Resistance to rust (*Puccinia arachidis* Speg.) identified in nascent allotetraploids cross-compatible with cultivated peanut (*Arachis hypogaea* L.)

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ABSTRACT

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

Keywords: Germplasm characterization, host plant resistance, introgression, *in vitro*, peanut breeding.

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

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fungicide applications (Smith and Littrell, 1980). Therefore, rust resistant, high-yielding peanut cultivars are an important part of an integrated pest management strategy to control rust in tropical countries as well as the U.S.

A major limitation to breeding rust-resistant cultivars is that only moderate levels of resistance have been identified in *A. hypogaea* germplasm (Power *et al.*, 2019). Fortunately, high resistance to rust has been identified in numerous wild *Arachis* species, including the readily usable A- and B-genome *Arachis* species, and resistance can be introgressed into cultivated peanut (Subrahmanyam *et al.*, 1982, 1983; Pande and Rao, 2001; Fávero *et al.*, 2009). Cultivated peanut is an allotetraploid (AABB; $2n=4x=40$) species and the majority of wild *Arachis* species are diploid ($2n=2x=20$); the most efficient way to introgress genes from wild *Arachis* species into cultivated peanut is to cross A- and B-genome species to produce allotetraploid interspecific hybrids that are cross-compatible to peanut. Then, the wild *Arachis* derived allotetraploids are backcrossed to peanut cultivars. Markers linked to rust resistance allow quick introgression as well as pyramiding of multiple QTLs. Rust resistant QTLs have been identified in the A-genome species *A. cardenasii* GKP 10017 and the B-genome species *A. magna* K 30097. One major QTL from each species is being used in peanut breeding programs to introgress rust resistance into peanut cultivars (Khedikar *et al.*, 2010; Sujay *et al.*, 2012; Leal-Bertioli *et al.*, 2015). For example, Gowda *et al.* (2002) released a rust resistant cultivated genotype 'GPBD 4] (ssp. *fastigiata* var. *vulgaris*) with bunch growth habit, in which resistance was derived from *A. cardenasii*. GPBD 4 had a mean rust score of three on a scale of one to nine, in which one was equivalent to no disease and nine was equivalent to 80 to 100% disease (Gowda *et al.*, 2002). While GPBD 4 still develops rust, it does so at far lower levels than most cultivated germplasm, and one rust resistance QTL derived from *A. cardenasii* but identified in a population derived from GPBD 4, has been shown to improve yields by 56 to 96% in rust infected environments (Gowda *et al.*, 2002; Varshney *et al.*, 2014). While progress towards resistant rust cultivars is being made, more major rust resistance QTLs need to be identified for further pyramiding in peanut cultivars to strengthen resistance and more importantly, to increase resistance durability to rust population pressures. This study identified rust resistance in newly synthesized allotetraploids that are cross compatible with cultivated peanut. The long-term goal of this study is to create rust resistant peanut cultivars that can protect yields and decrease the need for fungicides in the U.S. and to increase yields in tropical, developing countries for farmers who cannot afford or access costly fungicides.

Materials and Methods

Plant Materials

Diploid, wild *Arachis* species, *A. correntina* (Burkart) Krapov. and W.C. Gregory [PI 262808, GKP 9530 (*Cor*9530)], *A. duranensis* Krapov. and W.C. Gregory [PI 468197, GKBSPSc 30060 (*Dur*)], and *A. ipaënsis* Krapov. and W.C. Gregory [PI 468322, GKBSPSc 30076 (*Ipa*)] were used to generate the diploid hybrids, *IpaCor*2 and *IpaDur*4 in 2016 at North Carolina State University (NCSU). Wild *Arachis* species, *A. batizocoi* Krapov. and W.C. Gregory [PI 298639, K 9484 (*Bat*)], *A. cardenasii* Krapov. and W.C. Gregory [PI 261874, GKP 10017 (*Card*)], *A. correntina* (*Cor*9530) and [PI 262881, GKP 9548 (*Cor*9548)], *A. diogoi* [PI 331200, GK 10602 (*Dio*)], *A. duranensis* Krapov. and W.C. Gregory [V 14167 (*Dur*1), SeSn 2848 (*Dur*2), and K 7988 (*Dur*3)], *A. gregoryi* A. Gripp, C.E. Simpson, and J.F.M. Valls [PI 476116, VSGr 6389 (*Greg*)], *A. magna* Krapov., W.C. Gregory, and C.E. Simpson [PI 468337, K 30092 (*Mag*1) and PI 468340, K 30097 (*Mag*2)], *A. stenosperma* Krapov. and W.C. Gregory [V 10309 (*Sten*1) and PI 338280, HLK 410 (*Sten*2)], *A. valida* Krapov. and W.C. Gregory [PI 468154, KG 30011 (*Val*)] and *A. villosa* Benth. [V 12812 (*Villo*)] were used to create diploid hybrids *BatCor*, *BatDur*1, *BatDur*2, *BatSten*1, *GregSten*, *IpaCor*1, *IpaDur*1, *IpaSten*, *IpaVillo*1, *MagCard*, *MagDio*, *MagDur*, and *ValSten*1 at the University of Georgia (UGA) Athens Campus. All allotetraploids were derived from the diploid hybrids by colchicine treatment of F_1 hybrid cuttings at the UGA Athens Campus, except for *IpaDur*4 and *IpaCor*2, which were generated at the UGA Tifton Campus.

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

Table 1. Genetic materials tested in each rust bioassay and their abbreviations and ploidy level. Bolded genotypes were tested in both bioassays.

2017 Bioassay: Plant Materials	Abbreviation	Ploidy Level
A. hypogaea cv. Georgia Green	Georgia Green	Tetraploid
A. batizocoi K 9484	*Bat*	Diploid
A. cardenasii GKP 10017	***Card***	Diploid
A. correntina GKP 9530	***Cor*2**	Diploid
A. diogoi GK 10602	*Dio*	Diploid
A. duranensis V 14167	*Dur*1	Diploid
A. duranensis SeSn 2848	*Dur*2	Diploid
A. duranensis K 7988	*Dur*3	Diploid
A. gregoryi V 6389	*Greg*	Diploid
A. ipaënsis K 30076	***Ipa***	Diploid
A. magna K 30092	***Mag*1**	Diploid
A. magna K 30097	*Mag*2	Diploid
A. stenosperma V 10309	***Sten*1**	Diploid
A. valida KG 30011	***Val***	Diploid
A. villosa V 12812	*Villo*	Diploid
(*Bat*9484 x *Cor*9530) ^{2x}	*BatCor*^{2x}	Diploid
(*Ipa*30076 x *Cor*9530)^{2x}	*IpaCor*2^{2x}	Diploid
(*Ipa*30076 x *Sten*10309)^{2x}	*IpaSten*^{2x}	Diploid
(*Mag*30092 x *Card*10017)^{2x}	*MagCard*^{2x}	Diploid
(*Mag*30092 x *Dio*10602)^{2x}	*MagDio*^{2x}	Diploid
(*Bat*9484 x *Dur*14167)^{4x}	*BatDur*1	Tetraploid
(*Bat*9484 x *Dur*2848)^{4x}	*BatDur*2	Tetraploid
(*Bat*9494 x *Sten*10309)^{4x}	*BatSten*1	Tetraploid
(*Greg*6368 x *Sten*10309)^{4x}	*GregSten*	Tetraploid
(*Ipa*30076 x *Cor*9530)^{4x}	***IpaCor*2**_1[a]	Tetraploid
(*Ipa*30076 x *Cor*9548)^{4x}	*IpaCor*1	Tetraploid
(*Ipa*30076 x *Dur*14167)^{4x}	*IpaDur*1	Tetraploid
(*Ipa*30076 x *Villo*12812)^{4x}	*IpaVillo*1	Tetraploid
(*Mag*30097 x *Dur*7988)^{4x}	*MagDur*_1[b]	Tetraploid
(*Mag*30097 x *Dur*7988)^{4x}	*MagDur*_2[b]	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**_1[c]	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**_2[c]	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**_3[c]	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**_4[c]	Tetraploid
2020 Bioassay: Plant Materials	**Abbreviation**	**Ploidy Level**
A. hypogaea Runner 886	886	Tetraploid
A. correntina GKP 9548	*Cor*1	Diploid
A. correntina GKP 9530	***Cor*2**	Diploid
A. ipaënsis K 30076	***Ipa***	Diploid
A. duranensis K 30060	*Dur*4	Diploid
A. magna K 30092	***Mag*1**	Diploid
A. valida KG 30011	***Val***	Diploid
(*Ipa*30076 x *Cor*9530)^{4x}	***IpaCor*2**_2[a]	Tetraploid
(*Val*30011 x *Sten*10309)^{4x}	***ValSten*1**_5[b]	Tetraploid
(*Ipa*30076 x *Dur*30060)^{4x}	*IpaDur*4	Tetraploid

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

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

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

Rust Resistance Evaluation

For both experiments, seeds were coated in Captan + pentachloronitrobenzene + carboxin (Vitavax PC, Vitavax, Crompton, Middlebury, CT) and treated overnight in 0.5% ethephon [(2-chloroethyl) phosphonic acid] (Florel Growth Regulator, Lawn and Garden Products Inc., Fresno, CA) to break dormancy. Seeds were then planted in #123 7.62 cm round x 11.43 cm deep Jiffy Pots (Harris Seeds, Rochester, NY) and transplanted approximately one month later into 121.92 cm round x 27.94 cm deep pots filled with Promix growth medium (Premier Tech Horticulture, Quakertown, PA). Normal plant management was applied in the greenhouse except that fungicide treatments were withheld. One wk before each experiment, leaves infected with rust were collected from untreated border rows in Tifton, GA and rust spores were collected in sterile vials using a vacuum pump. Care was taken to avoid collecting late leaf spot spores. Spores were kept at 4 C until the day of inoculation.

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

by Leal-Bertioli *et al*. (2015): index was the number of lesions times a number that reflected lesion size/ reaction. $I = \Sigma(s * n)/LA$, where s = lesion size (1 = necrotic aborted lesion, 2–6 = ruptured, sporulating pustules, varying between 0.5 and 3 mm in diameter), n = number of lesions of a particular size, LA = leaf area (cm^2). Spore count and classification was performed with a stereoscope microscope, and leaf area was measured by scanning the leaves and then using Assess 2.0 (APS Press) for image analysis. The 2020 experiment was performed the same as the 2017 experiment, except 30 replications per genotype were tested, leaves were excised from five plants per genotype, mounted leaves were inoculated with a spore suspension of 0.025% Tween 20 (ThermoFisher Scientific, Waltham, MA) at 1.7×10^6 urediniospores/mL, and the experiment was ended 28 d after inoculation.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using R (R Core Team, 2021) in RStudio (RStudio, Inc.) using the package agricolae (de Mendiburu, 2021) to determine the genotype effect on rust resistance according to the following parameters: incubation period, IA, TLA, and SLA. Means of each parameter among the genotypes were separated based on the Tukey's Test ($\alpha = 0.05$) results with RStudio. *Greg* and *IpaDur*1 were excluded from incubation period analysis in the 2017 experiment because each had only one replication to develop rust pustules. Genotypes that presented no rust symptoms, and therefore had no incubation period, were artificially tabulated as 100 d after inoculation for statistical analysis.

Results

Significant genotypic effect on all rust resistance parameters was found for the 2017 and 2020 experiments (Table 2). Between both rust resistance experiments, 30 out of the 39 unique materials did not show rust symptoms and had no lesions at the end of each experiment (Table 3). In the 2017 experiment, the susceptible control, two wild *Arachis* species, *Greg* and *Ipa*, and three allotetraploids, *IpaCor*1, *IpaDur*1, and *IpaVillo*1, developed rust pustules. *Greg* and *IpaDur*1 only had one replication that developed rust pustules, so they were excluded from ANOVA analysis evaluating genotype effect on incubation time; however, these replications developed sporulating pustules, meaning they did not show a hypersensitive response. The susceptible cultivated control, Georgia Green, had the shortest incubation period of 14.33 d, while *Ipa, IpaCor*1, and *IpaVillo*1 had incubation periods within 15 to 16 d (Table 3). In the 2020 experiment, the susceptible control, one wild *Arachis* species, *Ipa,* and two allotetraploids, *IpaCor*2_2 and *IpaDur*4, developed rust pustules. The susceptible cultivated control 886 also had the shortest incubation period of 13.95 d, while *Ipa*, *IpaCor*2_2, and *IpaDur*4 had incubation periods between 14 and 15 d (Table 3).

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

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

[a]lesions/ leaf area (cm^2), TLA; sporulated lesions/leaf area (cm^2), SLA; susceptibility index /leaf area (cm^2), IA.

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

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

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

In 2017 rust experiment, the susceptible cultivated control Georgia Green had the highest IA score, but *Ipa*, with an IA score of 1.42, was not significantly different from Georgia Green (Table 3). *IpaCor*1, *IpaDur*1, and *IpaVillo*1 had IA scores of 0.92 or less, which were not significantly different from the highly resistant genotypes that did not develop rust pustules (Table 3). In the 2020 experiment, *Ipa* had the highest IA score of 1.94, which was significantly greater than all tested genotypes except the susceptible control, 886, which had an IA score of 0.76.

As expected, the susceptible control had the highest IA, TLA, SLA in the 2017 experiment. However, *Ipa* and *IpaCor*1 had similar TLA and SLA scores compared to the susceptible control. In the 2020 experiment, *Ipa* demonstrated greater susceptibility to rust with a TLA and SLA score twice as much as the susceptible control, although this difference was not significant. *IpaCor*2_2 showed a TLA and SLA comparable to the

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

2017: Genotype	Incubation Period	IA[a]	TLA	SLA
Georgia Green	14.33 b	3.15 a	0.84 a	0.82 a
Bat	∞ a	0.00 b	0.00 b	0.00 b
Card	∞ a	0.00 b	0.00 b	0.00 b
***Cor*2**	∞ a	0.00 b	0.00 b	0.00 b
Dio	∞ a	0.00 b	0.00 b	0.00 b
*Dur*1	∞ a	0.00 b	0.00 b	0.00 b
*Dur*2	∞ a	0.00 b	0.00 b	0.00 b
*Dur*3	∞ a	0.00 b	0.00 b	0.00 b
Greg	-	0.08 b	0.11 b	0.04 b
Ipa	15.00 b	1.42 ab	0.52 ab	0.47 ab
***Mag*1**	∞ a	0.00 b	0.00 b	0.00 b
*Mag*2	∞ a	0.00 b	0.00 b	0.00 b
***Sten*1**	∞ a	0.00 b	0.00 b	0.00 b
Val	∞ a	0.00 b	0.00 b	0.00 b
Villo	∞ a	0.00 b	0.00 b	0.00 b
BatCor^{2x}	∞ a	0.00 b	0.00 b	0.00 b
*IpaCor*2^{2x}	∞ a	0.00 b	0.00 b	0.00 b
IpaSten^{2x}	∞ a	0.00 b	0.00 b	0.00 b
MagCard^{2x}	∞ a	0.00 b	0.00 b	0.00 b
MagDio^{2x}	∞ a	0.00 b	0.00 b	0.00 b
*BatDur*1	∞ a	0.00 b	0.00 b	0.00 b
*BatDur*2	∞ a	0.00 b	0.00 b	0.00 b
*BatSten*1	∞ a	0.00 b	0.00 b	0.00 b
GregSten	∞ a	0.00 b	0.00 b	0.00 b
***IpaCor*2**_1	∞ a	0.00 b	0.00 b	0.00 b
*IpaCor*1	15.80 b	0.92 b	0.34 ab	0.34 ab
*IpaDur*1	-	0.71 b	0.22 b	0.22 b
*IpaVillo*1	16.00 b	0.52 b	0.17 b	0.17 b
*MagDur*_1	∞ a	0.00 b	0.00 b	0.00 b
*MagDur*_2	∞ a	0.00 b	0.00 b	0.00 b
***ValSten*1**_1	∞ a	0.00 b	0.00 b	0.00 b
***ValSten*1**_2	∞ a	0.00 b	0.00 b	0.00 b
***ValSten*1**_3	∞ a	0.00 b	0.00 b	0.00 b
***ValSten*1**_4	∞ a	0.00 b	0.00 b	0.00 b
2020: Genotype	**Incubation Period**	**IA**	**TLA**	**SLA**
886	13.95 b	0.76 ab	0.30 ab	0.30 ab
*Cor*1	∞ a	0.00 b	0.00 b	0.00 b
***Cor*2**	∞ a	0.00 b	0.00 b	0.00 b
Ipa	14.64 b	1.94 a	0.69 a	0.65 a
*Dur*4	∞ a	0.00 b	0.00 b	0.00 b
***Mag*1**	∞ a	0.00 b	0.00 b	0.00 b
Val	∞ a	0.00 b	0.00 b	0.00 b
***IpaCor*2**_2	14.69 b	0.68 b	0.28 ab	0.27 ab
***ValSten*1**_5	∞ a	0.00 b	0.00 b	0.00 b
*IpaDur*4	14.67 b	0.24 b	0.12 b	0.12 b

[a]TLA, SLA, and IA are abbreviations for total number of lesions/ leaf area (cm^2), number of sporulated lesions/leaf area (cm^2), and susceptibility index /leaf area (cm^2), respectively

susceptible control. While *IpaCor*2_2 demonstrated susceptibility in the 2020 experiment, *IpaCor*2^{2x} had no rust incidence in the 2017 experiment and *IpaCor*2_1 had no rust incidence in the 2017 experiment.

Discussion

All the wild peanut-derived allotetraploid genotypes that were not produced with *A. ipaënsis* as a parent showed promise as sources for rust resistance for peanut breeding programs, since they were highly resistant to rust, with no evidence of disease development in any assay. Therefore, these nine interspecific hybrids, *BatCor*, *BatDur*1, *BatDur*2, *BatSten*1, *GregSten*, *MagCard*, *MagDio*, *MagDur*, and *ValSten*1 made from 13 unique *Arachis* accessions are recommended to peanut breeding programs for rust resistance introgression. *BatSten*1 and *ValSten*1 were deposited in the USDA-ARS National Plant Germplasm System (Fort Collins, CO) and in the USDA Plant Genetic Resources and Conservation Unit (Griffin, GA), and seed is available for research purposes (Bertioli *et al*., 2021; Chu *et al*., 2021). The other allotetraploids will be deposited for public use after sufficient disease and insect resistance characterization has been completed.

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

Of the wild *Arachis* species tested, only one species, *Ipa*, the B-genome progenitor of peanut, was susceptible to rust. In the 2020 experiment, *Ipa* showed higher susceptibility than the susceptible control, 886, with greater IA, TLA, and SLA scores than 886, although, these differences were not significant. This affirms previous rust bioassays that found most *Arachis* species, especially those in section *Arachis*, to be highly resistant to rust (Subrahmanyam *et al*., 1983; Fávero *et al*., 2009). Like this study, Pande and Rao (2001), Fávero *et al*. (2009), and Leal-Bertioli *et al*. (2015) found *A. ipaënsis* to be as susceptible or more susceptible than cultivated peanut. Fávero *et al*. (2009) also found sporulation on six accessions of *A. stenosperma*, two accessions of *A. valida*, and one accession of *A. magna*, which were not tested in this study. So far, high resistance to rust has not been identified in *A. hypogaea* germplasm (Power *et al*., 2019; Subrahmanyam *et al*., 1982; Pande and Rao, 2001; Fávero *et al*., 2009). The lack of high resistance to rust in cultivated germplasm was contributed in part by having a highly susceptible progenitor as well as the ploidy barrier between allotetraploid peanut and its diploid, highly resistant *Arachis* relatives.

This study builds upon previous reports by identifying rust resistant allotetraploids that are cross compatible to *A. hypogaea* and are therefore valuable to breeding programs, instead of focusing solely on diploid, wild *Arachis* species. A few studies have identified rust resistance QTLs derived from the A-genome species *A. cardenasii* GKP10017 and the B-genome species *A. magna* K 30097 (Khedikar *et al*., 2010; Sujay *et al*., 2012; Leal-Bertioli *et al*., 2015). Sujay *et al*. (2012) identified five rust resistance QTLs in the same linkage group that explained up to 63% to 83% phenotypic variation; these QTLs likely originated from *A. cardenasii* GKP 10017. The populations used in Sujay *et al*. (2012) both have *A. hypogaea* GPBD 4 as a parent, which has *A. hypogaea* 'ICGV 86855] as a parent, which in turn originated from a cross between *A. hypogaea* and *A. cardenasii* GKP 10017 (Shirasawa *et al*., 2018). The QTL that explained the most rust phenotypic variation at 83% has been validated and introgressed into three cultivated varieties through marker-assisted backcrossing, improving yield by 56 to 96% in rust infected environments (Varshney *et al*., 2014). Leal-Bertioli *et al*. (2015) identified 13 rust resistance QTLs from *A. magna* K 30097. One of these QTLs contributed to four components of rust resistance, including IA, TLA, SLA, and incubation period, and explained up to 59% of phenotypic variation. Another resistance QTL explaining up to 35% of phenotypic variation was located in the same linkage group, just 25.4 to 33.1 cM away from the major QTL. These QTL identified by Leal-Bertioli *et al*. (2015) are distinct from those identified by Sujay *et al*. (2012) and can be pyramided into the same peanut cultivars to yield more effective and more robust resistance. All nine rust resistant allotetraploids identified in this study have unique *Arachis* parents compared to these two previous studies; therefore, any of these allotetraploids could be used to map new rust resistance QTL for further stacking of resistance QTLs in cultivated peanut. However, the greatest chance of identifying a major QTL would come from a mapping population using an allotetraploid that has a unique A- and B-genome species. For example, *BatCor*, *BatDur*1, *BatDur*2, *BatSten*1, *GregSten*, *MagDio,* and *ValSten*1 would be good candidates for future rust resistance QTL mapping.

One perceived limitation of this study was that both experiments were confined to *in vitro* bioassays using excised peanut leaves rather than having these *in vitro* experiments in addition to field evaluations. However, past rust field evaluations have been complicated by other fungal pathogens such as *Colletotrichum* spp., *Leptosphaerulina crassiasca* Sechet., early leaf spot*, Myrothecium roridum* Tode ex. Fr., and late leaf spot (Subrahmanyam *et al*., 1983; Sujay *et al*., 2012). Furthermore, while peanut rust is common in countries with warm, tropical climates, it only threatens U.S. peanut production every few years when brought by tropical storms (Bromfield, 1971; Subrahmanyam *et al*., 1985). When achieved, rust pressure is very inconsistent and fragmented within the same field, and results identifying resistant germplasm could easily result from avoidance of the pathogen instead of actual resistance. Lastly, the morphological differences, i.e., canopy structure, between wild *Arachis* species, allotetraploids, and cultivated peanut can add variations to pathogen pressure in the field. Despite these complications, Subrahmanyam *et al*. (1983) tested wild *Arachis* species and cultivated peanut germplasm in the field and *in vitro* and while complications in the field study from three other fungal pathogens were encountered, the reactions to rust were the same in the field and the *in vitro* experiments. Six of the wild *Arachis* accessions tested by Subrahmanyam *et al*. (1983) were also tested in this study and all were found to be highly resistant presenting no rust symptoms in both studies. Therefore, *in vitro* bioassays like this study have produced consistent results with previous studies, have similar results to field rust experiments, and have been used to map rust resistance

QTL, indicating *in vitro* bioassays are sufficient for identifying rust resistant germplasm (Subrahmanyam *et al*., 1983; Khedikar *et al*., 2010; Sujay *et al*., 2012; Leal-Bertioli *et al*., 2015).

Conclusions

This study built upon previous reports by testing rust resistance in numerous synthetic allotetraploids and diploid wild *Arachis* species. Nine allotetraploids demonstrated high levels of resistance to rust, making them a better source of rust resistance than cultivated peanut germplasm, which has only been found to have moderate levels of resistance. These allotetraploids are cross-compatible with peanut cultivars and thus, available as a genetic resource for peanut breeders. A few of these unique allotetraploids will be used to map rust resistance QTLs so that they can be introgressed into peanut cultivars along with the previously identified rust resistance QTLs from *A. cardenasii* GKP 10017 and *A. magna* K 30097. The long-term goal of this study is to create rust resistant peanut cultivars that can protect yields in the U.S. and to increase yields in tropical, developing countries for farmers that cannot afford, or do not have access, to costly fungicides.

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