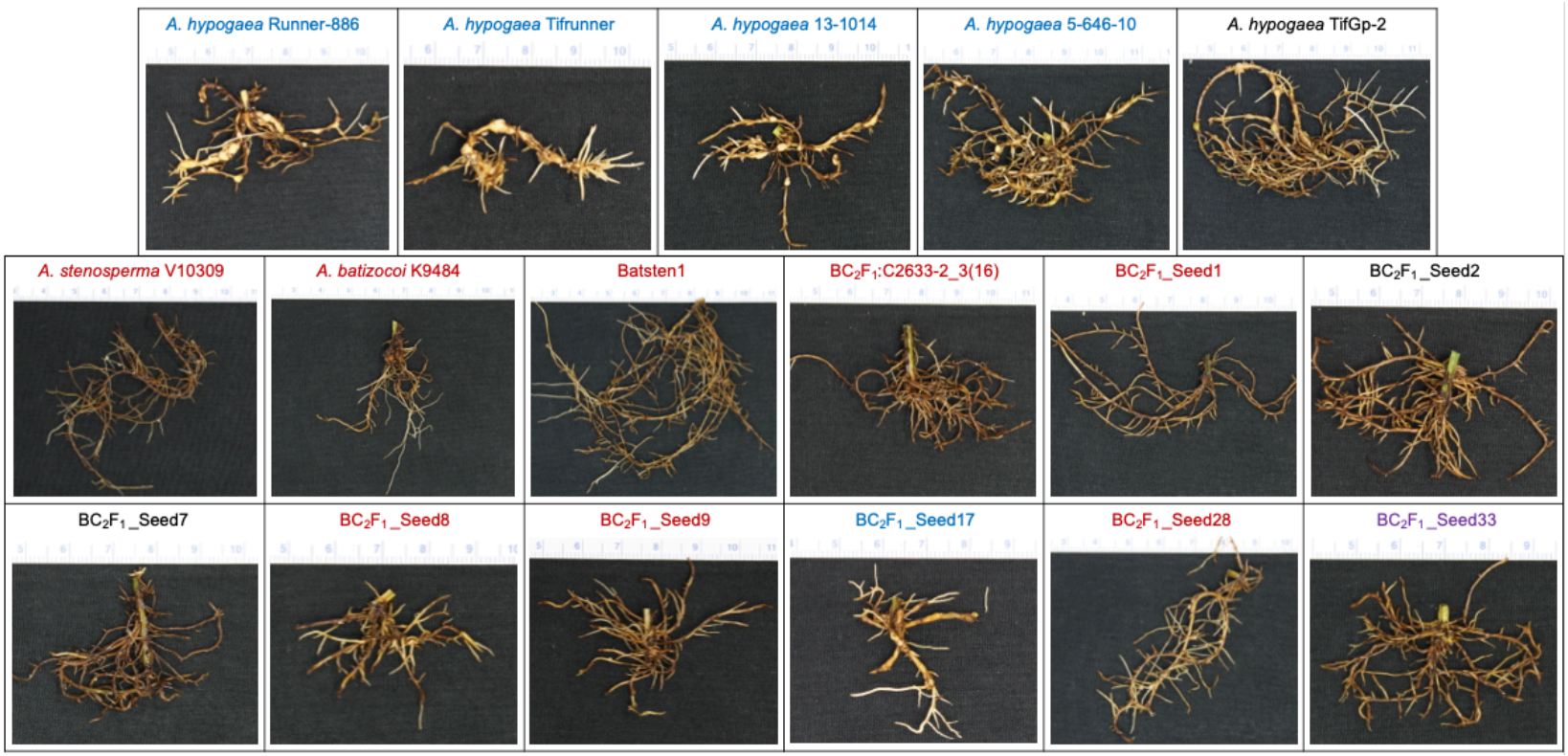

Figure 4.5. Images of root systems of BC_2F_1s and control genotypes at 36-48 DAI. Names in blue indicated the susceptible genotypes after nematode infection on the detached leaf bioassays; names in red indicate resistant genotypes; name in purple indicate a resistant BC_2F_1 line with a resistant recurrent parent 13-2113 on its pedigree; and names in black indicated genotypes not statistically test due to missing data. Ruler on top of each image in centimeters (cm).

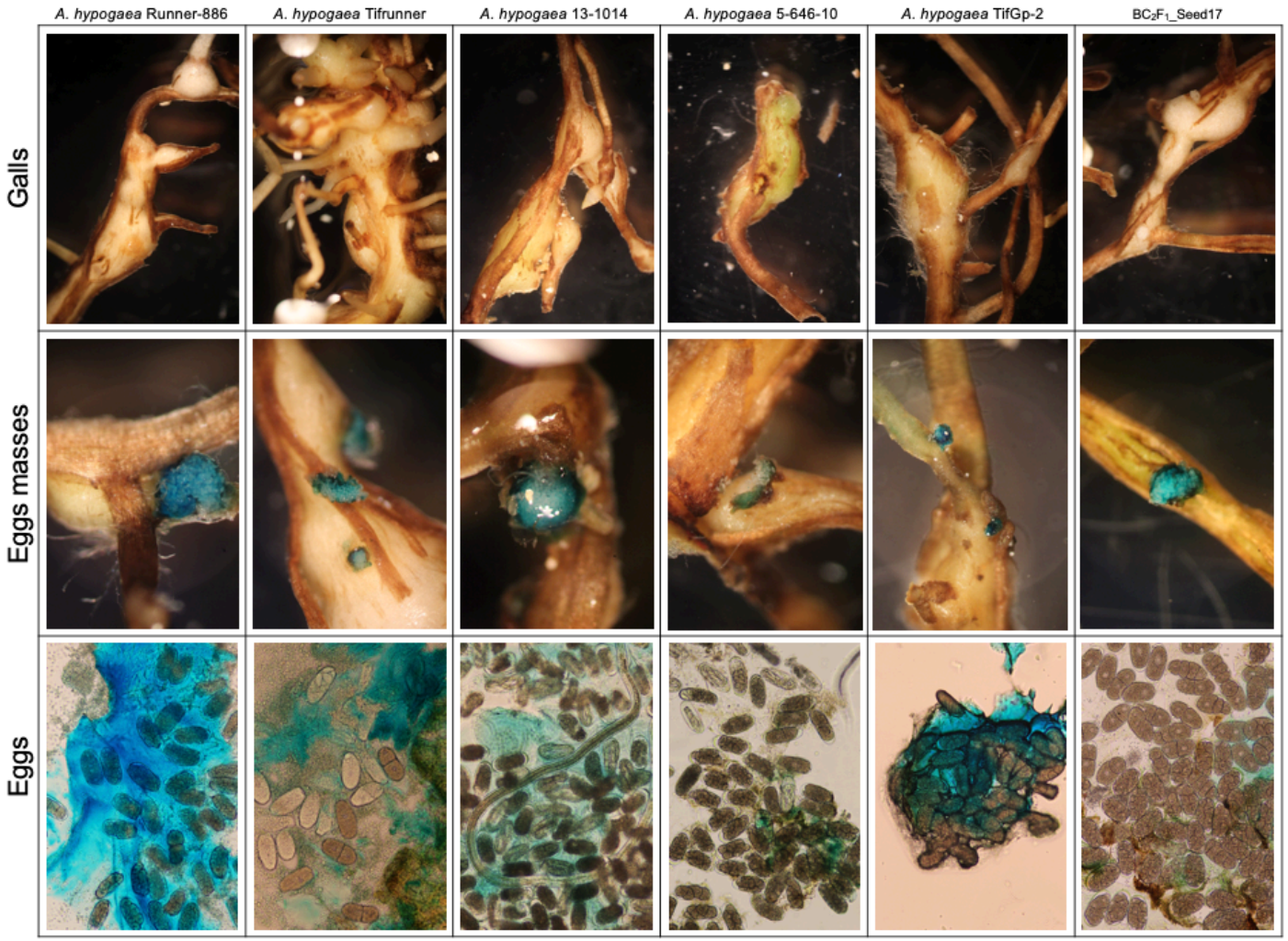

Figure 4.6. Images of galls and eggs observed in susceptible genotypes at 36-48 DAI. Galls observed under dissecting microscope (first row of images); Eggs masses stained with Erioglaucine solution observed under dissecting microscope (second row of images); Eggs observed under microscope at 10X magnification (third row of images).

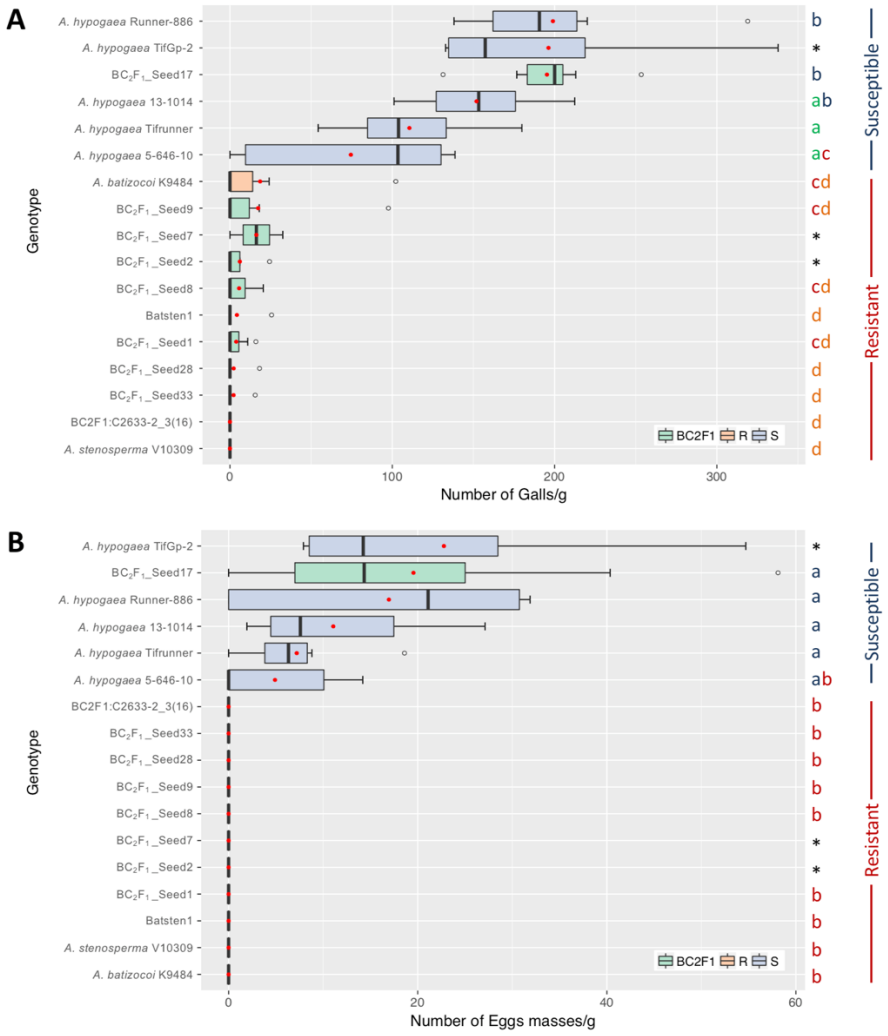

Figure 4.7. Boxplot diagrams for number of galls per gram of root (Number of Galls/g) **(A)** and number of eggs masses per gram of root (Number of Eggs masses/g) **(B)** of 9 BC_2F_1 lines, wild accessions, cultivated genotypes and induced allotetraploid BatSten1. Boxes with the same letter do not differ significantly ($P<0.05$). Light green boxes indicate BC_2F_1 lines, light orange boxes resistant controls (R) and light blue color represent susceptible cultivars (S). Black bars across boxes indicate the median and red dot the mean. Left and right ends represent the highest and lowest values. Circles represent outliers. Lines with "*" were not included in statistical analysis due to missing data.

Figure 4.8. Root images of resistant genotypes at 36-48 DAI observed under dissecting microscope. No galls or Eggs masses observed; Names in red represent lines confirmed resistant phenotypically; Name in purple indicate a resistant BC_2F_1 line with a resistant recurrent parent 13-2113 on its pedigree; and names in black indicated genotypes not statistically test due to missing data.

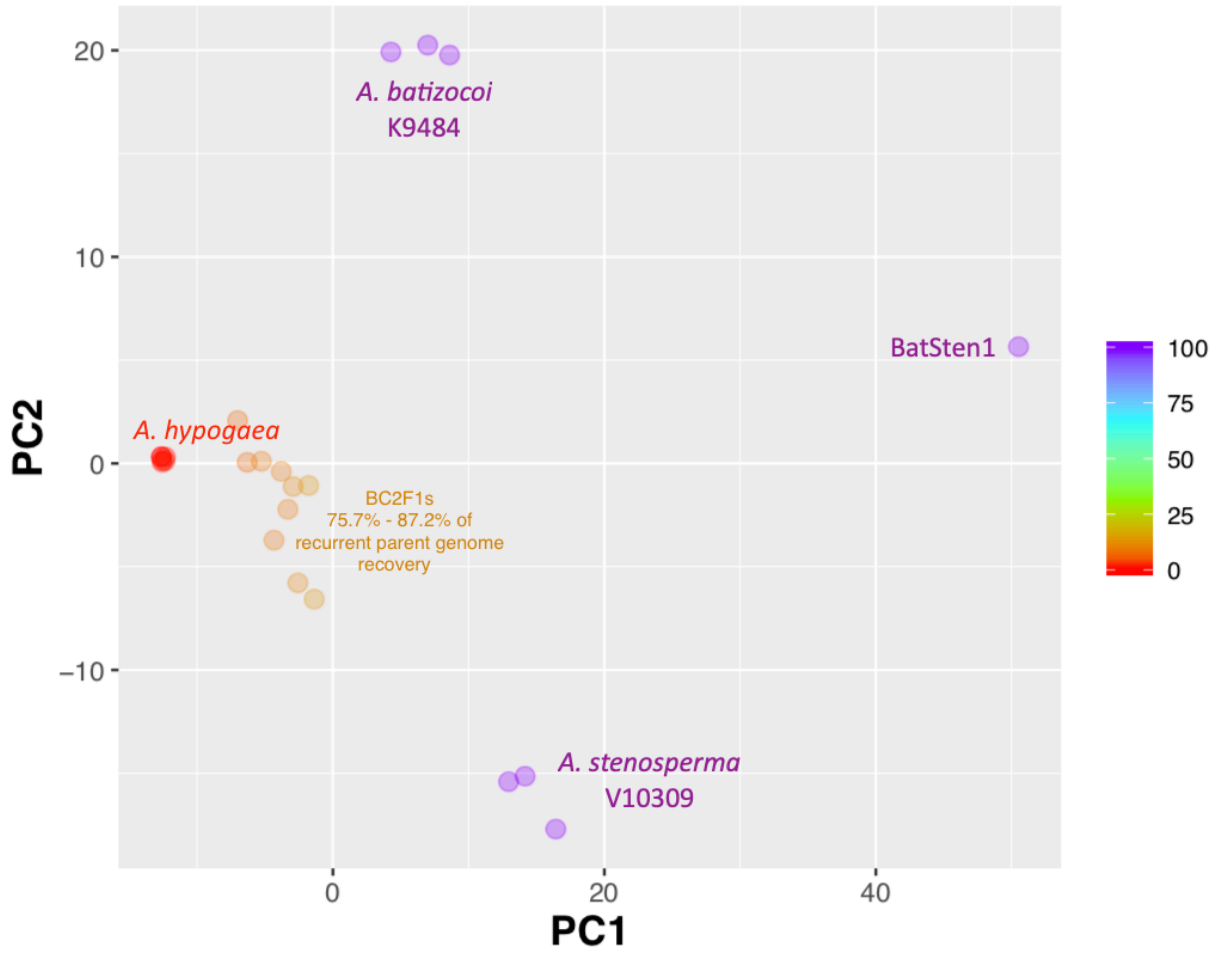

Figure 4.9. Principal components analysis (PCA) for high-throughput genotyping data of 10 BC_2F_1s lines (orange cluster), diploid controls *A. stenosperma*, *A. batizocoi*, and synthetic allotetraploid BatSten1 (purple clusters) and *A. hypogaea* genotypes (red cluster). Percentage of *A. hypogaea* genome is indicated for the BC_2F_1s cluster. The legend on the right side indicates the percentage of wild genome. Alleles were coded to maximize the difference between *A. hypogaea* and BatSten1 (see Methods for details).

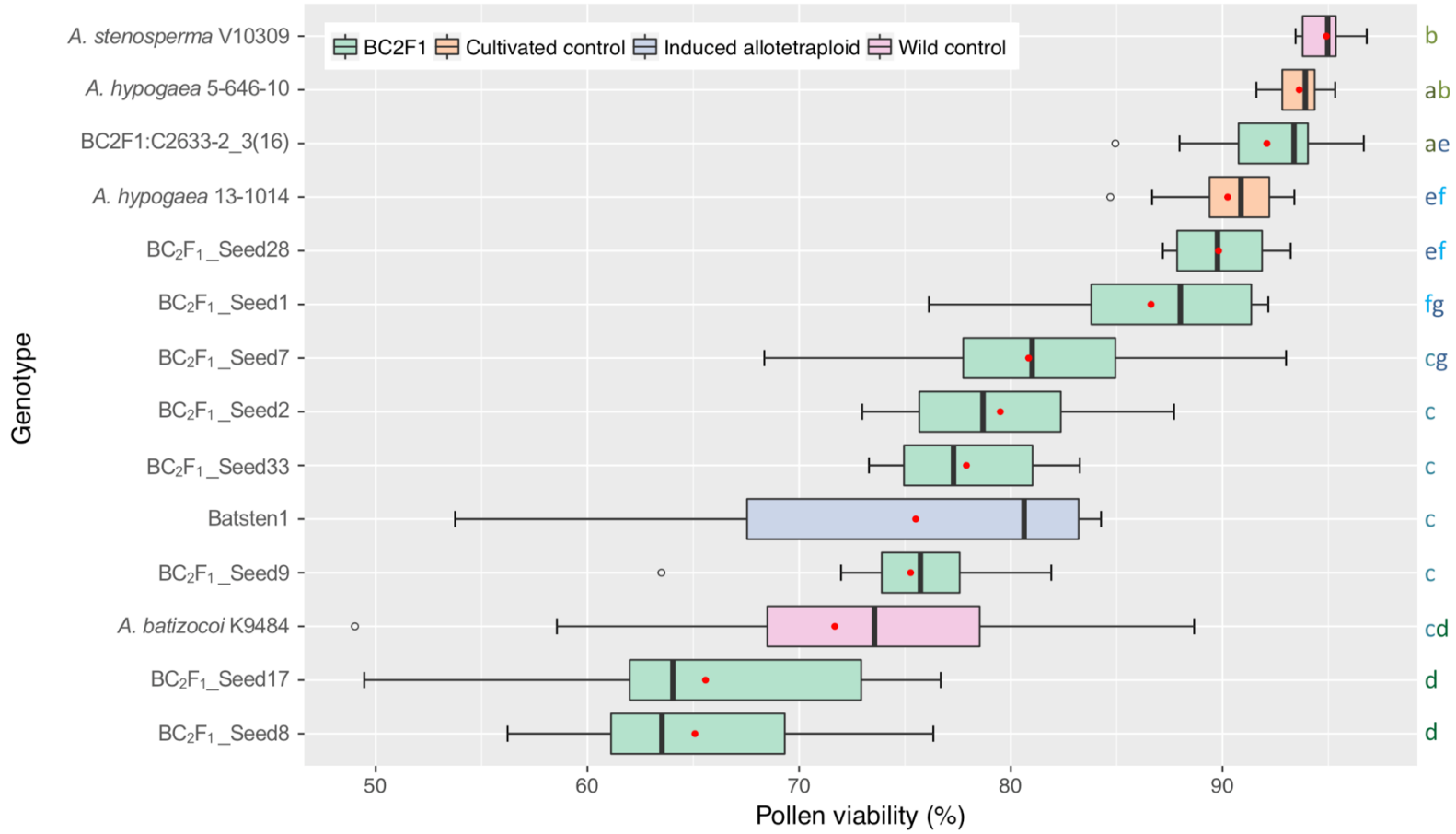

Figure 4.10. Boxplot diagram of estimated pollen viability (%) of 9 BC_2F_1 lines (light green), cultivated controls (light orange), induced allotetraploid BatSten1 (light blue) and wild accessions (light pink). Boxes with the same letter do not differ significantly ($P<0.05$). Black bars across boxes indicate the median and red dot the mean. Left and right ends represent the highest and lowest values. Circles represent outliers.

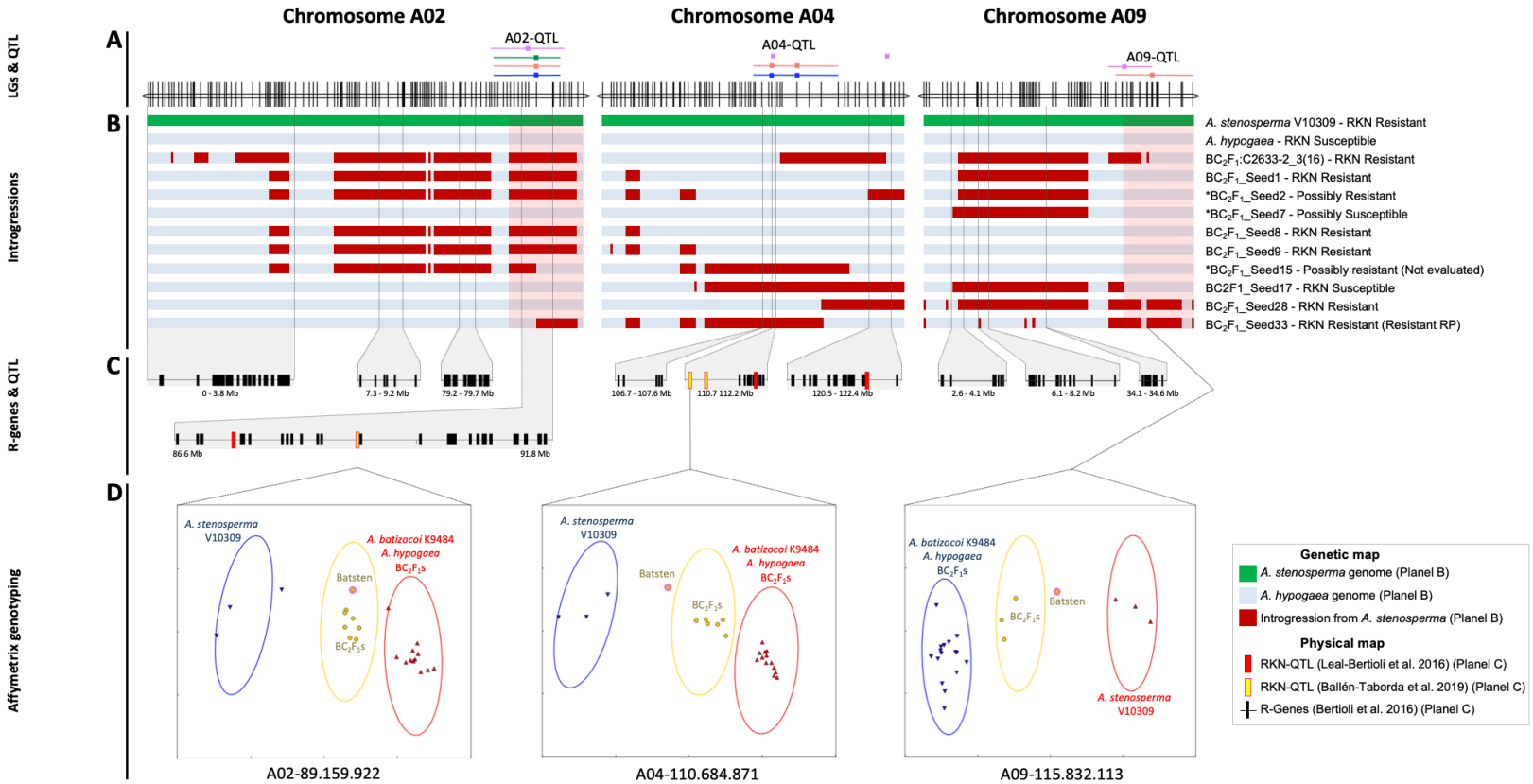
Chromosome A02
Chromosome A04
Chromosome A09
A
LGs & QTL
A02-QTL
A04-QTL
A09-QTL
B
Introgressions
A. stenosperma V10309 - RKN Resistant
A. hypogaea - RKN Susceptible
BC2F1:C2633-2_3(16) - RKN Resistant
BC2F1_Seed1 - RKN Resistant
*BC2F1_Seed2 - Possibly Resistant
*BC2F1_Seed7 - Possibly Susceptible
BC2F1_Seed8 - RKN Resistant
BC2F1_Seed9 - RKN Resistant
*BC2F1_Seed15 - Possibly resistant (Not evaluated)
BC2F1_Seed17 - RKN Susceptible
BC2F1_Seed28 - RKN Resistant
BC2F1_Seed33 - RKN Resistant (Resistant RP)
C
R-genes & QTL
0 - 3.8 Mb
7.3 - 9.2 Mb
79.2 - 79.7 Mb
106.7 - 107.6 Mb
110.7 112.2 Mb
120.5 - 122.4 Mb
2.6 - 4.1 Mb
6.1 - 8.2 Mb
34.1 - 34.6 Mb
86.6 Mb
91.8 Mb
D
Affymetrix genotyping
A. stenosperma V10309
A. batizocoi K9484
A. hypogaea
BC2F1s
Batsten
A02-89.159.922
A04-110.684.871
A09-115.832.113
Genetic map
A. stenosperma genome (Planel B)
A. hypogaea genome (Planel B)
Introgression from A. stenosperma (Planel B)
Physical map
RKN-QTL (Leal-Bertioli et al. 2016) (Planel C)
RKN-QTL (Ballén-Taborda et al. 2019) (Planel C)
R-Genes (Bertioli et al. 2016) (Planel C)

Figure 4.11. Graphical representation of root-knot nematode resistance introgression in chromosomes A02, A09 and A04 (associated with susceptibility) in peanut backcrossed lines (BC_2F_1). Linkage groups A02, A04 and A09 and genetic location of QTL previously characterized (Ballén-Taborda et al. Submitted; Leal-Bertioli et al. 2016) **(A)**; Introgressions from *A. stenosperma* in BC_2F_1 lines are indicated in red; *A. stenosperma* genome in green; *A. hypogaea* genome in light blue; On the side, the names of BC_2F_1s and their expected phenotype base on phenotyping and genotyping experiments (resistant and susceptible); "*" indicates genotypes not statistically test due to missing data or not evaluated, and the suggested phenotype base on genotype; Translucent red blocks at bottom of chr A02 and bottom of A09 represent key segments for nematode resistance **(B)**. Location of R-genes clusters: individual R-genes as black vertical lines (Bertioli et al. 2016) and physical position of QTL previously characterized as red (diploid) and yellow (tetraploid) (Ballén-Taborda et al. Submitted; Leal-Bertioli et al. 2016) **(C)**. Examples of Affymetrix genotyping plots of three markers located in QTL regions in A02, A09 and A04 (associated with susceptibility) that can be used to facilitate introgression of RKN resistance segments; BC_2F_1 lines with *A. stenosperma* alleles inside yellow cluster **(D)**.

Table 4.1. Average and standard deviation of BC_2F_1s lines, susceptible and resistant controls for RKN resistance.

Genotype	Genotype (Simplified name)	Type	Root weight (g)	Galls	Egg masses	Galls/g	Egg masses/g
A. hypogaea 5-646-10	*A. hypogaea* 5-646-10	Cultivated (Recurrent parent)	0.46 ± 0.20	43.14 ± 41.23	3.14 ± 4.34	74.49 ± 64.84 (ac)	4.91 ± 6.38 (ab)
A. hypogaea 13-1014	*A. hypogaea* 13-1014	Cultivated (Recurrent parent)	0.41 ± 0.19	57.13 ± 17.23	4.13 ± 3.87	151.91 ± 39.06 (ab)	11.07 ± 9.00 (a)
A. hypogaea Tifrunner	*A. hypogaea* Tifrunner	Cultivated control	0.21 ± 0.07	25.95 ± 16.38	1.50 ± 1.38	110.56 ± 44.95 (a)	7.21 ± 6.37 (a)
A. hypogaea RunnerIAC886	*A. hypogaea* Runner-886	Cultivated control	0.30 ± 0.18	54.71 ± 27.79	7.13 ± 7.47	198.98 ± 56.03 (b)	16.95 ± 14.82 (a)
A. hypogaea TifGp-2	*A. hypogaea* TifGp-2	Cultivated (Recurrent parent)	0.30 ± 0.26	51.67± 31.36	4.50 ± 2.38	196.27 ± 96.67 (*)	22.77 ± 21.95 (*)
A. stenosperma V10309	*A. stenosperma* V10309	Diploid wild species	0.08 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 (d)	0.00 ± 0.00 (b)
A. batizocoi K9484	*A. batizocoi* K9484	Diploid wild species	0.15 ± 0.08	1.76 ± 2.72	0.00 ± 0.00	18.59 ± 37.89 (cd)	0.00 ± 0.00 (b)
BatSten1	BatSten1	Induced allotetraploid	0.24 ± 0.11	0.83 ± 2.04	0.00 ± 0.00	4.27 ± 10.47 (d)	0.00 ± 0.00 (b)
BC_2F_1:C2633-2_3(16)	BC_2F_1:C2633-2_3(16)	Backcrossed line - BC_2F_1	0.28 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 (d)	0.00 ± 0.00 (b)
BC_2F_1_BRD_C0049_Seed1	BC_2F_1_Seed1	Backcrossed line - BC_2F_1	0.24 ± 0.08	0.86 ± 1.46	0.00 ± 0.00	3.84 ± 6.72 (cd)	0.00 ± 0.00 (b)
BC_2F_1_BRD_C0049_Seed2	BC_2F_1_Seed2	Backcrossed line - BC_2F_1	0.27 ± 0.05	2.08 ± 4.17	0.00 ± 0.00	6.09 ± 12.18 (*)	0.00 ± 0.00 (*)
BC_2F_1_BRD_C0049_Seed7	BC_2F_1_Seed7	Backcrossed line - BC_2F_1	0.21 ± 0.04	3.50 ± 4.95	0.00 ± 0.00	16.28 ± 23.02 (*)	0.00 ± 0.00 (*)
BC_2F_1_BRD_C0049_Seed8	BC_2F_1_Seed8	Backcrossed line - BC_2F_1	0.13 ± 0.04	0.86 ± 1.46	0.00 ± 0.00	5.61 ± 9.60 (cd)	0.00 ± 0.00 (b)
BC_2F_1_BRD_C0050_Seed9	BC_2F_1_Seed9	Backcrossed line - BC_2F_1	0.19 + 0.07	4.76 ± 10.12	0.00 ± 0.00	17.35 ± 36.01 (cd)	0.00 ± 0.00 (b)
BC_2F_1_BRD_C0055_Seed15	BC_2F_1_Seed15	Backcrossed line - BC_2F_1	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Not evaluated
BC_2F_1_BRD_C0055_Seed17	BC_2F_1_Seed17	Backcrossed line - BC_2F_1	0.29 ± 0.08	56.33 ± 15.47	6.50 ± 6.93	195.30 ± 34.51 (b)	19.55 ± 20.17 (a)
BC_2F_1_BRD_C0057_Seed28	BC_2F_1_Seed28	Backcrossed line - BC_2F_1	0.21 ± 0.11	1.00 ± 2.83	0.00 ± 0.00	2.28 ± 6.44 (d)	0.00 ± 0.00 (b)
BC_2F_1_BRD_C0058_Seed33	BC_2F_1_Seed33	Backcrossed line - BC_2F_1	0.23 ± 0.07	0.43 ± 1.14	0.00 ± 0.00	2.22 ± 5.88 (d)	0.00 ± 0.00 (b)

Notes: Galls, Total number of galls; Egg masses, Total number of egg masses; Galls/g, Total number of galls per gram of root; Egg masses/g, Number of egg masses per gram of root; Cells for Galls/g and Egg masses/g columns with the same letter do not differ significantly ($p<0.05$). No results are presented for BC_2F_1_Seed15 line due to TSWV infection before the set-up of the experiment. Lines with an "*" in cells for Galls/g and Egg masses/g columns where not included in statistical analysis due to missing data.

Table 4.2. Genotyping information of BC_2F_1s lines, susceptible and resistant controls. Presence/absence of segments for resistance in Chr A02, A09 and A04 and phenotype is detailed.

Genotype	Genotype (Simplified name)	Type	No. of reps	Presence/absence of *A. stenosperma* Resistance segments	Phenotype
A. hypogaea 5-646-10	*A. hypogaea* 5-646-10	Cultivated (Recurrent parent)	1	Absent	S
A. hypogaea 13-1014	*A. hypogaea* 13-1014	Cultivated (Recurrent parent)	1	Absent	S
A. hypogaea Tifrunner	*A. hypogaea* Tifrunner	Cultivated control	1	Absent	S
A. hypogaea RunnerIAC886	*A. hypogaea* Runner-886	Cultivated control	2	Absent	S
A. hypogaea TifGp-2	*A. hypogaea* TifGp-2	Cultivated (Recurrent parent)	1	Absent	S
A. stenosperma V10309	*A. stenosperma* V10309	Diploid wild species	3	A02 top; **A02 Bottom**; A04; A09 top; **A09 Bottom**	R
A. batizocoi K9484	*A. batizocoi* K9484	Diploid wild species	3	None reported	R
BatSten1	BatSten1	Induced allotetraploid	1	A02 top; **A02 Bottom**; A04; A09 top; **A09 Bottom**	R
BC_2F_1:C2633-2_3(16)	BC_2F_1:C2633-2_3(16)	Backcrossed line - BC_2F_1	1	A02 top-L; **A02 Bottom**; A04_bottom; A09 top; **A09 Bottom**	R
BC_2F_1_BRD_C0049_Seed1	BC_2F_1_Seed1	Backcrossed line - BC_2F_1	1	A02 top; **A02 Bottom**; A09 top	R
BC_2F_1_BRD_C0049_Seed2	BC_2F_1_Seed2	Backcrossed line - BC_2F_1	1	A02 top; **A02 Bottom**; A04; A09 top	Possibly R
BC_2F_1_BRD_C0049_Seed7	BC_2F_1_Seed7	Backcrossed line - BC_2F_1	1	A09 top	Possibly S
BC_2F_1_BRD_C0049_Seed8	BC_2F_1_Seed8	Backcrossed line - BC_2F_1	1	A02 top; **A02 bottom**	R
BC_2F_1_BRD_C0050_Seed9	BC_2F_1_Seed9	Backcrossed line - BC_2F_1	1	A02 top; **A02 bottom**	R
BC_2F_1_BRD_C0055_Seed15	BC_2F_1_Seed15	Backcrossed line - BC_2F_1	1	A02 top; A02 Bottom-sml; A04	Possibly R
BC_2F_1_BRD_C0055_Seed17	BC_2F_1_Seed17	Backcrossed line - BC_2F_1	1	A04; A09 top	S
BC_2F_1_BRD_C0057_Seed28	BC_2F_1_Seed28	Backcrossed line - BC_2F_1	1	A04; A09 top; **A09 Bottom**	R
BC_2F_1_BRD_C0058_Seed33	BC_2F_1_Seed33	Backcrossed line - BC_2F_1	1	**A02 Bottom**; A04; **A09 Bottom**	R

Notes: R/S: Results from the RKN resistance detached leaf bioassay; S, RKN susceptible phenotype; R, RKN susceptible phenotype; Possible R/S, suggested phenotype base on genotype only. Segments in bold highlighting the most important segments for nematode resistance according to Ballén-Taborda et al. Submitted (Fig. 4.11.B - Translucent red blocks).

Table 4.3. Average and standard deviation of BC_2F_1s lines, susceptible and resistant controls for pollen viability.

Genotype	Genotype (Simplified name)	Type	Viable pollen	Inviable pollen	Pollen viability (%)
A. hypogaea 5-646-10	*A. hypogaea* 5-646-10	Cultivated (Recurrent parent)	196.70 ± 35.40	11.61 ± 93.63	93.63 ± 1.17 (ab)
A. hypogaea 13-1014	*A. hypogaea* 13-1014	Cultivated (Recurrent parent)	174.16 ± 59.90	27.14 ± 90.24	90.24 ± 2.78 (ef)
A. stenosperma V10309	*A. stenosperma* V10309	Diploid wild species	114.25 ± 12.10	5.59 ± 94.90	94.90 ± 1.23 (b)
A. batizocoi K9484	*A. batizocoi* K9484	Diploid wild species	112.12 ± 64.70	64.12 ± 71.69	71.69 ± 11.45 (cd)
BatSten1	BatSten1	Induced allotetraploid	210.17 ± 154.90	198.12 ± 75.51	75.51 ± 11.01 (c)
BC_2F_1:C2633-2_3(16)	BC_2F_1:C2633-2_3(16)	Backcrossed line - BC_2F_1	902.23 ± 63.20	49.12 ± 92.10	92.10 ± 3.46 (ae)
BC_2F_1_BRD_C0049_Seed1	BC_2F_1_Seed1	Backcrossed line - BC_2F_1	253.29 ± 51.00	21.30 ± 86.62	86.62 ± 5.60 (fg)
BC_2F_1_BRD_C0049_Seed2	BC_2F_1_Seed2	Backcrossed line - BC_2F_1	218.52 ± 109.80	53.45 ± 79.50	79.50 ± 4.81 (c)
BC_2F_1_BRD_C0049_Seed7	BC_2F_1_Seed7	Backcrossed line - BC_2F_1	272.04 ± 113.20	142.53 ± 80.84	80.84 ± 7.05 (cg)
BC_2F_1_BRD_C0049_Seed8	BC_2F_1_Seed8	Backcrossed line - BC_2F_1	337.66 ± 176.10	135.08 ± 65.08	65.08 ± 6.05 (d)
BC_2F_1_BRD_C0050_Seed9	BC_2F_1_Seed9	Backcrossed line - BC_2F_1	131.39 ± 99.70	73.04 ± 75.27	75.27 ± 5.19 (c)
BC_2F_1_BRD_C0055_Seed15	BC_2F_1_Seed15	Backcrossed line - BC_2F_1	Not evaluated	Not evaluated	Not evaluated
BC_2F_1_BRD_C0055_Seed17	BC_2F_1_Seed17	Backcrossed line - BC_2F_1	270.52 ± 198.60	107.99 ± 65.58	65.58 ± 9.14 (d)
BC_2F_1_BRD_C0057_Seed28	BC_2F_1_Seed28	Backcrossed line - BC_2F_1	296.18 ± 67.70	41.95 ± 89.81	89.81 ± 2.29 (ef)
BC_2F_1_BRD_C0058_Seed33	BC_2F_1_Seed33	Backcrossed line - BC_2F_1	392.75 ± 153.40	82.46 ± 77.90	77.90 ± 3.68 (c)

Notes: Viable pollen - Total number of viable pollen grains; Inviable pollen - Total number of inviable pollen grains; Pollen viability (%) - Percentage of viable pollen grains. Pollen viability (%) column with the same letter do not differ significantly ($p<0.05$). No results are presented for BC_2F_1_Seed15 line since TSWV infection affected the production of flowers.

References

Arya SS, Salve AR, Chauhan S (2016) Peanuts as functional food: a review. J Food Sci Technol 53:31-41

Ballén-Taborda C, Chu C, Jackson S, Ozias-Akins P, Timper P, Holbrook C, Bertioli D, Leal-Bertioli S (Submitted) A new source of root-knot nematode resistance from Arachis stenosperma incorporated into allotetraploid peanut (Arachis hypogaea). Sci Rep

Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD, Cannon EK, Liu X, Gao D, Clevenger J, Dash S, Ren L, Moretzsohn MC, Shirasawa K, Huang W, Vidigal B, Abernathy B, Chu Y, Niederhuth CE, Umale P, Araujo AC, Kozik A, Kim KD, Burow MD, Varshney RK, Wang X, Zhang X, Barkley N, Guimaraes PM, Isobe S, Guo B, Liao B, Stalker HT, Schmitz RJ, Scheffler BE, Leal-Bertioli SC, Xun X, Jackson SA, Michelmore R, Ozias-Akins P (2016) The genome sequences of Arachis duranensis and Arachis ipaensis, the diploid ancestors of cultivated peanut. Nat Genet 48:438-446

Bertioli DJ, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, Leal-Bertioli SCM, Ren L, Farmer AD, Pandey MK, Samoluk SS, 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 KD, Korani W, Lanciano S, Lui CG, Mirouze M, Moretzsohn MC, Pham M, Shin JH, Shirasawa K, Sinharoy S, Sreedasyam A, Weeks NT, Zhang X, Zheng Z, Sun Z, Froenicke L, Aiden EL, Michelmore R, Varshney RK, Holbrook CC, Cannon EKS, Scheffler BE, Grimwood J, Ozias-Akins P, Cannon SB, Jackson SA, Schmutz J (2019) The genome sequence of peanut (Arachis hypogaea), a segmental allotetraploid. Nat Genet 51:877-884

Bertioli DJ, Seijo G, Freitas FO, Valls JFM, Leal-Bertioli SCM, Moretzsohn MC (2011) An overview of peanut and its wild relatives. Plant Gene Resour 9:134-149

Branch WD, Brenneman TB (2015) Registration of 'Georgia-14N' peanut. J Plant Regist 9:159-161

Burow MD, Simpson CE, Starr JL, Paterson AH (2001) Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (Arachis hypogaea L.). broadening the gene pool of a monophyletic polyploid species. Genetics 159:823-837

Bybd DW, Kirkpatrick T, Barker KR (1983) An improved technique for clearing and staining plant tissues for detection of nematodes. J Nematol 15:142-143

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803-814

Chu Y, Gill R, Clevenger J, Timper P, Corley Holbrook C, Ozias-Akins P (2016) Identification of rare recombinants leads to tightly linked markers for nematode resistance in peanut. Peanut Sci 43:88-93

Chu Y, Guimaraes LA, Wu CL, Timper P, Holbrook CC, Ozias-Akins P (2014) A Technique to Study Meloidogyne arenaria Resistance in Agrobacterium rhizogenes-Transformed Peanut. Plant Dis Report 98:1292-1299

Clevenger J, Chu Y, Arrais Guimaraes L, Maia T, Bertioli D, Leal-Bertioli S, Timper P, Holbrook CC, Ozias-Akins P (2017) Gene expression profiling describes the genetic regulation of Meloidogyne arenaria resistance in Arachis hypogaea and reveals a candidate gene for resistance. Sci Rep 7:1317

Clevenger JP, Korani W, Ozias-Akins P, Jackson S (2018) Haplotype-Based Genotyping in Polyploids. Frontiers in plant science 9:564

Collier R, Fuchs B, Walter N, Kevin Lutke W, Taylor CG (2005) Ex vitro composite plants: an inexpensive, rapid method for root biology. The plant journal 43:449-457

Danchin EG, Arguel MJ, Campan-Fournier A, Perfus-Barbeoch L, Magliano M, Rosso MN, Da Rocha M, Da Silva C, Nottet N, Labadie K, Guy J, Artiguenave F, Abad P (2013) Identification of novel target genes for safer and more specific control of root-knot nematodes from a pan-genome mining. PLoS Pathog 9:e1003745

Dickson DW, De Waele D (2005) Nematode parasites of peanut. In: Sikora RA, Bridge J (eds) Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI Publishing, Wallingford, UK, pp 393-436

Dong W, Holbrook CC, Timper P, Brenneman TB, Mullinix BG (2007) Comparison of methods for assessing resistance to meloidogyne arenaria in peanut. J Nematol 39:169-175

Fávero AP, Moraes SA, Garcia AAF, Valls JFM, Vello NA (2009) Characterization of rust, early and late leaf spot resistance in wild and cultivated peanut germplasm. Scientia Agricola 66:110-117

Feys BJ, Parker JE (2000) Interplay of signaling pathways in plant disease resistance. Trends Genet 16:449-455

Fonceka D, Tossim H-A, Rivallan R, Vignes H, Lacut E, de Bellis F, Faye I, Ndoye O, Leal-Bertioli SCM, Valls JFM, Bertioli DJ, Glaszmann J-C, Courtois B, Rami J-F (2012) Construction of Chromosome Segment Substitution Lines in Peanut (Arachis hypogaea L.) Using a Wild Synthetic and QTL Mapping for Plant Morphology. PLoS One 7:e48642

Frisch M, Bohn M, Melchinger AE (1999) Comparison of Selection Strategies for Marker-Assisted Backcrossing of a Gene. Crop Sci 39:1295-1301

Frisch M, Bohn M, Melchinger AE (2000) PLABSIM: software for simulation of marker-assisted backcrossing. J Hered 91:86-87

Gaaliche B, Majdoub A, Trad M, Mars M (2013) Assessment of Pollen Viability, Germination, and Tube Growth in Eight Tunisian Caprifig (Ficus carica L.) Cultivars. ISRN Agronomy 2013:1-4

Guimaraes LA, Pereira BM, Araujo ACG, Guimaraes PM, Brasileiro ACM (2017) Ex vitro hairy root induction in detached peanut leaves for plant-nematode interaction studies. Plant Methods 13:25

Guimaraes P, Brasileiro ACM, Proite K, Araujo A, Leal-Bertioli S, Pic-Taylor A, da Silva FR, Morgante C, Ribeiro S, Bertioli D (2010) A study of gene expression in the nematode resistant wild peanut relative, Arachis stenosperma, in response to challenge with Meloidogyne arenaria. Tropical Plant Biology 3:183-192

Halward T, Stalker T, LaRue E, Kochert G (1992) Use of single-primer DNA amplifications in genetic studies of peanut (Arachis hypogaea L.). Plant Mol Biol 18:315-325

Hasan MM, Rafii MY, Ismail MR, Mahmood M, Rahim HA, Alam MA, Ashkani S, Malek MA, Latif MA (2015) Marker-assisted backcrossing: a useful method for rice improvement. Biotechnol Biotechnol Equip 29:237-254

Hestop-Harrison JS (1992) Cytological techniques to assess pollen quality. In: Cresti M, Tiezzi A (eds) Sexual Plant Reproduction. Springer-Verlag, pp 41-38

Holbrook C, Ozias-Akins P, Chu Y, K. Culbreath A, Kvien C, Brenneman T (2017) Registration of ‘TifNV-high O/L’ peanut. J Plant Regist 11:228-230

Holbrook C, Stalker H (2003) Peanut breeding and genetic resources. Plant Breed Rev 22:297-356

Holbrook C, Timper P, Culbreath A, Kvien C (2008) Registration of 'Tifguard' peanut. J Plant Regist 2:391-410

Holbrook CC, Noe JP (1990) Resistance to Meloidogyne arenaria in Arachis spp. and the Implications on Development of Resistant Peanut Cultivars 1. Peanut Science 17:35-38

Hospital F (2003) Marker-assisted breeding. In: HJ N (ed) Plant molecular breeding. Blackwell Publishing and CRC Press, Oxford, Boca Raton, pp 30–59

Hospital F, Charcosset A (1997) Marker-assisted introgression of quantitative trait loci. Genetics 147:1469-1485

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 7:396-423

Jiang G-L (2015) Molecular Marker-Assisted Breeding: A Plant Breeder's Review. In: al. JMA-Ke (ed) Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools. Springer International Publishing Switzerland, Switzerland, pp 431-472

Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, Kikuchi T, Manzanilla-Lopez R, Palomares-Rius JE, Wesemael WM, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14:946-961

Kiyosawa S (1982) Genetics and Epidemiological Modeling of Breakdown of Plant Disease Resistance. Annu Rev Phytopathol 20:93-117

Kochert G, Stalker H, Gimenes M, Galgaro L, C. RL, Moore K (1996) RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, Arachis hypogaea (Leguminosae). American Journal of Botany 83:1281-1291

Korani W, Clevenger JP, Chu Y, Ozias-Akins P (2019) Machine Learning as an Effective Method for Identifying True Single Nucleotide Polymorphisms in Polyploid Plants. Plant Genome 12

Kruskal W, Wallis W (1952) Use of ranks in one-criterion variance analysis. J Amer Statist Assn 47:583-621

Leal-Bertioli SC, Jose AC, Alves-Freitas DM, Moretzsohn MC, Guimaraes PM, Nielen S, Vidigal BS, Pereira RW, Pike J, Favero AP, Parniske M, Varshney RK, Bertioli DJ (2009) Identification of candidate genome regions controlling disease resistance in Arachis. BMC Plant Biol 9:112

Leal-Bertioli SC, Santos SP, Dantas KM, Inglis PW, Nielen S, Araujo AC, Silva JP, Cavalcante U, Guimaraes PM, Brasileiro AC, Carrasquilla-Garcia N, Penmetsa RV, Cook D, Moretzsohn MC, Bertioli DJ (2015) 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. Ann Bot 115:237-249

Leal-Bertioli SCM, Moretzsohn MC, Roberts PA, Ballén-Taborda C, Borba TCO, Valdisser PA, Vianello RP, Araújo ACG, Guimarães PM, Bertioli DJ (2016) Genetic mapping of resistance to Meloidogyne arenaria in Arachis stenosperma: A new source of nematode resistance for peanut. G3: Genes, Genomes, Genetics 6:377-390

Lecomte L, Duffe P, Buret M, Servin B, Hospital F, Causse M (2004) Marker-assisted introgression of five QTLs controlling fruit quality traits into three tomato lines revealed interactions between QTLs and genetic backgrounds. Theor Appl Genet 109:658-668

Mallikarjuna N, Jadhav D, Reddy K, Husain F, Das K (2012) Screening new Arachis amphidiploids, and autotetraploids for resistance to late leaf spot by detached leaf technique. Eur J Plant Pathol 132:17-21

Michelotto MD, Barioni W, Jr., de Resende MD, de Godoy IJ, Leonardecz E, Favero AP (2015) Identification of Fungus Resistant Wild Accessions and Interspecific Hybrids of the Genus Arachis. PLoS One 10:e0128811

Minton NA, Baujard P (1990) Nematode parasites of peanut. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, Wallingford, UK, pp 285-320

Moens M, Perry RN, Starr JL (2009) Meloidogyne species - a diverse group of novel and important plant parasites. In: Perry RN, Moens M, Starr JL (eds) Root-knot nematodes. CAB International, Wallingford, UK, pp 1-17

Moretzsohn MC, Gouvea EG, Inglis PW, Leal-Bertioli SC, Valls JF, Bertioli DJ (2013) A study of the relationships of cultivated peanut (Arachis hypogaea) and its most closely related wild species using intron sequences and microsatellite markers. Ann Bot 111:113-126

Morgante C, Brasileiro ACM, Roberts PA, Guimaraes La, Aaujo A, Fonseca LN, Leal-Bertioli S, Bertioli D, Guimarães P (2013) A survey of genes involved in Arachis stenosperma resistance to Meloidogyne arenaria race 1. Functional Plant Biology 40:1298

Mupunga I, Mngqawa P, Katerere DR (2017) Peanuts, Aflatoxins and Undernutrition in Children in Sub-Saharan Africa. Nutrients 9

Nelson SC, Simpson CE, Starr JL (1989) Resistance to Meloidogyne arenaria in Arachis spp. germplasm. J Nematol 21:654-660

Noe JP, Holbrook CC, Minton NA (1992) Field Evaluation of Susceptibility to Meloidogyne arenaria in Arachis hypogaea Plant Introductions. J Nematol 24:712-716

Onkendi EM, Kariuki GM, Marais M, Moleleki LN (2014) The threat of root-knot nematodes (Meloidogyne spp.) in Africa: a review. Plant Pathology 63:727-737

Palloix A, Ayme V, Moury B (2009) Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. The New phytologist 183:190-199

Perry RN, Moens M, Starr JL (2009) Root-knot nematodes. CAB International, Wallingford, UK

Petrillo MD, Matthews WC, Roberts PA (2006) Dynamics of Meloidogyne incognita Virulence to Resistance Genes Rk and Rk in Cowpea. J Nematol 38:90-96

Proite K, Carneiro R, Falcão R, Gomes A, Leal-Bertioli S, Guimarães P, Bertioli D (2008) Post-infection development and histopathology of Meloidogyne arenaria race 1 on Arachis spp. Plant Pathology 57:974-980

Proite K, Leal-Bertioli SC, Bertioli DJ, Moretzsohn MC, da Silva FR, Martins NF, Guimaraes PM (2007) ESTs from a wild Arachis species for gene discovery and marker development. BMC Plant Biol 7

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. Theor Appl Genet 121:1033-1046

Settaluri VS, Kandala CVK, Puppala N, Sundaram J (2012) Peanuts and Their Nutritional Aspects¡ A Review. Food and Nutrition Sciences 3:1644-1650

Shanks JV, Morgan J (1999) Plant 'hairy root' culture. Curr Opin Biotechnol 10:151-155

Sharma HC, Pampapathy G, Dhillon MK, Ridsdill-Smith JT (2005) Detached leaf assay to screen for host plant resistance to Helicoverpa armigera. J Econ Entomol 98:568-576

Sikora RA (2018) Plant parasitic nematodes in subtropical and tropical agriculture, 3rd edition. edn. CABI, Boston, MA

Simpson C (2001) Use of Wild Arachis Species/Introgression of Genes into A. hypogaea L. Peanut Sci 28:114-116

Simpson C, C. Nelson S, L. Starr J, E. Woodard K, D. Smith O (1993) Registration of TxAG-6 and TxAG-7 peanut germplasm. Crop Sci 33:1418

Simpson C, L. Starr J, Church G, D. Burow M, H. Paterson A (2003) Registration of NemaTAM peanut. Crop Sci 43:1561

Simpson C, L. Starr J, R. Baring M, D. Burow M, M. Cason J, N. Wilson J (2013) Registration of 'Webb' peanut. J Plant Regist 7:265

Singh B, Singh U (1991) Peanut as a source of protein for human foods. Plant Food Hum Nutr 41:165-177

Starr J, R. Morgan E, E. Simpson C (2002) Management of the peanut root-knot nematode, Meloidogyne arenaria, with host resistance. Plant Health Prog

Stuthman DD, Leonard KJ, Miller-Garvin J (2007) Breeding Crops for Durable Resistance to Disease. Advances in Agronomy 95:319-367

Tameling WIL, Elzinga SDJ, Darmin PS, Vossen JH, Takken FLW, Haring MA, Cornelissen BJC (2002) The tomato R gene products I-2 and MI-1 are functional ATP binding proteins with ATPase activity. The Plant cell 14:2929-2939

Tareq W, Pandith S, Rana S, Bhat W, Dhar N, Razdan S, Chandra S, Kitchlu S, Sharma N, Lattoo S (2015) Promiscuous breeding behaviour in relation to reproductive success in Grewia asiatica L. (Malvaceae). Flora - Morphology Distribution Functional Ecology of Plants 211:62-71

Timper P, Dickson DW, Steenkamp S (2018) Nematode parasites of groundnut. In: Sikora RA, Coyne DL (eds) Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, pp 411-445

Vara Prasad PV, Craufurd PQ, Summerfield RJ (1999) Fruit Number in Relation to Pollen Production and Viability in Groundnut Exposed to Short Episodes of Heat Stress. Ann Bot 84:381-386

Wesemael W, Viaene N, Moens M (2011) Root-knot nematodes (Meloidogyne spp.) in Europe. Nematology 13:3-16

Williamson VM, Hussey RS (1996) Nematode pathogenesis and resistance in plants. The Plant cell 8:1735-1745

Appendix 4

Supplementary Script

Script 4.S1. Unix scripts for analysis and filtering of genotyping data for BC_2F_1 lines and controls.

#Input file:
Geno_BCLines_Diplo-Tetra1.txt

#Filtering and replace genotype by numbers BatSten1 (1), Runner-886 (2), different (3):

BatSten1 (Fourth column) ≠ Runner-886 (Fifth and sixth columns)
AA ≠ AB
AA ≠ BB
AB ≠ AA
AB ≠ BB
BB ≠ AA
BB ≠ AB

```
awk '{if($4=="AA" && $5== "AB" && $6== "AB") print $0}' Geno_BCLines_Diplo-Tetra1.txt | perl -lpe "s/AA/1/g" | perl -lpe "s/AB/2/g" | perl -lpe "s/BB/3/g" | perl -lpe "s/NoCall/-/g" > Geno_BCLines_Diplo-Tetra1_1.txt &

awk '{if($4=="AA" && $5== "BB" && $6== "BB") print $0}' Geno_BCLines_Diplo-Tetra1.txt | perl -lpe "s/AA/1/g" | perl -lpe "s/BB/2/g" | perl -lpe "s/AB/3/g" | perl -lpe "s/NoCall/-/g" > Geno_BCLines_Diplo-Tetra1_2.txt &

awk '{if($4=="AB" && $5== "AA" && $6== "AA") print $0}' Geno_BCLines_Diplo-Tetra1.txt | perl -lpe "s/AB/1/g" | perl -lpe "s/AA/2/g" | perl -lpe "s/BB/3/g" | perl -lpe "s/NoCall/-/g" > Geno_BCLines_Diplo-Tetra1_3.txt &

awk '{if($4=="AB" && $5== "BB" && $6== "BB") print $0}' Geno_BCLines_Diplo-Tetra1.txt | perl -lpe "s/AB/1/g" | perl -lpe "s/BB/2/g" | perl -lpe "s/AA/3/g" | perl -lpe "s/NoCall/-/g" > Geno_BCLines_Diplo-Tetra1_4.txt &

awk '{if($4=="BB" && $5== "AA" && $6== "AA") print $0}' Geno_BCLines_Diplo-Tetra1.txt | perl -lpe "s/BB/1/g" | perl -lpe "s/AA/2/g" | perl -lpe "s/AB/3/g" | perl -lpe "s/NoCall/-/g" > Geno_BCLines_Diplo-Tetra1_5.txt &

awk '{if($4=="BB" && $5== "AB" && $6== "AB") print $0}' Geno_BCLines_Diplo-Tetra1.txt | perl -lpe "s/BB/1/g" | perl -lpe "s/AB/2/g" | perl -lpe "s/AA/3/g" | perl -lpe "s/NoCall/-/g" > Geno_BCLines_Diplo-Tetra1_6.txt &
```

#Concatenate files:

```
head -6 Geno_BCLines_Diplo-Tetra1.txt > Geno_BCLines_Diplo-Tetra1_All.txt
cat Geno_BCLines_Diplo-Tetra1_1.txt Geno_BCLines_Diplo-Tetra1_2.txt Geno_BCLines_Diplo-Tetra1_3.txt Geno_BCLines_Diplo-Tetra1_4.txt Geno_BCLines_Diplo-Tetra1_5.txt Geno_BCLines_Diplo-Tetra1_6.txt >> Geno_BCLines_Diplo-Tetra1_All.txt
```

#Filter by genetic map (Ballén-Taborda et al. Submitted):

```
head -6 Geno_BCLines_Diplo-Tetra1_All.txt > Geno_BCLines_Diplo-Tetra1_All_BatStenMap.txt
for i in `cut -f 1 BatStenMap_MarkersList.txt` ; do grep "$i" Geno_BCLines_Diplo-Tetra1_All.txt >> Geno_BCLines_Diplo-Tetra1_All_BatStenMap.txt ; done
```

CHAPTER 5

CHARACTERIZATION OF HOMOEOLOGOUS GENE EXPRESSION BIAS IN AN INDUCED PEANUT ALLOTETRAPLOID

Introduction

Polyploidy or whole-genome duplication (WGD) is an important process in plant evolution shaping plant genomes and has been described in many plants lineages (Doyle et al. 2008; Jiao et al. 2011). Additional set of chromosomes can be derived from intraspecific genome duplication (autopolyploidy) or hybridization of divergent genomes and chromosome doubling (allopolyploidy) (Doyle et al. 2008; Yoo et al. 2014). In allopolyploidy, the coexistence of divergent genomes in a single nucleus is associated with non-mendelian genetic and epigenetic changes, and with effects on genome structure, gene expression and phenotype (Comai et al. 2003; Comai et al. 2000; Doyle et al. 2008; Jackson and Chen 2010; Yoo et al. 2014). This phenomenon was described by McClintock (1984) as "Genome shock" (McClintock 1984). Later, the term "transcriptome shock" was introduced (Buggs et al. 2011; Hegarty et al. 2006; Wu et al. 2016; Zhang et al. 2016). In addition to genome/transcriptome, other changes include sequence elimination (Han et al. 2005; Jackson and Chen 2010; Shaked et al. 2001; Skalicka et al. 2005; Tate et al. 2009), modification in epigenetic marks (Chen 2007; Comai et al. 2003; Doyle et al. 2008; Kovarik et al. 2008; Liu et al. 2009; Madlung et al. 2002; Rapp and Wendel 2005; Shaked et al. 2001; Soltis et al. 2010; Soltis and Soltis 2009; Wang et al. 2014), homoeologous genome interaction and exchange (Gaeta et al.

2007; Kovarik et al. 2008; Salmon et al. 2010; Szadkowski et al. 2010), chromosomal rearrangements (Madlung et al. 2005; Pontes et al. 2004), activation of transposable elements (TE) (Agren et al. 2016; Kashkush et al. 2003; Kraitshtein et al. 2010; Madlung et al. 2005; Mhiri et al. 2019; O'Neill et al. 1998; Wendel et al. 2016), variations in small RNA populations (Fu et al. 2016; Ha et al. 2009; Shen et al. 2014) and alterations in gene expression (gene loss, silencing and activation) (Comai et al. 2003; Comai et al. 2000; Hegarty et al. 2008; Liu et al. 2009).

In allopolyploids, the expression of some duplicated genes pairs (homoeologs) can deviate from parental additivity (average of parental gene expression) (Grover et al. 2012; Yoo et al. 2014). The expression of duplicated genes can be described with two concepts: 1) expression level dominance (ELD) that compares the total expression of genes in the allopolyploid, relative to the expression of the parental genomes; where, transgressive expression (novel expression patterns), total gene expression favoring one parental genome or additivity (average of the parental expression levels) can be observed; and 2) homoeolog expression bias (HEB) describes the preferential expression of one homoeolog gene relative to the other (biased or unbiased/additive); in other words, the contribution (unique/equal) of a homeolog gene to the transcriptome. Both ELD and HEB may be balanced among loci in the allotetraploid in relation to the diploid parents, or unbalanced when there is preference toward one of the parental genomes (Bottani et al. 2018; Grover et al. 2012; Rapp et al. 2009; Yoo et al. 2014; Yoo et al. 2013). These processes have been documented in a variety of plant species using both natural and newly formed allotetraploids, such *Coffea arabica* (Bardil et al. 2011) *Brassica napus* (Wu et al. 2018), *Mimulus* species (Edger et al. 2017), *Arabidopsis* (Chen 2007; Wang et

al. 2006), *Triticum* species (Chague et al. 2010; Li et al. 2014; Qi et al. 2012; Zhang et al. 2016), *Oryza sativa* (Xu et al. 2014), *Gossypium* (Flagel et al. 2008; Flagel and Wendel 2010; Rapp et al. 2009; Yoo et al. 2013), *Tragopogon* (Buggs et al. 2011) *Spartina* (Chelaifa et al. 2010) and *Senecio* (Hegarty et al. 2008; Hegarty et al. 2006).

The present work concerns the allotetraploid crop peanut, *Arachis hypogaea,* which is an allotetraploid species (AABB, 2n = 4x =40) with very narrow genetic diversity due to an inferred recent polyploid origin (Bertioli et al. 2016; Bertioli et al. 2019; Halward et al. 1992; Husted 1936; Kochert et al. 1996; Moretzsohn et al. 2013). For the peanut community, wild diploid species have been and are increasingly important to improve peanut performance under biotic and abiotic stresses (Bertioli et al. 2011; Holbrook and Stalker 2003; Simpson 2001; Stalker 1997; Stalker 2017). Transferring valuable alleles from wild species involves the hybridizing of wild diploid species followed by induced genome duplication using colchicine (Burow et al. 2001; Fonceka et al. 2009; Fonceka et al. 2012; Kumari et al. 2014; Leal-Bertioli et al. 2017; Leal-Bertioli et al. 2015b; Simpson 1991; Simpson et al. 1993; Simpson et al. 2003; Simpson and Starr 2001).

Previously, to incorporate root-knot nematode resistance from the wild relatives *Arachis stenosperma* into tetraploid peanut (Ballén-Taborda et al. Submitted; Leal-Bertioli et al. 2016), a synthetic allotetraploid BatSten1 ([*Arachis batizocoi* x *A. stenosperma*]$^{(2n=4x=40)}$) that incorporated this wild species was developed (Leal-Bertioli et al. 2015b). This wild-derived allotetraploid combines the A genome of *A. stenosperma* (Moretzsohn et al. 2013; Tallury et al. 2005) with the K genome of *A. batizocoi* (Moretzsohn et al. 2013; Robledo and Seijo 2010). This synthetic allotetraploid is here

used to explore changes at a genomic and transcriptomic that may have occurred as a consequence of uniting and the interaction of the A and K divergent genomes within a single nucleus. Using RNA sequencing (RNA-seq) we measured the homoeolog expression bias (HEB) to study unbalanced gene expression due to the interaction of the wild genomes coexisting within BatSten1. We found that ~ one-fourth of the homoeolog gene pairs showed evidence of biased gene expression with little difference between the two genomes (10.60% toward A-subgenome and 11.77% toward K-subgenome). A better understanding of these genetic changes is important for the transfer and use of desirable wild genes to improve cultivated peanut.

Materials and Methods

Plant materials

Wild *Arachis* accessions and genotypes were obtained from the wild peanut lab seed collection (https://wildpeanutlab.uga.edu/). In this study we included two diploid species *A. stenosperma* PI666100 (original collection voucher V10309) (2n = 2x =20, AA genome) and *A. batizocoi* PI298639 (original collection voucher K9484) (2n = 2x =20, KK genome); three generations (1^{st} - 2013, 2^{nd} - 2014 and 5^{th} - 2017) of the synthetic allotetraploid BatSten1 ([*A. batizocoi* x *A. stenosperma*]$^{(2n=4x=40)}$) (2n = 4x =40, KKAA genome) (Leal-Bertioli et al. 2015b); and the natural allotetraploid *A. hypogaea* cv. Tifrunner (2n = 4x =40, AABB genome) (Table 5.1), for which the genome sequence is available (Bertioli et al. 2019). Three plants per genotype were germinated and grown in a growth chamber under controlled conditions at ~27°C and 16 h photoperiod. After two months, three young leaves (first or second expanded) per plant per genotype were

harvested at a single time point into three independent 15 ml FALCON tubes and flash frozen in liquid nitrogen (thus 45 samples in total). Tissue was ground into a fine powder using a grinder (RETSCH MM301) and stored at -80°C for nucleic acid extraction (Fig. 5.1.A). Later, powder was transferred into two 2.0 ml tubes for DNA and RNA extraction (see next section) (Fig. 5.1.B). Once DNA and RNA were extracted, we pooled the nucleic acids from the three leaves per plant per genotype in equal amounts, to become one individual biological replicate. This was repeated for three biological replicates (plants). Note that RNA pooling was performed twice for mRNA and sRNA sequencing experiments (Fig. 5.1.C). In total, 15 samples per DNA, RNA and sRNA were prepared (3 biological replicates per 5 genotypes).

RNA extraction and sequencing

Total RNA was isolated from 45 collected leaves (three technical replicates (leaves) per three biological replicates (plants) per 5 genotypes (*A. stenosperma*, *A. batizocoi,* BatSten1 1st and 5th generation and Tifrunner) using Direct-zol RNA miniprep kits (Zymo Research) following the manufacturer's instructions. Quality and integrity (RIN ≥ 6.8) of isolated RNAs was inspected using the RNA 6000 Nano kit on the Agilent 2100 Bioanalyzer system (Agilent, Santa Clara, CA, USA), and quantified with the fluorometer QUBIT 2.0 using the RNA BR assay kit (Invitrogen).

Library construction and sequencing of pooled RNA samples was performed by Novogene sequencing company (https://en.novogene.com/). Briefly, library construction for 15 mRNA samples (3 biological replicates per 5 plant line, Table 5.2) was performed using the Illumina NEBNext Ultra II RNA Library preparation kit and mRNA sequencing

was completed using an Illumina HiSeq4000 sequencer. 250-300 bp insert cDNA library was selected and Paired-end reads of 2 x 150 bp in length was delivered. Due to genome size differences, 6G and 12G of paired-end reads were requested initially for the diploid and tetraploids species, respectively. Illumina reads were quality inspected and cleaned using TrimGalore v.0.6.1 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/), which executes Cutadapt (v.2.4) and FastQC (v0.11.8) for trimming and quality evaluation, respectively. Quality control and processing of raw reads included removal of: 1) adaptor sequence; 2) low quality reads; 3) low-quality bases from the ends; and 4) trimmed reads of length<75 bp. The high-quality reads that passed the quality control and filtering, were aligned to the non-coding (ncRNA) database from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Rfam (https://rfam.xfam.org/), and overrepresented sequences using Bowtie2 v.2.3.5.1 with default parameters and sensitive local alignment mode (Langmead and Salzberg 2012). Samtools v.1.9 with the "-f4" flag set up (Li et al. 2009) was used to recover unmapped reads exclusively, and reads that failed to align were converted into fastq format with bedtools v.2.28.0 and "bamtofastq" option (https://bedtools.readthedocs.io/en/latest/). Filtered data (Conesa et al. 2016) was used for homoeolog expression analysis (Table 5.2).

Homoeolog expression bias analysis

With the aim to study homoeolog expression in the synthetic allotetraploid BatSten1, we used HyLiTe v.2.0.1 (Hybrid Lineage Transcriptome Explorer) (Duchemin et al. 2015). SAM files were provided as input for the software. The SAM files were generated by mapping the cleaned RNA paired-end reads against a set of 44,436 *A.*

ipaensis K30076 transcript sequences with Bowtie2 (Langmead and Salzberg 2012). Sensitive local alignment was specified and one mismatch was allowed for the mapping analysis (Griffiths et al. 2019). HyLiTE was used to identify single nucleotide polymorphisms (SNPs) to differentiate parental genomes A and K, to categorize BatSten1's mRNA transcript sequences to a diploid parental type, and to provide read counts for A- homoeolog and K- homoeolog gene pairs (Duchemin et al. 2015; Griffiths et al. 2019). Estimated counts for homoeolog pairs were used for expression analysis using DESeq2 software following the standard procedure (Love et al. 2014). Benjamini & Hochberg (BH) multiple comparison correction was used and a significance threshold of adjusted P-value < 0.05 and <0.01 (Adj. P<0.05 and Adj. P<0.01) were set. To better understand gene expression bias in relation to their chromosomal position, the expression of A and K homoeologs of BatSten1 were plotted on the *A. ipaensis* chromosomal pseudomolecules.

Results and Discussion

Allopolyploidy has to deal with two or more set of divergent genomes and regulatory interactions (Doyle et al. 2008; Yoo et al. 2014) that could be responsible for many changes at the genetic and epigenetic level (Comai et al. 2003; Comai et al. 2000; Doyle et al. 2008; Jackson and Chen 2010; Yoo et al. 2014). Polyploidization is known to contribute new alleles and allelic combinations, phenotypic plasticity and enhanced ability to adapt to different environments (Adams and Wendel 2005; Wendel et al. 2016). At a gene expression level, the interaction of divergent parental genomes could result in

up- or downregulation of homoeolog genes (Buggs et al. 2011; Hegarty et al. 2006; Wu et al. 2016; Zhang et al. 2016).

For purpose of peanut improvement, the wild-derived synthetic allotetraploid BatSten1 was developed (Leal-Bertioli et al. 2015b). This allotetraploid combines the A genome of *A. stenosperma* (Moretzsohn et al. 2013; Tallury et al. 2005) and the K genome of *A. batizocoi* (Moretzsohn et al. 2013; Robledo and Seijo 2010). Given the synthetic origin of BatSten1, we were interested to study genomic and transcriptomic shock (Buggs et al. 2011; Hegarty et al. 2006; McClintock 1984; Wu et al. 2016; Zhang et al. 2016) as a consequence of the interaction of the divergent wild A and K genomes, and whether there were alterations in the expression of genes (e.g. disease resistance) (Yoo et al. 2013). Here, we present a preliminary study on homoeolog expression bias (HEB) in the induced allotetraploid BatSten1 using RNA sequencing (RNA-seq) data.

Samples used for the sequencing experiments included 3 biological replicates of 4 genotypes, *A. batizocoi* K9484, *A. stenosperma* V10309, BatSten1 [1st (2013), 2nd (2014) and 5th (2017) generations], and *A. hypogaea* cv. Tifrunner (Table 5.1). From the Illumina sequencing, the average total of raw paired-end reads was ~29 and ~49 million for diploid and tetraploid genotypes, respectively. After cleaning and filtering, ~18% of the reads were discarded resulting in ~23 and ~40 million for the diploid and tetraploid genotypes, respectively (Table 5.2).

Homoeolog expression bias in leaf transcriptome data

To study potential bias in homoeolog gene expression in leaf transcriptome data, cleaned reads from the 15 RNA samples were mapped onto the *A. ipaensis* K30076

transcript database, comprising 44,436 sequences. For *A. batizocoi*, *A. stenosperma*, BatSten1 and *A. hypogaea* Tifrunner, the average overall alignment rates to the *A. ipaensis* transcripts ranged from 84.2% to 85.5% (Table 5.2). Given the evolutionary distance between these species, these alignment rates were reasonable (Bechara et al. 2010; Bertioli et al. 2011) and were probably slightly lower due to reads from unannotated genes in the reference genome (Conesa et al. 2016).

A total of 1,442,450 SNP markers were identified using HyLiTe (Duchemin et al. 2015). Of these, 274,874 and 205,093 were diagnostic SNP markers for the A and K subgenomes in BatSten1, respectively. Also, 121,971 SNPs were unique for BatSten1, 15,217 for *A. stenosperma* and 15,091 for *A. batizocoi*. The rest of SNPs were classified as masked or not considered when at least one organism had poor coverage or were common between all the species (810,204). Using K and A diagnostic SNPs, of the 415,471,403 BatSten1 reads successfully mapped, 146,167,034 were classified as *A. stenosperma* reads (A genome) and 142,629,549 as *A. batizocoi* reads (K genome) (Table 5.3).

To explore homoeolog expression bias among homoeolog gene pairs in BatSten1 across genotypes and samples, read counts were retrieved for each homoeolog pair using HyLiTe and expression analysis with DESeq2 (Love et al. 2014). Of the 44,436 reference genes, 21,894 were expressed in BatSten1 (with nonzero read count). In total, 15,126 (69.09%) and 16,780 (76.64%) of the expressed homeolog genes maintained a balanced expression at 5% and 1% level of significance, respectively. 6,552 (29.93%, Adj. P <0.05) and 4,898 (22.37%, Adj. P <0.01) (Fig. 5.S1) homologous gene pairs displayed a biased expression in the synthetic allotetraploid BatSten1, similar to the proportions

observed in synthetic allopolyploid cotton (25.5%) (Yoo et al. 2013) and nascent allohexaploid wheat (27.0%) (Li et al. 2014), but lower than resynthesized allopolyploid *Brassica napus* (36.7%) (Wu et al. 2018) and synthetic allotetraploid peanut (43.91%) (Shin et al. *unpublished*, Bertioli lab).

Of note, homeologous pairs in BatSten1 showed a similar overall expression bias with a slight (~1-point) preference toward K-subgenome genes (10.60% vs 11.77% ($p<0.01$)) (Table 5.4). In other words, we found that duplicated gene pairs in the synthetic allotetraploid BatSten1 are mostly balanced in that most homoeologous genes show no expression bias and those that do are mostly equal from the two subgenomes (Fig. 5.S2). However, in few regions we found unbalanced homoeolog expression as observed in other allopolyploid species (Grover et al. 2012; Li et al. 2014; Wu et al. 2018; Yoo et al. 2013). We observed blocks of biased homoeolog gene expression in distal part of chromosomes B10 and B05 in BatSten1's 2nd and 5th generation, respectively (Fi. 5.S2.D,E). In B10 it was biased toward K-genome gene expression, whereas, in B05 it was biased toward the A-genome, consistent with previous study using RNA-seq data from the synthetic allotetraploid peanut (IpaDur) (Shin et al. *unpublished*) and with regions associated with homeologous recombination in *A. hypogaea* (Bertioli et al. 2016; Bertioli et al. 2019)

Reference gene sequences

We utilized the *A. ipaensis* transcripts as reference for this study for several reasons: 1) *A. ipaensis* genome is well characterize, annotated and transcript sequences are available (Bertioli et al. 2016), which is recommended (Duchemin et al. 2015); and 2) reference genomes do not have to be one of the studied species, but close enough to the

species of interest to be of use (Duchemin et al. 2015). Here, *A. ipaensis*, *A. stenosperma* and *A. batizocoi* are genetically related and phylogenetically clustered inside the section *Arachis* (Bechara et al. 2010; Bertioli et al. 2011). *A. stenosperma* (A genome) and *A. ipaensis* (B genome) have diverged genomes, and the K genome of *A. batizocoi* is closely related to the B genome of *A. ipaensis*, but considered distinct according to GISH and molecular phylogenetic evidence (Leal-Bertioli et al. 2015b; Moretzsohn et al. 2013). For future work, other reference databases (including *A. duranensis*) could be used for comparisons, but should be done with caution as one of the BatSten1's diploid parental (A and K genomes) could be closer to the reference than the other (Dr. Corrinne Grover - Iowa State University, personal comm.) (see "future work" section).

Future work

Based on these results, I am proposing several follow-up analyses to have a better understanding of homoeolog expression bias in the synthetic allotetraploid, and to explore other processes associated with genome and transcriptome shock.

To understand homoeolog expression bias due to the interaction of A and K subgenomes of BatSten1, DNA sequencing data will be also included to improve the SNP calling, particularly for genes with relatively low expression in the parental species. This with the aim to reduce the number of masked SNPs when there is poor coverage at the marker position (Duchemin et al. 2015). Also, other_transcripts datasets will be used as reference to confirm consistency of results, and to monitor that the biased expression is not due to the close genetic relationship of one of the BatSten1's parental genomes with the reference (e.g. *A. duranensis* V14167 transcripts or *A. hypogaea* cv. Tifrunner).

Additionally, other pipelines and software will be used for comparative purposes, and analyses including Tifrunner's cultivar sequencing data will be performed to compare results between the newly developed allotetraploid BatSten1 with this natural allotetraploid peanut cultivar.

In terms of gene expression analysis, a comparison of the homoeologous gene expression level in the allotetraploid BatSten1 relative to the corresponding parent's gene expression will be completed. This is described as expression level dominance (ELD) to assess whether the BatSten1's homoeolog expression versus diploid parent gene expression is statistically the same (inherited parental expression pattern) or deviated (novel bias in progeny), and to evaluate whether one of the genomes is dominant over the other. Also, metabolic/biochemical pathways enrichment analysis will be performed for the homoeolog gene pairs that are displaying biased expression.

Other analysis, will include the exploration of_sRNA population changes, specifically microRNA (miRNA) as they could be regulators of gene expression (Djami-Tchatchou et al. 2017; Wendel et al. 2016); and investigate the dynamics of transposable elements (TE) upon genome doubling when developing the synthetic allotetraploid BatSten1 (Wendel et al. 2016). Finally, using DNA sequencing data we will study genome rearrangements caused by homoeologous chromosome exchange (Bertioli et al. 2016; Clevenger et al. 2017; Leal-Bertioli et al. 2015a; Nguepjop et al. 2016).

Summary

In the present work we used RNA sequencing data of two diploid species (*A. stenosperma* and *A. batizocoi*) and a colchicine-derived allopolyploid BatSten1 to study

the effects of genome merger and chromosome doubling on gene expression of homoeolog gene pairs. In this preliminary study, we observed that approximately one-fourth of the homoeolog gene pairs showed expression bias in the synthetic allopolyploid BatSten1 (Adj. $P<0.01$) (Table 5.4). Further analysis with RNA, DNA and sRNA sequencing data will allow us to unravel and understand other processes affected by genome and transcriptome shock in the allopolyploid BatSten1 (see "future work" section above).

Acknowledgements

I would like to acknowledge Drs. Corrinne Grover, Jonathan Wendel and Chunming Xu for direction on the homoeolog expression bias analysis. To Emma Matthews and Victoria Morris for tissue collection, to Dr. Carolina Chavarro for advice with RNA extraction and quantification and to Brian Abernathy for assistance with Linux server.

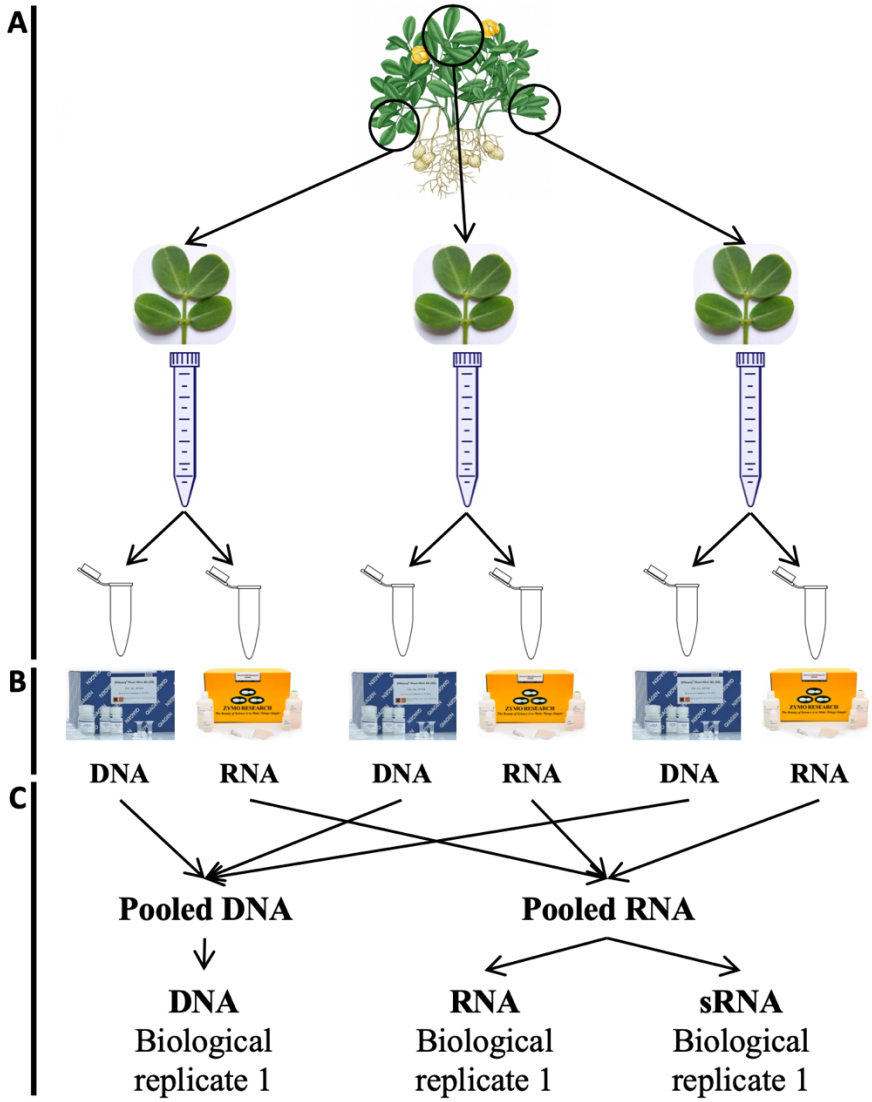

Figure 5.1. Leaf tissue collection procedure. This diagram describes the nucleic acid pooling for a single biological replicate. This was repeated for three different biological replicates (three plants per genotype). Three expanded leaves were harvested (technical replicate) into three different 15 ml tubes, frozen in liquid nitrogen and grinded. Later, tissue was transferred to two 1.5 ml tubes **(A)** for total DNA and total RNA extraction using the DNeasy *Plant* Mini *Kit* (QIAGEN) and Direct-zol RNA miniprep kit (Zymo Research), respectively **(B)**. DNA and RNA were pooled from three technical replicates to create one biological replicate **(C)**.

Table 5.1: Species/genotypes: ploidy, genome compositions and genome size.

Taxa	Accession	Ploidy	Genome composition	Gb
A. stenosperma	V10309	Diploid	AA	~1.25
A. batizocoi	K9484	Diploid	KK	~1.56
BatSten1 1st generation (2013) BatSten1 2nd generation (2014) BatSten1 5th generation (2017)	K9484 x V10309	Tetraploid	KKAA	~2.7
A. hypogaea	Tifrunner	Tetraploid	AABB	2.7

Notes: Gb, estimated genome sizes of BatSten1, *A. stenosperma* and *A. batizocoi* based on *A. hypogaea*, *A. duranensis* and *A. ipaensis* genome sizes, respectively (Bertioli et al. 2016; Bertioli et al. 2019).

Table 5.2: RNA Sequencing results.

Species/genotype - rep	Sample name	Seq requested	Total raw pairs	Discarded pairs	% Discarded pairs	Total cleaned pairs	% alignment to *A. ipaensis*
A. stenosperma V10309 - Rep 1	stenR1_R	6G - 20 M reads	27,785,977	4,029,404	14.5	23,756,573	84.0
A. stenosperma V10309 - Rep 2	stenR2_R	6G - 20 M reads	27,905,489	3,659,974	13.1	24,245,515	84.5
A. stenosperma V10309 - Rep 3	stenR3_R	6G - 20 M reads	32,383,456	5,979,614	18.5	26,403,842	84.0
A. batizocoi K9484 - Rep 1	batR1_R	6G - 20 M reads	30,618,932	8,141,827	26.6	22,477,105	83.3
A. batizocoi K9484 - Rep 2	batR2_R	6G - 20 M reads	27,016,846	9,155,024	33.9	17,861,822	81.0
A. batizocoi K9484 - Rep 3	batR3_R	6G - 20 M reads	30,946,153	6,260,204	20.2	24,685,949	83.3
BatSten1 1st generation (2013) - Rep 1	BS13R1_R	12G - 40 M reads	53,234,731	14,984,846	28.1	38,249,885	84.8
BatSten1 1st generation (2013) - Rep 2	BS13R2_R	12G - 40 M reads	52,446,396	7,237,138	13.8	45,209,258	86.7
BatSten1 2nd generation (2014) - Rep 1	BS14R1_R	12G - 40 M reads	55,995,235	18,690,592	33.4	37,304,643	84.0
BatSten1 5th generation (2017) - Rep 1	BS17R1_R	12G - 40 M reads	49,050,327	7,994,094	16.3	41,056,233	84.4
BatSten1 5th generation (2017) - Rep 2	BS17R2_R	12G - 40 M reads	52,247,376	9,086,479	17.4	43,160,897	84.9
BatSten1 5th generation (2017) - Rep 3	BS17R3_R	12G - 40 M reads	50,175,531	8,467,512	16.9	41,708,019	84.4
A. hypogaea cv. Tifrunner - Rep 1	TifR1_R	12G - 40 M reads	46,954,477	2,108,302	4.5	44,846,175	85.6
A. hypogaea cv. Tifrunner - Rep 2	TifR2_R	12G - 40 M reads	39,833,910	2,334,476	5.9	37,499,434	85.7
A. hypogaea cv. Tifrunner - Rep 3	TifR3_R	12G - 40 M reads	42,157,923	3,177,652	7.5	38,980,271	85.1

Notes: Species/genotype - rep, samples name including species, accession/genotype, generation (if applies) and biological replicate; Seq requested, RNA sequencing requested; Discarded pairs, removed low quality reads (short, unpaired, etc.), ncRNA and overrepresented sequences. % alignment to *A. ipaensis*, alignment overall rate to the *A. ipaensis* K30076 transcript sequences.

Table 5.3: HyLiTE RNA sequencing BatSten1 read assignment summary.

	Mapped reads	Assigned reads	Assigned reads - sten	Assigned reads - bat
BatSten1 1st generation (2013) - Rep 1	64,705,676	45,048,973	22,432,384	22,616,589
BatSten1 1st generation (2013) - Rep 2	78,137,476	53,218,328	26,668,729	26,549,599
BatSten1 2nd generation (2014) - Rep 1	62,174,451	43,299,231	21,420,099	21,879,132
BatSten1 5th generation (2017) - Rep 1	68,853,559	47,964,073	22,647,841	25,316,232
BatSten1 5th generation (2017) - Rep 2	72,017,225	50,736,927	26,540,974	24,195,953
BatSten1 5th generation (2017) - Rep 3	69,583,016	48,529,051	26,457,007	22,072,044
Total	**415,471,403**	**288,796,583**	**146,167,034**	**142,629,549**
A. hypogaea Tifrunner - Rep 1	76,806,138	22,408,311	12,619,131	9,789,180
A. hypogaea Tifrunner - Rep 2	64,252,731	19,014,020	10,619,079	8,394,941
A. hypogaea Tifrunner - Rep 3	66,338,960	19,338,383	10,958,249	8,380,134
Total	**207,397,829**	**60,760,714**	**34,196,459**	**26,564,255**

Notes: Mapped reads, umber of mapped reads in each sample onto the *A. ipaensis* K30076 transcript database; Assigned reads, number of assigned reads in each sample to a parental diploid genome; Assigned reads - sten, number of assigned reads in each sample to the *A. stenosperma* genome; Assigned reads - bat, number of assigned reads in each sample to the *A. batizocoi* genome. Summary for Tifrunner reads mapping is also shown but not discussed.

Table 5.4: Homoeolog expression bias (HEB) between 21,894 putative homoeologous gene pairs in the synthetic allotetraploid BatSten1 (calculated with DESeq2).

	Adj. P<0.05		Adj. P<0.01	
Biased	A-biased	B-biased	A-biased	B-biased
	3,203 (14.63%)	3,349 (15.30%)	2,320 (10.60%)	2,578 (11.77%)
Total biased	6,552 (29.93%)		4,898 (22.37%)	
Balanced	15,126 (69.09%)		16,780 (76.64%)	
Outliers	216 (0.99%)		216 (0.99%)	
Total	**21,894 (100%)**		**21,894 (100%)**	

Notes: Adj. P<0.05 and Adj. P<0.01, adjusted p-value less than 0.05 and 0.01, respectively. Balanced means no expression difference between the A and K copies; A-biased represent pairs significant higher expressed in A subgenome; K-biased represent pairs significant higher expressed in K subgenome; Percentages for each classification in parenthesis.

References

Adams KL, Wendel JF (2005) Polyploidy and genome evolution in plants. Current Opinion in Plant Biology 8:135-141

Agren JA, Huang HR, Wright SI (2016) Transposable element evolution in the allotetraploid Capsella bursa-pastoris. Am J Bot 103:1197-1202

Ballén-Taborda C, Chu C, Jackson S, Ozias-Akins P, Timper P, Holbrook C, Bertioli D, Leal-Bertioli S (Submitted) A new source of root-knot nematode resistance from Arachis stenosperma incorporated into allotetraploid peanut (Arachis hypogaea). Sci Rep

Bardil A, de Almeida JD, Combes MC, Lashermes P, Bertrand B (2011) Genomic expression dominance in the natural allopolyploid Coffea arabica is massively affected by growth temperature. New Phytol 192:760-774

Bechara MD, Moretzsohn MC, Palmieri DA, Monteiro JP, Bacci M, Martins J, Valls JFM, Lopes CR, Gimenes MA (2010) Phylogenetic relationships in genus Arachis based on ITS and 5.8S rDNA sequences. BMC Plant Biol 10:255

Bertioli DJ, Cannon SB, Froenicke L, Huang G, Farmer AD, Cannon EK, Liu X, Gao D, Clevenger J, Dash S, Ren L, Moretzsohn MC, Shirasawa K, Huang W, Vidigal B, Abernathy B, Chu Y, Niederhuth CE, Umale P, Araujo AC, Kozik A, Kim KD, Burow MD, Varshney RK, Wang X, Zhang X, Barkley N, Guimaraes PM, Isobe S, Guo B, Liao B, Stalker HT, Schmitz RJ, Scheffler BE, Leal-Bertioli SC, Xun X, Jackson SA, Michelmore R, Ozias-Akins P (2016) The genome sequences of Arachis duranensis and Arachis ipaensis, the diploid ancestors of cultivated peanut. Nat Genet 48:438-446

Bertioli DJ, Jenkins J, Clevenger J, Dudchenko O, Gao D, Seijo G, Leal-Bertioli SCM, Ren L, Farmer AD, Pandey MK, Samoluk SS, 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 KD, Korani W, Lanciano S, Lui CG, Mirouze M, Moretzsohn MC, Pham M, Shin JH, Shirasawa K, Sinharoy S, Sreedasyam A, Weeks NT, Zhang X, Zheng Z, Sun Z, Froenicke L, Aiden EL, Michelmore R, Varshney RK, Holbrook CC, Cannon EKS, Scheffler BE, Grimwood J, Ozias-Akins P, Cannon SB, Jackson SA, Schmutz J (2019) The genome sequence of peanut (Arachis hypogaea), a segmental allotetraploid. Nat Genet 51:877-884

Bertioli DJ, Seijo G, Freitas FO, Valls JFM, Leal-Bertioli SCM, Moretzsohn MC (2011) An overview of peanut and its wild relatives. Plant Gene Resour 9:134-149

Bottani S, Zabet NR, Wendel JF, Veitia RA (2018) Gene Expression Dominance in Allopolyploids: Hypotheses and Models. Trends Plant Sci 23:393-402

Buggs RJ, Zhang L, Miles N, Tate JA, Gao L, Wei W, Schnable PS, Barbazuk WB, Soltis PS, Soltis DE (2011) Transcriptomic shock generates evolutionary novelty in a newly formed, natural allopolyploid plant. Curr Biol 21:551-556

Burow MD, Simpson CE, Starr JL, Paterson AH (2001) Transmission genetics of chromatin from a synthetic amphidiploid to cultivated peanut (Arachis hypogaea L.). broadening the gene pool of a monophyletic polyploid species. Genetics 159:823-837

Chague V, Just J, Mestiri I, Balzergue S, Tanguy AM, Huneau C, Huteau V, Belcram H, Coriton O, Jahier J, Chalhoub B (2010) Genome-wide gene expression changes in genetically stable synthetic and natural wheat allohexaploids. New Phytol 187:1181-1194

Chelaifa H, Monnier A, Ainouche M (2010) Transcriptomic changes following recent natural hybridization and allopolyploidy in the salt marsh species Spartina x townsendii and Spartina anglica (Poaceae). New Phytol 186:161-174

Chen ZJ (2007) Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annu Rev Plant Biol 58:377-406

Clevenger J, Chu Y, Chavarro C, Agarwal G, Bertioli DJ, Leal-Bertioli SCM, Pandey MK, Vaughn J, Abernathy B, Barkley NA, Hovav R, Burow M, Nayak SN, Chitikineni A, Isleib TG, Holbrook CC, Jackson SA, Varshney RK, Ozias-Akins P (2017) Genome-wide SNP genotyping resolves signatures of selection and tetrasomic tecombination in peanut. Mol Plant 10:309-322

Comai L, Madlung A, Josefsson C, Tyagi A (2003) Do the different parental 'heteromes' cause genomic shock in newly formed allopolyploids? Philos Trans R Soc Lond B Biol Sci 358:1149-1155

Comai L, Tyagi AP, Winter K, Holmes-Davis R, Reynolds SH, Stevens Y, Byers B (2000) Phenotypic instability and rapid gene silencing in newly formed arabidopsis allotetraploids. Plant Cell 12:1551-1568

Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, Szcześniak MW, Gaffney DJ, Elo LL, Zhang X, Mortazavi A (2016) A survey of best practices for RNA-seq data analysis. Genome Biol 17:13-13

Djami-Tchatchou AT, Sanan-Mishra N, Ntushelo K, Dubery IA (2017) Functional Roles of microRNAs in Agronomically Important Plants-Potential as Targets for Crop Improvement and Protection. Front Plant Sci 8:378

Doyle JJ, Flagel LE, Paterson AH, Rapp RA, Soltis DE, Soltis PS, Wendel JF (2008) Evolutionary genetics of genome merger and doubling in plants. Annu Rev Genet 42:443-461

Duchemin W, Dupont PY, Campbell MA, Ganley AR, Cox MP (2015) HyLiTE: accurate and flexible analysis of gene expression in hybrid and allopolyploid species. BMC Bioinformatics 16:8

Edger PP, Smith R, McKain MR, Cooley AM, Vallejo-Marin M, Yuan Y, Bewick AJ, Ji L, Platts AE, Bowman MJ, Childs KL, Washburn JD, Schmitz RJ, Smith GD, Pires JC, Puzey JR (2017) Subgenome Dominance in an Interspecific Hybrid, Synthetic Allopolyploid, and a 140-Year-Old Naturally Established Neo-Allopolyploid Monkeyflower. Plant Cell 29:2150-2167

Flagel L, Udall J, Nettleton D, Wendel J (2008) Duplicate gene expression in allopolyploid Gossypium reveals two temporally distinct phases of expression evolution. BMC Biol 6:16

Flagel LE, Wendel JF (2010) Evolutionary rate variation, genomic dominance and duplicate gene expression evolution during allotetraploid cotton speciation. New Phytol 186:184-193

Fonceka D, Hodo-Abalo T, Rivallan R, Faye I, Sall MN, Ndoye O, Favero AP, Bertioli DJ, Glaszmann JC, Courtois B, Rami JF (2009) Genetic mapping of wild introgressions into cultivated peanut: a way toward enlarging the genetic basis of a recent allotetraploid. BMC Plant Biol 9:103

Fonceka D, Tossim H-A, Rivallan R, Vignes H, Lacut E, de Bellis F, Faye I, Ndoye O, Leal-Bertioli SCM, Valls JFM, Bertioli DJ, Glaszmann J-C, Courtois B, Rami J-F (2012) Construction of Chromosome Segment Substitution Lines in Peanut (Arachis hypogaea L.) Using a Wild Synthetic and QTL Mapping for Plant Morphology. PLoS One 7:e48642

Fu Y, Xiao M, Yu H, Mason AS, Yin J, Li J, Zhang D, Fu D (2016) Small RNA changes in synthetic Brassica napus. Planta 244:607-622

Gaeta RT, Pires JC, Iniguez-Luy F, Leon E, Osborn TC (2007) Genomic changes in resynthesized Brassica napus and their effect on gene expression and phenotype. Plant Cell 19:3403-3417

Griffiths AG, Moraga R, Tausen M, Gupta V, Bilton TP, Campbell MA, Ashby R, Nagy I, Khan A, Larking A, Anderson C, Franzmayr B, Hancock K, Scott A, Ellison NW, Cox MP, Asp T, Mailund T, Schierup MlH, Andersen SU (2019) Breaking Free: The Genomics of Allopolyploidy-Facilitated Niche Expansion in White Clover. Plant cell 31:1466-1487

Grover CE, Gallagher JP, Szadkowski EP, Yoo MJ, Flagel LE, Wendel JF (2012) Homoeolog expression bias and expression level dominance in allopolyploids. New Phytol 196:966-971

Ha M, Lu J, Tian L, Ramachandran V, Kasschau KD, Chapman EJ, Carrington JC, Chen X, Wang XJ, Chen ZJ (2009) Small RNAs serve as a genetic buffer against genomic shock in Arabidopsis interspecific hybrids and allopolyploids. Proc Natl Acad Sci U S A 106:17835-17840

Halward T, Stalker T, LaRue E, Kochert G (1992) Use of single-primer DNA amplifications in genetic studies of peanut (Arachis hypogaea L.). Plant Mol Biol 18:315-325

Han F, Fedak G, Guo W, Liu B (2005) Rapid and repeatable elimination of a parental genome-specific DNA repeat (pGc1R-1a) in newly synthesized wheat allopolyploids. Genetics 170:1239-1245

Hegarty MJ, Barker GL, Brennan AC, Edwards KJ, Abbott RJ, Hiscock SJ (2008) Changes to gene expression associated with hybrid speciation in plants: further insights from transcriptomic studies in Senecio. Philos Trans R Soc Lond B Biol Sci 363:3055-3069

Hegarty MJ, Barker GL, Wilson ID, Abbott RJ, Edwards KJ, Hiscock SJ (2006) Transcriptome shock after interspecific hybridization in senecio is ameliorated by genome duplication. Curr Biol 16:1652-1659

Holbrook C, Stalker H (2003) Peanut breeding and genetic resources. Plant Breed Rev 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 7:396-423

Jackson S, Chen ZJ (2010) Genomic and expression plasticity of polyploidy. Curr Opin Plant Biol 13:153-159

Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS, Soltis DE, Clifton SW, Schlarbaum SE, Schuster SC, Ma H, Leebens-Mack J, dePamphilis CW (2011) Ancestral polyploidy in seed plants and angiosperms. Nature 473:97-100

Kashkush K, Feldman M, Levy AA (2003) Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nature Genetics 33:102-106

Kochert G, Stalker H, Gimenes M, Galgaro L, Romero Lopes C, Moore K (1996) RFLP and cytogenetic evidence on the origin and evolution of allotetraploid domesticated peanut, Arachis hypogaea (Leguminosae). American Journal of Botany 83:1281-1291

Kovarik A, Dadejova M, Lim YK, Chase MW, Clarkson JJ, Knapp S, Leitch AR (2008) Evolution of rDNA in Nicotiana allopolyploids: a potential link between rDNA homogenization and epigenetics. Annals of botany 101:815-823

Kraitshtein Z, Yaakov B, Khasdan V, Kashkush K (2010) Genetic and epigenetic dynamics of a retrotransposon after allopolyploidization of wheat. Genetics 186:801-812

Kumari V, Gowda MVC, Tasiwal V, Pandey MK, Bhat RS, Mallikarjuna N, Upadhyaya HD, Varshney RK (2014) Diversification of primary gene pool through introgression of resistance to foliar diseases from synthetic amphidiploids to cultivated groundnut (Arachis hypogaea L.). Crop J 2:110-119

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357-359

Leal-Bertioli S, Shirasawa K, Abernathy B, Moretzsohn M, Chavarro C, Clevenger J, Ozias-Akins P, Jackson S, Bertioli D (2015a) Tetrasomic recombination is surprisingly frequent in allotetraploid Arachis. Genetics 199:1093-1105

Leal-Bertioli SC, Moretzsohn MC, Santos SP, Brasileiro AC, Guimaraes PM, Bertioli DJ, Araujo AC (2017) Phenotypic effects of allotetraploidization of wild Arachis and their implications for peanut domestication. Am J Bot 104:379-388

Leal-Bertioli SC, Santos SP, Dantas KM, Inglis PW, Nielen S, Araujo AC, Silva JP, Cavalcante U, Guimaraes PM, Brasileiro AC, Carrasquilla-Garcia N, Penmetsa RV, Cook D, Moretzsohn MC, Bertioli DJ (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. Ann Bot 115:237-249

Leal-Bertioli SCM, Moretzsohn MC, Roberts PA, Ballén-Taborda C, Borba TCO, Valdisser PA, Vianello RP, Araújo ACG, Guimarães PM, Bertioli DJ (2016) Genetic mapping of resistance to Meloidogyne arenaria in Arachis stenosperma: A new source of nematode resistance for peanut. G3: Genes, Genomes, Genetics 6:377-390

Li A, Liu D, Wu J, Zhao X, Hao M, Geng S, Yan J, Jiang X, Zhang L, Wu J, Yin L, Zhang R, Wu L, Zheng Y, Mao L (2014) mRNA and Small RNA transcriptomes

reveal insights into dynamic homoeolog regulation of allopolyploid heterosis in nascent hexaploid wheat. Plant Cell 26:1878-1900

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Subgroup GPDP (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078-2079

Liu B, Xu C, Zhao N, Qi B, Kimatu JN, Pang J, Han F (2009) Rapid genomic changes in polyploid wheat and related species: implications for genome evolution and genetic improvement. J Genet Genomics 36:519-528

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550

Madlung A, Masuelli RW, Watson B, Reynolds SH, Davison J, Comai L (2002) Remodeling of DNA methylation and phenotypic and transcriptional changes in synthetic Arabidopsis allotetraploids. Plant Physiol 129:733-746

Madlung A, Tyagi AP, Watson B, Jiang H, Kagochi T, Doerge RW, Martienssen R, Comai L (2005) Genomic changes in synthetic Arabidopsis polyploids. Plant J 41:221-230

McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792-801

Mhiri C, Parisod C, Daniel J, Petit M, Lim KY, Dorlhac de Borne F, Kovarik A, Leitch AR, Grandbastien MA (2019) Parental transposable element loads influence their dynamics in young Nicotiana hybrids and allotetraploids. New Phytol 221:1619-1633

Moretzsohn MC, Gouvea EG, Inglis PW, Leal-Bertioli SC, Valls JF, Bertioli DJ (2013) A study of the relationships of cultivated peanut (Arachis hypogaea) and its most closely related wild species using intron sequences and microsatellite markers. Ann Bot 111:113-126

Nguepjop JR, Tossim H-A, Bell JM, 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. Frontiers in plant science 7:1635-1635

O'Neill RJ, O'Neill MJ, Graves JA (1998) Undermethylation associated with retroelement activation and chromosome remodelling in an interspecific mammalian hybrid. Nature 393:68-72

Pontes O, Neves N, Silva M, Lewis MS, Madlung A, Comai L, Viegas W, Pikaard CS (2004) Chromosomal locus rearrangements are a rapid response to formation of the allotetraploid Arabidopsis suecica genome. Proc Natl Acad Sci U S A 101:18240-18245

Qi B, Huang W, Zhu B, Zhong X, Guo J, Zhao N, Xu C, Zhang H, Pang J, Han F, Liu B (2012) Global transgenerational gene expression dynamics in two newly synthesized allohexaploid wheat (Triticum aestivum) lines. BMC Biol 10:3

Rapp RA, Udall JA, Wendel JF (2009) Genomic expression dominance in allopolyploids. BMC Biol 7:18

Rapp RA, Wendel JF (2005) Epigenetics and plant evolution. New Phytol 168:81-91

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. Theor Appl Genet 121:1033-1046

Salmon A, Flagel L, Ying B, Udall JA, Wendel JF (2010) Homoeologous nonreciprocal recombination in polyploid cotton. New Phytol 186:123-134

Shaked H, Kashkush K, Ozkan H, Feldman M, Levy AA (2001) Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell 13:1749-1759

Shen Y, Zhao Q, Zou J, Wang W, Gao Y, Meng J, Wang J (2014) Characterization and expression patterns of small RNAs in synthesized Brassica hexaploids. Plant molecular biology 85:287-299

Simpson C (1991) Pathways for introgression of pest resistance into Arachis hypogaea L. Peanut Sci 18:22-26

Simpson C (2001) Use of Wild Arachis Species/Introgression of Genes into A. hypogaea L. Peanut Sci 28:114-116

Simpson C, C. Nelson S, L. Starr J, E. Woodard K, D. Smith O (1993) Registration of TxAG-6 and TxAG-7 peanut germplasm. Crop Sci 33:1418

Simpson C, L. Starr J, Church G, D. Burow M, H. Paterson A (2003) Registration of NemaTAM peanut. Crop Sci 43:1561

Simpson C, Starr J (2001) Registration of ‘COAN’ peanut. Crop Sci 41:918

Skalicka K, Lim KY, Matyasek R, Matzke M, Leitch AR, Kovarik A (2005) Preferential elimination of repeated DNA sequences from the paternal, Nicotiana tomentosiformis genome donor of a synthetic, allotetraploid tobacco. New Phytol 166:291-303

Soltis DE, Buggs RJA, Doyle JfJ, Soltis PS (2010) What we still don't know about polyploidy. Taxon 59:1387-1403

Soltis PS, Soltis DE (2009) The role of hybridization in plant speciation. Annu Rev Plant Biol 60:561-588

Stalker H (1997) Peanut (Arachis hypogaea L.). Field Crops Research 53:205–217

Stalker TH (2017) Utilizing Wild Species for Peanut Improvement. Crop Sci 57:1102

Szadkowski E, Eber F, Huteau V, Lode M, Huneau C, Belcram H, Coriton O, Manzanares-Dauleux MJ, Delourme R, King GJ, Chalhoub B, Jenczewski E, Chevre AM (2010) The first meiosis of resynthesized Brassica napus, a genome blender. New Phytol 186:102-112

Tallury SP, Hilu KW, Milla-Lewis SR, Friend S, Alsaghir M, Stalker H, Quandt D (2005) Genomic affinities in Arachis section Arachis (Fabaceae): Molecular and cytogenetic evidence. Theor Appl Genet 111:1229-1237

Tate JA, Joshi P, Soltis KA, Soltis PS, Soltis DE (2009) On the road to diploidization? Homoeolog loss in independently formed populations of the allopolyploid Tragopogon miscellus (Asteraceae). BMC Plant Biol 9:80

Wang H, Jiang J, Chen S, Qi X, Fang W, Guan Z, Teng N, Liao Y, Chen F (2014) Rapid genetic and epigenetic alterations under intergeneric genomic shock in newly synthesized Chrysanthemum morifolium x Leucanthemum paludosum hybrids (Asteraceae). Genome Biol Evol 6:247-259

Wang J, Tian L, Lee HS, Wei NE, Jiang H, Watson B, Madlung A, Osborn TC, Doerge RW, Comai L, Chen ZJ (2006) Genomewide nonadditive gene regulation in Arabidopsis allotetraploids. Genetics 172:507-517

Wendel JF, Jackson SA, Meyers BC, Wing RA (2016) Evolution of plant genome architecture. Genome Biol 17:37

Wu J, Lin L, Xu M, Chen P, Liu D, Sun Q, Ran L, Wang Y (2018) Homoeolog expression bias and expression level dominance in resynthesized allopolyploid Brassica napus. BMC Genomics 19:586

Wu Y, Sun Y, Wang X, Lin X, Sun S, Shen K, Wang J, Jiang T, Zhong S, Xu C, Liu B (2016) Transcriptome shock in an interspecific F1 triploid hybrid of Oryza revealed by RNA sequencing. J Integr Plant Biol 58:150-164

Xu C, Bai Y, Lin X, Zhao N, Hu L, Gong Z, Wendel JF, Liu B (2014) Genome-wide disruption of gene expression in allopolyploids but not hybrids of rice subspecies. Mol Biol Evol 31:1066-1076

Yoo MJ, Liu X, Pires JC, Soltis PS, Soltis DE (2014) Nonadditive gene expression in polyploids. Annu Rev Genet 48:485-517

Yoo MJ, Szadkowski E, Wendel JF (2013) Homoeolog expression bias and expression level dominance in allopolyploid cotton. Heredity 110:171-180

Zhang H, Gou X, Zhang A, Wang X, Zhao N, Dong Y, Li L, Liu B (2016) Transcriptome shock invokes disruption of parental expression-conserved genes in tetraploid wheat. Sci Rep 6:26363

Appendix 5

Supplementary Figure

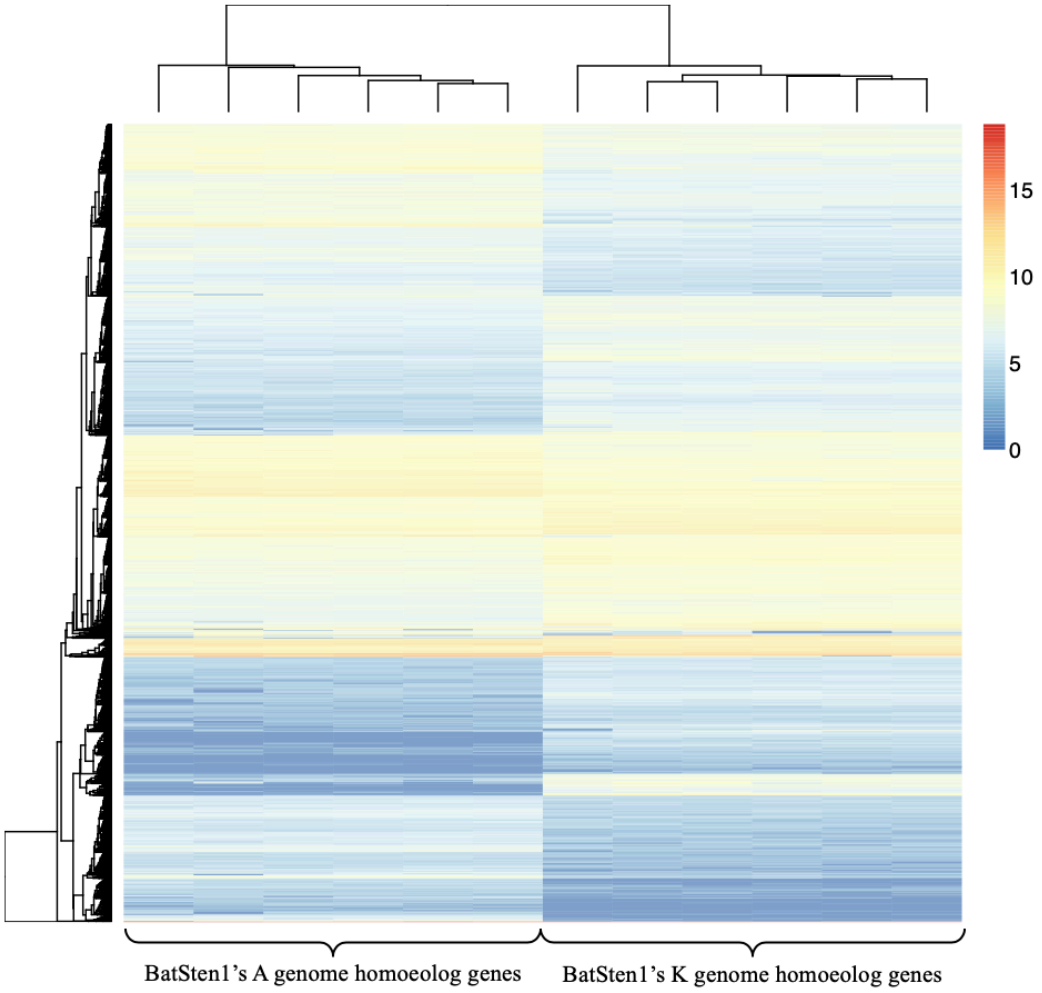

Figure 5.S1. Heatmap of log-transformed read counts for genes with adjusted p-values <0.01. 4,898 differentially expressed homoeolog genes in the synthetic allotetraploid BatSten1 sorted according to hierarchical clustering. BatSten1 A-genome assigned homoeolog genes (first six columns) and K-genome assigned homoeolog genes (last six columns) (Table 5.4).

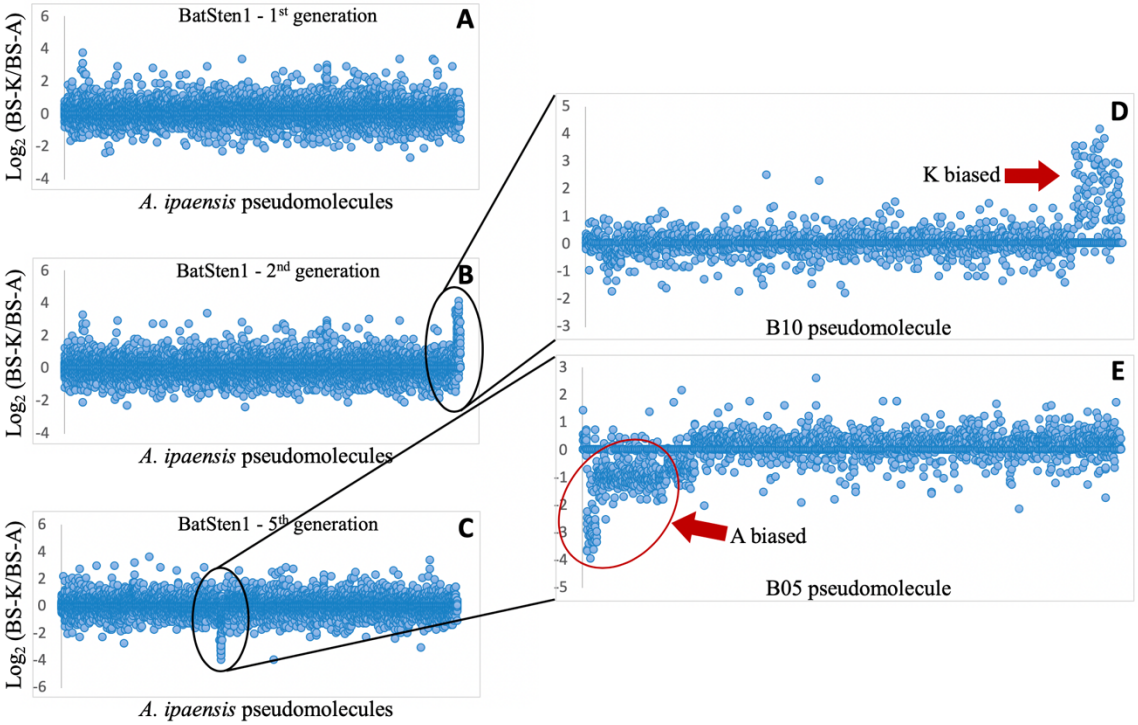

Figure 5.S2. Plots of BatSten1's homoeolog gene expression on *A. ipaensis* pseudomolecules. Log transformed ratio of BatSten1's K-homoeolog (BS-K) and A-homoeolog (BS-A) expression at the first **(A)** second **(B)** and fifth **(C)** generations across *A. ipaensis* K30076 genome. Close up to regions in chromosome B10 **(D)** and B05 **(E)** showing strong expression bias toward K and A genomes, respectively. Log_2 (BS-K/ BS-A) in y-axis and physical position in x-axis. Positive and negative values indicate homoeolog expression bias of K > A and K < A, respectively. Chromosomal regions showing homoeolog expression bias indicated by arrows.

CHAPTER 6

SUMMARY

Peanut (*Arachis hypogaea* L.) is an oilseed, food and fodder crop very important worldwide. Is an allotetraploid species (AABB, 2n = 4x = 40) characterized by a very low genetic diversity and is extremely susceptible to several diseases, including root-knot nematode (RKN) *Meloidogyne arenaria*. This pathogen is a highly destructive and can result in major yield losses. Above-ground symptoms include stunting, chlorosis and wilting, and underground the disease is characterized by formation of galls or "knots" in the roots and lesions in the pods and pegs.

Resistance to many pests/diseases is not found in cultivated peanut germplasm, but its wild relatives comprise a diverse genetic pool that can be utilized to enhance peanut performance under nematode pressure. To date, all modern commercial varieties harbor the RKN resistance from the wild species *A. cardenasii*. This introgression corresponds a large chromosome segment on A09 and is dominantly inherited. Even though this resistance has been durable thus far, it is important to find additional sources of resistance for the development of new high-yielding and nematode-resistant peanut cultivars, and to reduce the risk of resistance breakdown in the varieties currently used. For these reasons the present project was developed.

Strong resistance to RKN is present in the wild diploid relative *A. stenosperma* V10309 that can be used to develop new peanut cultivars with improved nematode

resistance. Previously, using diploid RIL mapping population (*A. duranensis* K7988 x *A. stenosperma* V10309) three QTLs controlling RKN resistance were discovered in *A. stenosperma*: a large-effect QTL on chromosome A02, together with minor effect QTLs on A04 and A09. Additionally, *A. batizocoi* K9484 harbors strong resistance to RKN, but no QTL have been discovered. Overcoming of the reproductive barrier between the diploid *A. stenosperma* and allotetraploid *A. hypogaea*, and for introgression of this new RKN resistance, a peanut compatible synthetic allotetraploid was developed, BatSten1 ([*A. batizocoi* K9484 x *A. stenosperma* V10309]$^{(2n=4x=40)}$).

The first question was whether or not the *A. stenosperma* RKN resistance alleles were transferrable, stable and expressed within the genetic background of tetraploid peanut. For this, an F_2 population was developed from a cross between peanut (cultivar Runner-886) with the induced allotetraploid BatSten1. This population was genotyped using the Axiom *Arachis* SNP array and phenotyped for nematode resistance. QTL mapping analysis allowed us to validate the major-effect QTL on chromosome A02 and the secondary QTL on A09, which was later confirmed in selected $F_{2:3}$ lines ($F_{2:3}$-7 and $F_{2:3}$-34). Both QTLs have with very strong effects on galling and egg production, and each contributing to a percentage reduction in nematode multiplication up to 98.2%. Conversely, we found in our study that putative QTL on A04 did not contribute to improve nematode resistance. The main QTL in A02 is located in a region rich in resistance-genes (R-genes), in particular a nearby TIR-NBS-LRR genes that could be involved in plant immune defenses. The QTL in A09 may correspond to the one from *A. cardenasii* (3.4 - 113.7 Mbp) though at a slightly different position on *A. duranensis*

pseudomolecules (peak ~116.3 Mbp). Thus, we concluded that a new and strong source of resistance to *M. arenaria* was discovered and validated in tetraploid peanut.

With the information described above, next goal was to incorporate this new RKN resistance alleles into cultivated peanut using marker-assisted backcrossing (MABC). Three breeding elite lines from Tifton were used as recurrent parents (RP) (5-646-10, 13-1014 and TifGp-2) and two $F_{2:3}$ lines ($F_{2:3}$-7 and $F_{2:3}$-34 derived from *A. hypogaea* cv. Runner-886 x BatSten1) harboring A02-QTL and A09-QTL were used as donor parents (DP). Three cycles of backcrossing were completed and foreground selection in each cycle was performed with 22 SNP markers.

As a validation step, phenotypic screening for resistance and genotypic characterization of nine BC_2F_1 lines was performed. Both phenotype and genotype data allowed us to validate and delineate the chromosomal segments in chromosomes A02 and A09 to ~8Mbp and ~6.5Mbp regions on the bottom of each, respectively. Subsequent steps included the genome-wide genotyping of 271 advanced backcrossed lines (BC_3F_1s) that allowed us to observe that: 1) the BC_3F_1 lines had a recurrent parent genome recovery (RPG) between 80.2% and 98.8% and retained between 1.1% and 19.1% of the wild donor genome; and 2) besides the RKN resistance in chr A02 and A09, there were other introgressions that may contribute to other agronomic traits of interest (e.g. leaf spot resistance, flower color, growth habit, etc.). These lines will be subjected to a further selections and advancement for germplasm release.

Finally in chapter 5**,** the wild-derived synthetic allotetraploid BatSten1 was used to explore changes at a genomic and transcriptomic level that may have occurred as a consequence of genome merger and the interaction of the A-genome of *A. stenosperma*

and K-genome of *A. batizocoi*. The understanding of RNA expression, small RNA population changes and reactivation of transposable elements is especially important as it could affect the expression of desirable wild genes transferred into peanut. Here, using RNA sequencing (RNA-seq) of two diploid species (*A. stenosperma* and *A. batizocoi*) and their colchicine-derived allopolyploid BatSten1, we measured the homoeolog gene expression bias (HEB) that may be a result of the interaction of the wild genomes coexisting within BatSten1. We found that about one-fourth of the homoeolog gene pairs showed evidence of biased gene expression, with 10.60% toward A-subgenome and 11.77% toward K-subgenome. We also observed a few blocks of biased homoeolog gene expression in distal part of chromosomes B10 and B05. Further analysis with RNA, DNA and sRNA sequencing data will allow us to unravel and understand other processes affected by genome and transcriptome shock in the allotetraploid BatSten1. A better understanding of these genetic changes is important for the transfer and use of desirable wild genes to improve the peanut crop.

This research allowed us to validate and transfer a new high value root-knot nematode (RKN) resistance derived from the diploid *A. stenosperma* into elite peanut background. We successfully developed advanced germplasm that incorporates *A. stenosperma* chromosome segments for strong defense against RKN. Additionally, diagnostic SNP markers are available to facilitate the introgression of QTLs into desired agronomically adapted peanut cultivars. This work is an important step to develop new high-yielding cultivars with strong and durable resistance to RKN, which will make a large impact on peanut cultivation. Expanding the gene pool will help ensure continued protection of the peanut crop from losses due to this pest. It will also enable lower inputs

of agrochemicals and fuel, reducing environmental impact, higher profitability and more stable peanut yields.