

Analysis of isoenzymatic variation in accessions of *Arachis pintoi* derived from its original germplasm collection

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Introduction

The genus *Arachis* has been originated probably in the Brazilian Central Plateau, in the northern portion of eastern Paraguay, where the most primitive species of the genus is found (Gregory et al., 1980). The genus comprises 69 species, which are distributed east of the Andes foothills, between the Amazonas and La Plata rivers (Krapovickas and Gregory, 1994). Among these species, *Arachis hypogaea* L. is commercially cultivated as a source of seeds with high levels of protein and good quality oil, while *A. glabrata* Benth., *A. pintoi* Krapov. & W. C. Gregory, and *A. repens* Handr. are cultivated as forage and ornamental species, providing exceptionally dense soil coverage.

According to Simpson et al. (1994) there is a large number of *Arachis* species that could be used as forage. Among these, *A. pintoi* had increasingly importance to pasture improvement in the tropics. Several cultivars of this species have been commercially released in the last 12 years (Argel and Villarreal, 1998).

Arachis pintoi has leaves with two pairs of leaflets, stoloniferous growth habit, and underground seed production, mostly to a depth of less than 10 cm (Krapovickas and Gregory, 1994). The underground fruit development in *Arachis* makes harder the germplasm collections, since the detection of seeds and

the estimation of their quantity are just possible after the soil under the plants is dug and sifted.

Arachis pintoi belongs to the section Caulorrhizae, has a chromosome number $2n = 20$ (Conagin, 1973), and shows high forage quality and acceptability to cattle grazing (Carulla et al., 1991; Lascano and Thomas, 1988). It has proved to be persistent and productive in tropical environments, adapted on acid soils, and tolerant to high aluminum saturation (Grof, 1985).

The original accession of *A. pintoi* was collected by Dr. Geraldo C. P. Pinto in April 1954, in the remote locality of Boca do Córrego, Bahia State, in a region covered by the Atlantic rain forest, in Brazil. The accession was initially grown in Cruz das Almas, also in the State of Bahia. After several years of increasing in a well isolated plot, at Cruz das Almas, subsamples were taken to Argentina and to United States in 1967, by Prof. Antonio Krapovickas and Dr. Walton C. Gregory, who made a voucher herbarium specimen, numbered GK 12787. From Argentina and United States, subsamples were progressively made available to several other countries, where accession GK 12787 was the only one of its species under cultivation. Several populations were derived from the original one, but no information was available on their genetic variability.

Isoenzymatic analysis is a useful method for species identification, to clarify taxonomic and evolutionary problems, to study genetic diversity and to identify distinct cultivars (Glaszmann, 1987; Kochko, 1987; Phillips et al., 1993; Ramírez et al., 1987) and has been successfully used with a wide range of plant species, including *Arachis* species (Cherry, 1975; Cherry and Ory, 1973; Grieshammer and Wynne, 1990; Klovová et al., 1983; Maass et al., 1993; Stalker et al., 1994). Other isoenzymatic analyses of seed proteins (Singh et al., 1991), and leaf tissue (Galgaro and Lopes, 1994; Galgaro et al., 1997; Lu and Pickersgill, 1993)

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have been used in *Arachis*, to evaluate phylogenetic relationships and genetic variability.

In spite of the fact that there are now many available accessions of *A. pinto*, most of the cultivars of this species released commercially until recently were derived from the original accession GK 12787, obtained at Boca do Córrego. Thus, this study aimed to evaluate the genetic basis of GK 12787 using isoenzymatic polymorphism.

Material and methods

Plant material

Seeds of *A. pinto* from nine distinct localities in six different countries were analyzed (Table 1). All of them, except V 13358, were originated from the accession collected in 1954 at the village of Boca do Córrego by Prof. Geraldo Pinto. Accession V 13358 was obtained in 1993 in a site that is believed to be where the first collection was made, near Boca do Córrego. This accession is based on seeds from 20 holes that were widely apart from each other.

Figure 1 shows the progressive increase and distribution as seeds of subsamples out of the original 1954 collection, as well as the sites of multiplication, numbering and naming of the nine accessions of *A. pinto* analyzed.

The seeds analyzed differed in age, varying from one to 12 years since harvest. Initially, these samples were used for analysis, as received at Cenargen, Brasília (Brazil).

Table 1. Countries, sites of collection or increase, and number of *Arachis pinto* samples (individual seeds) analyzed for each site.

Country	No. sites	No. samples (seeds)	Accession <i>A. pinto</i> identifications ^a
Argentina	1	26	GK 12787 CTES
Australia	2	24	CPI 58113
		80	cv. Amarillo
Bolivia	1	30	CIAT 17434 Bolivia
Brazil	3	55	VPzBmVaDb 13358
		26	VVeR 7529
		30	CIAT 17434 CPAC
Colombia	1	31	CIAT 17434
United States	1	25	GK 12787
Total	9	327	

a. Collectors: Bm = B. Maass; Db = M. D. Bechara; G = W. C. Gregory; K = A. Krapovickas; Pz = E. A. Pizarro; R = V. R. Rao; V = J. F. M. Valls; Va = S. E. S. Valente; Ve = Veiga; CIAT = International Center for Tropical Agriculture.

Isoenzymatic analysis

Several isoenzymatic systems were tested and 10 enzymes were chosen because they showed easy scoring patterns: aspartate aminotransferase (AAT), alcohol dehydrogenase (ADH), esterase (EST), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PDH), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (PGD), and phosphoglucoisomerase (PGI). Staining procedure and electrophoretic conditions was done according Soltis et al. (1983) and Stuber et al. (1998) with minor modifications. Isoenzymes were extracted from seeds that were soaked in fresh water for 2 days. The seeds were macerated with 300ml of cold extraction buffer, composed by 50 ml of TRIS-HCl 0.05M pH 8.3, 10 g of sucrose, 2.5 g of PVP 40, 0.25 ml of triton X 100 and 50 μ l of mercaptoetanol. The isoenzymes were separated in 13% partially hydrolyzed starch (penetrose) gel.

Data analysis

The isoenzymatic loci analyzed were inferred from the banding patterns observed, and from data of genetic control of the same enzymatic systems in other genera (Kephart, 1990).

Results and discussion

The inferences on genetic control—enzyme structure and number of loci—of the enzymes analyzed were based on studies developed for other species, including many of the section *Arachis* (Lu and Pickersgill, 1993).

Aspartate amine transferase system showed two bands in all the samples, which were assumed to be coded in two loci, which were homozygous for one allele. The other two bands detected in the gel were considered to be secondary bands, since they had fainter intensity. A monomorphic locus with one band was found in ADH and IDH. Leucine aminopeptidase (LAP) showed two bands, that were very close to each other, and had the same intensity. Lu and Pickersgill (1993) described similar results and they suggested the two bands observed were formed by isoenzymes coded in two distinct loci. Two activity zones were observed in MDH, the most cathodal zone being composed of three bands (two dark ones and a faint one), and the other zone by only one dark band. One band was found in the G6PDH in all the samples. This band was considered to be coded in one locus, where only one allele was detected. Two bands were observed in PGD, one darker than the other. Two bands were also observed in *Arachis* species evaluated for PGD locus (Lu and Pickersgill, 1993). Two bands were found in

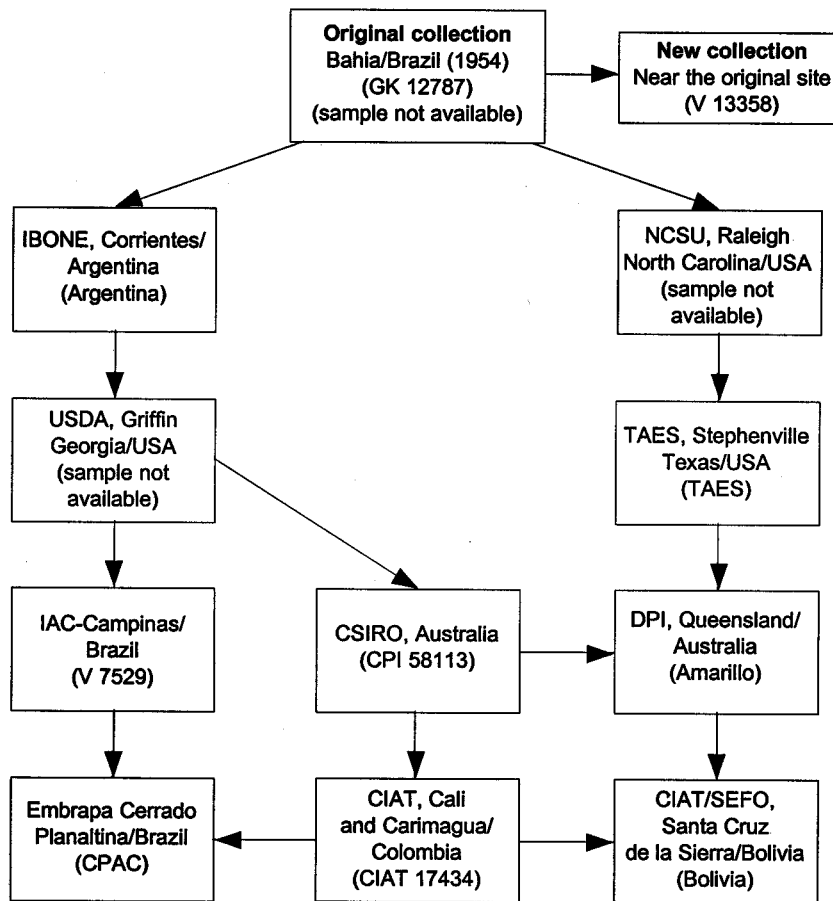


Figure 1. Progressive increase and distribution as seeds of subsamples out of the original 1954 collection, as well as the sites of multiplication, numbering, and naming of the nine accessions of *A. pintoii* analyzed.

PGI. Lu and Pickersgill (1993) found similar results for PGI, and proposed those two isoenzymes were coded in two different loci.

PGM and EST were the only system that showed polymorphism. In PGM, two putative alleles were observed in the same presumptive locus, resulting in patterns of one band or two bands. The band patterns observed fit in the descriptions for monomeric enzymes (Kephart, 1990). Lu and Pickersgill (1993) also found monomeric enzymes in a PGM loci for species of the section *Arachis*. Since changes in esterase activity associated with seed deterioration have been documented in the groundnut (*A. hypogaea*) by Aung and McDonald (1995) and it was known the seeds analyzed had different ages, the polymorphism found in EST was not taking in account and a new analysis for this enzymatic systems was performed using seeds of same age. The results showed the polymorphism detected for EST is due to the fact that the seeds were of different ages, and different EST genes were being expressed.

The polymorphism found in PGM system was only observed in the V 13358 accession. This accession is a sample with a structure different from the others. All other samples were originated from a single plant, introduced in Cruz das Almas by G. Pinto in 1954, which was taken and propagated in different localities. Accession V 13358 was collected in 1993, at what seems to be the original collection site, since detailed instructions for the location of the natural population of *A. pintoii* was kindly provided by Prof. G. Pinto. Seeds of accession V 13358 were dug and sifted from the soil at 20 points.

No genetic variation was detected within on among the accessions derived from accessions GK 12787. The absence of variation might be due to two reasons: (1) the fact that the original accessions made by Prof. G. Pinto has been based on very few or a single plants, and (2) the population where it was collected might have a very narrow genetic basis. The first hypothesis cannot be tested because a significant sample of the original accessions would have to evaluate and that is not available. On the other hand, the second hypothesis is

one of the probable reasons to the low polymorphism detected within and among the accessions since the results showed that the accession *A. pintoi* V 13358 had little variations in it and it was very similar to the other accessions, sharing the same alleles in all loci analyzed.

The low polymorphism in the location where *A. pintoi* V 13358 was collected can be due to the following factors: (1) that population was originated from few or even single floating plants or stolons spread down the Jequitinhonha river by floods, and (2) mechanisms that could increase the level of variation (gene flow, migration) do not happen often.

Heywood and Fleming (1986) suggested four explanations for the low allozyme variation in sympatric populations of three species of Piper (Piperaceae): (1) the populations may have been found relatively recently, (2) they may have been found by a small number of individuals, (3) there may be restricted gene flow between populations, and (4) the populations have suffered recent bottlenecks in numbers of individuals. All explanations do apply to the low allozyme variation in the cultivated subpopulations of *A. pintoi* deriving from the initial collection made in 1954. As such populations start out of the first increase in Cruz das Almas, not even 50 years ago; the original number of individuals was low, possibly a single one; all subsamples were grow isolated from other accessions; and, at each transfer from one institution to the next, the number of seeds was always low, as normally occurs in germplasm exchange. Reasons 2 and 3 are more likely to explain the low within-accession variability in nature, as documented for V 13358.

Just one genotype seems to be present, in the original accession of *A. pintoi* and its derived subpopulations, since no isoenzymatic variation has been found among them. However several cultivars have been released out of this accession, under distinct institutional frameworks, in different countries. Such cultivars may prove to be all quite similar to each other, from a genetic standpoint. Genetic improvement will probably be obtained, when the different accessions of *A. pintoi*, that have been intensively collected in the last decade (Valls and Pizarro, 1994) are incorporated in forage breeding programs. There is also a possibility that some of these new accessions are already fit for direct release as additional cultivars.

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Resumen

Mediante el análisis de polimorfismos enzimáticos se estudió la variabilidad genética en la accesión original de *Arachis pintoi* (recolectada en 1954 en Brasil) y en accesiones derivadas, y se compararon con la variabilidad entre nueve accesiones derivadas de la accesión original. Las accesiones habían sido multiplicadas en ocho localidades diferentes en varios países. Para evaluar la variabilidad presente en el sitio de origen se tomó una muestra estratificada en 20 lugares diferentes y muy próximos al sitio de origen probable. De las 10 enzimas analizadas, solamente dos revelaron polimorfismo: esterasa y fosfoglucomutasa. Como inicialmente fueron usadas muestras de semillas con amplias diferencias de edad entre ellas, el polimorfismo observado en las esterases pudo deberse a variaciones de origen epigenético y ambiental. Se realizó un segundo análisis de este sistema, usando semillas con germinación sincronizada durante una generación, bajo condiciones controladas. Este segundo análisis permitió observar patrones monomórficos de esterasa en todas las accesiones; por consiguiente, para las enzimas analizadas se detectó una alta uniformidad entre las accesiones estudiadas. La variación observada en la enzima fosfoglucomutasa sólo fue detectada en la muestra estratificada. La baja variación total observada pudo ser debida a dos razones: la colección de germoplasma original, que dio origen a la parcela de campo inicial, estaría representada por pocas plantas o por solamente una planta y todas las accesiones derivadas habrían sido originadas por un pequeño número de individuos; o el polimorfismo fue bajo en la población original, representada en este estudio por la accesión establecida a partir de semillas coleccionadas en 20 puntos en el sitio de origen natural.

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