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Analysis of Resistant Mechanisms in Groundnut Genotypes against Late Leaf Spot Disease

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Abstract: Forty two groundnut (*Arachis hypogaea*) cultivars were screened for resistance to *Phaeoisariopsispersonata* under glasshouse conditions. Among them, two germplasms (VG19561 and VG19654) were found to have resistance against late leaf spot. Biochemical parameters such as, phenylalanine ammonia-lyase, peroxidase, polyphenol oxidase and total phenols were estimated among the resistant germplasms and susceptible check, VRI2. biochemical analysis revealed the increased activities of the enzymes *viz*., phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, and phenolics in the resistant germplasms *viz*., VG19561 and VG19654 than the susceptible check, VRI 2.

Keywords: Groundnut, late leaf spot, *Phaeoisariopsispersonata*, resistant sources, plant defense enzymes

Introduction

Diseases are the limiting factors for the successful production of groundnut (Arachis hypogaea L.) crop. The foliar fungal diseaseslate leaf spot [*Phaeoisariopsispersonata* (Berk. & Curt) V. Arx] and rust (*Puccinia arachidis*Speg.) are the most widespread and destructive foliar diseases that causes significant yield losses in groundnut. The magnitude of yield losses caused by these diseases is very high ranging from 10 to 70% (Ghewande, 1990). However, the severity of each disease varies between localities and seasons. Repeated application of fungicide for the management of these diseases are discouraged by the farmers. Hence, there is a need to identify alternative method of disease management that are both economical and eco-friendly. These diseases damage the plant by reducing the leaf area available for

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photosynthesis and stimulating the leaflet abscission leading to heavy defoliation (Subrahmanyam et al., 1985).

Identification of resistant sources and knowledge of components and mechanism of resistance are the pre-requisite for the success of disease resistance breeding programs. Several sources of resistance to foliar diseases like, LLS and rust have been reported in groundnut (Anderson et al., 1993; Mehan et al., 1996).

Cultivation of resistant cultivars is the best strategy to overcome yield losses (Dwivedi et al., 1993). The presence of variation in biochemical characters play important role in disease resistance in groundnut (Jyosthna, et. al., 2004). Knowledge on components of resistance to LLS should facilitate the development of groundnut cultivars with enhanced resistance to this disease. Hence, the present investigation was undertaken to screen the advanced breeding lines and study the biochemical components of resistance to LLS disease.

Materials and Methods

Seed material

The seed materials (forty-two advanced breeding lines/germplasms and a susceptible check, VRI2) were obtained from Regional Research Station, Tamil Nadu Agricultural University, Vriddhachalam, Tamil Nadu, India (Table 1).

Screening of groundnut germplasms for resistance against late leaf spot and rust

Disease screening of groundnut germplasms for resistance to late leaf spot (LLS) was carried out under glasshouse conditions. The conidia of *Phaeoisariopsispersonata* was harvested and the concentration of the inoculum was adjusted to 20,000 spores/ml and sprayed over 45 days old seedlings. Immediately after inoculation, leaves were covered with

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a polythene cover and sprinkled with water to ensure wetness of the leaf surface during the night. Polythene cover was removed during the day time. The alternating wet and dry period treatments was repeated for 5 days. Plants were then kept in the glasshouse until the end of the experiment. The percentage of defoliation was recorded for LLS. Disease severity was assessed following 1 to 9 rating scale (Subramanyam et al., 1995).

Assay of defense related enzymes

Sample collection

Samples were collected from individual germplasm to study the induction of defense enzymes in response to pathogen attack in groundnut seedlings under glasshouse conditions. Leaves sprayed with fungal spores were collected at 0 h, 24 h, 48 h, 72 h, 96 h, and 120 h upto 7 days at 24 h interval.

Enzyme extraction

The leaf tissues collected from groundnut plants were homogenized with liquid nitrogen. One g of powdered sample was extracted with 2 ml of 0.1 M Sodium phosphate buffer 0.1 M (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min at 10,000 rpm. Protein extracts prepared from groundnut tissues were used for the assay of phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase enzymes.

Colorimetric assay

Assay of phenylalanine ammonia-lyase (PAL)

PAL activity (EC 4.3.1.5) was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 $M^{-1}cm^{-1}$ (Dickerson *et al.*,

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1984). Enzyme activity was expressed in fresh weight basis as nmol trans-cinnamic acid min^{-1} mg^{-1} of sample.

Peroxidase (PO)

Assay of PO (EC 1.11.1.7) activity was carried out as per the procedure described by Hammerschmidt *et al*. (1982). The reaction mixture consisted of 2.5 ml of a mixture containing 0.25% (v/v) guaiacol in 0.01 M sodium phosphate buffer, pH 6.0 and0.1 M hydrogen peroxide. Enzyme extract (0.1ml) was added to initiate the reaction, which was followed colorimetrically at 470 nm. Crude enzyme preparations were diluted to give changes in absorbance at 470 nm of 0.1 to 0.2 absorbance units/min. The boiled enzyme was used as blank. Activity was expressed as the increase in absorbance at 470 nm min^{-1} mg^{-1} of protein.

Assay of polyphenoloxidase (PPO)

The polyphenoloxidase(EC1.14.18.1) activity was determined as per the procedure given by Mayer *et al.* (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as change in absorbance $min^{-1}mg^{-1}$ of protein.

Total phenolic content

Phenol content was estimated as per the procedure given by Zieslin and Ben-Zaken (1993). One g of groundnut leaf tissue was homogenized in 10 ml of 80% methanol with pestle and mortar and agitated for 15 min at 70°C. One ml of the methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1 N) and the solution was kept at 25°C. After 3 min, one ml of saturated solution of sodium carbonate and one ml

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of distilled water was added and the reaction mixture was incubated for 1 h at 25°C. The absorption of the developed blue colour was measured using UV-Visible Spectrophotometer (Varian Cary 50, Victoria, Australia) at 725 nm. The content of the total soluble phenols was calculated according to a standard curve obtained from a Folin-Ciocalteau reagent with a phenol solution (C_6H_6O) and expressed as catechol equivalents mg^{-1} tissue weight.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). Data were subjected to analysis of variance (ANOVA) at two significant levels ($P< 0.05$ and $P< 0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

Results

Identification of resistant sources against LLS

Among the forty-two numbers of groundnut germplasms screened against late leaf spot during kharif 2020-2021, VG19561 and VG19654 were found to be resistant against late leaf spot with grade 2.5 as compared to other germplasms. Susceptible check (VRI 2) showed high disease severity with grade 9 (Table 1).

Activities of plant defense enzymes

The induced systemic resistance through biochemical analysis revealed the increased activities of the enzymes *viz.*, phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, and phenolics in the resistant germplasms *viz.*, VG19561 and VG19654 than the susceptible check, VRI 2.

Groundnut germplasms inoculated with spores of *P.personata* induced the plant to synthesize higher levels of PAL, PO, and PPO. However, enzyme activity was significantly lower in susceptible check, VRI 2. Upon inoculation with LLS pathogen, activities of PAL,

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PO, and PPO significantly increased in two germplasms, VG19561 and VG19654up to 5th day when inoculated with spores of *P.personata* and slowly declined thereafter when compared to susceptible check, VRI 2 (Fig. 1A-E).

In response to LLS infection, accumulation of phenolics was higher in two germplasms, VG19561 and VG19654 when compared to other germplasms. The phenol activity was comparatively lower in susceptible check, VRI 2 (Fig. 1D).

Discussion

Frequent application of chemicals may lead to development of resistance in the target organism (Smith and Littrell, 1980). Genotypes resistant to the disease would be economic and stable management practice (Wells et al., 1994). In the present study, VG19561 and VG19654 were found to be resistant as compared to other germplasms and susceptible check, VRI2.These findings are in accordance with Dubey et al. (1995) who reported 20 cultivars with tolerance, 7 moderately susceptible and 5 susceptible against late leaf spot and rust diseases.

The biochemical dynamics of parasitism and pathogenesis are triggered and controlled by a series of interactions between host and pathogen. Most of the research on disease resistance has shown that the plant uses its defense mechanism that is activated after infection to stop pathogen development in the host. Phenols have long been associated with passive and active defense responses of plants. In the present study, significant differences in synthesis of plant defense chemicals stomatal were observed between resistant germplasms (VG19561 and VG19654) and susceptible cultivar (VRI2) against invasion of pathogen The

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resistant cultivarsVG19561 and VG19654 has shown increased activities of PAL, PO, PPO and phenolics as compared to susceptible, VRI 2.

In inoculated leaves of resistant groundnut germplasms, quantity of phenols progressively increased compared to susceptible check. The post infectional increase in phenolic contents could be due to enhancement of synthesis, translocation of phenolics to the site of infection and hydrolysis of phenolic glycosides by fungal glycosidase to yield free phenols (Sharma et al., 1983).

Pathogenic infections of host plants after leading to alterations in the enzyme systems of infected plants have been reported by several investigators. In the present study, greatest increase of PAL, PO, and PPO activity was observed at 120 hours after inoculation in the resistant germplasms and then the activity started to decreases. Similarly, increase in PO and PPO activity upto eight days after inoculation and then decrease was observed in case of *Puccinia arachidis* affected groundnut leaves (Narayana Reddy and Khare, 1988). Increased activity of peroxidase upon infection might be essential for an additional deposition of lignin around the lesions induced by pathogens. The increased activity of PPO was reported due to either solubilization of polyphenolases from cellular compartments or activation of latent polyphenol oxidase (Robb et al., 1964). Similar increase in both PO and PPO enzymes following infection has also been reported in other host parasite combinations (Gangopadhyay and Lal, 1986).

Conclusion

Identified resistant genotypes can be used in the breeding programme for developing resistance against LLS in the desired cultivars.

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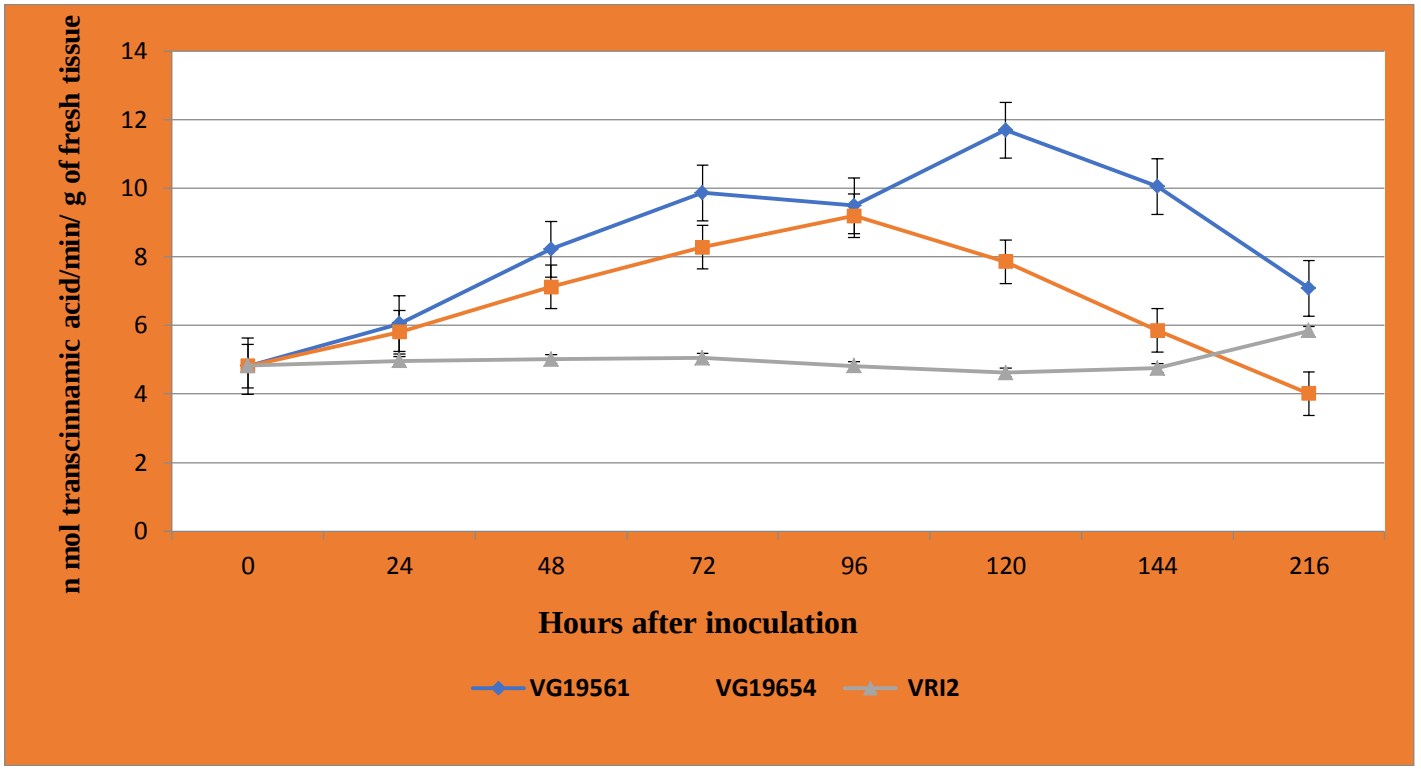
Figure 1. Induction of (A) phenylalanine ammonia-lyase, (B) peroxidase, (C) polyphenol oxidase, and (D) phenolics activities in groundnut genotypes inoculated with spores of *P. personata* against late leaf spot; the vertical bars indicate the standard error of three replications.

(A)



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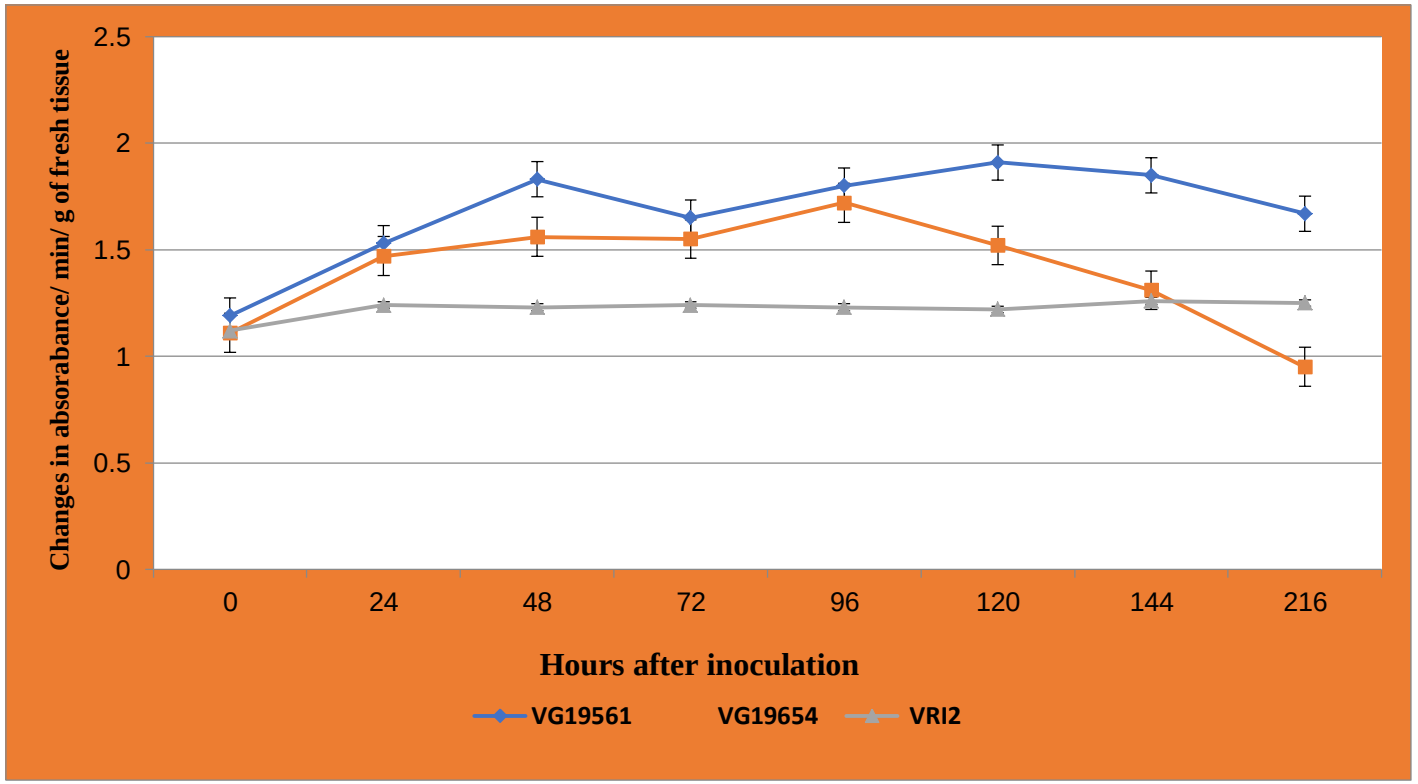
(B)



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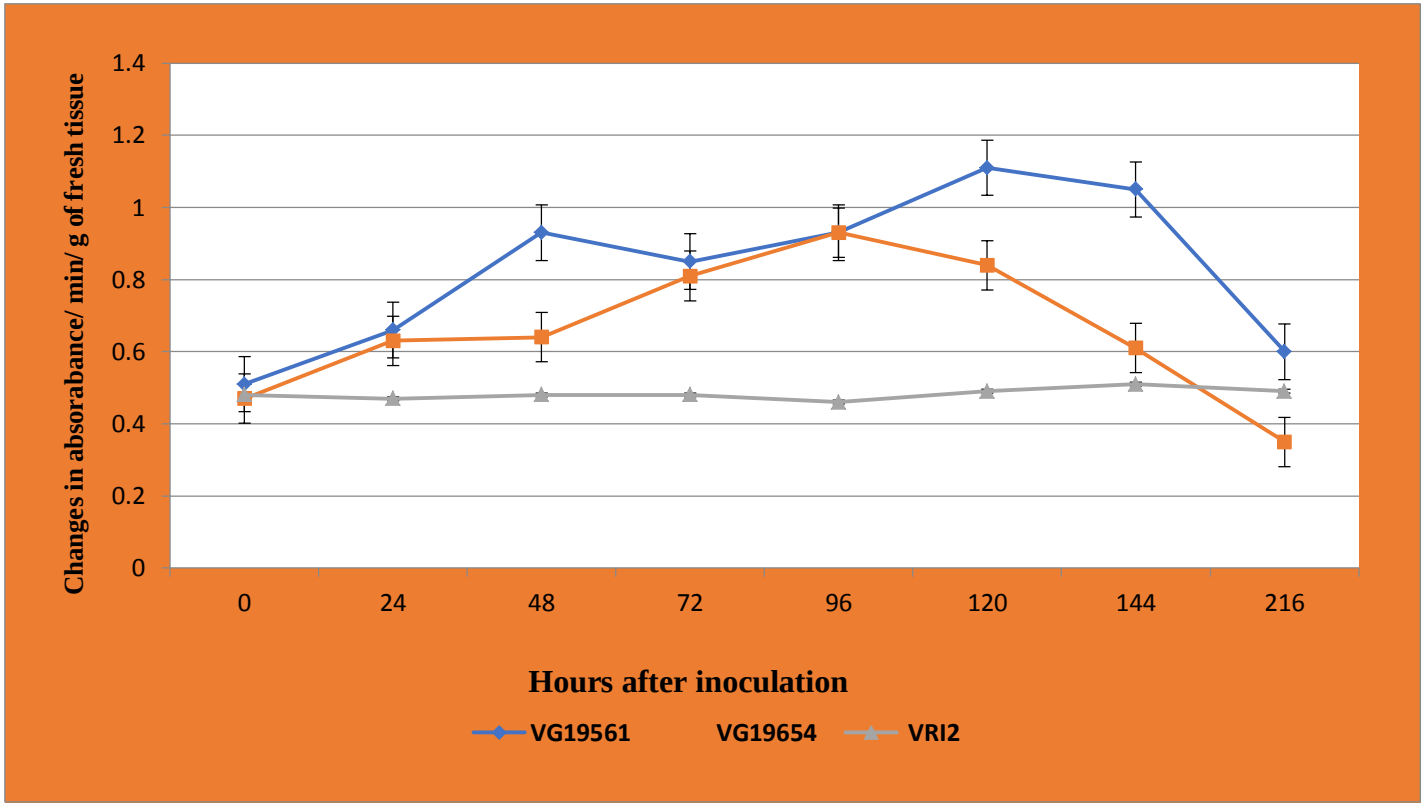

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(D)

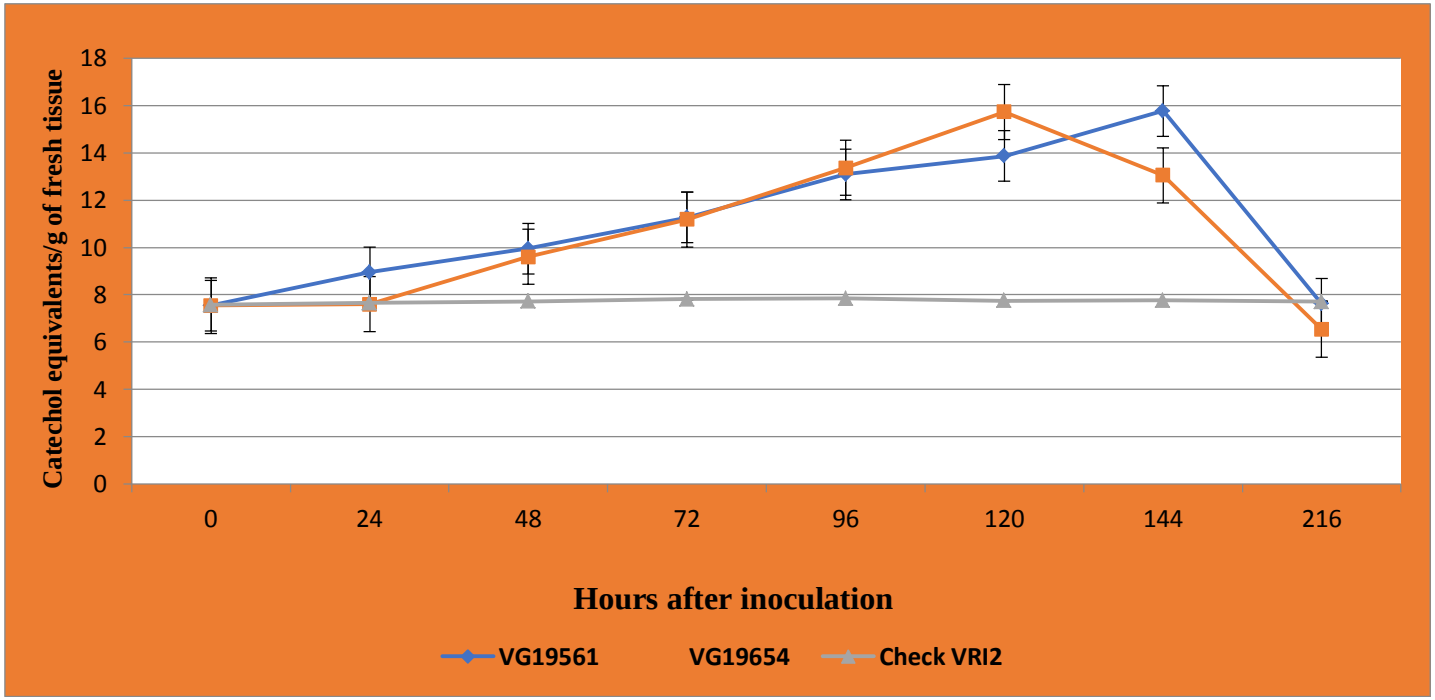

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Table 1. Screening of groundnut genotypes for their resistance against late leaf spot

S. No	Genotypes	LLS (Grade)
1.	VG17003	5.5 ^{b}
2.	VG17006	7.5 ^{d}
3.	VG17007	5.0 ^{b}
4.	VG17008	6.0^{bc}
5.	VG17009	6.0^{bc}
6.	VG17010	9.0^{ef}
7.	VG17013	9.0^{ef}
8.	VG17016	7.5 ^{d}
9.	VG17017	9.0^{ef}
10.	VG17018	8.5^{ef}
11.	VG17019	5.5 ^{b}
12.	VG17022	5.5 ^{b}
13.	VG17023	5.5 ^{b}
14.	VG17037	5.5 ^{b}
15.	VG17050	6.5^{bc}
16.	VG1705	9.0^{ef}
17.	VG18002	9.0^{ef}
18.	VG18049	9.0^{ef}
19.	VG18055	8.0 ^{cd}
20.	VG18058	9.0^{ef}
21.	VG18062	8.5^{ef}
22.	VG18076	9.0^{ef}
23.	VG18077	5.5 ^{b}

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24.	VG18081	5.5 [b]
25.	VG18089	6.0 [bc]
26.	VG18090	7.5 [d]
27.	VG18094	9.0 [ef]
28.	VG18096	9.0 [ef]
29.	VG18097	6.5 [bc]
30.	VG18098	5.5 [b]
31.	VG18100	5.5 [b]
32.	VG18111	7.0 [d]
33.	VG19002	9.0 [ef]
34.	VG19545	7.5 [d]
35.	VG19548	5.5 [b]
36.	VG19561	2.5 [a]
37.	VG19654	2.5 [a]
38.	VG19681	9.0 [ef]
39.	VG19719	9.0 [ef]
40.	VG19720	9.0 [ef]
41.	VG19721	8.5 [ef]
42.	VG19726	7.5 [d]
43.	VRI2	9.0 [ef]

Values are mean of two replications
Means followed by a common letter are not significantly different at 5% level by DMRT

