Genetic Parameter and Association Analysis for Resistance to *Sclerotium rolfsii* Sacc. in Groundnut (*Arachis hypogaea* L.)

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Evaluation of 165 groundnut genotypes and two check parents (TAG-24 and R-9227) was carried out under artificial inoculation conditions for stem rot, *Sclerotium rolfsii.* indicated significant difference among genotypes, season and genotypes x seasons interaction for disease, yield and yield-related parameters. The genotypic and phenotypic coefficient of variation was high (>20%) for secondary branches, disease incidence at 30, 60, 90 days after sowing and pod yield/plant, moderate (10-20%) for plant height, leaf length and leaf width and low (<10%) for oil content and test weight. Heritability was high (>60%) for most of the characters. Genetic advance was high (>20%) for plant height, secondary branches, pod yield per plant and disease at 30, 60 and 90 days after sowing. Diesease incidence at harvest was negatively associated with plant population, shelling percentage, test weight, primary branches, secondary branches, leaf length and leaf width. Among the parents, R-9227 showed resistance to stem and pod rot, where as TAG-24 showed susceptibility to stem and podrot incidence.

Key Words: Association analysis, Groundnut, Heritability, Resistance, *Sclerotium rolfsii,* Variability

Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most important oil seed crops and grain legumes grown worldwide. The groundnut seed has dual advantage of being important as a source of edible oil as well as protein. The exploitation of genetic resources from wild species is extremely difficult because of ploidy differences between cultivated tetraploid and diploid wild species coupled with compatibility barrier except with *Arachis* section. Obviously, poor soil fertility, and stem and pod rot disease caused by *Sclerotium rolfsii* Sacc.is one of the significant factors contributing to yield loss.

Only limited screening of germplasm for resistance has been attempted. There are very few reports of clear varietal differences for resistance to stem and pod rots. Although, no genotype is known to be immune or even highly resistant to *S. rolfsii*, several genotypes and advanced breeding lines have shown field resistance (Smith *et al.*, 1989; Grichar and Smith, 1992; Shokes *et al.*, 1993).

As in the case of many other diseases, breeding for disease resistance is the best way of controlling the *S. rolfsii*. To initiate breeding programme for resistance to any disease, understanding basic mechanism of disease resistance and its inheritance area pre-requisite. It is desirable to have a variety resistant to the disease, combined with other desirable yield characters. The knowledge of mode of inheritance and variability of resistance/susceptibility is essential to have effective selection programme. Estimate of gene effects will help in predicting the effectiveness of selection. The relative variance will decide the breeding procedure to be followed through the information available on the quantitative characters. Less information is available about the inheritance of *S. rolfsii* resistance as well as its association with other morphological traits.

The information searched thus far indicated the existence of variation in the pathogen. Further, pathogenic variability adds difficulties in effective management of this economically important disease. The situation also demands for the adoption of suitable breeding strategy in development of resistant varieties. Nevertheless, systematic study regarding the variability in the pathogen and other details are limited. Hence, to study the variability in *S. rolfsii* causing stem rot of groundnut, the present investigation was undertaken encompassing the objective of knowing the genetic parameter and association analysis for resistance to *S. rolfsii* in groundnut.

Material and Methods

The experimental material comprised of TAG-24 × R-9227 cross. This cross was made by using susceptible (TAG-24) and resistant (R-9227) parents in Randomized Complete Block Design. Hybridizations were forwarded to get F_1 (F_2 seeds) and F_2 generation (F_3 seeds) by selfing. The F_2 generation was advanced to F_3 through

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single seed decent method. Individual F_3 families were propagated as bulk in F_4 and F_5 generations. From F_5 generations, seeds were selected and evaluated in F_6 generations and artificial inoculation conditions were created during summer 2009.

S. rolfsii was isolated from infected groundnut plant grown in vertisols and mass multiplication was carried out on sand – corn meal medium (95:5) in order to get maximum sclerotial production (Abeygunwardhana and Wood, 1975). Two hundred gram of sand-corn meal medium was taken in 500 ml conical flasks and mixed with 30% distilled water and it was sterilized. The pure culture of isolated *S. rolfsii* was inoculated under aseptic conditions and incubated at 27 ± 1^0C for 30 days. These flasks were shaken frequently to get uniform growth of mycelium. The mass culture thus obtained was used for further studies. Inoculum containing mycelium and sclerotia along with corn meal and sand was applied to the soil surface around the base of the plants @ 125 g/2.5 m row, at 30 days after sowing or at flower initiation. Tie hopped sorghum stubbles (3–4 cm pieces) were scattered along the rows to enhance the fungal growth on soil. After two weeks, the inoculation was repeated. During summer season, the field was irrigated at five days interval until pod formation, to promote stem rot development. The observations were recorded for parameters like initial plant count, plant height, number of primary branches, number of secondary branches, leaf length and width, pod yield/plant, test weight, shelling (%), oil content (%), disease incidence (%) at various stages of crop growth and at harvest. Statistical analysis was done on mean, range, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance as per cent over mean (GAM) by using GENERS SPAR statistical package.

Results and Discussion

Plant breeders are mainly concerned with identification of important factors limiting productivity and formulation of appropriate breeding strategies to develop suitable genotypes. The average yield of groundnut in India (926 kg/ha) remains well below the levels achieved in countries like USA (2995 kg/ha). Several reasons are attributed for low yield level. Lack of improved high yielding cultivars, low soil fertility, uneven rainfall distribution, continuous cropping without crop rotation, low plant population and incidence of pests and diseases are major limiting factors in most of the groundnut growing regions.

Among the biotic stresses, stem and pod rot disease is predominant, accounting for yield losses to the extent of 10 – 25% and up to 80% in severely infected fields. Only limited resistance screening of germplasm has been attempted as there are very few reports of clear varietal difference with respect to resistance to stem and pod rot. None of the lines tested were completely resistant to stem and pod rot. It is unlikely that high degree of resistance to a highly necrotrophic pathogen such as *S. rolfsii* will be available. It would be desirable to select or develop lines that possess moderate resistance to *S. rolfsii* and resistance to other economically important diseases of groundnut.

The mean performance of segregating progenies with respect to 11 quantitative characters in *kharif* and nine characters in *summer* were studied. Plant height is the important component character in groundnut. The mean value of plant height was higher in case of F_5 population. It is because of good rainy condition for crop growth in *kharif* (F_5) compared to *summer* (F_6) population.

Secondary branches, leaf length, leaf width and mean values were similar or nearer in both F_5 and F_6 population. Hence, there was no difference among the genotypes. It is, thus, not advisable to use this parameter as a selection criteria.

In case of F_6 population, the mean value of disease incidence was low at 30 days after sowing (DAS), high at 60 DAS and moderate at 90 DAS. The range of disease incidence at 30 DAS, 60 DAS and 90 DAS was widened for this trait in F_6 population, because of application of artificial inocula, creation of artificial humidity and temperature by covering thin plastic sheet over experimental plot which favoured the spread of disease. It could be seen in the present study that there has been a change in mean value that accompanied the change in range also. This was evident for disease at harvest in both F_5 and F_6 progeny. The increase in mean value with increased upper unit of range of a character could offer better scope for selection.

The mean of test weight, shelling percentage, oil content, and pod yield per plant of *kharif* population were higher than those of *summer* population. It is indicated that during *summer* yield loss is greater compared to that during *kharif* season because high temperature and lack of irrigation leads to more root and pod rot disease.

Another measure of variation *i.e.* range displayed shift in its limits due to the trend of mean value. The range value widened for traits like plant height, pod-yield, shelling percentage and test weight during both the seasons indicated the release of concealed variability. Thus it has led to opening up broad spectrum of variation in above mentioned traits. The release of hidden variability could be of great use in improving the related traits through modifying breeding approaches (Singh and Balyan, 1988; Sharma *et al.*, 1995; Singh and Sahu, 1981).

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Range of some traits like oil content, leaf length, and leaf width did not show any change, there by, indicating the operation of strong linkage between the traits. Under such circumstances a stringent selection following inter mating of high extreme segregants in the population would bring about breakage of tight-linked genes (Mather and Jinks, 1971). Narrow range was observed for plant height, plant population, primary branches and secondary branches.

Total variability observed in a crop can be divided into genotypic (Vg) and can be interpreted in terms of GCV and PCV (Table 1, 2 and 3). Coefficient of variance for F_5 and F_6 indicated the presence of less amount of variation for characters other then shelling percentage, disease incidence at harvest, pod weight per plant (GCV 10-20%) and high variability (>20%) (Badwal *et al.*, 1967; Nadaf and Habib, 1987). A comparison of GCV and PCV in F_5 and F_6 indicate estimated PCV were generally higher than GCV for all the characters. This may be due to involvement of high environmental and genotype × environmental interaction effects in character expression (Kaushik *et al.*, 1996).

High magnitude GCV and PCV was observed in case of secondary branches in F_5 and pod yield/plant, disease incidence at 30 DAS, 60 DAS and 90 DAS was widened for this trait in F_6 population. Higher magnitude of PCV and GCV for these characters in both F_5 and F_6 populations indicate the presence of high degree of variability and better scope for improvement.

Table 1. Genetic components of variance for various parameters of *Kharif* 2008

Characters	*Kharif* 2008					
	Mean	Range	PCV	GCV	h^2	GAM
Plant population	22.31	14.00-26.50	13.60	5.53	16.60	4.03
Plant height	21.75	15.62-30.97	13.99	12.69	82.30	20.50
Primary branches	6.39	4.50-8.75	15.67	6.57	17.60	8.60
Secondary branches	1.45	0.5-5.0	52.88	52.62	99.00	87.58
Leaf length	4.06	3.05-5.45	13.39	7.03	27.60	9.11
Leaf width	1.73	1.27-2.45	17.22	7.36	18.30	15.60
Test weight	43.75	33.00-53.50	14.72	7.64	26.90	7.74
Shelling (%)	69.10	57.67-76.87	16.78	7.48	19.90	5.70
Oil content (%)	43.14	39.10-46.93	3.90	2.98	58.30	4.68
Disease incidence at harvest	11.07	0.00-40.00	68.94	17.22	62.00	8.85
Pod weight/ plant	11.17	7.29-19.45	38.80	35.80	85.20	65.75

PCV=Phenotypic coefficient of variation, GCV=Genotypic coefficient variation, h^2=Heritability, GAM=Genetic advance over mean

In F_5 population the leaf width, leaf length, test weight, shelling percentage has shown moderate PCV and low GCV for both characters was observed. Improvement of GCV in F_5 population can be brought about by hybridization or selection in advanced generations.

Oil content of F_5 and shelling percentage in F_6 generation showed low PCV and GCV indicates that this character showed genetically much variation. Disease incidence at harvest in F_5 population exhibited high PCV and moderate GCV was observed. It indicates greater phenotypic variation compared to genotypic variation. Higher magnitude of PCV for this character indicates the presence of high degree of variability and better scope for improvement, but in F_6 population, high magnitude of PCV and low magnitude of GCV. This indicated the presence of high degree of variability and better scope for improvement.

In F_5 and F_6 populations, pod weight per plant with higher magnitude of PCV and GCV was obsened. Higher PCV and GCV were observed in F_5 population compared to F_6 owing to disease incidence in the F_6 population compared to F_5 population and resulting less yield in F_6 population.

Table 2. Genetic components of variance for various parameters of *summer* 2009 season

Characters	TAG-24		R-9227		*Summer* 2009			
	Mean	Range	Mean	Range	PCV	GCV	h^2	GAM
Plant population	25.50	25.00-26.00	19.00	14.24	13.03	5.12	15.50	4.60
Pod weight per Plant	10.25	9.60-10.90	11.10	10.4-11.80	23.55	20.58	76.40	35.45
Shelling percentage	76.37	76.25-76.50	72.25	72.00-72.50	7.82	3.95	25.60	9.14
Test weight	43.50	43.00-44.00	43.50	43.00-44.00	12.09	4.86	16.20	8.19
Oil content	44.68	44.51-44.85	41.85	41.33-42.37	3.86	3.02	61.10	5.81
Disease incidence at 30 DAS	0.50	0.00-1.00	0.55	0.00-1.00	88.66	87.82	98.10	180.80
Disease incidence at 60 DAS	4.00	1.00-7.00	1.50	1.00-2.00	82.92	81.39	96.40	164.70
Disease incidence at 90 DAS	3.00	2.00-4.00	0.00	0.00-0.00	61.61	61.49	99.60	126.90
Disease incidence at harvest	19.88	11.76-28.00	6.26	0.00-12.50	22.38	6.36	81.00	3.71

PCV=Phenotypic coefficient of variation, GCV=Genotypic coefficient variation, h^2=Heritability, GAM=Genetic advance over mean

The genotypes differed significantly for most of the parameters and reaction to disease. This indicated usefulness of this material and their appropriateness in the study. Over seasons, significant seasonal variation and genotypic × season interaction was observed for majority of the traits due to impact of environment and genotype × environment interaction. Wynne and Isleib (1978) reported extensive prevalence of G × E interaction for productivity traits, while Knauff and Gorbert (1993), and Wynne and Isleib (1978) indicated for physical traits. In general, variance estimate were relatively high in individual season as compared to pooled data indicating the role of G × E interactions.

Heritability estimates were useful while making selection based on phenotype. Nevertheless, as pointed out by Johnson *et al.* (1955), it would be limited as it is prone to change with fluctuation in season, environment, material *etc.* So, the estimation of heritability, thus, has a role in determining the effectiveness of selection in both early and advanced segregating generations. These are considered in conjunction with predicted genetic advance as suggested by Panse and Sukhatme (1967).

The high heritability with high genetic advance as percent over mean (GAM) were observed for traits like plant height, secondary branches, pod yield per plant, disease incidence at 30 DAS, 60 DAS and 90 DAS in both F_5 and F_6 populations. This indicated that there was lower environmental influence on the expression of these characters and it was governed by additive gene action and hence one can practice selection. Similar findings were reported by Reddy *et al.* (1985) and Hazara and Basu (2000) in okra.

Further, selection can be more effective in F_6 population because it exhibited higher variability for yield components as compared to F_5 which displayed low to moderate variability for various characters as these were showing less segregation for different characters. The F_5 population showed higher magnitude of variability, heritability and GAM for yield.

Low heritability and low GAM was observed in plant population, primary branches, test weight and shelling percentage in both F_5 and F_6 populations.

High heritability and low GAM was observed with respect to disease incidence at harvest and also for oil content in both F_5 and F_6 populations. It indicates that, these were controlled to a greater extent by non-additive gene action; it could be attributed to low genetic variability. Low GAM reflects increased effect of environment on these traits and thus selection procedures involving progeny testing may be followed to improve them.

Genetic correlation among different characters of a plant often arises because of linkage or pleiotropy (Horland and Csinos, 1939). Hence, the study of character association through correlation will help in selecting the yield attribute. The association between two characters can be ascertained by phenotypic correlation which

Table 3. Genetic components of variance for various parameters over seasons

Characters	Pooled analysis					
	Mean	Range	PCV	GCV	h^2	GAM
Plant population	20.89	11.0-28.0	13.11	4.81	13.5	3.64
Pod weight/Plant	11.52	3.93-30.40	13.26	1.27	23.52	2.17
Shelling (%)	63.53	30.50-78.70	11.26	2.41	0.46	1.05
Test weight	42.62	26.50-60.0	12.07	2.48	0.42	1.05
Oil content	43.15	38.52-49.16	4.25	3.47	6.64	5.81
Disease at harvest	41.60	0.00-100	29.15	0.08	51.65	0.84

PCV Phenotypic coefficient of variation, GCV Genotypic coefficient of variation, h^2=Heritability, GAM=Genetic advance over mean

Table 4. Genotypic and phenotypic correlations of *kharif* 2008

Character	PP	PH	PB	SB	LL	LW	TW	SP	OC	DIAH	PWP
Plant population	1.000	-0.307*	0.348*	-0.073	-0.942*	-0.951*	-0.344*	0.184*	0.372*	0.483*	-0.332*
		-0.117**	0.016	-0.025	-0.164*	-0.131**	-0.061	0.071	0.080	0.206*	-0.152**
Plant height		1.000	-0.151**	0.057	0.354*	0.248*	0.155**	0.201*	-0.165*	-0.708*	-0.304*
			-0.049	0.051	0.200*	0.090	0.083	0.059	-0.129**	-0.124**	-0.239*
Primary branches			1.000	-0.078	-0.662*	-0.589*	-0.340*	-0.107	0.030	0.083	0.267*
				-0.036	-0.099	-0.091	0.058	-0.027	0.078	-0.031	0.086
Secondary branches				1.000	0.062	-0.166 *	0.017	-0.036	0.135**	-0.098	-0.114**
					0.032	-0.069	0.005	-0.024	0.107	-0.013	-0.107
Leaf length					1.000	0.566*	-0.098	0.048	-0.202*	-0.339*	-0.325*
						0.617*	0.030	0.004	-0.024	-0.090	-0.133**
Leaf width						1.000	0.324*	-0.196*	0.006	0.108	-0.187*
							0.037	-0.097	0.033	0.005	-0.006
Test weight							1.000	0.623*	0.109	-0.225*	0.051
								0.269*	0.033	0.039	0.058
Shelling (%)								1.000	0.114**	-0.533*	0.351*
									0.014	-0.046	0.224*
Oil content (%)									1.000	-0.576*	0.146**
										0.102	0.092
Disease incidence at harvest										1.000	-0.346*
											-0.330*
Pod weight/plant											1.000

** Significance at 5% and 1% Propability
PP= Plant population; PH= Plant height; PB= Primary branches; SB= Secondary branches; LL= Leaf length; LW= Leaf width; TW= Test weight; SP= Shelling percentage; OC= Oil content; DIAH= Disease incidence at harvest; PWP= Pod weight/plant

is determined by measurement of two characters in a number of individuals of the segregating population.

In the present study, phenotypic and genotypic correlations were studied for pod weight per plant and its component traits in F_5 and F_6 populations. Phenotypic correlation of plant population, primary branches, test weight, shelling percentage and oil content exhibited positive significant association with pod weight per plant. Similar results were reported for primary branches (Badwal *et al.,* 1967; Sharma and Varshney, 1990; Nagabhushanam and Prasad, 1992), for shelling percentage (Pushkaran and Nair, 1993; Sharma *et al.,* 1995) and 100-seed weight (Sarala and Gowda, 1998; Nagda *et al.,* 2001) and were positively correlated with yield (Tables 4 and 5).

In all the populations, pod weight per plant had negative correlation with plant population, plant height (except F_6) and disease incidence at harvest. This indicates the possibility of identifying and isolating genotype with lesser plant population and lower disease incidence.

The characters *viz*., plant population, plant height, primary branches, secondary branches, leaf length, leaf width, test weight, shelling percentage, oil content and disease incidence at harvest not only exhibited significant association with pod weight per plant, but also showed significant positive association among themselves.

This character can thus be considered while selecting plants for high yield in F_5 and F_6 populations.

In case of F_6 population, disease incidence at 30 DAS was negatively correlated with plant population and with no significant effect on oil content, shelling percentage and pod weight per plant. Similar results were reported by Grichar and Smith (1992). Disease incidence at 30, 60 and 90 DAS was negatively correlated with plant population. This indicates that the disease incidence leads to reduction in plant population and yield components (Smith *et al.,* 1989).

The 100-seed weight was positively correlated with shelling percentage. Similar results were observed by Ramanathan and Raman (1968). Test weight had significant positive correlation with shelling percentage and oil content. Similar results were reported for shelling percentage (Ramanathan and Raman, 1968; Sangha, 1973) and disease incidence at harvest was positively correlated with plant population and negatively correlated with plant height, number of primary branches, oil content and pod yield per plant. Similar results were reported by Nagaraj (2003).

Table 5. Genotypic and phynotypic correlations of Summer 2009

Character	PP	PWP	SP	TW	OC	DI 30	DI 60	DI 90	DIAH
Plant population (PP)	1.000	-0.702** -0.288**	0.352** 0.060	-0.139 -0.021	-0.035 0.020	-0.563** -0.217**	-0.690** -0.249**	-0.613** -0.241**	-0.116 -0.200**
Pod weight/plant (PWP)		1.000	-0.174** -0.011	0.048 0.035	-0.160* -0.138	-0.029 -0.025	0.049 0.047	-0.032 -0.028	0.179** 0.040
Shelling percentage (SP)			1.000	-0.402** 0.069	-0.016 0.057	-0.167** -0.084	-0.041 -0.026	0.015 0.003	-0.058 0.098
Test weight (TW)				1.000	-0.295** -0.078	0.044 0.009	0.014 0.006	-0.029 -0.011	-0.497** 0.001
Oil content (OC)					1.000	-0.044 -0.042	-0.078 -0.047	0.113 0.082	0.133 0.012
Disease incidence at 30 days (D 30)						1.000	-0.178** -0.173**	-0.224** -0.222**	-0.009 -0.273**
Disease incidence at 60 days (D 60)							1.000	-0.300** -0.294**	-0.458** -0.400**
Disease incidence at 90 days (D90)								1.000	-0.100 -0.308**
Disease incidence at harvest (DIAH)									1.000

** Significance at 5% and 1% Propability

PP= Plant population; PWP= Pod weight per plant; SP= Shelling percentage; TW= Test weight; OC= Oil content; DI 30= Disease incidence at 30 days; DI 60= Disease incidence at 60 days; DI 90= Disease incidence at 90 days; DIAH= Disease incidence at harvest

Conclusion

It can be concluded that the studies clearly demonstrated genotypic difference in crop susceptibility to disease incidence and its parameters. As revealed by PCV, GCV, Heritability (h^2), GAM, this variation was highly heritable and thus existance of scope of selection. Among different characters, disease incidence at harvest and yield per plant exhibited substantial genotype x seasonal interaction as revealed by depressed genetic components of variance in pooled analysis. This indicates need for caution in selection for yield potential and disease incidence. In case of groundnut, *S. rolfsii* is a major disease which can cause up to 100% yield losses. There is a need for breeding for resistance to *S. rolfsii* since it is showing continuous negative association with all components of yield in groundnut.

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