First Report of Successful Hybridization between 
*Arachis vallsii* and *Arachis dardani*

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**Core Ideas**
- Development of drought tolerance has become a priority in many crops, including peanut.
- Germplasm collection data show there is variation among wild relatives of peanut for drought tolerance.
- This research represents the first report of a successful hybridization of section *Arachis* with section *Heteranthae*.

**ABSTRACT**
In this study, a hybrid of the bridge species *Arachis vallsii* and *A. dardani* was created to initiate an introgression pathway for movement of possible drought tolerance into the cultivated peanut (*A. hypogaea* L.). *Arachis vallsii* is now accepted as belonging to section *Arachis* by most taxonomists who work with the genus. *Arachis dardani* is a member of section *Heteranthae*. Based on conformation of intermediate, seed, leaf, and flower morphology of the hybrid, this article represents the first report of successful hybridization between section *Arachis* (*A. vallsii*) and species of section *Heteranthae* (*A. dardani*).

Peanut, *Arachis hypogaea* L., is an allotetraploid species (2\(n = 4x = 40\)) that has been cultivated for approximately 3500 yr (Singh and Simpson, 1994). Today it is grown throughout the temperate and tropical part of the world and is an important international crop (Kochert et al. 1991; Krapovickas and Gregory, 1994). In the United States, approximately 740,000 ha of peanut were harvested in 2017, with an average yield of 4680 kg ha\(^{-1}\) (USDA, 2017). In 2016 the estimated farm value of US production was more than US$1.2 billion, with peanut being listed as the third most valuable cash crop in the United States when considering net revenue (Schnepf, 2016).

Groundwater depletion and climate change have led to an increased interest in drought tolerance. The High Plains of Texas are an excellent example of the increasing concern over drought and groundwater levels. Most of the high plains of Texas irrigates out of the Ogallala aquifer (Chaudhuri and Ale, 2014). Although recharge rates vary due to several factors including type of landcover, soil type, and impermeable cover, they have not been sufficient to maintain water levels. In the past 70 yr, median water levels in the Ogallala aquifer in the Texas Panhandle have dropped between 25 and 67 m, primary due to irrigated agriculture (Chaudhuri and Ale, 2014). Given that the population of Texas is estimated to double by 2060, this fact will put further pressure on an already decreasing water supply (Texas Water Development Board, 2012). Consequently, the development of drought tolerance has become a priority in many crops, including peanut.

Germplasm collection data indicates that there is variation among the wild relatives of peanut for drought tolerance (Krapovickas and Gregory, 2007; Valls and Simpson, 2005). However, these wild relatives are diploid (2\(n = 2x = 20\)) and are not readily hybridized with cultivated peanut due to ploidy and species divergence (Kochert et al., 1991, 1996). These differences mean that the introgression of traits from wild relatives requires interspecific hybridization and chromosome doubling techniques to make genes available to the cultivated peanut.

The diversity of the genus makes introgression challenging. The genus *Arachis* contains nine taxonomic sections, of which section *Arachis* is the largest and includes the cultigen *A. hypogaea*. All of the species in section *Arachis* are cross compatible with *A. hypogaea* (Krapovickas and Gregory, 2007). However, the transfer of traits from other sections is more complex. Herein we report the first successful hybridization of *A. vallsii* and *A. dardani* (Krapov. and W.C. Gregory). The purpose for making this cross is to eventually extract drought tolerance traits (Krapovickas and Gregory, 1994, 2007) from *A. dardani* and move them into the cultivated peanut (*A. hypogaea*).
MATERIALS AND METHODS

The wild species *A. vallsii* (VSW 9902-1) was used as the female of the cross and is found in the Mato Grosso do Sul of Brazil in periodically flooded grasslands (Krapovickas and Gregory, 2007). In its native environment, the lateral branches grow along the top of tall native grasses and can produce pegs that will descend up to 1 m to reach the soil surface (C.E. Simpson, personal communication, 2017). This species was chosen because it has been successfully crossed with species of sections *Caulorrhizae* (Krapov. and W.C. Greg.), *Arachis*, *Procumbentes* (Krapov. and W.C. Greg.), and *Erectoides* (Krapov. and W.C. Greg.) (Simpson et al., 2018). This ability has led to its assignment to section *Arachis* (Lavia, 1999, 2001; Lavia et al., 2009; Teixeira, 2003; Peñaloza, 1999; Simpson et al., 2018) and has led to extensive use as a bridge species.

*Arachis dardani* is a member of section *Heteranthae* (Krapov. and W.C. Greg.). This species is found in the northeast region of Brazil where it typically grows in wooded Caatinga shrublands. The Caatinga has a shallow stony soil and only two defined seasons per year, a short wet season, and an extended dry season (Krapovickas and Gregory, 2007). The area is considered a dry forest region and receives, in some cases, <250 mm of annual precipitation (Selge et al., 2007). The species was of interest for its drought tolerance as it is adapted to extreme environmental conditions (Krapovickas and Gregory, 2007). Two accessions, GK 12946 and VKVeV 7215, were used as the male in a cross.

Crossing between the two species was conducted in both the spring (April–June) and fall (September–November) of 2013–2017 in greenhouses located at the Texas A&M AgriLife Research and Extension center at Stephenville. Crossing was conducted in an IBG greenhouse operating on a Wadsworth Step-50 temperature control system. The system operated where the heaters cycle on if the temperature drops below 21°C and the cooling system cycles on if the temperature exceeds 32°C. A target of 20 to 30 pegs was sought for each crossing block. The crossing procedure is a variation of the method reported by Norden (1980), were utilized for crossing, where the anthers were removed manually the evening before pollination. Due to the long lateral branches that are characteristic in the Southwest United States growing region.

Peg formation and seed set were very low (see below) for the cross. Differences were observed in given years where wet and rainy conditions were present during the crossing programs, which is conductive to successful pollination. Analysis of variance for seed production revealed differences among the crossing blocks containing GK 12946 and V 7215 (Table 1). The average successful pollination to seed percent was 2.45% for GK 12946 and 17.9% for V 7215. Although pollinations resulted in pegs from both males, the pegs from crosses using V 7215 emerged in 5 to 7 d, which was significantly earlier than pegs from crossing with GK 12946, which normally required 21 to 30 d for emergence. The 3- to 7-d time period was similar to self-pollinations of *A. vallsii*.

Putative hybrid seed was germinated, and seedlings were produced from several different crossing blocks. Hybridization was confirmed based on several morphological parameters. First, growth patterns of the crosses for leaf, pod, and seed morphology were intermediate between the two-parent species (Fig. 1). Further,

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Table 1. A table with the production of nine crossing blocks of *A. vallsii* x *A. dardani* with LSD grouping for seed produced.

<table>
<thead>
<tr>
<th>Crossing block</th>
<th>Male</th>
<th>Pollinations</th>
<th>Pegs</th>
<th>Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>13X</td>
<td>12946</td>
<td>136</td>
<td>5</td>
<td>3b</td>
</tr>
<tr>
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<td>12946</td>
<td>83</td>
<td>6</td>
<td>0b</td>
</tr>
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<td>251</td>
<td>36</td>
<td>8b</td>
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<tr>
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<td>12946</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
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<td>12946</td>
<td>133</td>
<td>13</td>
<td>6b</td>
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<td>212</td>
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<tr>
<td>17FX</td>
<td>7215</td>
<td>170</td>
<td>36</td>
<td>30a</td>
</tr>
</tbody>
</table>

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The results for the crossing block male 13X with Pollinations, Pegs, and Seed production revealed differences among the crossing blocks containing GK 12946 and V 7215 (Table 1). The average successful pollination to seed percent was 2.45% for GK 12946 and 17.9% for V 7215. Although pollinations resulted in pegs from both males, the pegs from crosses using V 7215 emerged in 5 to 7 d, which was significantly earlier than pegs from crossing with GK 12946, which normally required 21 to 30 d for emergence. The 3- to 7-d time period was similar to self-pollinations of *A. vallsii* or *A. dardani*. Putative hybrid seed was germinated, and seedlings were produced from several different crossing blocks. Hybridization was confirmed based on several morphological parameters. First, growth patterns of the crosses for leaf, pod, and seed morphology were intermediate between the two-parent species (Fig. 1). Further,
these plants flowered, and pollen evaluated using aceto-carmine staining, which indicated that all pollen was completely sterile. Given the divergence among the two species, pollen fertility was not expected based on experience in other interspecific hybrids. This research represents the first report of a successful hybridization of section *Arachis* with section *Heteranthae*. Current research is focused on attempting to double the chromosome complement, which represents the next step in the introgression process.

### CONCLUSIONS

Traditional gene introgression is currently the only commercially acceptable method available for movement of genes between wild and cultivated peanut. This research represents the first report of the use of section *Arachis* (*A. vallsii*) as a bridge species in crosses involving species of the *Heteranthae* section of the genus *Arachis*. This hybrid opens potential new pathways in which genes can potentially be transferred not only from *A. dardani* but from other species in the section into cultivated peanut (*A. hypogaea*).

Although initial attempts to establish the pathway have been successful, further work to improve fertility and introgression is needed. Alternatively, this species represents, at a minimum, a potential reservoir for the characterization of drought-tolerant genes in *Arachis*. Once the genes are identified, they are potential candidates for modification using the gene editing technologies. All research conducted in this project fits into the long-term goals including development and release of varieties with traits introgressed from *A. dardani*.

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**REFERENCES**


