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Biochemical defense mechanism in groundnut genotypes against rust caused by *Puccinia arachidis* Speg.

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Abstract
Biochemical defense response in groundnut genotypes infected with *Puccinia arachidis* (Speg.) causing rust at different growth stage was studied in present investigations. Twelve genotypes *viz.* ICG 13165, ICG 13160 (wild resistant), RHRG-6083, GPBD-4, KDG-128, ICG-11426, ICG-12672, ICGV-86590, ICGV-94118, ICGV-96283 (cultivated resistant) and SB- XI and JL- 24 (highly susceptible) were selected. The results revealed that all the genotypes showed variable disease severity. It was observed that in all stages of pathogen infection, disease severity and characteristic symptoms were more prominent in susceptible genotype than the other ten. The biochemical analysis of leaves of different varieties of groundnut revealed the content of chlorophyll, phenol and the activity of peroxidase and polyphenol oxidase were found maximum in resistant genotypes while total sugar content was found maximum in susceptible genotypes than in resistant at all the growth stages. There was reduction in chlorophyll and reducing sugar content and the level of reduction of these biochemical constituents were less in resistant genotypes than susceptible genotypes, while total sugar and total phenol and the activity of peroxidase and polyphenol oxidase increased after inoculation in all the groundnut genotypes and were more in resistant genotypes than susceptible ones. The results indicated that factors conditioning the host response to *P. arachidis* might be the outcome of complex biochemical changes operated in host genotypes.

Keywords: Puccinia arachidis, biochemical changes, defense mechanism

Introduction

Groundnut (*Arachis hypogaea* L.) is one of the important legume oilseed crops of the world. It originated in South America, where the genus *Arachis* is widely distributed. The groundnut mostly grown in India and China. India is the second largest producer of groundnut after China with area 5.86 m. ha. and production 8.26mt. with average productivity of 1411 kg/ha in 2010-11 (Anon.; 2014) [1].

Groundnut (*Arachis hypogaea* L) is presently cultivated in more than 80 countries from 40^{o} N to 40^{o} S in tropical and warm temperate regions of the world.

Like any other economically important crops, groundnut is also susceptible to many diseases caused by fungi, bacteria, viruses and nematodes. Fungal foliar diseases such as early leaf spot (ELS) caused by *Cercospora arachidicola* Hori., late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk and Curtis) Van Arx., web blotch (WB) caused by *Phoma arachidicola* Marasas, Pauer and Boerema, rust caused by *Puccinia arachidis* Spegazinni, collar rot caused by *Aspergillus niger* Van Tieghem, root rot caused by *Macrophomina phaseolina* (Tassi) Goid and stem rot caused by *Sclerotium rolfsii* Sacc. are very important diseases on groundnut. Occurrence of these diseases results in reduction of quality and hamper yield up to 50-70 per cent (Pretorius, 2005, Subrahmanyam *et al.*, 1980) [7, 13].

The rust of groundnut is caused by *Puccinia arachidis* Speg. It has been reported from all groundnut growing areas of the country. It is severe in south India. But now, it is prevalent throughout India. The combined infection of rust and leaf spots cause losses to the extent of 53 per cent in pod yield and 27 per cent in kernel weight. Rust alone reduces the pod yield by 50 per cent. In addition to the direct yield losses, rust can lower down seed quality by lowering seed size and oil content. Early and late leaf spots coincide with rust and cause loss in quite great magnitude (Subrahmanyam *et al*., 1992) [12]. Rust can be readily recognised as orange coloured pustules (Uredinia) that appear at the lower leaf surface and rupture to expose masses of reddish brown uredospores. Pustules appear first on the lower surface and in highly susceptible cultivars the original pustule may be surrounded by colonies of secondary pustules. Pustules may also appear on the upper surface of the leaf. Under favourable conditions the disease may lead to defoliation and almost total destruction of the crop.

Groundnut rust is managed mainly by chemical methods. However, repeated application of fungicides can cause environmental pollution and a gradual loss in disease resistance to the pathogen. Therefore, the best way to manage this disease is development of resistant varieties. This would be effective in decreasing cultivation cost, environmental safety in addition to the management of the disease. The genetic makeup and enzymatic studies of the causal pathogen can help us to obtain a greater insight in the nature and mechanism of disease resistance, which can be successfully utilized in resistance breeding programme.

However, the effects of rust on the biochemical parameters in genotypes of groundnut differing rust resistance is not well understood and could contribute to better identification of improved cultivars in breeding programs. The presence of variation in biochemical characters play important role in disease resistance in groundnut (Jyosthna, *et. al.*, 2004) [4]. The aim of this research was to explore the possibility of biochemical changes for defense in the groundnut genotypes infected by rust caused by *P. arachidis*.

Materials and Methods

The analysis of biochemical parameters were carried out in the Department of Plant Pathology MPKV, Rahuri. Twelve genotypes *viz.* ICG 13165 (*Arachis batizocoi)* and ICGV 13160 (*A. cardenasi*) (wild resistant), RHRG-6083, GPBD-4, KDG-128, ICG-11426, ICG-12672, ICGV-86590, ICGV-94118, ICGV-96283 (cultivated resistant) and SB- XI and JL-24 (highly susceptible) were selected for the biochemical studies. These selected genotypes sown in 12" plastic pots in triplicate under glass house conditions. Prior to sowing these pots were filled with sterilized soil, sand and compost mixture. Three plant per pot were maintained for further studies. After 30 days of sowing the plants were inoculated with rust pathogen. One separate set of pots was maintained as uninoculated control. Sampling was done at an interval of 5 days from the date of inoculation i.e. 30 DAS and continued till 25 days after inoculation under glass house conditions. Similar sampling procedure was adopted for uninoculated control. The third leaves from apex of the rust pathogen inoculated plants were collected and used for estimation of biochemicals. The collected leaf samples were placed in ice box containing ice cubes and brought to the laboratory for estimation of different biochemicals. The experiment was laid out in a factorial randomized block design (FRBD) with three replications. Critical differences were calculated at 5% probability level if significance.

Estimation of chlorophyll

Chlorophyll content was determined by Arnon (1949) adding half g fresh leaf sample from each test genotype was used for chlorophyll estimation. Leaves were cut in to small pieces and homogenized with 80 per cent acetone. The extract was filtered through Whatman No.1 filter paper and washed 2-3 times using 80 per cent acetone. Finally the volume of the extract was made up to 50 ml in volumetric flask. The absorbance of the extract was read at 645 and 663 nm in UV-Spectrophotometer. The blank (control) reading was recorded on 80 per cent acetone only. Total chlorophyll, chlorophyll 'a' and 'b' was calculated by using the formulae given below and expressed in mg/g fresh weight of leaf.

$$\text{Chlorophyll 'a' (mg/g)} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times \frac{V}{1000} \times \frac{1}{W}$$

$$\text{Chlorophyll 'b' (mg/g)} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times \frac{V}{1000} \times \frac{1}{W}$$

$$\text{Total Chlorophyll (mg/g)} = (20.2 \times A_{645} + 8.02 \times A_{663}) \times \frac{V}{1000} \times \frac{1}{W}$$

Where,

A_{645}	=	Absorbance of the extract at 645 nm
A_{663}	=	Absorbance of the extract at 663 nm
W	=	Fresh weight of the sample (g)
V	=	Final volume of the chlorophyll extract

Estimation of reducing sugar

The reducing sugars were estimated based on 'the protocol described by Somogyi (1952) [10]. The absorbance of blue colour was read at 620 nm and standard curve was prepared by using glucose.

Estimation of total sugar

Totalsugar content was determined by (Thimmaiah, 2004) [18] adding 0.5 g of the sample was taken in test tube. Hydrolyzed it by keeping in a boiling water bath for 3 hr with 5 ml of 2.5 N HCL and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence ceases. Volume was made to 100 ml and centrifuged. Pipetted 0.2 ml of the supernant in test tube and final volume was made to 1 ml with distilled water in each tube. Added 1 ml phenol solution to each tube. Five ml of 96% sulphuric acid was added in each tube and shaken well. The tubes were placed in a water bath at 25-30 ^{0}C for 20 min. after 10 min shaking. Observations were taken at 490 nmand standard curve was prepared by using glucose.

$$\text{Total Sugars in the sample (\%)} = \frac{\text{Sugar value from the graph}}{\text{Aliquot sample used (ml)}} \times \frac{\text{Total volume of Extract}}{\text{Wt. of sample}} \times \frac{1}{1000}$$

Estimation of total phenols

Total phenol content was determined by using Folin-Denis reagent as described by Swain and Hills (1959) [16]. Groundnut leaf sample of 0.5 g from each genotype was separately macerated in a mortar and pestle in 10 ml of distilled water and phenols were extracted by keeping the tubes boiling in hot water bath for 30 min. The contents were centrifuged at 10000 x g for 15 min. the extraction was repeated two more times and the supernatant was diluted to 10 ml. The concentration of total phenolics was calculated from a standard curve of tannic acid and expressed as mg 100 g^{-1} on a fresh weight basis.

Estimation of peroxidase (PO)

The peroxidase activity from the leaf portion was assayed by the method of Maco *et al.* (1968) [6]. A known quantity (0.5 g) of groundnut leaf sample was macerated separately with 6 ml of 0.1 M phosphate buffer in pre-chilled mortar and pestle. The homogenate was centrifuged at 15000 x g at 4 ^{0}C for 30 min. One ml supernatant was diluted to 10 ml with distilled

water and was used as the enzyme source. The assay mixture of peroxidase contained 3.6 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml of 0.005 M hydrogen peroxide, 1 ml of 0.01 M pyrogallol and 1 ml of well diluted enzyme extract. The absorbance was read at 420 nm on a Spectronic-20 Spectrophotometer for every 30 sec. up to 3 min.
One unit of peroxidase activity was determined as an increase in O.D. by 0.001. The enzyme activity was calculated for one g of sample and expressed as unit min^{-1} fresh weight.

Estimation of Polyphen oloxidase (PPO)
The Polyphenol oxidase activity from the leaf portion was assayed by the method of Linskens *et al.* (1964) [5]. The enzyme extract was prepared as described under the assay of peroxidase and was used as the enzyme source. The assay mixture of polyphenol oxidase contained 2.0 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml of 0.01 M pyrogallol and 1 ml of properly diluted enzyme extract. The absorbance was read at 420 nm on a Spectronic-20 for every 30 sec. up to 3 min.
One unit of polyphenol oxidase activity was calculated as the change in absorbance units min^{-1} mg^{-1} fresh weight.

Results and Discussion
Effect on chlorophyll content
Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of ten resistant and two susceptible genotypes were estimated under rust free and rust inoculated conditions. From the data presented in Table 1 it was observed that the chlorophyll 'a' content of resistant genotypes was higher as compared to susceptible lines. Under uninoculated conditions the chlorophyll 'a' content increased with growth of seedlings, irrespective of resistance or susceptibility. The initial chlorophyll 'a' content was high (0.51 mg to 0.72 mg per g of fresh weight of leaves) in resistant genotypes increased up to 1.43 to 1.76 mg per g of fresh weight in resistant genotypes. While in susceptible genotypes the low initial chlorophyll content of 0.38 mg and 0.41 mg per g of fresh weight increased to only 1.17 and 1.19 mg per g of fresh weight in JL-24 and SB-XI, respectively. The rate of increase in chlorophyll 'a' was higher in resistant than in susceptible genotypes under uninoculated conditions over 25 days of incubation period.
Rust infection process induced significant alteration in the chlorophyll 'a' content in all stages of observations. In the resistant genotype the chlorophyll 'a' content increased continuously. In case of susceptible line, however, the chlorophyll 'a' content declined after 15 days of inoculation and reduction was more than 35 per cent at 25 days after inoculation.
The same trend was observed in the changes in chlorophyll 'b' content as observed in chlorophyll 'a' content. Rust inoculations reduced chlorophyll 'b' content significantly in susceptible genotypes. Chlorophyll 'b' content started declining from 15 days after inoculation in susceptible genotypes. The reduction in chlorophyll 'b' was 64 and 51 per cent in SB-XI and JL- 24, respectively (Table 2).
The evaluation of total chlorophyll revealed the same trend as observed in chlorophyll 'a' and 'b' in susceptible as well as resistant genotypes under rust free conditions. The difference in total chlorophyll content at all stages within the resistant and susceptible genotypes were significant. The initial high total chlorophyll content of resistant genotypes ranged from 0.72 mg to 1.04 mg per g of fresh weight and raised from 1.91 mg to 2.31 mg per g of fresh weight of leaves at the end of sampling. After inoculation the initial total chlorophyll content declined 1.76 mg from 2.08 mg per g of fresh weight at the end of sampling showing slow decline in total chlorophyll as disease progress. As compared to this the susceptible genotypes show drastic alterations (Table 3).
Rust inoculations increased the total chlorophyll in all the genotypes up to 25 days in resistant and up to 15 days in susceptible genotypes. Rust inoculations decreased the total chlorophyll content drastically in susceptible lines and moderately in the resistant lines. Nearly 42 per cent reduction was evident at the final evaluation in the total chlorophyll content of susceptible genotypes as against 4 to 13 per cent in resistant genotypes.

Effect on Sugar content
The data on the reducing sugar and the total sugar content in resistant and susceptible genotypes of groundnut influenced by the rust infection showed that under rust free condition the difference in the reducing sugar between resistant genotypes were non-significant. The reducing sugar content of the susceptible genotypes was higher than the resistant genotypes at 0 stage but subsequently it was found to significantly lower than the resistant genotypes.
Rust inoculation drastically declined the reducing sugar content in susceptible genotypes 15 days after inoculation, while in the resistant genotypes decrease in reducing sugar initiated at 20 days after inoculation but the rate of reduction was slightly more in susceptible lines. The rate of reduction of reducing sugars was more (55 per cent) in susceptible genotypes as compared to (45- 52 per cent) resistant genotypes (Table 4).
The total sugar content of susceptible genotypes was higher than the resistant genotypes. The initial high total sugar content of susceptible lines increased from 698.20and 720.12 µg/g fresh wt and 2306.89to 2462.20 µg/g fresh wt in SB-XI and JL- 24, respectively within 10 days. Subsequently there was reduction in the total sugars irrespective of genotypes (Table 5).
The rust inoculations brought about significant reduction in the total sugar level of resistant genotype at the initial stage after inoculation i.e. up to 10 DAI but later on there was very high accumulation of sugars reaching to double the quantity in uninoculated samples. In susceptible lines the pathogen brought about significant accumulations of total sugars from the very beginning 83 percent which reached to the level of 120 per cent at the end of sampling. The physiology and biochemistry of rust infection in groundnut was also studied by a number of workers *viz.*, Sudhagar *et al.*, 2000 [14]. Jyosthana *et al.,* 2004 [4]; Rathnakumar *et al.,* 2004 [8].

Effect on total phenol content
The data on total phenol content of the resistant and susceptible genotypes under rust free and rust pathogenesis conditions revealed that under rust free conditions the total phenol content of resistant genotypes was significantly higher (50 per cent) than the susceptible genotypes at all the stages of observations. It was observed that quantity of total phenol accumulated increased in the seedling with the growth of plants irrespective of the genotypes and the inoculations (Table 6).
Rust infection enhances the accumulations of total phenol in the leaf tissues. However, the phenol accumulation was more than 100 per cent in susceptible genotypes, after the symptoms were fully expressed. But in the resistant genotypes increase in total phenol content was only to the tune of 67 to

92 per cent. The level of total phenols increased after rust inoculation in both the resistant and susceptible genotypes. These results are in agreement with those reported by Reddy and Khare (1988) [9], who reported that phenol contents in leaves increased after the inoculation of urediospore suspension on groundnut variety Jyoti (highly susceptible) and ICG 1697 a resistant genotype.

Effect on peroxidase and polyphenol oxidase activity

Data on peroxidase activity revealed that it was very high in the resistant tissues than the susceptible one. It was more than two times in resistant lines than the susceptible genotypes at the end of sampling.

The rust infection however, enhanced the activity of peroxidase irrespective of genotypes. In susceptible line peroxidase were enhanced nearly 3 times as compared to 80 per cent increase in resistant lines. In uninoculated condition the peroxidase content in resistant lines was ranged from 5.60 to 5.86 units/g/min at 0 days which is reach to 10.10 to 11.00 units/g/min at 25 days while, upon inoculation the high accumulation is observed in the susceptible genotypes upto 20 days i.e. from 5.48 to 17.49 units/g/min in SB XI and 5.41 to 17.41 units/g/min in JL 24 (Table 7). Similar type of results also reported by Subba Rao (1988) [11] who recorded highest peroxidase activity in the resistant genotype and lowest in susceptible genotypes.

It was evident that differences in the polyphenol oxidase activity between the resistant genotypes were marginal but PPO activity in resistant lines was more than susceptible ones. The PPO activity was generally increased by the rust infection in all the genotypes. However, the activity was found to be fairly low in the susceptible genotypes than the resistant ones (Table 8).

Similar type of results were reported by several other workers *viz.*, Velazhahan and Vidhyasekaran, 1994 [19]; Hasabnis, 1998 [3]; Jyosthana *et al.*, 2004 [4]; Rathnakumar *et al.*, 2004 [8]; Sunkad and Kulkarni, 2006 [15]; Tashildar *et al.*, 2012 [17].

Based on the present findings, it may be concluded that high level of chlorophyll, phenol, and the activity of peroxidase and polyphenol oxidase of resistant genotypes appeared to be the important biochemical constituents which may impart resistance against rust infection. Post inflectional response of the metabolites under consideration in the susceptible genotype seems to be associated with its susceptible response. Further studies in this direction may provide information regarding host-pathogen interaction which can be utilized for resistance breeding for the development of desirable trait by incorporating resistance in promising but susceptible genotype of groundnut.

Table 1: Influence of rust on the chlorophyll 'a' content (mg/g fresh wt.) of resistant and susceptible genotypes.

Sr. No.	Genotype	DAI	0	5	10	15	20	25
1.	ICG-13165	UNI	0.51	0.82	0.93	1.06	1.18	1.43
		INO	0.51	0.80	0.91	1.01	1.11	1.29
2.	ICG-13160	UNI	0.54	0.86	0.93	1.09	1.21	1.49
		INO	0.54	0.82	0.89	1.03	1.14	1.29
3.	RHRG-6083	UNI	0.66	0.95	1.09	1.17	1.36	1.65
		INO	0.66	0.91	1.01	1.13	1.26	1.45
4.	KDG-128	UNI	0.72	0.98	1.10	1.27	1.39	1.76
		INO	0.72	0.90	1.03	1.17	1.27	1.51
5.	GPBD-4	UNI	0.61	0.88	1.06	1.18	1.27	1.52
		INO	0.61	0.83	1.00	1.18	1.20	1.58
6.	ICG-11426	UNI	0.62	0.91	0.98	1.22	1.36	1.56
		INO	0.62	0.88	0.99	1.12	1.30	1.52
7.	ICG-12672	UNI	0.68	0.86	1.03	1.18	1.39	1.55
		INO	0.68	0.85	1.02	1.12	1.29	1.49
8.	ICGV-94118	UNI	0.64	0.93	1.05	1.15	1.34	1.56
		INO	0.64	0.89	1.01	1.11	1.25	1.46
9.	ICGV-96283	UNI	0.65	0.96	1.09	1.17	1.37	1.63
		INO	0.65	0.91	1.04	1.12	1.32	1.58
10.	ICGV-86590	UNI	0.68	0.93	1.09	1.18	1.29	1.50
		INO	0.68	0.90	1.04	1.11	1.24	1.40
11.	SB-XI	UNI	0.41	0.53	0.76	0.93	1.05	1.19
		INO	0.41	0.47	0.70	0.89	0.81	0.77
12.	JL-24	UNI	0.38	0.49	0.74	0.86	1.01	1.17
		INO	0.38	0.41	0.64	0.83	0.72	0.69
SE±			0.037	0.058	0.026	0.069	0.066	0.075
CD 5%			N.S.	N.S.	N.S.	0.210	0.185	0.210

Table 2: Influence of rust on the chlorophyll 'b' content (mg/g fresh wt.) of resistant and susceptible genotypes.

Sr. No.	Genotype	DAI	0	5	10	15	20	25
1.	ICG-13165	UNI	0.21	0.26	0.31	0.37	0.41	0.48
		INO	0.21	0.23	0.28	0.31	0.38	0.44
2.	ICG-13160	UNI	0.22	0.27	0.31	0.38	0.45	0.52
		INO	0.22	0.25	0.29	0.32	0.39	0.47
3.	RHRG-6083	UNI	0.31	0.34	0.38	0.45	0.53	0.59
		INO	0.31	0.33	0.36	0.41	0.48	0.52
4.	KDG-128	UNI	0.33	0.36	0.41	0.46	0.50	0.55
		INO	0.33	0.34	0.38	0.43	0.49	0.52
5.	GPBD-4	UNI	0.30	0.33	0.38	0.44	0.51	0.56

		INO	0.30	0.33	0.36	0.41	0.46	0.50
6.	ICG-11426	UNI	0.24	0.29	0.35	0.39	0.46	0.59
		INO	0.24	0.28	0.32	0.36	0.41	0.50
7.	ICG-12672	UNI	0.28	0.34	0.39	0.45	0.49	0.54
		INO	0.28	0.34	0.39	0.45	0.49	0.54
8.	ICGV-94118	UNI	0.31	0.34	0.40	0.43	0.50	0.56
		INO	0.31	0.33	0.39	0.42	0.47	0.53
9.	ICGV-96283	UNI	0.24	0.30	0.33	0.40	0.47	0.55
		INO	0.24	0.28	0.33	0.37	0.42	0.49
10.	ICGV-86590	UNI	0.27	0.31	0.36	0.42	0.49	0.58
		INO	0.27	0.31	0.35	0.40	0.44	0.49
11.	SB-XI	UNI	0.11	0.16	0.21	0.27	0.35	0.42
		INO	0.11	0.14	0.18	0.23	0.18	0.15
12.	JL-24	UNI	0.18	0.21	0.25	0.29	0.35	0.39
		INO	0.18	0.21	0.23	0.28	0.25	0.19
SE±			0.044	0.034	0.041	0.052	0.060	0.065
CD 5%			N.S.	N.S.	0.120	0.151	0.181	0.189

Table 3: Influence of rust on the total chlorophyll content (mg/g fresh wt.) of resistant and susceptible genotypes.

Sr. No.	Genotype	DAI	0	5	10	15	20	25
1.	ICG-13165	UNI	0.72	1.08	1.25	1.43	1.59	1.91
		INO	0.72	1.03	1.19	1.32	1.48	1.73
2.	ICG-13160	UNI	0.76	1.13	1.24	1.47	1.66	2.02
		INO	0.76	1.07	1.18	1.36	1.53	1.76
3.	RHRG-6083	UNI	0.96	1.29	1.47	1.62	1.89	2.25
		INO	0.96	1.24	1.38	1.54	1.74	1.97
4.	KDG-128	UNI	1.04	1.33	1.52	1.73	1.89	2.31
		INO	1.04	1.24	1.41	1.60	1.76	2.03
5.	GPBD-4	UNI	0.91	1.21	1.45	1.62	1.78	2.08
		INO	0.91	1.30	1.36	1.60	1.76	2.08
6.	ICG-11426	UNI	0.86	1.20	1.33	1.61	1.82	2.15
		INO	0.86	1.16	1.31	1.49	1.71	2.01
7.	ICG-12672	UNI	0.97	1.19	1.42	1.63	1.88	2.10
		INO	0.97	1.20	1.41	1.56	1.77	2.03
8.	ICGV-94118	UNI	0.95	1.27	1.45	1.58	1.84	2.13
		INO	0.95	1.22	1.40	1.53	1.72	1.99
9.	ICGV-96283	UNI	0.89	1.25	1.42	1.57	1.84	2.18
		INO	0.89	1.19	1.37	1.50	1.74	2.07
10.	ICGV-86590	UNI	0.95	1.24	1.45	1.60	1.78	2.08
		INO	0.95	1.22	1.39	1.51	1.68	1.89
11.	SB-XI	UNI	0.53	0.69	0.97	1.20	1.41	1.61
		INO	0.53	0.61	0.88	1.12	0.99	0.92
12.	JL-24	UNI	0.56	0.70	0.99	1.15	1.36	1.56
		INO	0.56	0.62	0.87	1.11	0.97	0.88
SE±			0.050	0.068	0.038	0.094	0.086	0.086
CD 5%			N.S.	N.S.	0.116	0.285	0.256	0.258

Table 4: Influence of rust on the reducing sugar content (µg/g fresh wt.) of resistant and susceptible genotypes.

Sr. No.	Genotype	DAI	0	5	10	15	20	25
1.	ICG-13165	UNI	120.01	360.04	530.01	621.10	720.01	542.04
		INO	120.01	314.11	424.26	557.44	479.07	295.01
2.	ICG-13160	UNI	116.49	356.12	521.41	629.26	718.46	568.29
		INO	116.49	309.46	422.49	542.49	460.49	292.46
3.	RHRG-6083	UNI	131.98	371.58	539.08	658.04	736.76	661.98
		INO	131.98	325.95	444.04	602.98	501.04	312.98
4.	KDG-128	UNI	136.31	376.31	546.29	648.26	739.31	579.34
		INO	136.31	329.26	445.23	595.34	536.26	318.31
5.	GPBD-4	UNI	139.02	379.02	551.97	646.97	752.03	624.02
		INO	139.02	336.97	456.12	621.02	524.04	323.31
6.	ICG-11426	UNI	137.01	377.23	547.01	650.43	724.96	656.95
		INO	137.01	337.04	446.96	640.01	537.01	323.25
7.	ICG-12672	UNI	134.21	374.22	544. 21	659.23	745.10	669.41
		INO	134.21	327.24	439.16	617.24	530.16	316.21
8.	ICGV-94118	UNI	135.37	375.37	553.41	652.36	744.35	649.37
		INO	135.37	330.40	443.40	590.37	490.40	316.40
9.	ICGV-96283	UNI	133.21	373.21	550.24	656.24	743.16	630.24
		INO	133.21	332.16	441.21	589.21	503.21	309.21
10.	ICGV-86590	UNI	131.95	371.26	541.95	644.95	736.94	609.93

		INO	131.95	328.91	439.89	624.05	546.26	325.42
11.	SB-XI	UNI	151.99	296.43	456.96	602.07	652.99	456.79
		INO	151.99	263.96	359.04	528.97	380.81	199.00
12.	JL-24	UNI	159.99	312.91	460.01	616.05	659.38	463.05
		INO	159.99	289.05	373.97	533.69	384.15	204.59
SE±			0.0335	0.029	0.030	0.028	0.030	0.030
CD 5%			NS	0.084	0.086	0.081	0.085	0.087

Table 5: Influence of rust on the total sugar content (µg/g fresh wt.) of resistant and susceptible genotypes.

Sr. No.	Genotype	DAI	0	5	10	15	20	25
1.	ICG-13165	UNI	520.01	926.40	1624.01	1549.70	1478.50	1165.35
		INO	520.01	748.54	1530.40	1941.15	1848.70	1720.04
2.	ICG-13160	UNI	526.99	948.70	1654.52	1496.93	1234.94	1174.99
		INO	526.99	742.78	1422.65	2016.40	1805.62	1782.96
3.	RHRG-6083	UNI	590.40	1186.50	1847.78	1698.00	1000.20	988.40
		INO	590.40	1098.45	1784.60	2289.67	2007.40	1910.40
4.	KDG-128	UNI	536.01	1136.90	1829.65	1628.13	1246.01	1185.99
		INO	536.01	1065.89	1803.40	2156.40	2012.46	1988.01
5.	GPBD-4	UNI	539.02	1187.40	1840.91	1660.40	1155.48	1095.40
		INO	539.02	1089.74	1671.97	2375.40	2115.02	1891.97
6.	ICG-11426	UNI	542.01	1242.80	1857.01	1621.04	1300.53	1250.40
		INO	542.01	1124.46	1768.40	2162.01	2119.24	1745.10
7.	ICG-12672	UNI	638.40	1302.60	1978.04	1624.01	1242.26	1181.04
		INO	638.40	1075.49	1675.01	2159.99	2016.04	1899.01
8.	ICGV-94118	UNI	546.97	1151.20	1852.97	1537.97	1300.40	1196.00
		INO	546.97	1121.20	1680.07	2074.23	1998.74	1998.40
9.	ICGV-96283	UNI	534.01	1137.40	1978.40	1623.33	1254.04	1185.01
		INO	534.01	1107.40	1664.96	2268.21	2112.01	1912.99
10.	ICGV-86590	UNI	539.05	1204.50	1850.01	1634.95	1252.01	1194.05
		INO	539.05	1163.40	1675.05	2259.22	2017.48	1893.48
11.	SB-XI	UNI	720.12	1576.95	2306.89	1998.76	1609.62	1563.96
		INO	720.12	1394.70	2124.50	2344.91	3055.00	3669.99
12.	JL-24	UNI	698.20	1687.50	2462.20	1948.40	1532.40	1469.45
		INO	698.20	1412.50	2218.00	2355.53	3162.50	3784.54
SE±			0.033569	0.028	0.030	0.029	0.029	0.029
CD 5%			NS	0.082	0.085	0.085	0.084	0.084

Table 6: Influence of rust on the total phenol content (µg/g fresh wt.) of resistant and susceptible genotypes.

Sr. No.	Genotype	DAI	0	5	10	15	20	25
1.	ICG-13165	UNI	680.01	1255.04	2003.01	2373.01	2594.45	2698.04
		INO	680.01	2255.24	3497.26	4200.04	4392.00	4507.35
2.	ICG-13160	UNI	685.99	1257.04	2007.64	2260.00	2575.05	2596.99
		INO	685.99	2261.00	3396.40	4227.04	4411.99	4516.98
3.	RHRG-6083	UNI	512.00	1012.00	1656.29	2237.04	2326.98	2450.99
		INO	512.00	1987.40	3233.23	3840.98	4000.00	4252.04
4.	KDG-128	UNI	550.01	1128.22	1671.21	1993.00	2130.01	2271.04
		INO	550.01	2050.04	3100.00	4060.00	4262.99	4369.01
5.	GPBD-4	UNI	662.02	1045.25	1792.27	2036.43	2251.27	2460.02
		INO	662.02	1945.50	3170.28	4171.03	4363.00	4479.00
6.	ICG-11426	UNI	661.01	1136.01	1742.00	2257.01	2339.99	2548.00
		INO	661.01	2062.96	3283.29	3870.04	4266.48	4489.01
7.	ICG-12672	UNI	646.01	1356.00	1675.21	2138.96	2600.00	2650.00
		INO	646.01	2045.80	3349.26	4056.94	4356.99	4468.01
8.	ICGV-94118	UNI	650.97	1196.40	1860.25	2234.09	2445.04	2464.00
		INO	650.97	2073.44	3462.22	4157.23	4266.26	4475.97
9.	ICGV-96283	UNI	663.01	1226.04	1984.26	2160.04	2446.03	2686.01
		INO	663.01	2161.28	3565.21	4172.23	4363.01	4493.96
10.	ICGV-86590	UNI	678.05	1202.99	1902.30	2271.05	2575.01	2485.05
		INO	678.05	2179.22	3674.26	4291.03	4476.02	4605.01
11.	SB-XI	UNI	250.99	375.86	578.40	987.40	1245.00	1360.00
		INO	250.99	875.40	1355.24	1798.00	1960.00	2758.00
12.	JL-24	UNI	262.99	412.00	526.40	1158.99	1049.62	1289.05
		INO	262.99	750.40	1435.00	1585.00	2046.00	2648.93
SE±			0.033569	0.028	0.030	0.029	0.030	0.031
CD 5%			NS	0.081	0.086	0.082	0.087	0.089

Table 7: Influence of rust on the peroxidase content (units/g/min) of resistant and susceptible genotypes

Sr. No.	Genotype	DAI	0	5	10	15	20	25
1.	ICG-13165	UNI	5.60	7.00	8.16	11.00	12.36	10.10
		INO	5.91	10.66	13.01	15.06	18.52	17.41
2.	ICG-13160	UNI	5.86	6.84	7.97	10.90	12.21	10.50
		INO	5.86	11.61	12.96	14.86	17.86	17.36
3.	RHRG-6083	UNI	5.76	6.66	7.96	9.75	11.00	9.95
		INO	5.76	11.00	12.86	15.00	16.58	16.41
4.	KDG-128	UNI	5.50	6.61	8.04	10.76	11.50	10.20
		INO	5.76	11.51	12.86	14.63	17.32	17.26
5.	GPBD-4	UNI	5.83	7.50	8.15	10.50	12.06	10.65
		INO	5.83	10.65	12.93	16.20	17.76	17.33
6.	ICG-11426	UNI	5.80	6.33	8.11	10.00	11.90	9.50
		INO	5.80	11.55	12.90	15.00	17.01	17.30
7.	ICG-12672	UNI	5.84	6.98	8.10	10.81	12.50	10.03
		INO	5.84	10.00	12.94	14.81	17.84	17.34
8.	ICGV-94118	UNI	5.74	6.53	7.90	9.00	12.20	9.93
		INO	5.74	11.49	12.84	15.65	17.74	17.24
9.	ICGV-96283	UNI	5.83	6.70	7.97	9.50	11.95	11.00
		INO	5.83	11.20	12.93	14.50	16.86	16.50
10.	ICGV-86590	UNI	5.85	7.25	8.09	10.00	11.65	10.04
		INO	5.85	11.60	12.95	13.92	17.74	17.35
11.	SB-XI	UNI	5.02	6.50	7.20	8.00	6.30	4.42
		INO	5.48	8.73	12.00	13.51	17.49	14.90
12.	JL-24	UNI	5.41	6.00	6.50	7.80	6.90	5.30
		INO	5.41	8.62	11.50	13.65	17.41	14.87
SE±			0.0335	0.037031	0.038616	0.039721	0.034244	0.061
CD 5%			NS	0.105296	0.109804	0.112946	0.097373	0.173

Table 8: Influence of rust on the Polyphenol oxidase content (units/g/min) of resistant and susceptible genotypes.

SN	Genotype	DAI	0	5	10	15	20	25
1.	ICG-13165	UNI	8.89	9.66	11.16	13.50	12.50	8.50
		INO	8.89	11.66	12.67	16.00	13.20	11.22
2.	ICG-13160	UNI	9.94	10.92	12.01	13.91	11.48	8.39
		INO	9.94	11.00	12.98	17.16	14.83	11.16
3.	RHRG-6083	UNI	9.25	10.61	11.92	13.60	12.18	9.10
		INO	9.25	10.98	13.39	17.93	15.00	13.65
4.	KDG-128	UNI	9.77	10.62	12.05	13.49	12.12	9.12
		INO	9.77	11.69	14.60	16.99	13.12	11.10
5.	GPBD-4	UNI	9.87	10.66	12.19	13.29	12.22	8.44
		INO	9.87	12.05	13.55	17.09	15.00	13.65
6.	ICG-11426	UNI	9.84	10.29	12.15	13.27	12.19	10.02
		INO	9.84	12.50	14.50	17.06	15.50	12.78
7.	ICG-12672	UNI	9.79	10.44	12.12	13.26	12.14	8.54
		INO	9.79	12.00	15.00	17.01	14.68	12.00
8.	ICGV-94118	UNI	9.85	10.64	12.01	13.38	13.20	9.40
		INO	9.85	12.05	15.53	18.06	15.60	12.79
9.	ICGV-96283	UNI	9.93	10.80	12.07	13.45	11.28	8.26
		INO	9.93	11.20	13.61	16.93	14.52	12.81
10.	ICGV-86590	UNI	10.12	10.93	12.18	13.48	12.29	10.42
		INO	10.12	11.00	15.06	17.50	14.83	12.91
11.	SB-XI	UNI	8.50	9.39	10.75	11.08	10.78	7.23
		INO	8.50	10.02	11.75	13.45	12.78	9.23
12.	JL-24	UNI	8.40	9.20	10.12	11.42	10.03	7.50
		INO	8.40	9.84	11.12	14.02	12.03	10.50
SE±			0.0335	0.03419	0.042323	0.042213	0.043167	0.0344
CD 5%			NS	0.097219	0.120343	0.120032	0.122745	0.0980

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