

Original Research Article

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Erysiphe polygoni Induced Morpho-physiological and Biochemical Changes in Blackgram (*Vigna mungo*)

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ABSTRACT

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Morpho-physiological and biochemical changes was carried out for 16 germplasm lines of *Urdbean* against disease powdery mildew caused by *Erysiphe polygoni*, where KUP-34 recorded as significantly high in leaf thickness, phenol content, trichome density, and lowest in total sugars, stomatal size, reducing sugars and non-reducing sugars compared to highly susceptible genotype LBG-623.

Introduction

Black gram is essential seed crop and the best source of phosphoric acid in the pluses and is an significant dietary protein level (Duffus and Slaughter, 1980). It's seed has the highest protein level (25g/100g) with other aminoacids and minerals such as potassium, calcium, iron, niacin, thamine and riboflavin. *Urdbean* is an mini-fertilizer factory, which rehabilitates soil fecundity by raising atmospheric nitrogen, generates an equivalent of 'N' of approximately 22 kg per hectare (Rachie and Roberts, 1974). Blackgram genesis from Central Asia and currently found

in tropical and subtropical areas around Africa, Asia, and Madagascar (Arora *et al.*, 1989). It was domesticated in the neighbouring regions of South Asia. Globally, the production of black gram from the main producer countries like India, Myanmar or Thailand amounts to around 8.5 million tonnes.

The cultivated in an area of 3.56 Mt and 655 kg/ha was such as a third important pluses crop in a India. (Department Of Farming and Co-Operation, The Government of India, 2018) in region of 5.44Mh, with output.

The objective of the current research was to check the effect of powdery mildew on different physio-biochemical traits in resistant and susceptible pea genotypes.

The possibility of these traits *viz.*, electrolyte leakage, reducing sugars, non-reducing sugars, total sugars and plant dry weight as selection criteria for powdery mildew resistance was also assessed.

Materials and Methods

The experiment was conducted during *rabi* 2015-16, Agricultural College Farm and Department of Plant Pathology, Agricultural College, Bapatla, Guntur District. Geographically the Agricultural College Farm, Bapatla is situated at an altitude of 5 m above the mean sea level and at 80° 30' E Longitude and 15° 54' N Latitude and seven km away from the coast of Bay of Bengal.

Morphological Characters of Selected Blackgram Genotypes

Estimation of Leaf Thickness (mm)

A total of 16 genotypes indicating all categories of reactions were selected from the genotypes evaluated during *kharif* 2015-16,(data not shown) and were sown in three replications of 5 m each at Agricultural college, Bapatla, Department of plant pathology during *rabi* 2015-16. Three plants were selected at random from each genotype at 45 DAS.

Three leaves from each plant were selected randomly from each plant to measure leaf thickness by using Venier callipers (Perez *et al.*, 2013). Same genotypes and similar sampling method have been used for estimation of stomatal frequency, trichome density, and biochemical analysis.

Estimation of Stomatal Frequency (number of stomata/mm²)

Healthy trifoliate leaf of from 45 days old have been collected and then were smeared with synthetic gum. It's gum is being allowed to dry, flakes have been peeled and mounted on microscope glass slide. Number of stomata per mm² was counted using ocular micrometer with 40 X objective lens (Varadarajan and Wilson, 1973).

Estimation of Trichome Density (5 mm dia leaf disc)

Circular leaf discs with such a diameter 5 mm have been made from punching machine soaked in saffron dye for 5-10 min which were used to validate the number of hairs using stereo zoom microscope as outlined by Tagger and Gill (2012).

Biochemical Analysis

Glassware and sterilization

The glassware used in this study *viz.*, Borosil, Petri plates, conical flasks, test tubes, pipettes, measuring cylinders, beakers, micropipettes and microtips have been used in this study. Glassware was washed first with detergent powder and then rinsed under tap water. Subsequently they was kept overnight in cleaning solution prepared by mixing 75 g of potassium dichromate, 500 ml of concentrated sulphuric acid and 1000 ml of distilled water and rinsed with tap water followed by distilled water and analytical or reagent grade chemicals were used in the latter study.

Estimation of Total phenols

Total phenols were estimated by Folin-Ciocalteu Reagent method (Malick and Singh, 1980).

Estimation of total sugars

Total sugars were estimated following Anthrone method (Hodge and Hofreiter, 1962).

Estimation of reducing sugars

Reducing sugars estimation was carried out by Dinitrosalicylic acid method (Miller, 1959).

Estimation of non-reducing sugars

The non-reducing sugar quantity was determined by deducting the reducing sugar content from that of the total soluble sugars

Statistical Analysis

The data obtained from all the experiments were statistically analyzed following the standard procedures (Gomez and Gomez, 1984).

Morphological characters was measured by concerned apparatus as mentioned above, as well as biochemical analysis measured by spectrophotometer and estimated according their formulas.

Results and Discussion

Among morphological characters, features of stomata, cuticle and trichome morphology can impact disease resistance (Niks and Rubiales, 2002).

Morphological and Vegetative Characters in Selected Blackgram Genotypes

Leaf thickness (μm)

Significantly highest leaf thickness was observed in highly resistant genotypes KUP-34 (201.4 μm), KUP-40 (191.3 μm) and four

moderately resistant genotypes KUP-12 (176.2 μm), KUP-6 (173.6 μm), KUP-11 (171.7 μm), KUP-31 (167.3 μm) which were on a par.

Two susceptible genotypes KUP-39 (134.8 μm), KUP-25 (135.7 μm) and four moderately susceptible genotypes *viz.*, KUP-27 (138.1 μm), KUP-30 (139.9 μm), KUP-4 (143.1 μm) and KUP-5 (141.8 μm) showed significantly less leaf thickness compared to highly resistant category and were on par. Highly susceptible genotype LBG-623 (107.3 μm) which showed lowest leaf thickness which was on par with one susceptible genotype KUP-37 (118.6 μm) (Table.1).

High degree of resistance and moderate resistance to powdery mildew in the blackgram genotypes can be attributed to higher leaf thickness. Cuticle thickness in phlox found to be more in resistant genotype Texas 4n than susceptible genotype Oklahoma as reported by Andrew *et al.*, (1982). Leaf and cuticular or epidermal thickness have also been associated with powdery mildew resistance (Commenil *et al.*, 1997).

Stomatal frequency (per mm^2)

Significantly lowest stomatal frequency was observed in highly resistant genotypes KUP-34 (88.64/ mm^2), KUP-40 (99.24/ mm^2) and were on a par with moderately resistant genotypes KUP-12 (104.55/ mm^2), KUP-6 (107.58/ mm^2), KUP-11 (106.82/ mm^2), KUP-31 (109.09/ mm^2). Highly susceptible genotype LBG-623 (193.94/ mm^2) and one susceptible genotype KUP-37 (184.85/ mm^2) were found to have highest stomatal frequency and were on a par (Fig. 2 and Plate 1). Stomatal frequency was observed to be an important character in determining the resistance in the studied genotypes. Dhanumjayrao *et al.*, (2006) observed high

variation in stomatal density in grape genotypes against powdery mildew.

Trichome density (5mm diameter disc)

Significantly higher trichome density was observed in highly resistant genotypes KUP-34 (62.33), KUP-40 (59.11) and were on a par with each other. Four moderately resistant genotypes viz., KUP-12 (42.56), KUP-6 (41.11), KUP-11 (36.00), KUP-31 (38.78) and two moderately susceptible genotypes KUP-4 (36.56), KUP-15 (39.44) showed on a par trichome density with that of resistant genotypes. Rest of the moderately susceptible genotypes were significantly low trichome density and were on a par. The highly susceptible genotype LBG-623 (19.33) was found to possess significantly lowest trichome density and was on par with one susceptible genotype KUP-37 (25.0) (Fig. 3 and Plate 2).

In highly resistant and moderately resistant genotypes, the trichome density was found to be significantly highest which implies that trichome density can a morphological character contributing for the resistance of blackgram genotypes to powdery mildew.

Trichomes play an important role by inhibiting penetration of the pathogen into the host plant by keeping the pathogen away from the infection courts (Horsfall and Diamond, 1960). Similarly, Martin and Glover (2007) found that trichomes can act as physical barriers to infection. High frequency of trichomes can also prevent mycelial penetration and infection of other biotrophic fungi (Shalik, 1985). From the work of Kortekamp and Zyprian (1999), it appears that increased number of hydrophobic pubescences may repel water from leaf surfaces thus preventing successful penetration. Alternatively, a high trichome number may simply reduce the frequency of germ tube contact points that can lead

penetration (Niks and Rubiales, 2002). The results strongly suggested that morphological characters showed resistance to powdery mildew has existed among genotypes.

Studies on Biochemical Variability in Selected Black gram Genotypes

Total Phenols content (mg/100 mg)

Significantly difference in phenol content was observed in between two highly resistant genotypes KUP-34 (0.912 mg/100 mg) and KUP-40 (0.861 mg/100 mg) followed by a moderately resistant genotype KUP-12 (0.678 mg/100 mg). Susceptible and highly susceptible genotypes showed lowest phenol content and there are on a par. Overall differential phenol contents was observed among highly resistant and moderately resistant genotypes and moderately susceptible and susceptible genotypes (Fig 4).

Total phenol content has a role to play in resistance mechanism. Concentration of phenolic compounds was usually higher in resistant genotypes than in susceptible genotypes of different crop plants (Arora and wagle, 1985, Saini *et al.*, 1988). Parashar and Sindhan (1986) noted higher content of total phenols in stem and leaf of powdery mildew resistant varieties of pea than susceptible. Kalia and Sharma (1988) found higher levels of phenolics and phenol oxidising enzymes in resistant cultivars of pea (P 185 and P 6583) than susceptible, the correlation between the biochemical parameters and disease index were also high. Hattappa *et al.*, (2003) noticed that the biochemical changes in mulberry (*Morus alba*) leaves infected with *Phyllactinia corylea* causing powdery mildew the total chlorophyll, reducing sugar and protein content of mulberry leaves decreased with increased infection by the fungus. Helal *et al.*, (1978) reported that the resistance to *E. cichoracearum* in the cucumber variety

Poinsettia was due to a high concentration of phenols which hindered infection and a low concentration of sugars prevented establishment of the pathogen in the host tissues.

Total soluble sugars

Total Sugars content (mg/100 mg)

Significantly lowest sugar content was observed in highly resistant genotypes KUP-34 (4.48 mg/100 mg) KUP-40 (4.62 mg/100 mg) and were on a par in their total sugar content with moderately resistant genotypes *viz.*, KUP-12 (4.63 mg/100 mg), KUP-6 (4.65 mg/100 mg), KUP-11 (4.66 mg/100 mg) and KUP-31 (4.74 mg/100 mg). Highest total sugar content was observed in highly susceptible genotype LBG-623 (7.39 mg/100 mg) (Fig.5). Resistance of genotypes was inverse to the total sugar content.

Reducing sugars content (mg/100 mg)

Highly resistant genotypes KUP-34 (2.39 mg/100 mg) and KUP-40 (2.36 mg/100 mg) did not significantly differ in their reducing sugar content and were on par with moderately resistant genotypes, KUP -12 (2.70 mg/100 mg), KUP -6 (2.67 mg/100 mg), KUP -11 (2.67 mg/100 mg) and KUP-31 (2.82 mg/100 mg) in their reducing sugar contents. Moderately susceptible genotypes *viz.*, KUP-15 (2.89 mg/100 mg) and KUP -18 (2.71 mg/100 mg) observed to have reducing sugar content on par with highly resistant and moderately resistant genotypes and one highly susceptible genotype LBG-623 (4.10 mg/100 mg) were on par in their reducing sugars content. Highly resistant genotypes showed lowest reducing sugar contents (Fig.6)

Non-reducing sugars (mg/100 mg)

Highly resistant genotypes KUP-34 (1.96 mg/100 mg), KUP-40 (2.26 mg/100 mg)

observed to have lowest non-reducing sugars, they did not differ significantly in their non-reducing sugar content and were on par with moderately resistant genotypes *viz.*, KUP-12 (1.94 mg/100 mg), KUP-6 (1.64 mg/100 mg), KUP-11 (2.00mg/100 mg) and KUP-31 (1.92 mg/100 mg) in non-reducing sugar content and with highly susceptible genotypes LBG-623 (3.39 mg/100 mg) for the non-reducing sugar content (Fig.7).

Non reducing sugar content in the genotypes showed an inverse relation with resistance to powdery mildew. Early decades, Muhammad and Ali (2014) found that incidence of powdery mildew in pea induces changes in reducing sugars, non-reducing sugars, total sugars powdery mildew resistant and susceptible peas genotypes. Dakshayani *et al.*, (2005) reported that the susceptible genotypes Chinamung, Pusa Baisakhi and TM-98-50 recorded higher levels of sugars compared to the TARM-18. Parashar and Sindhan (1986) reported that powdery mildew resistant pea varieties (P185 and P388) had higher contents of total phenols in stem and leaf and low concentration of total sugars and reducing sugars, than susceptible varieties. Gawande *et al.*, (2002) carried out a biochemical study on reducing, non-reducing and total sugars and found that resistant genotypes had lower total sugars content before and after infection. Guleria *et al.*, (1997) reported the post-infection of powdery mildew decrease the reducing sugar content in the leaves of both resistant (DPP68 and JP71) and susceptible cultivars (Bonneville and Lincoln) in pea.

Stomatal Frequency and Trichome Density

Braun (1987b) revealed that some lines in the mildew-susceptible germplasm of mulberry of which RFS-135, Mother graft, Shrim-5 and Mizuzawa) have a smaller stomatal density, the number of stomata per unit area of leaf surface and stomatal index were positively

correlated with powdery mildew resistance. Eighty per cent of the resistant germplasm were characterized by high trichome densities and a high stomatal density and stomatal index. There are some significant genotypic effects of stomatal frequency on penetration by powdery mildew pathogens (Lima *et al.*, 2010). Chattopadhyay *et al.*, (2011) evaluated 30 lines of mulberry with contrasting susceptibilities to powdery mildew (15 resistant and 15 susceptible), susceptible genotypes showed significant more stomatal index, stomatal area and less trichome

density. Whereas, resistant group was distinguished by 17.4 % lower stomatal density, 12.5% smaller stomatal index per unit leaf area, 20.0 % greater trichome density and 18.0% higher stomatal area compared with the susceptible group. Trichome density was negatively correlated with disease severity index and with the accumulative area under disease progression curves (AUPDC). Georgiev *et al.*, (2013) found positive relation between the degree of pubescence and resistance to powdery mildew under natural conditions.

Table.1 Biochemical characters in selected blackgram genotypes

Sl.No.	Genotypes	Disease reaction	Total phenols (mg/100mg)	Total sugars (mg/100mg)	Reducing sugars (mg/100mg)	Non-reducing sugars (mg/100mg)
1	KUP-34	HR	*0.912 ^a	4.48 ^e	2.39 ^c	1.96 ^c
2	KUP-40	HR	0.861 ^b	4.62 ^e	2.36 ^c	2.26 ^{bc}
3	KUP-12	MR	0.678 ^c	4.63 ^e	2.70 ^{bc}	1.94 ^c
4	KUP-6	MR	0.617 ^d	4.65 ^e	2.67 ^{bc}	1.64 ^c
5	KUP-11	MR	0.581 ^d	4.66 ^e	2.67 ^{bc}	2.00 ^c
6	KUP-31	MR	0.487 ^e	4.74 ^e	2.82 ^{bc}	1.92 ^c
7	KUP-15	MS	0.475 ^e	5.97 ^{bcd}	2.89 ^{bc}	3.08 ^a
8	KUP-18	MS	0.448 ^{ef}	5.90 ^{bcd}	2.71 ^{bc}	3.19 ^a
9	KUP-4	MS	0.422 ^f	5.83 ^d	3.01 ^b	3.16 ^a
10	KUP-5	MS	0.381 ^g	5.84 ^d	2.82 ^{bc}	3.02 ^a
11	KUP-30	MS	0.365 ^{gh}	5.96 ^{bcd}	2.89 ^{bc}	3.07 ^a
12	KUP-27	MS	0.358 ^{ghi}	5.97 ^{bcd}	2.96 ^{bc}	3.02 ^a
13	KUP-25	S	0.328 ^{hij}	6.93 ^{abc}	3.87 ^a	3.05 ^a
14	KUP-39	S	0.317 ^{ij}	7.01 ^{ab}	4.00 ^a	2.91 ^{ab}
15	KUP-37	S	0.308 ^j	6.91 ^{abcd}	4.05 ^a	2.86 ^{ab}
16	LBG 623	HS	0.299 ^j	7.39 ^a	4.10 ^a	3.39 ^a

* In the column means followed by common letter are not significantly different at 5% level by DMRT

Fig.1 Variation of leaf thickness (µm) in selected blackgram genotypes

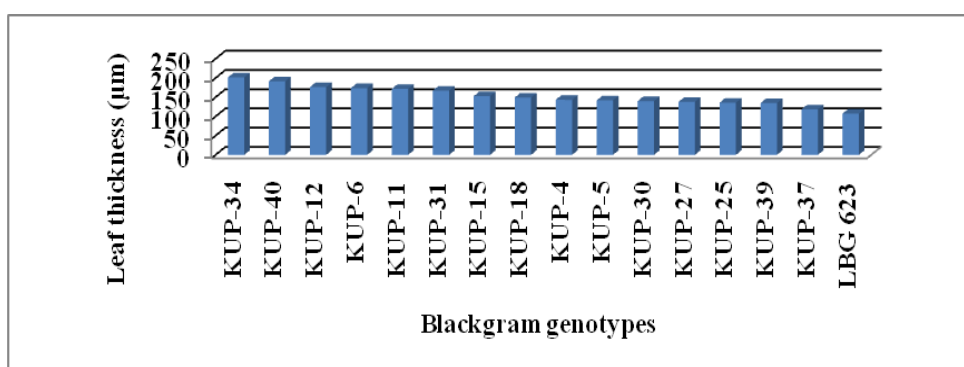


Fig.2. Variation in stomatal frequency (no of stomata mm²) in selected blackgram genotypes

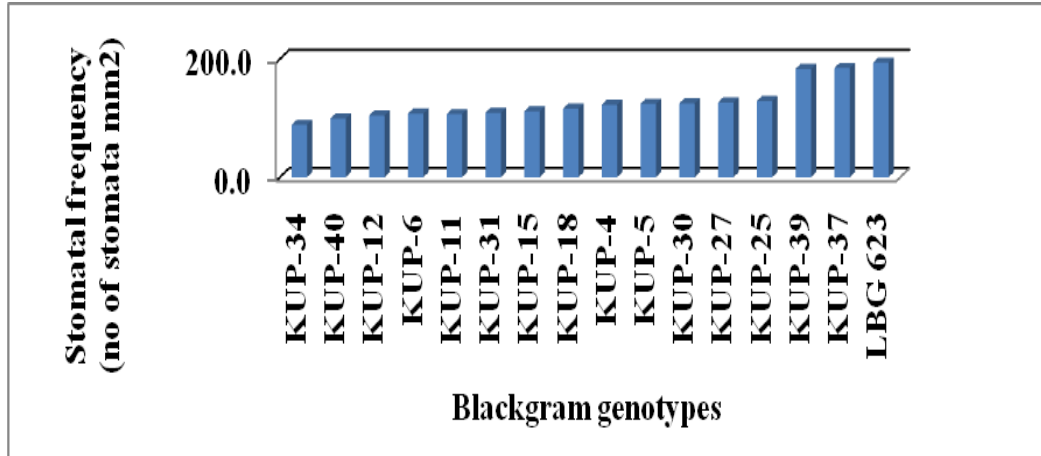


Fig.3 Variation of trichome density (5mm dia leaf disc) in selected black gram genotypes

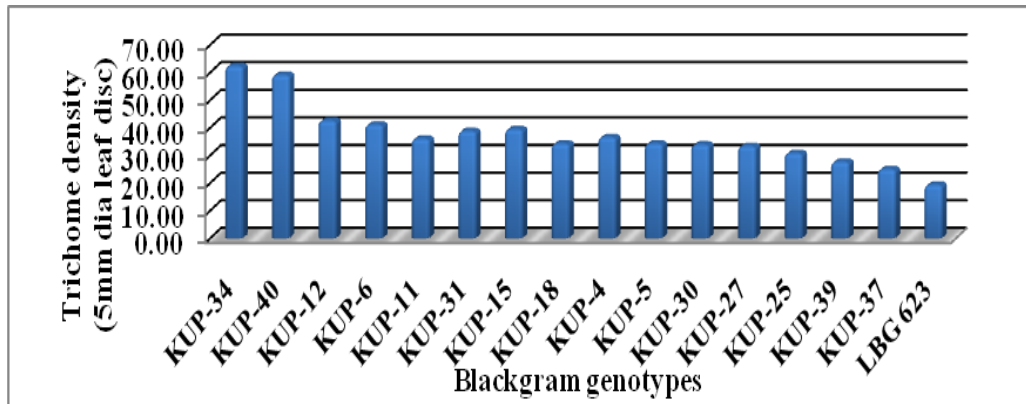


Fig.4 Variation in total phenols (mg/100 mg) on selected blackgram genotypes

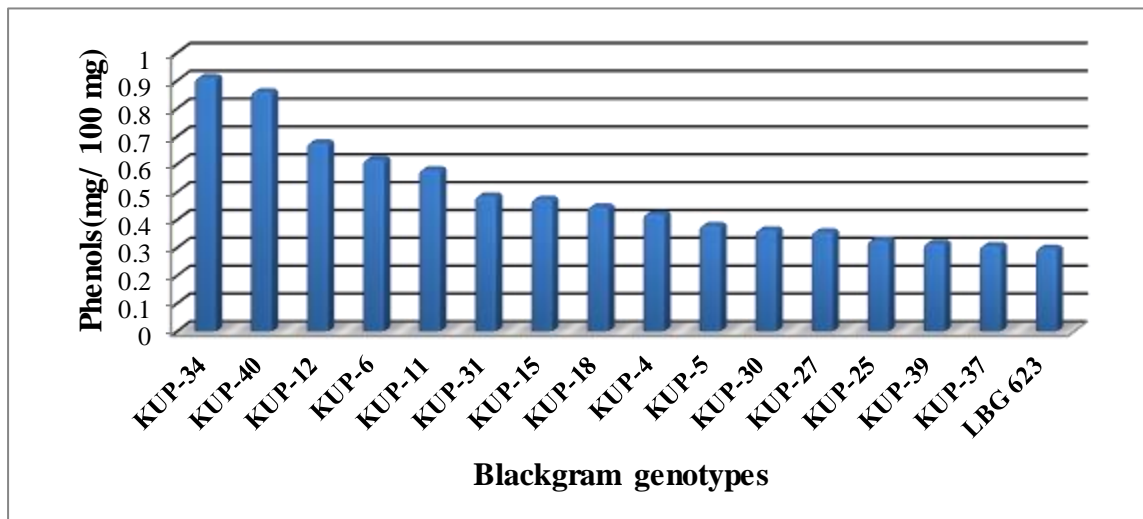


Fig.5 Variation in total sugars (mg/100 mg) on selected blackgram genotype

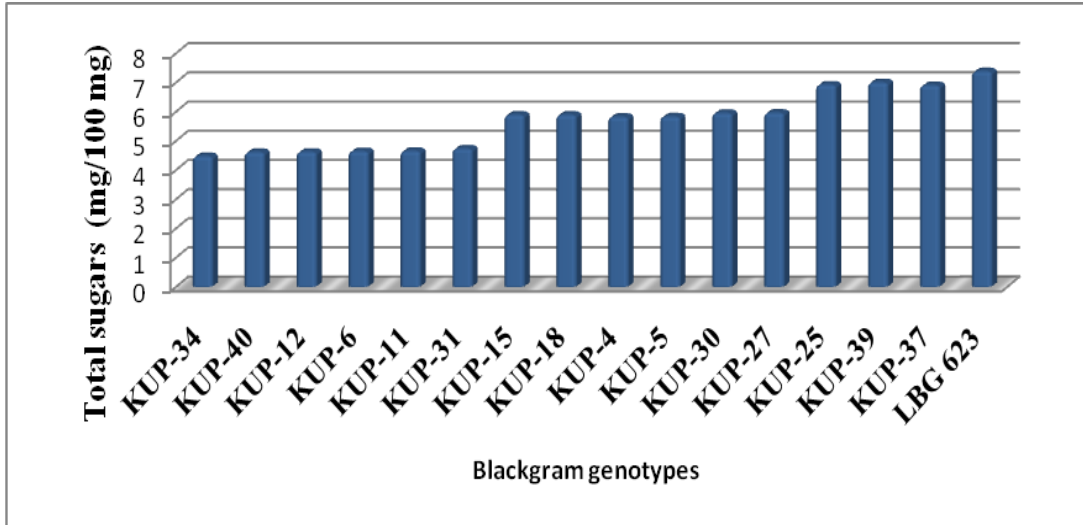


Fig.6 Variation of reducing sugars (mg/100 mg) in selected blackgram genotypes

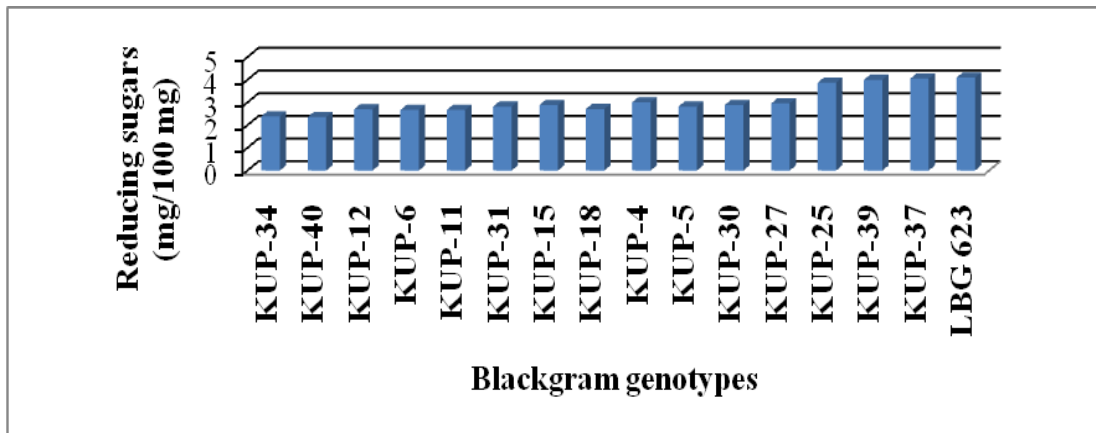


Fig.7 Variation of non-reducing sugars (mg/100 mg) in selected blackgram genotypes

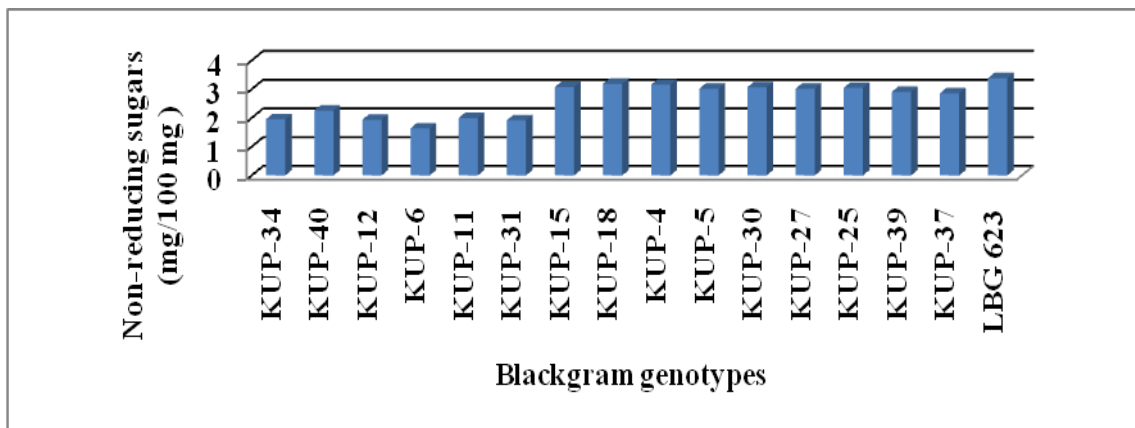


Plate.1 Variation in stomatal frequency in powdery mildew disease resistant (KUP-34) and susceptible (LBG-623) black gram genotypes

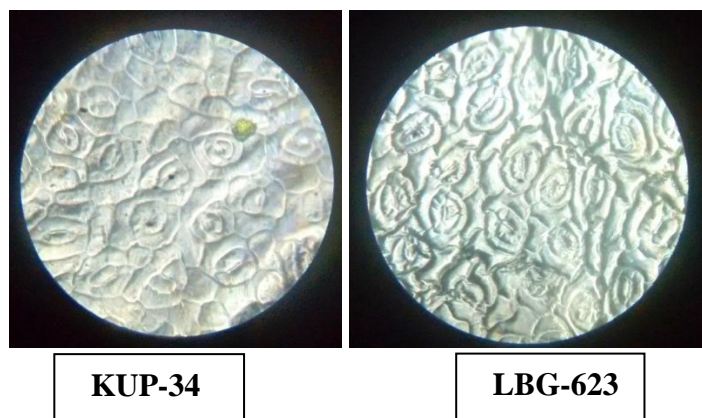
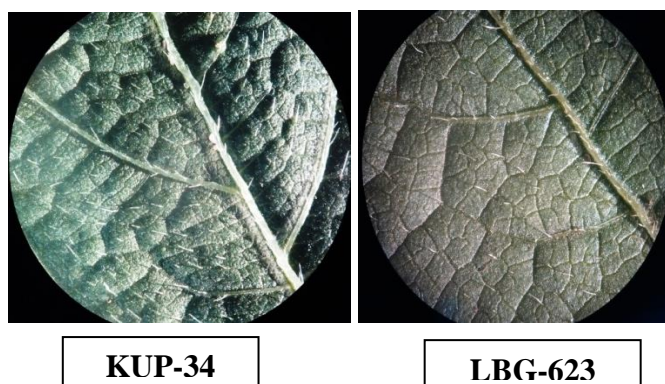


Plate.2 Variation in trichome density in powdery mildew disease resistant (KUP-34) and susceptible (LBG-623) black gram genotypes



Biochemical Characters for Disease Resistance

Involvement of phenolic compounds in many aspects of plant parasite relationship other than plant protection has been reported by Friend in 1979. The role of phenolics in the resistance mechanisms in plants has been reviewed by several workers (Allen, 1959; Agrios, 2005). Concentration of phenolic compounds was usually higher in resistant genotypes than in susceptible genotypes of different crop plants (Arora and Wagle, 1985 and Saini *et al.*, 1988). Mandahar and Garg (1975) observed that okra leaves infected with powdery mildew (*E. cichoracearum*) had higher reducing sugars content than healthy

leaves. Helal *et al.*, (1978) reported that the resistance to *E. cichoracearum* in the cucumber variety Poinsettia was due to a high concentration of phenols which hindered infection and a low concentration of sugars prevented establishment of the pathogen in the host tissues. Guleria *et al.*, (1997) reported the post-infection decrease the reducing sugar content in the leaves of both resistant (DPP68 and JP71) and susceptible cultivars (Bonnevillie and Lincoln) of pea against powdery mildew. Sridhan and Parashar (1984) found higher content of total phenols, O-dihydric phenols, P, K, Zn, and Cu but lower of N, Mn, and Fe in the foliage of resistant and moderately resistant varieties of pea compared to susceptible. Parashar and

Sindhan (1986) noted higher content of total phenols and Orthodihydro-phenols and lower of total sugars and reducing sugars in stem and leaf of powdery mildew of resistant varieties of pea than susceptible. Kalia and Sharma (1988) found higher levels of phenolics and phenol oxidising enzymes in resistant cultivars of pea (P 185 and P 6583) than susceptible cultivars, the correlation between the biochemical parameters and disease index were also high. Sharma *et al.*, (1996) found higher total phenol content in powdery mildew resistant varieties of pea *viz.*, JP 179, JM 5, PMR 3 AND PMR 4.

Gawande *et al.*, (2002) carried out biochemical study on reducing, non-reducing and total sugars and total phenols before and after powdery mildew infection in seven *mungbean* genotypes found that resistant genotypes had higher total phenols before and after infection. The total phenols were positively correlated with resistance. Whereas, sugars were negatively associated with disease resistance. Dakshayani *et al.*, (2005) reported that the susceptible genotypes Chinamung, Pusa Baisakhi and TM-98-50 recorded higher levels of sugars compared to the TARM-18. Muhammad and Ali (2014) found that incidence of powdery mildew in pea induces changes in reducing sugars, non-reducing sugars, total sugars powdery mildew resistant and susceptibility of peas genotypes.

In conclusion the 15 blackgram genotypes, significantly highest leaf thickness was observed in highly resistant genotypes KUP-34 (201.4 μm), KUP-40 (191.3 μm). Highly susceptible genotype LBG-623 (107.3 μm) which showed lowest leaf thickness which was on par with one susceptible genotype KUP-37 (118.6 μm). Significantly lowest stomatal frequency was observed in highly resistant genotypes KUP-34 (88.64/ mm^2), KUP-40 (99.24/ mm^2). Highly susceptible genotype LBG-623 (193.94/ mm^2) and one

susceptible genotype KUP-37 (184.85/ mm^2) were found to have highest stomatal frequency. Significantly higher trichome density was observed in highly resistant genotypes KUP-34 (62.33), KUP-40 (59.11) and were on a par with each other. The highly susceptible genotype LBG-623 (19.33) was found to possess significantly lowest trichome density. Significantly higher phenol content was observed in highly resistant genotypes KUP-34 (0.912 mg/100 mg) and KUP-40 (0.861 mg/100 mg) and one moderately resistant genotype KUP-12 (0.678 mg/100 mg). Highly susceptible genotype LBG-623 recorded the lowest total phenol content (0.299 mg/100 mg).

Significantly lowest sugar content was observed in highly resistant genotypes KUP-34 (4.48 mg/100 mg) KUP-40 (4.62 mg/100 mg) and were on a par. Highest total sugar content was observed in highly susceptible genotype LBG-623 (7.39 mg/100 mg). Highly resistant genotypes KUP-34 (2.39 mg/100 mg) and KUP-40 (2.36 mg/100 mg) showed lowest reducing sugars and there on a par. Highly resistant genotypes KUP-34 (1.96 mg/100 mg), KUP-40 (2.26 mg/100 mg) showed observed to have lowest non reducing sugars, they did not differ significantly in their non-reducing sugar content.

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