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Genetic model of Inheritance of Late leaf spot resistance in Groundnut (*Arachis hypogaea*. L)

Tukaram Sadgar[1*], Viju Amolic[2], G. C. Shinde [3], S. R. More [4], R. T. Gaikwad[5], M. R. Patil[6], Puja Mate[1] and Ramchandra Navatre[1]

[1]Ph.D. Research Scholar, Department of Agricultural Botany, PGI, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar (Maharashtra), India.
[2]Professor & Head Department of Agril. Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar (Maharashtra), India.
[3]Assistant Professor of Botany, PGI, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar (Maharashtra), India.
[4]Associate Professor, Plant Physiology, PGI, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar (Maharashtra), India.
[5]Associate Professor, Plant Pathology, PGI, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar (Maharashtra), India.
[6]Associate Professor, Statistic, PGI, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar (Maharashtra), India.

(Corresponding author: Tukaram Sadgar)*
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ABSTRACT: Groundnut late leaf spot disease caused by *Phaeoisariopsis personata* (Berk. & Curt.) is an most destructing biotic constraint for the groundnut production. Early leaf spot and late leaf spots, together can cause losses in pod yield of upto 60 percent and reduce the quality of the pod and fodder. To fulfill the need and challenges of edible oil demand and saving the expenditure on import of foreign edible oil, we need to increase the oilseed production through minimizing the biotic and abiotic stress losses. Identification and transfer of resistance source gene to develop advance breeding lines is one of the primary objective for resistance breeding in groundnut. Present experiment was carried out to understand the inheritance pattern of late leaf spot disease by using disease scoring scale at 90 to 100 days stage of maturity. The resistant parent Phule Unnati was crossed with susceptible SBXI recipient parent and different advance generations were scored as per 0 to 9 scale. The screening of generations like Parent (P_1, P_2) and generations like F_1, F_2, BC_1 and BC_2 was done at open field condition to know the disease reaction. The segregation pattern of F_2 and back crossed generation revealed that resistance to late leaf spot is controlled by a single recessive gene and segregated in 15(Susceptible):1(Resistant) ratio. The 15:1 ratio indicates that the gene interaction involved for disease resistance was duplicate type. Genetics of late leaf spot disease resistance in groundnut and will aid groundnut breeders to develop a strategic late leaf spot disease resistance breeding program and to map the genes governing resistance.

Keywords: Late leaf spot, duplicate gene interaction, F_2 segregating population.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) (2n = 40) is an important legume crop rich in oil, protein, vitamins and other micronutrients. Groundnut is an important oilseed crop in India which occupies first position in terms of area and second position in terms of production after soyabean. China ranks first in groundnut production with 17.57 million tonnes followed by India 6.73 million tonnes at second position in production. According to the all India *kharif* crop coverage report, Government of India, as on 30th September 2022, groundnut was sown in around 45.59 lakh hectares as compared to last year (49.44 lakh ha). Among the states, Gujarat stood first in area coverage with 17.09 lakh ha followed by Rajasthan (7.90 lakh ha), Andhra Pradesh (5.47 lakh ha), Madhya Pradesh (4.50 lakh ha), Karnataka (3.73 lakh ha) and Telangana (0.08 lakh ha), (Groundnut outlook 2023).

According to Okello *et al*. (2010), the most serious fungal diseases that cause foliar diseases and are primarily responsible for the economic yield loss of groundnuts are late leaf spot and rust. The most serious diseases are *Cercosporidium personatum* (Berk and Curtis.) late leaf spot and *Puccinia arachidis* Speg rust. When environmental conditions are favorable for the disease's development, the combined effect of these factors can increase the disease's incidence to the point where susceptible cultivars suffer yield losses of more than 60 percent. As a result of these multiple interactions, various changes may occur in the plants or

in the related microorganisms responsible for the joint infection.

All above ground plant parts are susceptible to late leaf spot disease, with the leaves being more severely affected. It is simple to differentiate between the leaf symptoms caused by the pathogen C. *personata* (Sexual stage: M. *berkeleyii*) based on appearance, spot color, and shape. Lesions are also produced by both fungi on the petiole, stem, and pegs. Both species produce lesions that converge when infection worsens and leaves with severe spots prematurely shed. When infections are severe, the yield and quality of nuts are significantly decreased.

Fungicide application is an effective method to control the disease, but the production cost would be increased by 10 per cent. Sources of resistance against them have been identified in some genotypes of cultivated groundnuts. Transfer of resistance in cultivated varieties is thus the cheapest method of disease control as there won't be any need of extra inputs to the farmer. Thus, it is necessary to ascertain the pattern of inheritance of this disease for effective transfer of resistance into cultivated varieties. Genetic studies on late leaf spot and rust resistance suggest that resistance is naturally complex and polygenic and probably governed by several recessive genes. Motagi, 2001, Dwivedi *et al*., 2002)., Kumar *et al*., (2016) conducted an experiment to study the inheritance of late leaf spot disease resistance in groundnut based on F_2 population of 15 crosses. Earlier researchers investigated the inheritance of late leaf spot on groundnut Motagi *et al*., (2000), Nevill (1980). The genetic analysis for resistance to late leaf spot disease was carried out by Janila *et al*. (2016) study was conducted on, JL 24 × ICG 11337, JL 24 × ICG 13919 and ICG11337 × ICG 13919 and their reciprocals, at ICRISAT, Patancheru, India. Breeding efforts to develop late leaf spot disease resistant groundnut varieties have resulted in the development of high yielding varieties with moderate levels of disease resistance. There is a need to improve late leaf spot disease resistance levels further so that new varieties can withstand disease pressure using marker based resistance gene identification and transfer Pandey *et al*. (2023), particularly in disease epidemics or disease endemic areas. Breeders will be able to design an effective breeding strategy if they have a solid understanding of resistance genetics. The currently available interspecific groundnut derivatives have a high level of resistance to late leaf spot disease Pooniya *et al*. (2020), Ramakrishnan *et al*., (2020) & Kurella *et al*. (2022), acceptable pod and seed traits, and good agronomic potential, but they mature late. They provide an excellent opportunity to boost resistance levels in breeding populations. The goal of this study was to determine the genetic basis of late leaf spot disease resistance in interspecific groundnut derivatives under both field and controlled conditions.

MATERIAL AND METHODS

Groundnut genotypes and pathogen spread seeds of groundnut genotypes were taken from AICRP on summer groundnut, MPKV, Rahuri, India. The parent SBXI was local susceptible parent used as recipient parent for LLS resistance reaction study and Phule unnati as donor parent for LLS resistance. The crosses were made to develop advance generations like F_1, F_2, BC_1 and BC_2. The parents along with different generations were grown in Randomized block design at field condition to screen their reaction to leaf spot disease. The disease pressure were created by using infected row technique method of disease spread. The border row of susceptible SBXI genotype were grown around the plot and after every fourth row of plot SBXI planted to spread the disease properly in the field. Disease incidence was recorded 90 to 100 days after inoculation as per cent infected plants. The disease was scored using 0 to 9 disease scale given by Subrahmanyam *et al*., (1983). The plants showing 0 to 3 scale categorized as resistant and above 5 scale it was considered as susceptible plants. The scale is mainly based on amount of disease incidence and leaf area damage.

Generation of progenies segregating for late leaf spot resistance.

The crosses were made in *kharif* - 2021 season to develop F_1 generation, In next season F_2 population were developed by selfing and back crossed progenies were developed by crossing F_1 with both the parents.

Table 1: Material used for experiment.

Generation	Cross
P_1	SB-XI
P_2	Phule Unnati
F_1	SB-XI × Phule Unnati
F_2	F_1 selfed
BC_1	F_1 × SB-XI
BC_2	F_1 × Phule Unnati

Sowing, inoculation and disease evaluation: For the inheritance study, seed of parents and F_1, F_2, BC_1, and BC_2; generations of each cross were sown in field. For the allelism study, seed of parents and F_1, and F_2 generations of each S × R cross were sown in field as described above for the inheritance study. The 100 days old plants of parents and six F_1, F_2, BC_1, BC_2, populations were spray inoculated with an aqueous sporangial suspension (approximately 1 > 10 ml) of isolates of Susceptible parent. Observations on individual plants were recorded at 100 days after sowing; the plants showing LLS symptom: were classified as Susceptible and healthy plants were categorized as Resistant.

Statistical analysis: The observed ratios of Resistant to Susceptible plants in the segregating generations (F_2 and BC,) in the greenhouse were compared with theoretical ratios using x^2 test. The x^2 test ($P < 0.05$ was used to test the segregation ratio of the phenotypic classes.

Plate1: General Field view of Experimental plot.

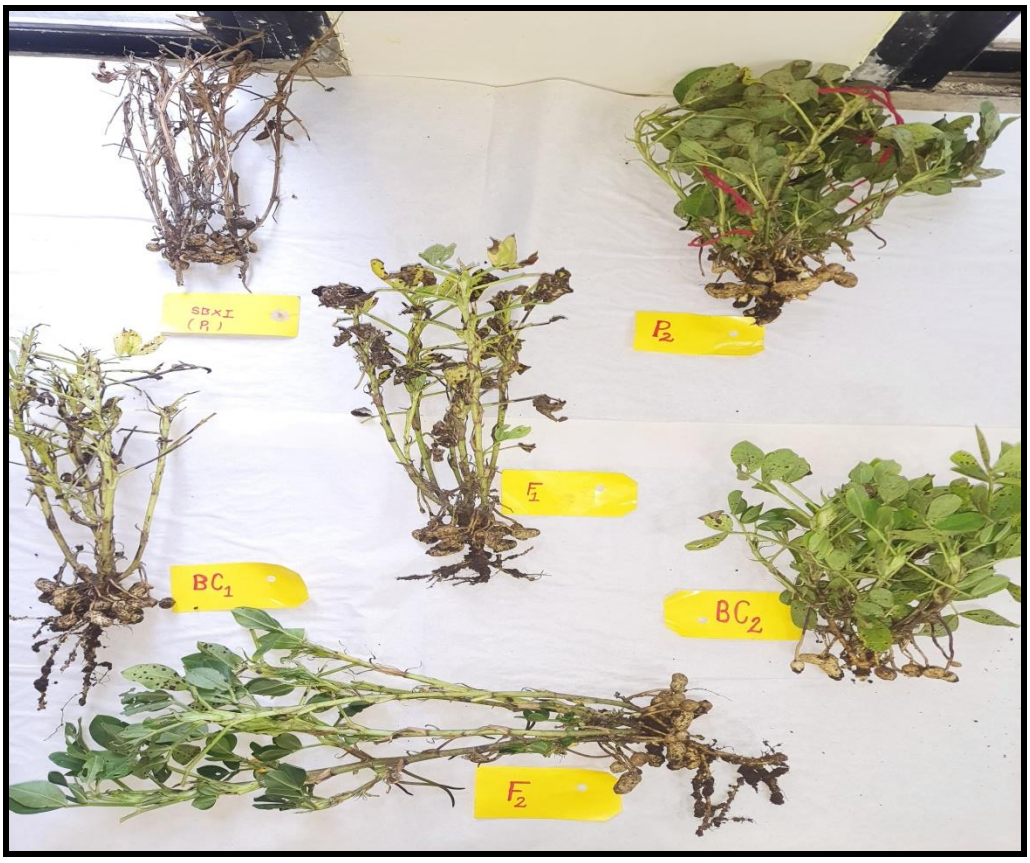

Plate 2: Late leaf spot disease incidence distribution in parents and their generations.

RESULTS AND DISCUSSION

In the present investigation, late leaf spot susceptible (SBXI) and one resistant parents (Phule unnati) were selected for study. From the two selected parent F_1 their F_2, BC_1 and BC_2 generations were developed subsequently. The experiment was conducted along with the F_1, F_2, BC_1 and BC_2 generations and parents were scored for their reaction to late leaf spot under field conditions.

The results revealed that in cross SBXI x Phule unnati all plants of the susceptible parent SBXI (field condition 50 plants) showed susceptibility to late leaf spot (score ≥3), while for resistant parent Phule unnati all plants (field condition 50 plants) were resistant (score of ≤ 2). Similarly, all plants of F_1 of cross were

susceptible (score of ≥3) under field (50 plants) conditions.

In, F_2 generation of cross, 412 plants were screened at field condition, of which 386 plants showed susceptible nature (score of ≥3) and 26 plants showed resistance (score ≤3). The segregation of F_2 at field condition showed good fit to the digenic ratio of 15:1. The chi-square value were non-significant.

Table 2: Inheritance of late leaf spot resistance in groundnut in different generations.

Parent/ cross	Gene Action	No. of Observed plants			No. of expected plants		Expected ratio (15:1)		χ^2
Parents		S	R	Total	S	R	S	R	
	SBXI	50	00	50	-	-	-	-	-
	Phule Unnati	00	50	50	-	-	-	-	-
Cross-I (S× R) SBXI × Phule Unnati	F_1	50	00	50	-	-	-	-	
	F_2	386	26	412	386.25	25.75	15	1	(NS)
	BC_1	37	00	37	37	-	1	0	(NS)
	BC_2	33	16	49	36	13	3	1	(NS)

(S: Susceptible, R: Resistant, χ^2: Chi square)

Table 3: Percent damage caused and inherited by late leaf spot disease in different generations.

Sr. No.	Generation	100 DAS	Total no. of plant affected	Late leaf spot (%)
1	P_1	50	**50**	**100**
2	**P_2**	50	**00**	**00**
3	**F_1**	50	**50**	**100**
4	**F_2**	412	**386**	**93.68**
5	**BC_1**	37	**37**	**100**
6	**BC_2**	49	**15**	**30.61**

(P: Parent, BC: Backcross, DAS: Days after sowing, %: per cent)

BC_2 population of cross showed segregation with respect to late leaf spot resistance and susceptibility, while in BC_1 all plants at field conditions were found susceptible. In BC_1 of cross, out of 37 plants. All plants were found susceptible and 00 were resistance. At field condition, 49 plants of BC_2 of cross were screened, out of that 16 plants showed resistance and 33 showed susceptibility for Late leaf spot, and chi-square value were non-significant. The segregation showed goodness of fit of 3:1 ratio for field conditions for BC_2 population of cross which confirms the digenic ratio of 15:1 for late leaf spot inheritance.

The development of late leaf spot disease is a complex character and it depends up on the genetic potential of thr genotype to resist the pathogen development. In the present investigation cross involving two diverse parents *viz*., SB-XI × Phule Unnati (S × R) were studied for inheritance of Late leaf spot resistance in six generations *viz*; P_1, P_2, F_1, F_2, BC_1 and BC_2. For recording Late leaf spot 0-9 scale was used for numerical rating (Subrahmanyam *et al*., 1983), based on this rating percent disease intensity (PDI) was worked out as per the formula given by Wheeler (1969). The response of the parents demonstrated that the parents participated in the current experiment are genetically varied for leaf spot disease resistance. The F_1 of the cross (SB-XI × Phule Unnati) showed upto 100 per cent disease susceptible reaction, which indicated that the resistance is governed by recessive gene, because the F_1 of the cross showing susceptible reaction. To test the genetic ratio, the digenic F_2 ratio segregated in the pattern of 15 susceptible: 1 resistance gave best goodness of fit for the cross SB-XI × Phule Unnati, which was further confirmed by evaluation of backcross progeny. The duplicate type of gene action was earlier reported by Pasupuleti *et.al*. (2012). The similar result were obtained by Motagi *et al*. (2000, 2013), Sapam Kumar (2016), Janila *et al*. (2013). Similar results were also reported by Vasanti and Reddy (1997), Tiwari *et al*. (1984), Nigam *et al*. (1984), Cook who observed resistance governed by duplicate recessive genes.

CONCLUSIONS

The present investigation indicated better combinations like cross SBXI × Phule Unnati, associated with late leaf spot resistance. The parent Phule Unnati were found to be superior parents in contributing to late leaf spot resistance as well as pod yield and its component traits. The identified late leaf spot resistant plants obtained in F_2 shall be utilized for development of resistant variety in groundnut. The results of the present study have important implications for breeding programs which aim to deploy LLS resistance genes or stack different genes conferring resistance to different pathotypes of LLS into elite cultivars. According to Thakur *et al*. (2008), pyramiding of genes is a strategy to develop varieties with durable DM resistance in cereal crop. The stacking of resistance genes with major effects delays the appearance of new races of the pathogen. The basis for this stability of resistance is the decrease in pathogen fitness when a number of virulence genes are necessary to overcome the resistance of the host (Van der Plank, 1984). Therefore, a potential strategy in order to maintain disease resistance for a long period of time would be the introgression of several resistance genes in a single variety. The data obtained in the present study

demonstrated that the breeder should choose a number of sources having different resistance genes for gene pyramiding, in order to put together in the best possible combination of genes in new cultivars. Therefore, these varieties expressing durable resistance would be resistant to a large number of pathotypes of the pathogen over a long period of time. However, more studies are required to identify different resistance genes (non-allelic) for their spatial and temporal deployment.

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