

Molecular genetic relationships among *Arachis diogeni* and *A. chiquitana* accessions

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Based on morphological characters and cross compatibility relationships, the genus *Arachis* is classified into nine sections: *Arachis*, *Caulorhizae*, *Erectoides*, *Extranervosae*, *Heteranthae*, *Procumbentes*, *Rhizomatosae*, *Trierectoides* and *Triseminatae*. In total, 72 species are distributed in the nine sections.

Arachis diogeni is a member of section *Arachis*. There are numerous accessions of *A. diogeni* and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) holds two accessions of *A. diogeni*, ICG 4983 and ICG 8962. *Arachis chiquitana* is a wild species belonging to section *Procumbentes*. There are four known accessions of *A. chiquitana* and ICRISAT holds three accessions, ICG 11560, ICG 13181 and ICG 13241 (Stalker et al. 2003) (Table 1).

DNA variation has been shown to be very informative in unraveling the evolutionary history of a plant. Simple sequence repeat (SSR) markers were chosen for the study as they are highly polymorphic between species, and more importantly between individuals within a species and populations (Akkaya et al. 1992, Saghai-Marooof et al. 1994). Seventeen SSR markers were selected randomly for molecular characterization/fingerprinting of two accessions of *A. diogeni* and three accessions of *A. chiquitana* in order to ascertain whether the morphologically different *A. chiquitana* accession ICG 11560 may be an accession of *A. diogeni* (= *A. chacoense*). Young leaves from glasshouse grown plants were harvested, frozen in liquid nitrogen and DNA extracted using Qiagen mini DNA isolation kit. SSR analysis was carried out following the method standardized for groundnut (Ferguson et al. 2004). The seventeen SSR markers

which showed polymorphism between *A. diogeni* and *A. chiquitana* used in the study were 2E06, 4H11, 3B10, 4G2, 7H09, 3A06, 4C11, 4H02, 2F07, 2H11, 3F07, 4F07, 4F08, 5G09, 7G11, 13D01A and 13D01B. The amplified fragments were scored as 1 for the presence and 0 for the absence of a band. The binary data was converted into similarity matrix using Dice coefficient. The similarity matrix was subjected to multi-dimensional scaling (MDS) and unweighted pair-group method using arithmetic averages (UPGMA)-based cluster analysis to assess inter-relationships among accessions (Fig. 1).

Two accessions of *A. chiquitana*, ICG 13181 and ICG 13241, formed a distinct group. *Arachis chiquitana* accession ICG 11560 did not group closely with the other *A. chiquitana* accessions, but showed more closer relationship with them than with the *A. diogeni* accessions ICG 4983 and ICG 8962. The two accessions of *A. diogeni*, ICG 4983 and ICG 8962, grouped together and were separated from the group formed by section *Procumbentes* members. These results show that *A. chiquitana* accessions in general and ICG 11560 in particular, are not related to accessions of *A. diogeni*. In another experiment similar results were obtained when RAPDs (random amplified polymorphic DNAs) were used to distinguish wild *Arachis* species (Mallikarjuna et al. 2005).

The similarity-index-based methods (MDS and UPGMA-based clustering) suffer from a number of deficiencies (Pritchard et al. 2000). These, among others, include the lack of quantitative assessment of confidence in the clusters obtained. Therefore, to further confirm the conclusions reached above, the multi-locus genotype data on the five accessions were subjected to Bayesian

Table 1. Wild *Arachis* species used in simple sequence repeat (SSR) analysis.

Name	Accession no.	Collector no.	PI no.	Section
<i>A. diogeni</i>	ICG 4983	Coll. No. 10602	PI 276235	<i>Arachis</i>
<i>A. diogeni</i>	ICG 8962	Coll. No. 30106	PI 468354	<i>Arachis</i>
<i>A. chiquitana</i>	ICG 11560	Coll. No. 36025	PI 476004	<i>Procumbentes</i>
<i>A. chiquitana</i>	ICG 13181	Coll. No. 36028	PI 476007	<i>Procumbentes</i>
<i>A. chiquitana</i>	ICG 13241	Coll. No. 36031	PI 476010	<i>Procumbentes</i>

model-based analysis as suggested by Pritchard et al. (2000) using STRUCTURE 2.0 software (<http://pritch.bsd.uchicago.edu>). The Bayesian approach quantifies the probability of a particular accession, *a priori* known to belong to a particular section, belonging to that section based on the multi-locus genotype data. It is evident from the results in Table 2 that the accessions in the two sections are clearly separated from each other as the probability of an accession belonging to the section, to which it is known to belong to, is very high for *Arachis* accessions and is nearly one for the *Procumbentes* accessions.

During the collection of *A. chiquitana* from the Chiquitos province of Brazil, accessions Coll. No. 36025 (ICG 11560) and Coll. No. 36028 (ICG 13181) were collected at a closer distance to each other than the accession Coll. No. 36027. There is some doubt regarding Coll. No. 36027; it might indeed be an accession of *A. diogeni*. Accession Coll. No. 36027, not present in ICRISAT genebank collection, was not used in the crossability experiments nor in the molecular analysis experiments. The results of the molecular analysis of the other two accessions clearly show the identity of the *A. chiquitana* accessions ICG 11560, ICG 13181 and

Table 2. Ancestry of wild groundnut accessions as inferred from Bayesian model-based analysis.

Accession no.	<i>Arachis</i> species	Section	Probability of belonging to the section <i>Arachis</i>	Probability of belonging to section <i>Procumbentes</i>
ICG 4983	<i>A. diogeni</i>	<i>Arachis</i>	0.946	0.054
ICG 8962	<i>A. diogeni</i>	<i>Arachis</i>	0.958	0.042
ICG 11560	<i>A. chiquitana</i>	<i>Procumbentes</i>	0.003	0.997
ICG 13181	<i>A. chiquitana</i>	<i>Procumbentes</i>	0.002	0.998
ICG 13241	<i>A. chiquitana</i>	<i>Procumbentes</i>	0.003	0.997

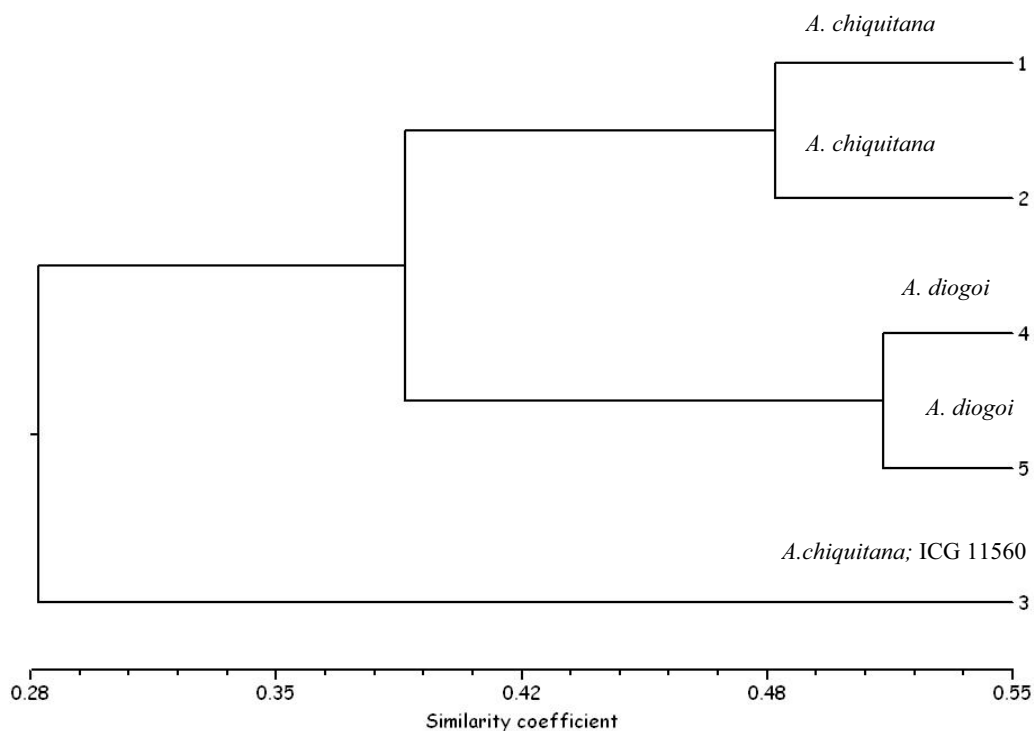


Figure 1. Dendrogram showing relationship between *Arachis chiquitana* and *A. diogeni* accessions.

ICG 13241 and that they are different from *A. diogeni* accessions ICG 4983 and ICG 8962. Accession ICG 11560 was used in the crossing experiments at ICRISAT and fertile hybrids were produced (Mallikarjuna, in press).

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