Indian J. Genet., 72(3): 384-389 (2012)

Short Communication

Genetic diversity in Bolivian landrace lines of groundnut (*Arachis hypogaea* L.)

Fatema Husain and Nalini Mallikarjuna*

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324

(Received : October 2011; Revised : May 2012; Accepted: June 2012)

Abstract

Bolivian landraces of groundnut were analyzed for morphological and molecular diversity as well as for late leaf spot resistance, an important fungal disease of groundnut. The study revealed large diversity with respect to seed color, size and weight. There was variation for oleic and linolic acid contents ratio too. Molecular studies using SSR markers showed large diversity between Bolivian landrace lines and improved cultivars of groundnut. Late leaf spot resistance studied through detached leaf bioassay showed moderate to high levels of resistance, which can be exploited for the improvement of cultivated groundnut.

Key words: Groundnut, Bolivian landrace, morphological diversity, molecular diversity, LLS resistance.

Landrace refers to a population that has its own morphological and genetical integrity and develops naturally from wild species without systematic selections by breeders. A significant proportion of the world's farmers grow landraces, they reflect the cultural identity of the people and harbor a diversity that is of interest for future breeding work. The landraces used in this study are from Bolivia in South America, one of the seven gene center of diversity. Although cultivated groundnut (*Arachis hypogaea* L.) exhibits a considerable amount of variability for various morphological, physiological, and some agronomic traits, little variation has been detected at the molecular level. Whereas, landraces have diverse morphological as well as variable genetic base [1]. As landraces have a long history of specific adaptation to low-input agriculture and have maintained a considerable amount of genetic variability, landraces can be a useful breeding material in stress environments and for farmers with low input agriculture.

Groundnut is susceptible to a range of diseases caused by bacteria and fungi. Among the fungal diseases, late leaf spot (LLS) caused by *Phaeoisariopsis personata* is widespread globally and cause yield losses ranging from 0-50 percent or even more [2], varying considerably from place to place and between the seasons. Losses due to leaf spots of around 10 % have been estimated even with the use of fungicide. Although some progress has been made in developing varieties with resistance to LLS, the improvements that can be made by breeders are limited by the availability of genes within cultivated *A. hypogaea*. The landrace germplasm used in this study are tetraploid, and hence should be a straight forward process to recover tetraploid progeny. Morphological and molecular diversity studies have shown that Bolivian landraces are diverse when compared to cultivated germplasm used in the study. It can be speculated that utilization of such germplasm can broaden the genetic base of groundnut.

The study included 11 accessions of Bolivian landraces obtained from USDA-ARS, Georgia, USA., and 3 accessions of cultivated groundnut for morphologically characterization (Table 1).

*Corresponding author's e-mail: n.mallikarjuna@cgiar.org
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
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Table 1. Morphological studies in Bolivian Landrace lines

Landraces/ accessions	Seed length	Seed width	100-seed weight in gm	Leaf height	Leaf width	O/L	Pod beak	Pod reticulation	Pod length	Pod width	Seed colour	Primary seed colour	Secondary seed colour	Seeds per plant
PI 161298	1.6	0.99	58.08	40.3	17.8	1.40	Slight	Slight	3.4	1.5	Variegated	Red	white	35
PI 261906	1.7	1.05	69.7	40.33	21.2	1.28	Absent	Slight	3.2	1.6	Single	Red	none	41
PI 331332	1.6	1	29.4	43.5	22.8	1.32	Slight	Slight	3	1.5	Single	Red	none	40
PI 339967	1.3	0.9	68.91	42.3	21.9	Not studied	Absent	Moderate	3	1.4	Single	Red	none	38
PI 339972	1.4	1	53.1	41.1	20.9	1.53	Slight	Deep	3.3	1.3	Variegated	Red	white	37
PI 468270	1.5	1	89	41	24	1.38	Slight	Deep	3.6	1.7	Variegated	Red	white	45
PI 475971	1.5	1.1	54.71	45.6	23.9	1.74	Slight	Deep	3.8	1.6	Single	Tan	none	42
PI 475972	1.6	1.06	39.1	46.6	22.4	2.00	Absent	Deep	3.4	1.5	Single	Tan	none	44
PI 497358	1.3	0.83	85.4	44	21	1.65	Absent	Deep	3	1.5	Single	Tan	none	39
PI 497412	1.5	0.9	50.2	42.1	20.1	1.38	Absent	Deep	3.2	1.5	Single	Tan	none	41
PI 540866	1.6	1	46.68	40.2	20.7	1.82	Absent	Deep	3.1	1.4	Single	Tan	none	48
TMV2	1.3	0.8	32.5	39.3	22.1	0.97	Absent	Moderate	2.8	1.6	Single	Tan	none	48
JL 24	1.3	0.8	37	43.22	23.01	0.94	Slight	Slight	2.9	1.3	Single	Tan	none	43
ICGS 44	1.7	0.8	44	44.5	22.89	0.94	Absent	Moderate	2.8	1.6	Single	Tan	none	51

Observations on selected morphological characters were recorded according to agro-morphological and taxonomic descriptors [3]. Data on traits were recorded on ten randomly selected plants from each accession (Fig. 1). All the genotypes had orange colour flower which two seeds per constricted pod.

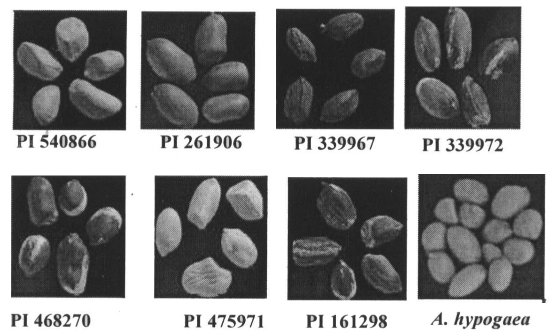

Fig. 1. Variation in seed coat colour

Molecular diversity study was carried out using 10 accessions of Bolivian landraces along with 3 accessions of *A. hypogaea.* The CTAB procedure was adopted for isolation of DNA. PCR amplification of microsatellite loci using 21 primer pairs for Bolivian landraces was carried out. Amplified products were pooled as per multiplex plan and separated on an ABI 3700 fragment analyzer. The results were evaluated using the software package Genotyper 3.7 (Applied Biosystem Foster City, CA, USA). Genetic polymorphism was measured in terms of number of alleles per locus, expected and observed heterozygosity, average genetic distance between accessions (Dg) and the polymorphic information content (PIC) using Power marker V3 [4].

Genetic diversity analysis was carried out by using the program DARwin [5]. A dissimilarity matrix was calculated by using simple matching coefficient with 90% of minimal proportion of valid data required for each unit pair and 4000 replicate bootstrapping. Factorial analysis of the samples was performed based on dissimilarity matrix of the data. The dendrogram was built by using the NJ method (UPGMA) developed by Saitou and Nei [6]. Screening for late leaf spot (LLS) was carried out following the method described by Mallikarjuna *et al*. [7].

Growth habit was found to be annual in the Bolivian landraces as well as in the cultivated groundnut.

Similarly stem branching pattern was found to be alternate as in the cultivars. Stem pigmentation was absent and stem hairiness was present both in the landraces and in the cultivars. Leaf shape was elliptic in all the accessions studied. Flower color was orange across all the accessions. Leaf height ranged from 40.2 mm to 46.6 mm in the landraces, whereas it ranged from 39.3 mm to 44.5 mm in the cultivars. Pod beak was found to be either slight or completely absent in all the accessions studied. Pod constriction was found to be slight in all the accessions, whereas reticulation was slight to deep in the Bolivian landraces and was either slight or moderate in the cultivars. Pod length ranged from 3 cm to 3.8 cm in the landrace accessions and 2.8-2.9 in cultivars. Pod width ranged from 1.3 cm to 1.7 cm in the landrace accessions and 1.3-1.6 in the cultivars. An average of two seeds per pod was found all across the accessions. Seed coat color was observed to be tan of all the accessions of cultivated groundnut. Bolivian landraces were found to have variable seed coat color ranging from single color varieties to variegated colored varieties. PI 161298, PI 339972 and PI 468270 were variegated with red and white seed color, whereas other accessions were either red or tan in color. Seed length varied from 1.3 cm to 1.7 cm all across the accessions, whereas seed width varied from 0.8 cm to 1.06 cm all across. As compared to the cultivated accessions, 100 seeds weighed more in the Bolivian landraces. It was found to be 32.5 g to 44 g in the cultivated species as compared to 29.4 g to 85.4 g in the Bolivian Landraces. O/L ratio was calculated by measuring the oleic and linolic acid content of the seeds. It was found to be higher (1.28-2.00) than the cultivated groundnut (0.94-0.97). An average yield of 40.9 pods per plant was observed in the landrace accessions as compared to an average of 47.3 pods per pot in the cultivated groundnut (Table 1).

A total of 21 SSR markers were used to study diversity within Bolivian landraces and to check how diverse they were from the cultivated groundnut. The allele number ranged from 2 (8E12) to 12 (TC6E01)

Table 2. Genetic diversity between the accessions as revealed by SSR markers

Marker	Major allele frquency	Genotype no.	Sample size	No. of obs.	No. of allele	Availability	Gene diversity	Hetero-zygosity	PIC value
1.70E+04	0.4583	4	12	12	4	1.00	0.6563	0.9167	0.5949
TC11H06	0.4091	7	12	11	6	0.91	0.7397	0.2727	0.7033
TC6E01	0.2083	7	12	12	12	1.00	0.8819	1.0000	0.8709
19B1	0.6667	4	12	12	4	1.00	0.5139	0.0000	0.4760
TC6H03	0.3636	7	12	11	8	0.91	0.7975	0.1818	0.7754
5D5	0.3333	8	12	12	10	1.00	0.8056	0.7500	0.7828
8.00E+12	0.9167	2	12	12	2	1.00	0.1528	0.1667	0.1411
TC4F12	0.4167	5	12	12	5	1.00	0.6944	0.0000	0.6437
1.30E+10	0.5000	4	12	12	5	1.00	0.6285	0.7500	0.5653
TC1A02	0.3750	6	12	12	7	1.00	0.7743	1.0000	0.7456
18C5	0.6667	5	12	12	5	1.00	0.5278	0.0000	0.5025
1B9	0.4583	4	12	12	5	1.00	0.6597	1.0000	0.6007
7H6	0.7500	3	12	12	3	1.00	0.4028	0.0000	0.3633
2D12B	0.5000	6	12	12	8	1.00	0.6979	0.2500	0.6702
15C12	0.3333	6	12	12	6	1.00	0.7500	0.1667	0.7090
TC3E02	0.8333	3	12	12	3	1.00	0.2882	0.0833	0.2640
TC6G09	0.8750	2	12	12	2	1.00	0.2188	0.2500	0.1948
TC7H11	0.2917	10	12	12	10	1.00	0.8507	0.7500	0.8363
TC1E01	0.2083	8	12	12	7	1.00	0.8403	0.6667	0.8199
TC11A04	0.4167	6	12	12	8	1.00	0.7535	0.9167	0.7250
Mean	0.4991	5.35	12	11.9	6	0.99	0.6317	0.4561	0.5992

to an average of 5.9 mm in TMV2. Infection frequency ranged from 0.23 in PI 540866 to 1.38 in PI 475972 as compared to 1.8 in the cultivar. Average incubation period ranged from 13 DAI in PI 331332 to 20 DAI in PI 468270, and 7 DAI in TMV2. Average latent period ranged from 22.33 DAI in PI 331332 and PI 339967 to 27.97 in PI 475971 as compared to 12 DAI in TMV2 (Table 4).

Morphological diversity with respect to seed size and color was evident the Bolivian landrace lines. The O/L ratio in all the landrace lines was higher than that of cultivated groundnut. This might be one of the traits that cultivated groundnut would benefit if transferred. The seed weight in Bolivian landrace lines was generally higher than the cultivated groundnut used in the study. This may again be a desirable trait for confectionary

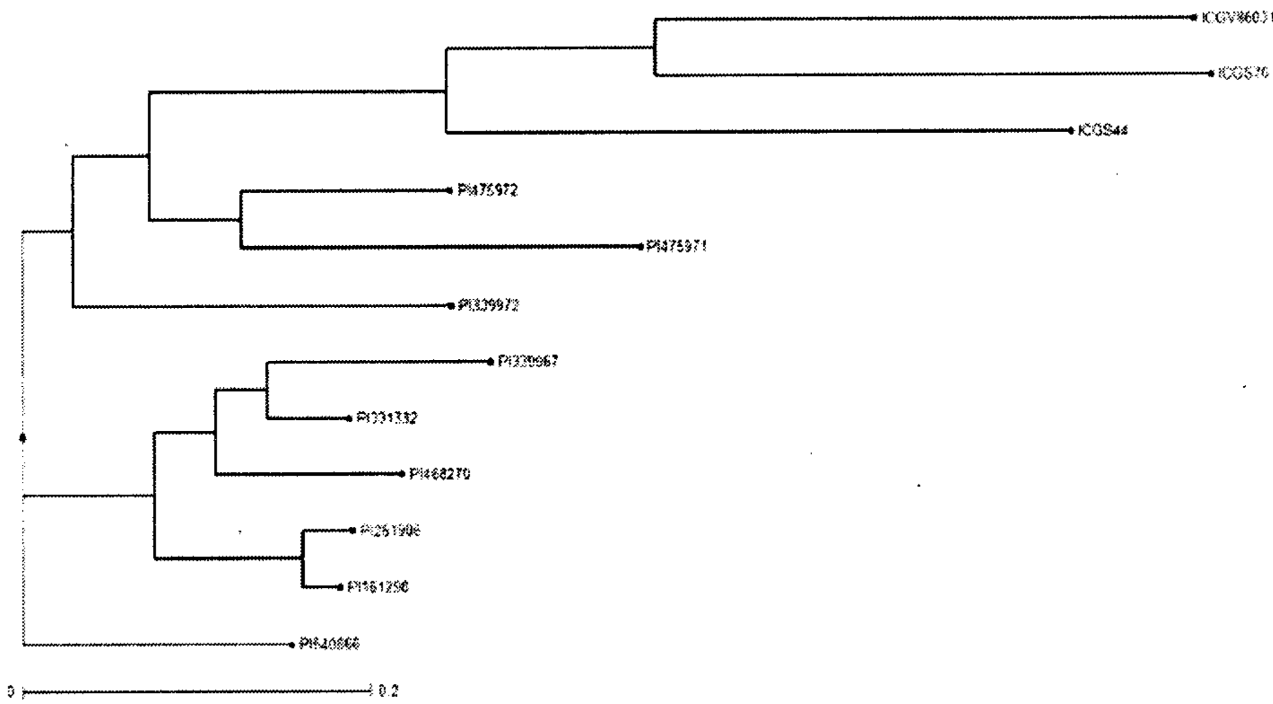

Fig. 2. NJ tree showing molecular diversity in Bolivian landraces

per locus for all genotypes, with an average of 6 alleles per locus. The average PIC value being 0.59, was lowest with 8E12 (0.1) and highest with TC6E01 (0.87) (Table 2). Gene diversity ranged from 0.15 with 8E12 to 0.88 by TC6E01, whereas heterozygosity ranged from 0.0 with 18C5 and 7H6 to 1.00 with TC6E01, TC1A02 and 1B9 (Table 2). Genetic distances were calculated using Power Marker and was found to be in the range of 0.63 to 0.92 between the landrace lines and the cultivated groundnut, whereas it ranged from 0.1 to 0.62 between the landrace lines (Table 3). A NJ tree was constructed based on genetic distances (Fig. 2). Cluster I comprised PI 339972, PI 475971 and PI 475972 along with the cultivated groundnut as a separate sub-cluster. Whereas in Cluster II, PI 339967 clustered with PI 331332, PI 468270, PI 261906 and PI 161298. PI 540866 formed an out cluster from other accessions. Genetic distance was found to be in the range of 0.63 to 0.92 between the landrace accessions and the cultivated groundnut, whereas it ranged from 0.1 to 0.62 between the landrace lines (Table 3).

Ten Bolivian landrace lines and their hybrids were also screened for LLS. They showed a wide range of variation in their reaction to the disease. Among the components of resistance, lesion number on 30th day after inoculation (DAI) ranged from 4 in PI 161298 and PI 261906 to 21.7 in PI 331332 as compared to 59.4 in TMV2. Leaf Area Damage (LAD) on 30th DAI ranged from 2 % in PI 540866 to 18.7 % in PI 497412, whereas LAD was 85 % in TMV2. Lesion diameter ranged from 1.5 mm in PI 161298 to 3.1 in PI 331332 as compared

Table 3. Genetic distances between the accessions

OTU	PI540866	PI161298	PI261906	PI475972	PI339972	PI331332	PI468270	PI339967	PI475971	ICGS44	ICGS76
PI161298	0.3250										
PI261906	0.3500	0.0500									
PI475972	0.3250	0.4500	0.4500								
PI339972	0.4250	0.4250	0.4250	0.3250							
PI331332	0.4000	0.2000	0.2500	0.5250	0.4500						
PI468270	0.3421	0.2895	0.2632	0.4474	0.4474	0.1579					
PI339967	0.4000	0.2750	0.2750	0.5500	0.5000	0.1750	0.2895				
PI475971	0.4250	0.5500	0.5750	0.3500	0.5750	0.6250	0.5789	0.6250			
ICGS44	0.8000	0.8000	0.7750	0.7750	0.8500	0.8250	0.7895	0.8000	0.7250		
ICGS76	0.8500	0.9250	0.9250	0.7750	0.9250	0.9250	0.8684	0.9250	0.8250	0.7250	
ICGV86031	0.8684	0.8421	0.8421	0.8684	0.8421	0.8421	0.8611	0.8421	0.8947	0.8684	0.6316

Table 4. Components of Late Leaf Spot disease resistance in Bolivian landrace lines

Genotype	IP	LP	LAD30	LN30	L diameter	IF
PI 161298	14	22.79	3.7	4	1.53	0.32
PI 261906	19.67	23.6	3	4	1.71	0.27
PI 331332	13	22.33	12.3	21.7	3.16	1.03
PI 339967	13	22.33	6.3	13.3	2.84	0.67
PI 468270	20	25.6	5.3	4.3	2.42	0.26
PI 475971	17	27.97	2.3	5.3	1.91	0.23
PI 475972	17	23.7	2.3	8.7	1.7	1.38
PI 497358	14.67	23	12	15.7	2.73	1.02
PI 497412	13	22.34	18.7	27	2.33	1.04
PI 540866	14	25	2	5.7	2.5	0.23
TMV2	7	12	85	59.4	5.93	1.8
Mean	14.76	22.79	13.9	15.4	2.62	0.75
SED	3.009	1.44	8.23	8.43	0.564	0.534
LSD	6.2	3.26	17.16	17.7	1.8	1.123
CV%	25	7.8	72.4	67.1	26.4	87.1
F value	0.02	<0.001	<0.001	<0.001	<0.001	0.089

IP : Incubation period; LP : Latent period; LAD : Leaf area damage; LN : Lesion number; LD : Lesion diamter; IF : Infection frequency

groundnuts where large seed size is preferred over small seed size. The presence of colored seed coat may be a desired trait as flavonoids are known to be present in colored seed coat and many of the flavonoids have antioxidative properties [8]. The diversity present was also evident when molecular analysis was carried out. Genetic diversity has been previously documented in landraces from Brazil [1]. The present investigation shows genetic diversity between Bolivian Landrace lines and cultivated groundnut to be large enough to benefit cultivated groundnut with wider genetic diversity.

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