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Evaluation of Groundnut Segregating Population for Resistance to *Sclerotium rolfsii*. using an Efficient Field Screening Technique

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ABSTRACT

Background: Stem rot of groundnut caused by *Sclerotium rolfsii* Sacc. is one of the major constraint to groundnut production in many countries and yield losses upto 10-25% were recorded. Screening of groundnut genotypes for resistance to stem rot under field conditions is complicated by the non-uniform spatial distribution of the pathogen.

Methods: A total of 165 segregants derived from TAG 24 and R 9227 were evaluated for resistance to stem and pod rot during rainy and summer seasons by using sick plot technique. The field is artificially inoculated at 30 days interval.

Result: Among different parameters, maximum phenotypic variability and heritability were observed for disease at 30, 60, 90 days after sowing. The strong negative associations were observed for disease incidence and plant population and positive association were observed for test weight and pod weight per plant. The higher number of superior segregants was observed for pod weight per plant followed by oil content and test weight as compared to both the parents. Generally, high frequencies of desirable segregants were observed for oil content combined with pod weight per plant followed by test weight and pod weight per plant. Out of 165 lines, only six lines showed moderate resistance to *Sclerotium rolfsii* with good yield attributing characters, further these lines can be utilized in future breeding programme.

Key words: Disease incidence, Groundnut, Pod weight per plant, *Sclerotium rolfsii.*

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the major economic oilseed crops in globe. It is an annual legume crop grown primarily for high quality edible oil. Groundnut kernel rich in energy source, that supply about 5.6 and 5.8 calories per gram of kernel in the raw and roasted forms, respectively with good source of nutritional values *viz.*, 44-50% of oil, 25-33% protein, 18% carbohydrates, high nitrogen content (7.0-8.0%), mineral (calcium, magnesium and iron) and vitamins (B_1, B_2 and Niacin) (Pasupuleti, 2013). In India, the groundnut is being cultivating in 5.22 mha with annual production of 7.41 MT and 1418 kg/ha of productivity (Anon, 2017). Karnataka is one among the major groundnut producing states with an annual production of 0.39 MT from 0.57 mha with the productivity of 729 kg/ha (Anonymous, 2017). In spite of good production the groundnut needs an immediate attention to address the inevitable constraints such as poor soil fertility, abiotic and biotic stress factors. Among biotic stresses stem and pod rot disease caused by *Sclerotium rolfsii* Sacc. is one of the significant factors contributing to 10 to 25% annual yield loss (Chapin, 2010). However, the fungus is being managed by chemical and other agronomic practices but the fungus survive in the soil for a long period of time because of wide host range. Hence, it demands frequent managing practices which increases cost of cultivation. Therefore, it is essential to develop Sclerotium wilt resistant cultivars that may reduce the cost of cultivation (Cuong, 2011). Hence, evaluation of genotypes for resistance to *Sclerotium rolfsii* would be more reliable to select some of the resistant lines for their further use in resistance breeding programme (Rakholiya and Jadeja, 2010). The present study aimed to develop and select the *Sclerotium rolfsii* resistant cultivars from the cross between adoptive but susceptible cultivar TAG 24 and stem rot resistant donor parent R 9227.

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MATERIALS AND METHODS

The experiments were conducted in two seasons (rainy and summer, 2017-18) at University of Agricultural Sciences, Dharwad. The material comprised of TAG 24 × R 9227 cross, this cross was made by using susceptible and adopted variety (TAG 24) and resistant (R 9227) parents. Hybrids 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 single seed decent (SSD) method. Individual F_3 families were propagated as bulk in F_4 and F_5 generations. From F_5 generation selections were made and selected plants/ progenies were evaluated in F_6 generation and artificial inoculation conditions were created during rainy and summer seasons. Selected progenies/lines were forwarded for large scale trials and then released as cultivar. Progeny rows which showed negative interactions to pod yield, such progeny rows will be used back in a crossing programme to cross with F_{1s} (TAG 24 × R 9227) and further selection will be followed (Fig 1). *S. rolfsii* was isolated from the diseased groundnut plant grown in vertisols. The standard procedure (Bagwan, 2011; Bekriwala *et al*., 2016) to obtain pure culture and for mass multiplication sand-corn meal medium was used in the proportion of 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 per cent of distilled water and it was sterilized in autoclave at 121°C with 1.1 kg/cm^2 pressure for 20 minutes. The pure culture of isolated *S. rolfsii* was inoculated separately for flasks under aseptic condition and incubated at 27±1°C for 30 days. 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 at 125 g/2.5 m row at 30 days after sowing or at flower initiation. Chopped sorghum stubbles (3-4 cm

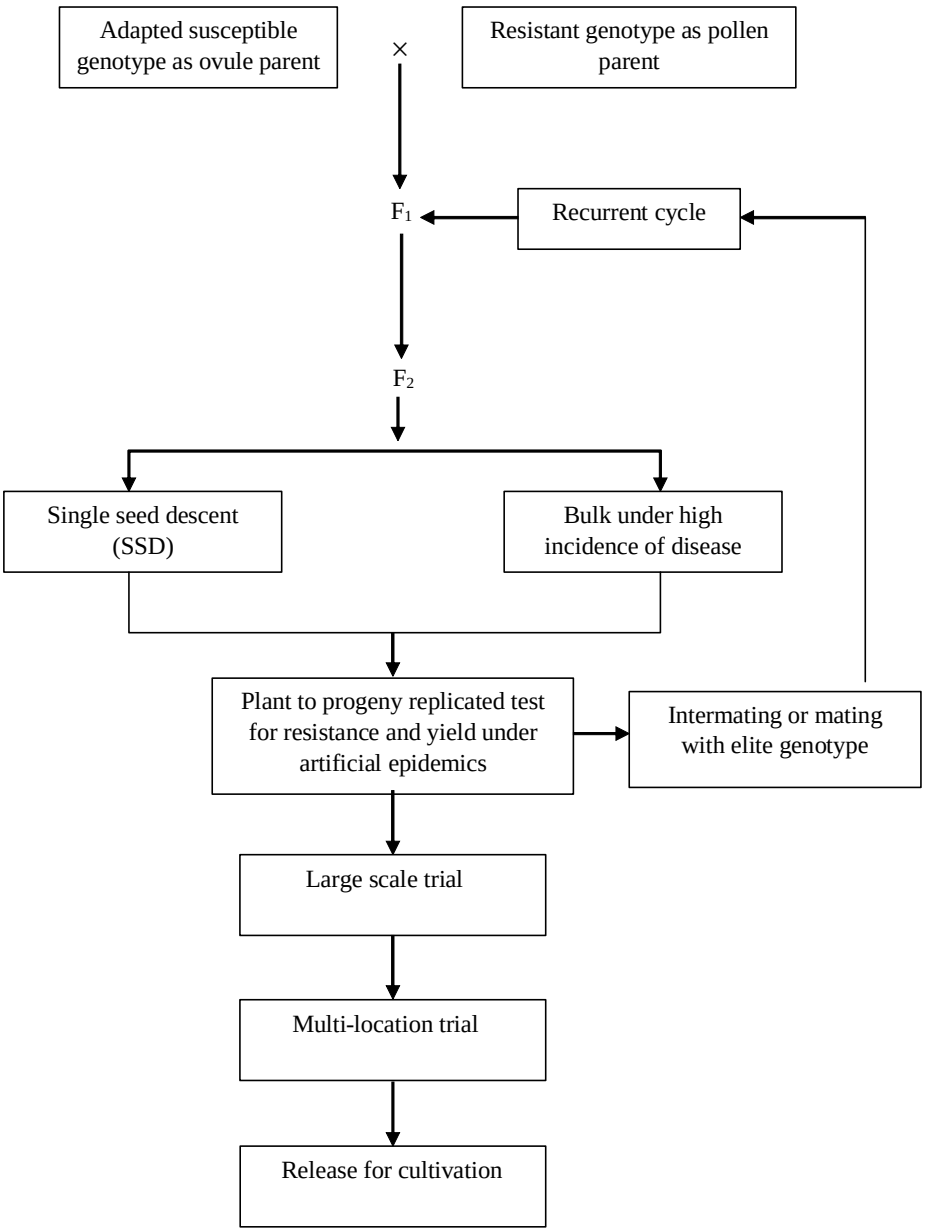

Fig 1: Strategy of selection for resistance to *Sclerotium rolfsii.*

pieces) were scattered along the rows to enhance the fungal growth on soil and after two weeks the inoculation was repeated (Pujer *et al*., 2013). During summer season, the field was irrigated at five days intervals until pod formation to promote stem rot development (Arpita and Kenchanagoudar, 2018; Pujer *et al*., 2011). The irrigation interval was increased by 15 days interval to promote pod infection. In this experiment 165 segregants (F_5 and F_6 generations) derived from TAG 24 × R 9227 cross were screened for resistance to stem and pod rot using sick plot technique (Rangaswami, 1972) over two seasons (rainy and summer). Observations on productivity parameters *viz*., pod yield per plant (g/plant), test weight (g), shelling outturn (%), oil content (%) and disease parameters *viz*., Sclerotium disease incidence (%) at various stages of crop growth (30, 60, 90 days after sowing and at harvest) were recorded.

Infection of disease was successful in summer compared to rainy. It is because of some of climatic variation and low inoculum load in rainy season. During summer, optimum temperature and soil moisture manipulated through irrigation and covering thin plastic sheet over an experiment plot (Bera *et al*., 2016) particularly from peg initiation to until harvest, which has favored *S. rolfsii* infection.

Statistical analysis

Statistical analysis was carried out using SPAR software. Standard statistical procedures were adopted for calculating the mean, range and various genetic parameters like phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) in a broad sense and genetic advance as per cent of mean (GAM). The range of coefficient of variation (CV) was categorized as per Sivasubramanian and Madhavamenon (1973): below 10%- Low coefficient of variation; 10-20%- Medium coefficient of variation; above 20%- High coefficient of variation. Heritability was calculated as suggested by Robinson *et al.* (1949), and the range was classified as: less than 30%- Low heritability; 30%-60%- Moderate heritability; more than 60% - High heritability. Similarly, the range of genetic advance as per cent of mean (GAM) was grouped as: less than 10%- Low GAM; 10%-20%- Medium GAM; more than 20%- High GAM (Johnson *et al*., 1955).

RESULTS AND DISCUSSION

The plant improvement activities through breeding contemplate an eventual boost in genetic potential for yield. Since, yield is polygenically controlled and highly influenced by environment, selection based on yield alone is not effective. The breeder hence develops into proposition of selecting for high yield indirectly through yield associated and highly heritable characters after eliminating environmental components of phenotypic variance. An attempt to improve a character by selection would be futile unless a major portion of variation is heritable which depends entirely on the magnitude of genetic variability in the source progeny. In respect to yield and its components, most of genetic variability available today in plant collection is the result of spontaneous mutation, recombination and exposure to natural selection over centuries. Various crop plants have molded themselves to the needs of nature through forces of evolution. As time passed and man's pursuit for better genotypes progressed, the concept of hybridization evolved as a means to generate more variability through recombination.

Analysis of variance revealed significant differences among the genotypes (g) for all the parameters over season(s) indicating existence of variability for most of characters (Table 1). Pooled analysis of variance found that significant seasonal variation and also exhibited significant G × S interaction for all characters, emphasizing the requirement for multi seasonal/location testing.

The 165 progenies along with their parents were evaluated over two seasons under artificial inoculated conditions. There was significant variance among genotypes between seasons as well as genotype × season interaction for population stand, disease incidence and yield and yield related parameters.

High coefficient of variation observed for different disease intervals explained the less impact of environment on expression of disease. This condition helped for selection of progenies with good resistance source. Moderate coefficient of variation for traits such as pod yield per plant, shelling outturn and test weight indicating that there is influence of environment and oil content showed low coefficient of variation less more influence of environment (Table 2).

Among various disease and productivity parameters studied, high heritability for disease incidence at different intervals was observed. This showed the potentiality for selection under disease epidemics condition. Moderate heritability for yield per plant and productivity parameters such as test weight (g), shelling outturn (%) and oil content (%) shows low heritability revealing that the character is highly influenced by environmental factor and genetic improvement through selection will be difficult due to masking effect of environmental on the genotypic effects.

Among different parameters, variation was highly heritable for yield per plant, disease incidence parameters potential for selection under disease epidemics. But, substantial proportion of genotype × season interaction for pod yield per plant and diseases at harvest indicated a need for caution in selection for this character.

Genetic correlation between different characters of plant often arises because of either linkage or pleiotropy (Horland, 1939). Hence, the study of character association through correlation will help in selecting the yield attribute. Disease incidence at different intervals showed significant negative association to pod yield, indicating selection of disease resistance progenies will have negative impact on pod yield per plant. In early selection cycles pod yield with moderate

Table 1: Pooled analysis of variance over seasons for disease and productivity traits.

Source of variance	d.f	Variables (MSS)								
		Plant population	Test weight (g)	Shelling percentage (%)	Oil content (%)	Pod weight/ plant(g)	Disease at 30 days(D 30)	Disease at 60 days(D 60)	Disease at 90 days(D 90)	Disease at harvest
Season (S)	1	1382.5**	856.5**	20297.0**	0.25	26.79**	425810.10**	624581.18**	754872.20**	622416.12**
Genotype (G)	166	10.96*	42.13*	79.95**	8.95**	23.89**	108.35*	132.83*	154.76*	160.53*
S × G	166	6.93*	37.68*	70.58**	0.0037	25.24**	121.39*	151.43*	174.59*	182.93*
Pooled error	332	6.48	25.35	48.92	1.13	2.33	97.22	121.35	134.49	147.02

*, ** - indicate the significance at 5% and 1% probability, respectively.

resistance can be the criteria for selection. However in the present population about six superior segregant with tolerance to *Sclerotium rolfsii* and desirable yield and yield attributes than the adaptive and susceptible parent/variety TAG 24 (Pujer *et al.,* 2013; Arpita and Kenchanagoudar, 2018). Positive association between disease at harvest and yield related parameters (oil content, shelling percentage and test weight), indicated the undesirable association thus emphasizing a need for intermating among selected lines to break these undesirable associations (Pujer *et al*., 2011) (Table 3).

Disease incidence at 30 days was negatively correlated with plant population and no significant effect with oil content, shelling percentage and pod weight per plant (Pujer *et al*., 2013). Test weight exhibited positive significant association with pod weight per plant (Zaman *et al*., 2011; Pujer *et al*., 2011; Pujer *et al*., 2013).

Selection of superior segregants from the crossed population was rather more for the pod weight per plant and oil content (Table 4). This may be because of quantitative inheritance with modifier gene enhance the phenotypic expression of gene at loci controlling resistance (Thirumalaisamy *et al*., 2014). Twenty six lines were observed to be superior segregant (15.76) for pod weight per plant and 21 lines were observed as superior segregant (12.73%) for oil content, 19 lines were observed to be superior segregant (11.52%) for test weight as compare both the parents, this may be because of the parents involved in the cross exhibiting superior for these characters. 8 lines were observed as superior segregant (4.85) in shelling percentage, very less number of superior segregants (3.6%) was observed in case of disease at harvest *i.e*., 6 lines was recorded (Pujer *et al*., 2013).

These six lines (21, 77, 109, 25, 165 and 36) compare with other productive characters are presented in Table 5, these lines can be used for the further breeding programme for development of new variety with high level disease resistance and other productive traits (Divya Rani *et al*. 2018). Among these lines, the line 21 had maximum shelling out turn (%) and lower disease incidence at harvest and line 77 had lower disease incidence at harvest, high test weight and oil content compared to both the parents. Performance of superior lines for disease at harvest is compare with its productivity parameters like pod yield per plant, oil content, shelling percentage and test weight. The disease incidence performance of 165 recombinant inbreed line along with two parents is presented in (Fig 2).

The high frequency of distribution was observed for oil content combined with pod weight per plant (0.030) and medium to low frequency of segregants were observed in case of test weight is combined with shelling percentage (0.0060), oil content (0.012) and pod weight per plant (0.018), respectively. Very low frequency of segregant was observed in shelling percentage combined with oil content (0.006) and shelling percentage combined with disease at harvest (0.006) (Table 6).

Table 2: Genetic components of variance for various parameters over the season.

Character	Mean	Range	PCV	GCV	h^2	GAM
Plant population	20.89	11.00-28.00	13.11	4.81	13.5	3.64
Pod yield per plant	11.52	3.93-30.40	13.26	1.27	23.52	2.17
Shelling outturn	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 30 days	15.89	0.00-50.0	58.66	7.82	58.10	8.80
Disease at 60 days	22.59	0.00-60.0	52.92	1.39	56.40	6.70
Disease at 90 days	30.41	0.00-80.0	41.61	1.49	59.60	3.90
Disease at harvest	41.60	0.00-100	29.15	0.08	51.65	0.84

Table 3: Genotypic and phenotypic correlation coefficients over season.

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

*, ** - indicate the significance at 5% and 1% probability, respectively.

Table 4: Percentage of superior segrgeants over the parents (TAG-24 and R 9227).

Characters	TAG 24	R 9227	Number of lines	% of superior segregants
Test weight	42.56	46.28	19	11.52
Shelling percentage	65.30	69.52	8	4.85
Oil content	44.68	41.85	21	12.73
Pod weight/plant	14.18	10.74	26	15.76
Disease at harvest	42.17	26.88	6	3.60

Table 5: Performance of superior segregants for disease resistance and productivity parameters.

Genotype Superior lines	Disease at harvest (%)	Pod weight per plant (g)	Oil content (%)	Test weight (g)	Shelling out turn (%)
21	19.79	10.05	43.22	49.00	74.50
77	20.04	10.80	44.33	50.00	64.69
109	22.04	8.35	42.08	41.50	69.43
25	24.72	9.35	41.43	46.50	69.80
165	24.92	8.80	43.42	38.50	73.13
36	25.92	18.55	41.00	45.50	68.50
Parents					
TAG-24	42.17	10.25	44.68	43.50	76.38
R-9227	26.88	11.10	41.85	44.90	72.25

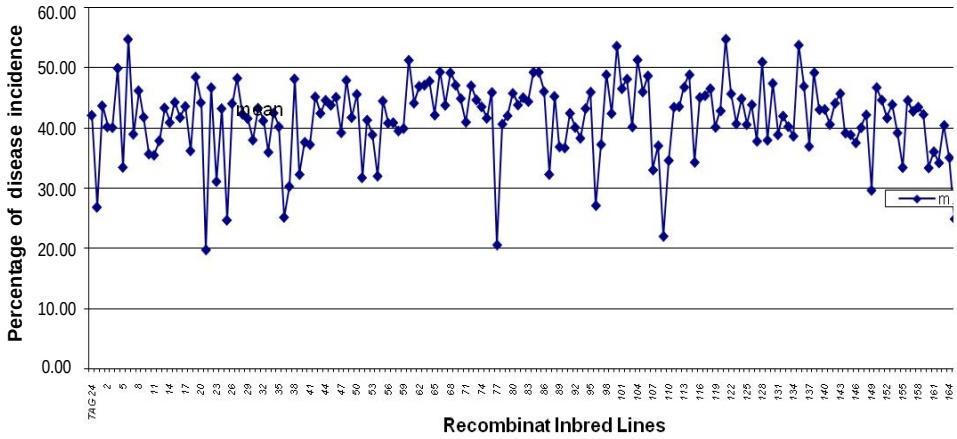

Fig 2: Performance of Individual genotype disease incidence over season.

Table 6: Frequency distribution of superior recombinant with respect to disease resistance and productivity parameters.

Combination	Superior lines	Frequency
Test weight + Shelling percentage	1	0.006
Test weight + Oil content	2	0.012
Test weight + Pod weight/plant	3	0.018
Shelling percentage + Oil content	1	0.006
Shelling percentage + Disease at harvest	1	0.006
Oil content+ Pod weight/plant	5	0.030

CONCLUSION

Potential variability was evident among the cross for resistance but there was less potential in generating resistant segregants combining other desirable traits. These need for large population and intermating among the selected segregants which will help to identify resistant genotypes to necrotopic pathogen like *Sclerotium rolfsii*. The quantitative and partial nature of resistance makes the resistance breeding. The recovery of very less number of superior breeding lines indicated the importance of evaluating the breeding lines for disease resistance along with the yield in the earlier generation itself. Instead of crossing susceptible and resistant parents, mating of resistant genotype may enhance the level of resistance besides recovery of high frequency of resistant progeny. The selected superior lines for sclerotium resistance with desirable agronomic attributes can be utilized in future breeding programme. Line number 36 is better for adaptive traits than susceptible parent (TAG 24), hence it can be tested in multi location trait to test adoptability across the locations.

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