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Genotypic Variation for Late Leaf Spot and *Aspergillus* Colonization in Mini Core Set of Groundnut (*Arachis hypogaea*)

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ABSTRACT

Core collection could greatly increase the utilization of germplasm resources. Groundnut mini core subset was evaluated for resistance against late leaf spot and seed colonization by *Aspergillus flavus*. High level resistance for late leaf spot was observed in sixteen accessions including ICG 12625, ICG 15419, ICG 12697, ICG 12682, ICG 4716, ICG 76, ICG 8760, ICG 2857, ICG 4412, ICG 9905, ICG 12672, ICG 13787, ICG 3027, ICG 532, ICG 6706 and ICG 14475. While the high level of resistance to seed colonization by *A.flavus* was observed in ICG 6027, ICG 3673, MN 1-35, ICG 14985, ICG 8760 and ICG 13787. The accessions namely ICG 8760 and ICG 13787 were found to possess high level of resistance to both late leaf spot and *Aspergillus* colonization and could be utilized in multiple disease resistance breeding programme.

Key words : *Aspergillus* colonization, Groundnut, Late leaf spot, Mini core.

Groundnut is an important source of oil and protein. It is native of South America where the greatest diversity is found (Krapovickas, 1969).Gregory and Gregory (1976) recognized the Chaco region between Bolivia and North Western Argentina as the prime center of diversity of cultivated groundnut. Breeding of groundnut cultivars with high yield potential and resistance to various biotic and abiotic constraints is the main objective to enhance the productivity in most of the groundnut improvement programmes.

The foliar disease such as Cercospora or Tikka leaf spots (Early & Late Leaf Spot) are the important fungal diseases of groundnut. In India, Late Leaf Spot is more predominant compared to Early Leaf spot because of its fast spreading nature. This disease is causing substantial yield loss as well as reduce the quality of the produce. Aflatoxin contamination is another wide spread serious problem. The aflatoxin producing fungus *A. flavus* and *A. paraciticus* can invade seed in the field before harvest and during post harvest drying and curing. It makes the produce unfit for consumption and is a major impediment for export of groundnuts. There is a need to develop and identify new germplasm combining high level of resistance to multiple diseases. Although progress has been made to increase yield in cultivars, satisfactory resistance to most diseases has not been achieved because of limited genetic resources within the *A.hypogaea* gene pool.

To overcome the need for large scale evaluation of germplasm collections against various biotic and abiotic stresses, Frankel (1984) proposed the concept of core collection (10% of an entire collection) to minimize repetitiveness within the collection and to represent the genetic diversity of a crop species. Upadhyaya *et al.,* (2001) have developed a groundnut core collection consisting of 1704 entries using data of taxonomical, geographical and morphological descriptors. The peanut core collection has been very effective in enhancing the utilization of peanut genetic resources (Holbrook, 1999). However, an even smaller subset of germplasm is needed for traits, which are difficult and / of expensive to measure. Upadhyaya *et al.,* (2002) suggested a mini core (10% of core collection, 1% of an entire collection) approach. A groundnut mini core consisting of 188 accessions along with three breeding lines and four cultivars were evaluated to identity sources of multiple disease resistance in the mini core subset of the groundnut germplasm against late leaf spot, *Aspergillus* seed colonization for use in disease resistance breeding programmes to develop resistant cultivars.

MATERIAL AND METHODS

The experimental material comprised of 188 diverse accessions of mini core which includes fastigiata (33), Vulgaris (71), Peruviana (2), Aequatoriana (1), hypogaea runner (33), hypogaea bunch (48), three breeding lines (R 9227, MN 1-28 and MN 1-35) and four cultivars (GPBD-4, TAG 24, JL 24 and M28-2). The mini core accessions were evaluated in a Lattice square design with two replications at Botanical garden of University of Agricultural Sciences, Dharwad. The inter and intra row spacing of 30 cm X 10 cm was adopted in sowing of experimental material. Need based plant protection measures were adopted to raise a good crop.

Evaluation for late leaf spot:

Artificial epiphytotic conditions were created to enhance the disease development. When the crop was at 30 days, old Late Leaf Spot spore inoculum was sprayed regularly at an interval of 3 days. The accessions were scored at 70 days and 90 days for late leaf spot infection using modified 0-9 point scale given by Subbarao *et al.,* (1990) based on the extent of leaf area damage as given in Table 1.

Evaluation for resistance to seed colonization by *Aspergillus flavus* :

The pure culture of *A. flavus* strain *Af* 11-4, a highly aggressive and toxigenic strain was used for *In vitro* seed colonization (Thakur *et al.*, 2000) . Fifty sound mature seeds from each genotype were surface sterilized with 0.1% aqueous solution of Mercuric Chloride for 2 minutes and washed in two changes of distilled water. Each seed was uniformly wounded by pricking with sterile needle to facilitate invasion of fungal spores. Seeds were kept in petriplates and were inoculated with spore suspension ($1x10^6$ spores/ml). The petriplates were incubated at 25°C and at a relative humidity of 95% for 10 days. Individual seeds were scored for the extent of seed surface colonization using 1-4 scale of colonization severity as given by Thakur *et al* (2000) and detailed in Table 2.

Disease reaction:

Reaction of mini core accessions to late leaf spot was categorized as resistant (disease score: 1-3 scale), moderate (disease score: 3.1-6 scale) and susceptible (disease score: 6.1-9). Reaction to *Aspergillus* seed colonization was categorized as resistant (1-2 scale), moderate (2-3 scale) and susceptible (3-4 scale).

RESULTS AND DISCUSSION

A mini core collection of groundnut with 188 accessions represents the total diversity contained in the entire collection. The mini core subset composition reflected the predominance of Asian entries in the core subset. In the mini core subset, the number of entries included were 63 (33.51%) from Asia, 58 (30.85%) from America, 41(21.81%) from Africa, 23(12.23%) from other countries and 3(1.60%) from Europe (Upadyaya et al, 2001). South America, which is the primary center of diversity (Krapovickas, 1969) accounted for 29 (15.43%) entries in the mini core subset.

Evaluation for late leaf spot resistance:

The mean disease score in subsp. *fastigiata* viz. Valencia (5.19) and Spanish bunch (5.47) were slightly higher as compared to subsp *hypogaea* viz., Virginia runner (3.91) and Virginia bunch (4.04). Late leaf spot disease score ranged from 1.67 (MN 1-35) to 7.33 (ICG 397) in mini core and it was also recorded in four botanical varieties i.e., in Valencia (3.67 to 7.33), Spanish bunch (2.55 to 7.33), Virginia runner (2.83 to 5.68), and Virginia bunch (2.5 to 6.26). Among the checks GPBD-4 recorded the highest resistance (1.62) followed by mutant (2.67) while TAG 24 was highly susceptible (8.84). The accessions Viz., ICG 12625 (2.33) of Valencia; ICG 15419 (2.50), ICG 12697 (2.50), ICG 12682 (2.83) and ICG 4716 (3.00) of Spanish bunch; ICG 76 (2.83), ICG 8760 (2.84), ICG 2857 (3.00), ICG 4412 (3.00) and ICG 9905 (3.00) of Virginia runner; ICG 12672 (2.50), ICG 13787 (2.50), ICG 3027 (2.83), ICG 532 (2.83), ICG 6706 (3.00) and ICG 14475 (3.00) of Virginia bunch revealed resistance against late leaf spot. The genotypes MN 1-28 (1.84) and MN 1-35 (1.67) of Valencia were on par with GPBD-4 (1.62).

Evaluation for *Aspergillus* seed colonization resistance:

In the min core, *Aspergillus* seed colonization ranged from 1 (ICG 8760) to 4. The

Table 1. Modified 9-point scale used for field-screening of groundnut genotypes for resistance to late leaf spot.

Disease Score	Description	Disease severity (%)
1	No disease	0
2	Lesions present largely on lower leaves, no defoliation	1-5
3	Lesions present largely on lower leaves, very few on middle leaves, defoliation of some leaflets evident on lower leaves	6-10
4	Lesions on lower and middle leaves but severe on lower leaves; defoliation of some leaflets evident on lower leaves	11-20
5	Lesions present on all lower and middle leaves, over 50 percent defoliation of lower leaves	21-30
6	Severe lesions on lower and middle leaves, lesions present but less severe on top leaves, extensive defoliation of lower leaves, defoliation of some leaflets evident on middle leaves.	31-40
7	Lesions on all leaves but less severe on top leaves, defoliation of all lower and some middle leaves	41-60
8	Defoliation of all lower and middle leaves, severe lesions on top leaves, some defilation of top leaves evident.	61-80
9	Almost all leaves defoliated, leaving bare stems, some leaflets may remain, but show severe leaf spots	81-100

Table 2. Seed surface colonization and severity scale (Thakur *et al*., 2000).

Scale	Description
1	<5 per cent seed surface colonized with scanty mycelia growth and scanty sporulation.
2	5-26 per cent seed surface colonized with good mycelia growth and scanty sporulation
3	26 – 50 per cent seed surface colonized with good mycelia growth and good sporulation
4	>50 per cent seed surface colonized with heavy sporulation

accessions, ICG 6027, ICG 3673, MN 1-35 (1.02 to 1.16) of Valencia type, ICG 14985(1.08) of Spanish bunch, ICG 8760 (1.0) of Virginia runner, and ICG 13787 (1.10) of Virginia bunch were found resistant by recording lower infection than the resistant check TG 49 (1.16). While, moderate resistance to seed colonization was observed in ICG 12625 (2.04) and ICG 1862 (2.16) of Spanish bunch, ICG 76 (2.40) and ICG 2381 (2.32) of Virginia runner, ICG 6402 (2.24) and ICG 12276 (2.29) of Virginia bunch and MN 1-28 of Valencia (2.08). Many genotypes were susceptible to *Aspergillus* seed colonization and were on par with the susceptible check TMV-2 (4.0). The accessions namely ICG 8760 of Virginia runner, ICG 13787 of Virginia bunch have shown the high level of resistance to both late leaf spot as well as *Aspergillus* seed colonization.

In general, Spanish bunch accessions recorded high kernel yield followed by Valencia, Virginia runner and Virginia bunch types in the decreasing order. The promising superior resistant accessions identified were ICG 12625 of Valencia, ICG 8760 and ICG 769 of Virginia runner and ICG

Table 3. List of superior genotypes identified for resistance to Late Leaf Spot and *Aspergillus s*eed colonization in minicore of groundnut.

S.No	Disease Resistance Trait	Botanical types							
		Valencia		Spanish Bunch		Virginia Runner		Virginia Bunch	
		Genotype	Origin	Genotype	Origin	Genotype	Origin	Genotype	Origin
1.	Late Leaf Spot	ICG 12625 (2.33)	Ecuador	ICG 4716 (3.00)	Unknown	ICG 76 (2.83)	India	ICG 532 (2.83)	Unknown
				ICG 12682 (2.83)	India	ICG 2857 (3.00)	Argentina	ICG 3027 (2.83)	India
				ICG 12697 (2.50)	India	ICG 4412 (3.00)	USA	ICG 6706 (3.00)	USA
				ICG 15419 (2.50)	Ecuador	ICG 8760 (2.84)	Zambia	ICG 12672 (2.50)	Bolivia
						ICG 9905 (3.00)	Zambia	ICG 13787 (2.50)	Nigeria
								ICG 14475 (3.00)	Nigeria
2.	*Aspergillus* seed colonization	ICG 3673 (1.16)	Korea	ICG 1862 (2.16)	Unknown	ICG 76 (2.40)	India	ICG 6402 (2.24)	Unknown
		ICG 6027 (1.16)	Sudan	ICG 14985(1.08)	Unknown	ICG 2381 (2.32)	Brazil	ICG 13787 (1.10)	Nigeria
		ICG 12625(2.04)	Ecuador			ICG 8760 (1.0)	Zambia		
						ICG 12276 (2.29)	Bolivia		
3.	Both Late Leaf Spot and *Aspergillus* seed colonization	ICG 12625	Ecuador			ICG 76	India	ICG 13787	Nigeria
						ICG 8760	Zambia		
4.	Yield perplant (g)	ICG 332 (20.50)	Brazil	ICG 1862 (33.50)	Unknown	ICG 8760 (20.31)	Zambia	ICG 8285(19.46)	USA
		ICG 5221 (19.76)	Argentina	ICG 3240 (30.14)	Uganda	ICG 11219 (18.59)	Mexico		
		ICG 6027 (21.90)	India	ICG 4716 (28.76)	Unknown				
		ICG 6201 (20.0)	Cuba	ICG 4750 (26.04)	Paraguay				
		ICG 13856 (18.6)	Uganda	ICG 11687 (27.96)	India				
		ICG 15042 (19.5)	Unknown	ICG 12697 (22.76)	India				
		ICG 15309 (21.0)	Brazil	ICG 14985 (22.04)	Unknown				

13787 of Virginia bunch (Table 3). These resistant genotypes could be utilized in hybridization programme with high yield accessions of Spanish bunch Viz., ICG 1862, ICG 3240, ICG 4716 and ICG 11687 so as to breed for high yielding multiple disease resistance genotypes.

LITERATURE CITED

Frankel O H 1984 Genetic perspective of germplasm conservation. pp: 161-170.

Gregory W C and Gregory M P 1976 Groundnuts in evolution of crop plants (ed. N W Simmonds), Longman Group Ltd., London, pp. 151-154.

Holbrook C C 1999 Testing and utilization of a core collection for the U.S germplasm collection peanut. In: R.C. Johnson and T. Hodgkin (ed), core collection for today and tomorrow. International Plant Genetic Resources Institute, Rome, Italy. pp: 68-73.

Krapovickas A 1969 The origin, variability and spread of the groundnut (*Arachis hypogaea*) (English translation). In: Ucko, P.J. and Falk, I.S., The domestication and exploitation of plants and animals. *Gerald Duckwoth Co.Ltd., London,* pp: 424-441.

Subbarao P V, Subrahamanyam P and Reddy P M 1990 A modified nine point disease scale for assessment of rust and late leaf spot of groundnut. In: Second International Congress of French Phytopathological Society, 28-30 November 1990, Montpellier, and France. pp. 25.

Thakur R P, Rao V P, Reddy S V and Ferguson M 2000 Evaluation of wild *Arachis* germplasm accession for *in vitro* seed colonization and Aflatoxin production by *Aspergillus flavus*. *International Arachis Newsletter*, 20, pp: 44-46.

Upadhyaya H D, Ortiz R and Singh S 2002 Developing a min core of peanut for Utilization of genetic resources. *Crop Science*, 42: 2150-2156.

Upadhyaya H D, Ortiz R, Bramel P J and Singh S 2003 Development of a ground nut core collection using taxonomical, geographical and morphological descriptors. *Genetic Resources and Crop evolution*, 50: 139-148.

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