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POTENTIAL SOURCES OF RESISTANCE TO MULTIPLE BIOTIC STRESSES IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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ABSTRACT

The cultivated groundnut is an important oilseed crop of the world. Several biotic stresses reduce groundnut yields considerably. Cultivation of resistant varieties is an ecologically sound and economically viable approach. But the occurrence and intensity of these stresses vary in space and time necessitating the use of multiple stress resistant genotypes. In the present study, 39 diverse groundnut genotypes were assessed for different biotic stresses under epiphytotic conditions. Interspecific derivatives, ICGV 87165 and ICGV 86699 were resistant to late leaf spot (<4.5), rust (3.0), stem and pod rot (< 5 %) and bud necrosis (< 33%) but were late in maturity (120 days). While, ICGV 86590 was resistant to rust (4.0), Stem and pod rot (14.35 %), *Spodoptera* (42.17 %) and bud necrosis (26.92 %) and matured in 105 days. Pedigree of multiple stress resistant genotypes revealed contribution of wild species for resistance to many biotic stresses. Significant negative correlations between resistance and maturity both at phenotypic (-0.262 to -0.584) and genotypic (-0.229 to -0.557) level indicated late maturing nature of resistant germplasm. The identified sources are potential genotypes to transfer multiple stress resistance into popular, high yielding, early maturing but susceptible cultivars.

INTRODUCTION

The cultivated groundnut (*Arachis hypogaea* L.) is an important oilseed crop of the world grown on 25.44 million ha with a production of 45.23 million tons and productivity of 1.77 tons per ha (FAOSTAT, 2014). India stands first in groundnut area (5.25 m ha), while second in production (9.47 m t) after China (17.01 m t). Though, India is one of the leading producers of the crop, the productivity is low (<1000 kg/ha) as compared to other major producers like USA (3393 kg/ha) and China (3143 kg/ha) (Damodaram and Hegde, 2000). In India, about 80 per cent of the crop is grown under rainfed situation wherein, many biotic stresses damage the crop and limit the productivity (Nigam, 2000). The two important foliar diseases viz., late leaf spot caused by *Phaeoisariopsis personata* Berk & Curt. V. Arx. and rust caused by *Puccinia arachidis* Speg. normally occur together and can cause yield loss up to 70 per cent (Subrahmanyam *et al.*, 1980). These diseases also have an adverse influence on the recovery of pods and on quality of pods, seeds and haulms. The stem and pod rot caused by *Sclerotium rolfsii* Sacc. commonly occurs and yield losses usually range from 10-25 per cent, but may reach up to 80 per cent in severely infected fields (Mehan and McDonald, 1990). Bud necrosis caused by tomato spotted wilt virus is a severe problem in dry regions and may result in yield reduction up to 80 per cent (Chohan, 1974). The yield losses due to defoliating insect, tobacco cutworm (*Spodoptera litura* F.) range from 13 to 71 per cent (Amin, 1983).

Though, both genetic and non-genetic solutions are available to manage these constraints, the non-genetic solutions are uneconomic under the rainfed agro-ecology due to low yield levels and resource limitations of the farmers. Further, non-genetic solutions may not always be eco-friendly as there is increased concern about pesticide residues in foods and environment. Moreover, the non-genetic solutions like chemicals (systemic, contact and combination of fungicides) are very effective under in vitro conditions (Rakholiya, 2015). Under the circumstances, genetic solution through resistant cultivars holds out a better promise. The occurrence and intensity of different biotic stresses vary in space and time and hence, resistance to any one stress may hinder the wider adaptation of genotypes. Several genotypes have been identified as resistant to individual biotic stresses like late leaf spot and rust (Motagi *et al.*, 2014), Stem and pod rot (Mehan *et al.*, 1995), *Spodoptera* (Rajendraprasad *et al.*, 2000) and bud necrosis (Sunkad *et al.*, 2012) but efforts on identifying multiple stress resistant genotypes are meager. Therefore, need arises to search for multiple disease and pest resistant genotypes.

In groundnut, early maturing Spanish bunch cultivars are most popular and widely cultivated in India, but they are susceptible to various diseases and suffer heavy yield loss. Concerted efforts have been made through hybridization, mutation and use of wild species to develop disease and pest resistant genotypes. In the present study, a systematic effort was made to assess a wide array of genetic material from different research centers to identify multiple disease and pest resistant germplasm. The source and breeding approaches employed in generating such genetic resources is discussed.

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MATERIALS AND METHODS

Thirty-nine genotypes of diverse origin, belonging to Spanish bunch, Virginia bunch and Valencia botanical groups were used in the study (Table 1). They were assessed separately in each trial for reaction to late leaf spot, rust and stem and pod rot, tobacco cutworm during rainy season under artificial epiphytotics at Dharwad, Karnataka, India. The same material was also screened for bud necrosis during rainy season at Raichur, Karnataka, a hot spot location for the disease.

Genotypes were sown in 3 rows plot of 5 m length with 30 x 10 cm spacing under randomized block design with 2 replications. The seeds were treated with Tricoderma @ 4g/Kg seeds in all the experiments except the trial for stem and pod rot resistance screening. Fertilizer was applied @ 10:25:10 Kg NPK/ha.

The artificial epiphytic for late leaf spot and rust was created through spreading inoculum (affected leaves collected and stored from previous season) and providing alternate wetting and thawing condition for disease build up. The genotypes were scored based on field disease score (1–9 scale) wherein, 1 = 0 % affected and 9 = 100 % leaf area affected (Subbarao *et al.*, 1990). The field disease score was mainly based on the extent of leaf area damaged. For late leaf spot, the extent of defoliation was also incorporated into the scale (Subrahmanyam *et al.*, 1995). Genotypes were scored one week before harvest. No fungicidal spray was given to control late leaf spot and rust.

Artificial epiphytic for stem and pod rot was created by spreading the chopped pieces of *Sclerotium* infected plants and by providing irrigation for proper mycelial growth. The disease incidence was scored by counting dead or wilted plants showing sclerotia and/or heavy mycelial growth on stem or collar and pods (Mehan *et al.*, 1995). The trial was protected from late leaf spot and rust with Chlorothalonil @ 1.15 kg/ha.

Egg masses of tobacco cutworm (*Spodoptera*) were pinned on to the leaves of 50 days old crop. Visual assessment of the leaf area damaged from the top five leaflets was made as the damage was confined to young leaves. In each plot, five random plants were assessed for leaf area damage and average was computed. The visual assessment was done at 70 DAS, when highest damage of *Spodoptera* was noticed. The trials were protected from late leaf spot and rust by spraying Chlorothalonil.

In the trial for screening of bud necrosis, neither fungicide nor insecticide was applied to the crop. Plants showing severe symptoms of bud necrosis *viz.*, general chlorosis, necrosis of the terminal apices and buds in addition to general stunting were counted one week before harvest and expressed as percentage of total plants in a plot.

The damage due to different stresses was normally distributed in the germplasm which was equally grouped into three categories *viz.*, resistant, moderately resistant and susceptible (Table 2). The genotypes showing resistance to more than one stress were considered multiple stress resistant.

RESULTS AND DISCUSSION

Analysis of variance revealed highly significant genotypic

variation for response to all the biotic stresses. Extent of variability was relatively more for rust and stem and pod rot as revealed by phenotypic coefficient of variation (Table 2). Heritability ranged from 51.6 to 86.1 for different stresses indicating predominantly heritable nature of the variation indicating scope for selection of the resistant genotypes. Based on frequency, interspecific derivatives formed the major source of resistance to rust (86 %) and *Sclerotium* (71 %); mutants were major sources for late leaf spot, *Spodoptera* and bud necrosis diseases (75 % in each). Least frequency (<33 %) of resistant sources for various biotic stresses were found in the ruling cultivars (Table 3).

Positive correlations among different stresses (Table 4) indicated the potential of germplasm for multiple stress resistance. Association was significant between late leaf spot and rust (0.410 and 0.349), rust with *Sclerotium* (0.418 and 0.319) and bud necrosis (0.360 and 0.273), *Sclerotium* with bud necrosis (0.474 and 0.375), *Spodoptera* and bud necrosis (0.654 and 0.379) both at phenotypic and genotypic levels, respectively. Significant negative association (-0.351 to -0.584) between resistance to different stresses (except *Spodoptera*) and maturity revealed the late maturing nature of resistant germplasm (Table 4).

Inter-specific derivatives constituted the excellent sources of resistance to late leaf spot, rust and *Sclerotium* (Table 5). But all of them except GPBD 4, matured late (>110 days). Early maturing mutants formed a better source of resistance to late leaf spot, *Spodoptera* and bud necrosis. Many advanced breeding lines were resistant to *Sclerotium*, *Spodoptera* and bud necrosis. The cultivated varieties though matured early were susceptible to different stresses.

The inter-specific derivatives, ICGV 86699, ICGV 87165 and ICGV 93023 were resistant to late leaf spot (<5.0), rust (<4.0) and bud necrosis (<32.93%) (Table 4). The pedigree of these genotypes comprised of wild species *A. duranensis*, *A. batizocoi* and *A. cardenasii* which are known to be resistant/immune to rust and late leaf spot (Subrahmanyam *et al.*, 1983; Amin, 1985; Stalker and Beute, 1993). NC 2 (Cook, 1981) in the pedigree of ICGV 86699 and ICGV 87165 contributed resistance to *Sclerotium* in these genotypes. But, they matured late (>120 days) and not suitable for direct cultivation due to low shelling out turn and undesirable pod and kernel features (Nigam *et al.*, 1991).

The interspecific derivative from second cycle of hybridization *viz.*, GPBD 4 matured early (105 days) and combined desirable agronomic features (data not shown) of Spanish bunch types revealing the possibility of breaking negative association between resistance and maturity. Resistance to late leaf spot and rust in GPBD 4 and to late leaf spot, rust and *Sclerotium* in B 37c traced to ICGV 86855 and ICGV 87165, respectively, in their pedigree. GPBD 4 has been registered as valuable germplasm (Gowda, *et al.*, 2001) and released for cultivation in southern zone of India (Gowda, *et al.*, 2002).

Multiple resistant germplasm NC Ac 343 (Campbell, 1971) has shown resistance to *Sclerotium* (6 %), *Spodoptera* (36 %) and bud necrosis (27 %) in the present study also, but matured late (120 days). The insect resistance of this germplasm is traced to PI 121067 in its parentage (Isleib and Wynne, 1992). The advanced breeding lines *viz.*, ICGV 86031 was resistant to

Table 1: Pedigree of groundnut germplasm used in the study

Genotype	Botanical Group	Pedigree	Source
Interspecific derivatives			
1. ICGV 86699	VB	[(<i>A. batizocoi</i> × <i>A. duranensis</i>) × <i>A. hypogaea</i> (Cv. NC 2)]	ICRISAT, India
2. ICGV 87165	VB	[<i>A. hypogaea</i> var. <i>fastigiata</i> (PI 261942) × <i>A. cardenasii</i>]	ICRISAT, India
3. ICGV 88256	VB	(ICGV 87165 × (Robut 33-1 × NC Ac 316))	ICRISAT, India
4. ICGV 93023	VB	[(Robut 33-1 × NC Ac 2214) × Cyto 213-2]	ICRISAT, India
5. A 30b	VB	KRG 1 × ICGV 87165	Karnataka, India
6. B 37c	SB	JL 24 × ICGV 87165	Karnataka, India
7. GPBD 4	SB	KRG 1 × ICGV 86855 (<i>A. hypogaea</i> × <i>A. cardenasii</i>)	Karnataka, India
Advanced breeding lines			
1. ICGV 86031	SB	F 334 A-B-14 × NC Ac 2214	ICRISAT, India
2. ICGV 87264	SB	Manfredi × NC Ac 17133RF	ICRISAT, India
3. ICGV 87807	VL	[(MK 374 × Robut 33-1) × FESR 2]	ICRISAT, India
4. ICGV 90266	VB	[(J11 × (M 13 × NC Ac 2214)) × ICG 2271]	ICRISAT, India
5. ICGV 91173	VB	[(NC Ac 343 × NC Ac 2214) × ICG 5240]	ICRISAT, India
6. ICGV 91177	VB	(F 334 A-B-14 × NC Ac 2232) × ((TMV 7 × FSB 7-2) × NC Ac 2214)	ICRISAT, India
7. ICGV 91180	VB	[(TMV 2 × FSB 7-2) × NC Ac 2232] × (F 334 A-B-14 × NC Ac 2214)	ICRISAT, India
8. ICGV 92188	VB	[(Robut 33-1 × (M 13 × Nc Ac 2214)) × JL 24	ICRISAT, India
9. ICGV 93008	VB	[(Mani Pintar × (Robut 33-1 × NC Ac 2232))] × ICG 2320	ICRISAT, India
10. ICGV 93020	SB	[(Manfredi 68 × NC Ac 343) × ((Mani Pintar × (Robut 33-1 × NC Ac 2232)))]	ICRISAT, India
11. ICGV 93021	VB	[(F 334 A-B-14 × N C Ac 2214) × 9/136]	ICRISAT, India
12. ICG 2271	VB	NC Ac 343 (NC Bunch × PI 121067)	North Carolina, USA
13. ICG 1697	VL	NC Ac 17090	North Carolina, USA
14. ICGV 96262	VB	89 R/52-8 × PI 270806	ICRISAT, India
15. ICGV 96266	VB	ICGV 86577 × ICGV 86594	ICRISAT, India
16. Dh 73	SB	D h 3-30 × ICGV 87264	Karnataka, India
17. R 8972	SB	ICGS 59 × NC Ac 2240	Karnataka, India
18. R 9214	SB	(ICGS 7 × NC Ac 2214) × ICGV 86031	Karnataka, India
19. R 9227	SB	(ICGS 7 × NC Ac 2214) × ICGV 86031	Karnataka, India
Mutants			
1. VL 1	VL	EMS mutant of Dharwad Early Runner (DER)	Karnataka, India
2. 28-2	SB	EMS mutant of Valencia 1 (VL 1)	Karnataka, India
3. 45	SB	EMS mutant of Valencia 1 (VL 1)	Karnataka, India
4. 110	SB	EMS mutant of Valencia 1 (VL 1)	Karnataka, India
Cultivated varieties			
1. ICGV 86590	VL	X14-4-B-19B × PI 259747	ICRISAT, India
2. K 134	SB	Kadiri 3 × JL 24	Kadiri, India
3. KRG 1	SB	Selection from Argentina	Karnataka, India
4. JL 24	SB	Selection from EC 94943	Maharashtra, India
5. TMV 2	SB	Mass selection from "Gudhiatham bunch"	Tamilnadu, India
6. Dh 8	SB	Selection from RS 144	Karnataka, India
7. Dh 40	SB	Dh 3-30 × TGE 2	Karnataka, India
8. R 8808	SB	ICGS 11 × Chico	Karnataka, India
9. TAG 24	SB	TGS 2 × TGE 1	BARC, India

SB – Spanish bunch, VB – Virginia bunch, VL – Valencia

Table 2: Components of variation for reaction to various stresses and maturity in groundnut germplasm and basis for classification of genotypes

Biotic stress		Late leaf spot	Rust	<i>Sclerotium</i>	<i>Spodoptera</i>	Bud necrosis
Components	Maximum	8.0	7.5	45.2	80.7	69.6
	Minimum	4.0	3.0	0.0	34.3	16.6
	Mean	6.5	5.2	18.3	56.7	36.9
	PCV	37.9	55.0	70.4	25.7	39.7
	H	84.9	86.1	81.2	76.3	51.6
Parameter		Field disease score	Field disease score	% damage	% damage	% damage
Category	Resistant	< 5.0	< 4.0	< 15	< 50	< 35
	Moderately resistant	5.1 to 6.5	4.1 to 6.0	16 to 30	51 to 65	36 to 50
	Susceptible	> 6.5	> 6.0	> 30	> 65	> 50

PCV: Phenotypic coefficient of variation H - Heritability

Spodoptera (41 %) and bud necrosis (27 %), while, R 8972 was resistant to rust (4.0) and *Sclerotium* (11 %) and matured

early (101 days) along with desirable agronomic features of Spanish groundnuts. They had insect resistant germplasm

Table 3: Frequency of genotypes resistant to various stresses in different categories of groundnut germplasm

Genotypes	Late leaf spot	Rust	<i>Sclerotium</i>	<i>Spodoptera</i>	Bud necrosis
Inter-specific derivatives (7)	5 (71)	6 (86)	5 (71)	0 (0)	3 (43)
Advanced breeding lines (19)	2 (11)	6 (32)	11 (58)	8 (42)	13 (68)
Mutants (4)	3 (75)	1 (25)	1 (25)	3 (75)	3 (75)
Ruling cultivars (9)	0 (0)	1 (11)	2 (22)	1 (11)	3 (33)
Total (39)	10 (26)	14 (36)	19 (49)	12 (31)	22 (56)

Figures in parenthesis represent the number of genotypes in each category / per cent of total lines in each category

Table 4: Correlation among various stresses and maturity in groundnut germplasm

Stresses/Maturity	Late leaf spot	Rust	<i>Sclerotium</i>	<i>Spodoptera</i>	Bud necrosis	Maturity
Late leaf spot	1.000	0.410**	0.243	0.202	0.234	-0.584**
Rust	0.349*	1.000	0.418**	-0.238	0.360*	-0.532**
<i>Sclerotium</i>	0.172	0.391*	1.000	0.198	0.474**	-0.582**
<i>Spodoptera</i>	0.152	-0.146	0.166	1.000	0.654**	-0.262
Bud necrosis	0.174	0.273	0.345*	0.379*	1.000	-0.486**
Maturity	-0.537**	-0.494**	-0.525**	-0.229	-0.351*	1.000

*, ** - Significant at 5 % and 1 % level of probability, respectively; The phenotypic and genotypic correlation coefficients are represented above and below the diagonal, respectively

Table 5: Performance of groundnut germplasm for different biotic stresses and maturity

Genotype	Late leaf spot	Rust	<i>Sclerotium</i>	<i>Spodoptera</i>	Bud necrosis	Days to maturity
ICGV 86699	5.0*	3.0*	4.38*	61.67	32.93*	122
ICGV 87165	4.5*	3.0*	0.00*	52.00	16.64*	121
ICGV 88256	6.5	4.0*	0.00*	65.67	36.84	122
ICGV 93023	5.0*	4.0*	15.65	62.50	19.38*	121
A 30 b	5.5	5.0	12.15*	50.17	39.24	121
B 37c	4.0*	3.0*	14.36*	72.00	39.58	115
GPBD 4	4.0*	3.0*	28.79	80.67	51.07	105
ICGV 86031	7.5	6.5	25.69	41.00*	26.58*	101
ICGV 87264	5.5	4.0*	32.79	50.00*	26.10*	105
ICGV 87807	6.5	4.0*	11.40*	49.33*	51.86	119
ICGV 90266	6.5	4.0*	5.00*	60.44	25.91*	122
ICGV 91173	7.5	5.0	25.24	55.50	24.93*	121
ICGV 91177	5.0*	5.0	9.61*	58.33	31.73	122
ICGV 91180	6.5	6.5	16.67	34.33*	22.19*	121
ICGV 92188	7.0	4.0*	2.33*	54.33	21.15*	121
ICGV 93008	6.5	3.0*	21.92	53.33	47.13	121
ICGV 93020	7.5	5.0	12.07*	55.33	40.15	121
ICGV 93021	6.5	5.0	6.68*	46.17*	34.67*	121
NC Ac 343	7.5	5.5	6.25*	35.67*	27.02*	120
NC Ac 17090	7.5	6.5	7.16*	46.83*	50.26	105
ICGV 96262	4.5*	7.0	6.85*	54.67	31.34*	122
ICGV 96266	5.5	5.0	31.69	58.33	39.54	121
Dh 73	7.5	5.0	29.93	59.33	30.28*	105
R 8972	7.5	4.0*	10.75*	74.33	37.72	101
R 9214	8.0	6.5	19.02	41.00*	31.90*	101
R 9227	7.5	5.5	3.94*	60.00	25.69*	105
VL 1	8.0	4.0*	2.63*	59.44	28.60*	105
28-2	4.5*	5.5	34.72	36.33*	49.31	107
45	5.0*	7.0	30.51	34.33*	35.00*	107
110	4.5*	7.0	25.63	45.00*	27.69*	107
ICGV 86590	8.0	4.0*	14.35*	42.17*	26.92*	105
K 134	8.0	6.5	33.68	70.67	55.00	101
KRG 1	8.0	5.5	39.62	82.33	52.49	101
JL 24	8.0	7.0	23.64	77.83	52.59	101
TMV 2	8.0	7.0	27.87	73.33	54.86	101
Dh 8	7.0	7.5	12.39*	63.00	69.58	101
Dh 40	8.0	6.5	45.17	83.67	63.22	101
R 8808	8.0	6.5	24.83	67.00	32.20	101
TAG 24	8.0	7.5	36.65	41.33*	29.63*	101
Mean	6.6	5.2	18.33	56.65	36.89	112

*- indicate resistance

NC Ac 2214 and NC Ac 2240, respectively in their pedigree (Campbell *et al.*, 1976, Dwivedi *et al.*, 1986). The genotypes, R 9214 and R 9227 were resistant to *Spodoptera/Sclerotium* and bud necrosis and they are derived from ICGV 86031 and NC Ac 2214. NC Ac 17090 was resistant to *Sclerotium* and *Spodoptera* was also reported earlier as rust resistant (Subrahmanyam *et al.*, 1982) was susceptible in the present study, which could be ascribed to physiological responses arising from changes in latitudes of the locations (Nigam *et al.*, 1991; Wynne, *et al.*, 1991).

Among the mutants, VL 1 was resistant to rust (4.0), *Sclerotium* (3 %) and bud necrosis (29%), while, 28-2, 45 and 110 were resistant to late leaf spot (< 5.0) and *Spodoptera* (< 45 %). In addition, the latter two genotypes were also resistant to bud necrosis (< 35 %). These mutants were derived from a taxonomical variant DER (Gowda *et al.*, 1991) on mutagenesis with EMS. Resistance to late leaf spot in these mutants was due to elimination of suppressor of resistance through EMS mutagenesis of original parent DER and gain of functional mutation i.e., duplicate recessive resistance genes for late leaf spot in VL 1 (Motagi *et al.*, 2000). Mutant 28-2 has been registered as leaf spot, armyworm and thrips resistant germplasm (Gowda *et al.*, 1998) and released for cultivation in Karnataka (Gowda *et al.*, 2002).

Among the released varieties, ICGV 86590 was resistant to rust (4.0), *Sclerotium* (14 %), *Spodoptera* (42 %) and bud necrosis (27 %) and it has been registered for its multiple stress resistance (Reddy *et al.*, 1993). Its resistance to rust could be traced to PI 259747 (Anderson *et al.*, 1993) in its parentage. But because of low shelling out turn and poor pod features (data not shown), it is not popular among the farmers.

Most of the varieties released in India are selections from local land races or direct introductions. Only limited use of resistant germplasm is made in crop improvement through hybridization. The present study shows the diversified resistant sources for various biotic stresses. These could be utilized in resistance breeding programmes to produce stable and high yielding resistant lines. It is also evident from the above discussion that, resistance to different biotic stresses is distributed among various categories of genotypes under Spanish background. These genotypes could be used in intermating to incorporate multiple biotic stress resistance in groundnut. This pre-breeding would facilitate further genetic enhancement of germplasm retaining their desirable agronomic features.

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