A-5845
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RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume Issue : ()

Scouting for Multiple Disease Resistance in Urdbean

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10.18805/IJARe.A-5845

ABSTRACT

Background: Dry root rot (DRR), yellow mosaic disease (YMD) and stem necrosis diseases are inflicting the economic losses in urdbean. Information on the interrelation of morphological and biochemical parameters of the urdbean cultivars with the disease resistance is scarce.

Methods: Thirty nine cultivars of urdbean were screened in the field for their resistance to DRR, YMD and stem necrosis diseases during *Rabi*, 2019-2020. The incidence of the diseases is compared with the morphological characters *viz.,* trichome density, leaf thickness, root thickness and biochemical composition *viz.,* total free amino acids, total soluble sugars and phenol content of cultivars.

Result: Cultivar GBG 103 was found resistant to DRR and YMD with the maximum amount of trichome density. No cultivar was found resistant to stem necrosis under field conditions. Correlation analysis revealed the positive correlation of phenol content with the DRR incidence; trichome density and total soluble sugars with YMD incidence. Albeit the leaf thickness and total free amino acid content of the cultivars were negatively correlated with the incidence of the DRR, YMD and stem necrosis diseases.

Key words: Dry root rot, Resistance, Stem necrosis, Urdbean, Yellow mosaic virus.

INTRODUCTION

Urdbean [*Vigna mungo* (Linnaeus) Hepper] is one of the predominantly grown legume crops in India. In the present scenario, yields of urdbean were being declined by the incidence of various viral and fungal diseases. Among them, dry root rot caused by *Rhizoctonia bataticola* (sclerotial stage) and *Macrophomina phaseolina* (pycnidial stage), yellow mosaic disease (YMD) caused by mungbean yellow mosaic virus (MYMV) or mungbean yellow mosaic India virus (MYMIV) or both, leaf curl caused by groundnut bud necrosis virus (GBNV) and stem necrosis caused by tobacco streak virus (TSV) were responsible for cent percent yield loss in urdbean when infected at early stages of growth (Ladhalakshmi *et al*. 2005; Malathi and John, 2009; Biswas *et al*. 2009, Reddy *et al*. 2014). The MYMV is transmitted by *Bemisia tabaci* (whitefly) whereas, GBNV and TSV were transmitted by *Thrips* spp. All the 3 viruses were transmitted in a persistent manner (Prasada Rao *et al*. 2003; Biswas *et al*. 2009). The disease management practices (physical, chemical, and biological) were being followed for reducing the disease incidence despite high input costs. The inclusion of host plant resistance in integrated disease management is a sustainable and effective way of combating plant diseases. Lab and field screening techniques facilitate the identification of resistant germplasm which could be further utilized in the breeding programme for developing cultivars with disease resistance (Pande *et al*. 2010). Henceforth, scouting for new disease-resistant sources requires to be augmented.

Aside from identifying the source of resistance, the reason for resistance should be clear. Hence, that particular trait of resistance could be used in laboratory screening. Morphological parameters such as trichome density and biochemical parameters *viz.,* total sugar, phenols and phytic acid contents were found to be related to the incidence of MYMV (Tamilzharasi *et al*. 2018; Mantesh *et al*. 2020). The inter-relation of disease resistance with morphological and biochemical parameters of the host plant is an enigma. Hence, the present study was designed to amalgamate the morphological and biochemical basis of the urdbean germplasm with disease resistance.

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How to cite this article: Kumar, N.K , Kumar, A.R.N, Reddy, B.V.B. and Prasanthi, L. (2022). Scouting for Multiple Disease Resistance in Urdbean. Indian Journal of Agricultural Research. DOI: 10.18805/IJARe.A-5845.

Submitted: 09-06-2021 **Accepted:** 25-02-2022 **Online:** 19-04-2022

MATERIALS AND METHODS

The experiment was conducted during *Rabi,* 2019-2020 in the fields of Regional Agricultural Research Station, Tirupati, Andhra Pradesh, India with 39 cultivars of urdbean obtained from Pulse Breeder, RARS, Tirupati.

Disease screening

Disease screening was carried out by sowing the cultivars in a single row of 3 m length at 30 × 10 cm spacing in a randomized block design (RBD) with 2 replications.

Dry root rot (DRR)

The cultivars were screened for their tolerance to dry root rot in the sick plot (Nene *et al*. 1979). Susceptible cultivar TBG 104 is used as the check variety. The crop was sown in the second fortnight of January, 2020 to expose the plant to dry conditions after flowering. The inoculum was prepared in the laboratory on sorghum seeds and uniformly spread in the sick plot from one year before laying out the experiment. The disease incidence was recorded at the time of harvest and calculated by using the following formula,

$$\text{Per cent disease incidence} = \frac{\text{Number of plants infected}}{\text{Number of plants assessed}} \times 100$$

For examining the infection within the root cells, the root portion of the infected plants was cross-sectioned and viewed under the stereo zoom microscope (Olympus, SZ×10, Japan).

Yellow mosaic disease (YMD)

The cultivars were screened for their resistance to YMD under natural conditions. The experiment was laid out in the second fortnight of January. For every 5 cultivars, 1 row each of resistant check cv. TBG 104 and susceptible check cv. LBG 623 were sown. Insecticidal spray was avoided to allow the infestation by whitefly. The disease incidence was recorded at every 10 days interval from 30 to 90 days after sowing (DAS) by using the modified MULLaRP scale (0-9 scale) proposed by Alice and Nadarajan, (2007).

Stem necrosis

The infector border row technique using *Parthenium hysterophorus* (Vemana *et al*. 2016) was followed to screen the cultivars for their resistance to stem necrosis. Urdbean genotypes were sown during the flowering stage of the infector border to facilitate exposure of urdbean seedlings to the pollen of infector row. The cultivar SRI was used as the susceptible check. The disease incidence was recorded at 30 and 50 DAS.

Morphological parameters

Trichome density

Trichome density was measured in the detached leaves by taking leaf bits of 1 cm^2. The excised bits were placed under the stereo zoom microscope to visualize the density and mentioned as number per cm^2.

Estimation of leaf thickness and root thickness

The nondestructive method is followed to measure the leaf thickness by using the Vernier calipers. Similarly, the root thickness was measured by using the Vernier calipers in the excised roots. The root thickness was measured at the tip, middle and at the point below collar region and the mean was computed. The leaf and root thickness was measured in 10 plants per genotype and is expressed in mm.

Biochemical parameters

The total free amino acids, total soluble sugars (carbohydrates), phenols present in the leaf samples were estimated by following the protocols given by Sadasivam and Manickam, 1992. Total soluble sugars and phenols were expressed in gram per gram (g g^{-1}) of the tissue. Whereas, total free amino acids were expressed in µg g^{-1}.

Statistical analysis

The field data were analyzed in an RBD using univariate one way ANOVA. Analysis of the statistical data was carried out using SPSS statistical software version 20.0 (IBM, 2012). Correlation studies were conducted using Microsoft Excel, 2019 version.

RESULTS AND DISCUSSION

DRR incidence

The cultivars were screened for their tolerance to DRR disease under sick plot facility. The presence of *R. bataticola* infection was confirmed by observing the presence of numerous black colured micro sclerotia on the infected rottened roots. Among the screened cultivars, cv. GBG 103 exhibited the lowest disease incidence (5%), followed by GBG 1 (8.0%), LBG 827 (8.5%) and GBG 12 (8.7%). Whereas, the highest incidence of DRR was recorded in IPU 12-30 (45.2%) and TJU 258 (42.9%). The results of the present study are similar to the results obtained by Dambal *et al*. (2019) where 75 urdbean germplasm were screened for their tolerance to DRR under natural conditions and observed no immune variety. In a separate study, mungbean genotypes were screened for their field resistance to DRR and identified 3 resistant genotypes namely KM 4-59, KM 4-44 and MSJ-118 (Choudhary *et al*. 2011). The environment and aggressiveness of the pathogen influences the disease incidence in plants (Vale *et al*. 2001). Henceforth, the field screening for resistance to DRR for multiple seasons is warranted for identifying the sources for dry root rot resistance. The roots of the infected plants were cross-sectioned and observed for the progress of infection in the stele. Black coloured discolourations were observed in the phloem cells of the stele, which indicated the movement of the pathogen within the phloem (Table 1, Fig 1).

YMD incidence

Fourteen cultivars namely LBG 806, LBG 811, LBG 823, GBG 45, PU 13- 15, VBG 12-111, DKU 82, IPU 13-3, DKU 99, IPU 2-43, GBG 1, VBG 12-062, IPU 12-30, ABG 3 were free from disease (0 score). Whereas, 3 cultivars namely LBG 645, LBG 623, PBG 32 exhibited the highest disease (8 score). In a similar study on field screening of 36 urdbean germplasm for YMD resistance, 3 germplasm (PU31, KUG 216 × SPS 5 and KUG 216 × PU40) were identified to be resistant with 0 score (Hari *et al*. 2018). In a separate study,

the 20 urdbean genotypes were screened for their resistance to YMD and identified 4 resistant genotypes namely RSU 03, RSU 06, TU 22 and PU 31 (Raman *et al*. 2019). In India, cv. PU 31 is widely cultivated for high yielding potential and YMD resistance. However, in the present study minute yellow specks were observed on the leaves of the resistant cv. PU 31. The development of symptoms in the resistant varieties warrants future studies on the identification and inclusion of the prevailing MYMV strains in the disease resistance screening programmes.

Stem necrosis incidence

The cultivars were screened for stem necrosis resistance by *Parthenium* infector border row technique. The field screening data manifested the minimum stem necrosis incidence in cv. VBG 12-062 (67%). Comparably, Vemana

Table 1: Disease incidence, morphological and biochemical parameters of urdbean cultivars.

Genotypes	Dry root rot (%)	Yellow mosaic disease(score)	Stem necrosis (%)	Trichome density (cm^{-2})	Leaf thickness (mm)	Root thickness (mm)	Total free amino acids ($\mu g\ g^{-1}$)	Total soluble sugars ($g\ g^{-1}$)	Phenols ($g\ g^{-1}$)
LBG 808	17.1	1	80.9	9.00	0.23	1.59	400.00	0.113	13.63
LBG 806	20.9	0	93.3	12.33	0.27	1.82	534.10	0.043	13.17
LBG 811	13.3	0	96.8	17.00	0.25	1.36	515.83	0.102	12.68
LBG 796	12.8	2	97.1	16.67	0.26	1.63	503.40	0.077	11.81
LBG 823	18.9	0	95.1	8.33	0.33	0.95	590.33	0.048	11.50
LBG 827	8.5	1	88.3	10.33	0.27	1.29	431.83	0.091	10.83
GBG-12	8.7	1	81.5	15.67	0.27	1.33	353.33	0.074	9.03
GBG-45	27.3	0	97.2	4.00	0.33	1.56	365.00	0.095	5.74
GBG-47	10.3	1	96.9	11.33	0.28	1.04	281.77	0.125	7.43
LBG 752	20.7	6	92.1	9.33	0.18	0.92	301.70	0.099	8.61
PU 31	16.0	1	96.6	11.00	0.24	1.60	385.77	0.042	8.97
PBG 32-1	25.9	2	95.1	13.33	0.18	1.66	238.33	0.074	8.74
PBG 32-2	30.4	3	93.5	15.67	0.28	1.26	275.33	0.169	7.94
PU 13-15	22.6	0	97.1	10.33	0.21	1.36	238.43	0.012	9.42
VBG 12-034	21.6	3	96.5	18.67	0.24	1.44	359.23	0.033	8.29
VBG 12-111	15.4	0	96.7	14.33	0.18	1.81	238.33	0.067	8.20
DKU 82	26.7	0	90.4	17.33	0.21	1.23	405.80	0.045	8.35
IPU 13-3	27.6	0	92.0	7.67	0.26	1.15	348.30	0.051	9.28
DKU 99	19.4	0	93.2	12.33	0.27	1.68	381.73	0.029	10.93
IPU 2-43	25.7	0	95.7	6.00	0.18	1.36	359.07	0.059	12.74
TBG 104	33.9	1	97.4	23.33	0.26	0.87	398.30	0.058	10.11
COBG 653	16.5	7	96.4	18.33	0.21	1.19	316.67	0.144	8.51
LBG 645	22.8	8	96.9	17.33	0.18	0.99	345.77	0.051	9.03
GBG-1	8.0	0	96.1	22.00	0.19	0.58	375.67	0.044	7.32
VBG 12-062	12.1	0	67.0	17.33	0.21	0.74	387.47	0.045	4.86
LBG 788	26.1	1	93.5	20.67	0.24	0.63	349.20	0.092	11.23
ADBG 13-023	15.8	7	88.3	15.00	0.25	1.18	402.53	0.174	15.38
TJU 258	42.9	4	96.4	14.67	0.18	0.78	345.00	0.053	16.06
OBG 39	18.4	1	91.8	11.33	0.27	0.61	400.00	0.059	11.82
IPU 12-30	45.2	0	82.9	12.33	0.24	0.82	375.00	0.065	15.04
ABG-1	32.0	6	94.6	16.67	0.25	0.90	370.33	0.203	9.69
ABG-3	13.5	0	97.0	20.00	0.22	0.81	366.70	0.088	8.12
TBG-125	16.1	2	92.5	20.67	0.30	0.72	315.77	0.152	11.23
TBG 129-1	28.0	3	91.9	19.67	0.27	0.72	372.50	0.135	10.28
TBG 130	12.2	1	91.1	19.33	0.25	0.65	422.50	0.073	11.23
GBG-103	5.0	1	97.3	21.67	0.26	1.22	339.20	0.188	12.40
SRI	17.4	1	97.5	23.67	0.23	1.15	330.77	0.237	12.49
LBG 623	21.1	8	97.1	23.00	0.22	0.99	355.80	0.165	8.58
PBG-32	31.3	8	97.8	21.00	0.17	1.06	366.70	0.082	9.24
CD (0.05)	-	-	-	3.15	0.03	0.268	25.47	0.019	1.50
CV %	-	-	-	12.37	6.48	11.576	4.18	12.827	8.79

et al (2016) screened the groundnut cultivars for identifying the resistant sources of peanut stem necrosis disease (PSND) caused by TSV and identified the minimum disease incidence (61.9%) in genotype NRCG2976. Necrotic streaks were noticed on the veins of the leaves, petiole, and stems of the infected plants (Fig 2). Conclusively, no genotype was found resistant to stem necrosis disease. There is an urgent need for the development of urdbean cultivars with resistance to stem necrosis. Hence, future studies are required for the identification of stem necrosis resistant sources in urdbean.

Morphological parameters

The morphological parameters *viz.,* trichome density, leaf lamina thickness, and root thickness were examined to study their involvement in disease resistance.

Leaf lamina thickness and trichome density

The host plant preference by *B. tabaci*, the vector of YMD depends on the thickness, shape and hairiness of the leaves (Berlinger, 1986). The literature survey indicated the positive correlation of whitefly incidence with the trichome density and leaf lamina thickness (Hasanuzzaman *et al*. 2016). In the current study, leaf lamina thickness was found to be significantly different among the 39 cultivars (Table 1). The maximum leaf thickness was exhibited by 3 cultivars namely LBG 823 (0.33 mm), GBG 45 (0.33 mm) and TBG 125 (0.30 mm). The lowest leaf lamina thickness was examined in cv. PBG 32 (0.17 mm) in which the highest YMD incidence was recorded. The results are in contrast with Taggar and Gill (2012) where the leaf lamina thickness and area were correlated positively with the population of *B. tabaci.*

Among the 39 cultivars screened, trichome density (leaf hairiness) was found to be significantly different from each other. Eight cultivars *i.e*. SRI (23.67), TBG 104 (23.33), LBG 623 (23.0), GBG 1 (22.0), GBG 103 (21.67), PBG 32 (21.0), TBG 125 (20.67) and LBG 788 (20.67) contained maximum number of trichomes (Table 1). However, 2 cultivars *i.e*. GBG 45 (4.0) and IPU 2-43 (6.0) contained a minimum number of trichomes. Feeding and oviposition of the *B. tabaci* increases with the availability of larger leaf areas with a minimum number of trichomes. The trichome density of the urdbean cultivars was negatively correlated with the population of *B. tabaci* (Chand *et al*. 1980; Lakshminarayan *et al*. 2008; Taggar and Gill, 2012). Similarly in the present study cv. SRI exhibited the maximum trichome density (23.67), which might be the reason for resistance to YMD.

Root thickness

The thickness of the roots was examined to evaluate its contribution in tolerance to DRR disease. The root thickness of common bean cultivar FR266 is positively correlated with the tolerance to *Fusarium solani* f.sp. *phaseoli* (Snapp *et al*. 2003). In the current study, the thickness of root varied significantly among the 39 cultivars (Table 1). The maximum root thickness was recorded in 8 cultivars *i.e*. LBG 806 (1.82 mm), VBG 12-111 (1.81 mm), DKU 99 (1.68 mm), PBG 32-1 (1.66 mm), LBG 796 (1.63 mm), PU 31 (1.60 mm), LBG 808 (1.59 mm) and GBG 45 (1.56 mm). The highest root thickness of 1.82 mm was recorded in cv. LBG 806, where the dry root rot incidence is 20.9%. Similar results were obtained in screening the common bean cultivars for their resistance to Fusarium root rot (Cichy *et al*. 2007). From the current investigation, it was conspicuous that the root

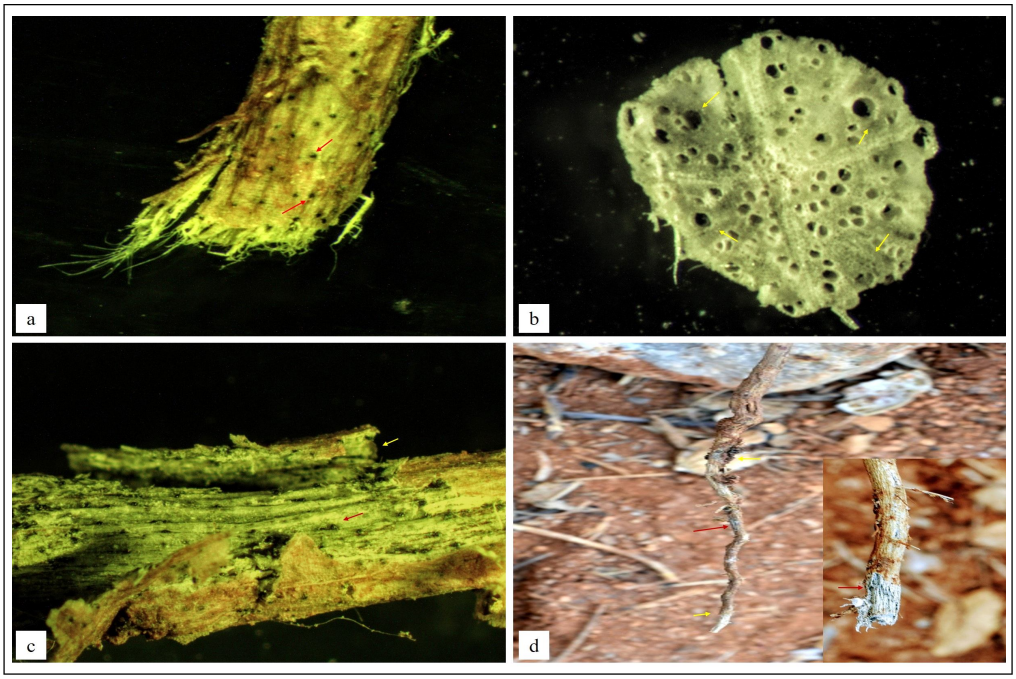

Fig 1: Incidence of dry root rot in urdbean. Microsclerotia on the infected roots (a, c), black discoloured phloem cells in the stele of the infected root (b), presence of microsclerotia on root tissues (d).

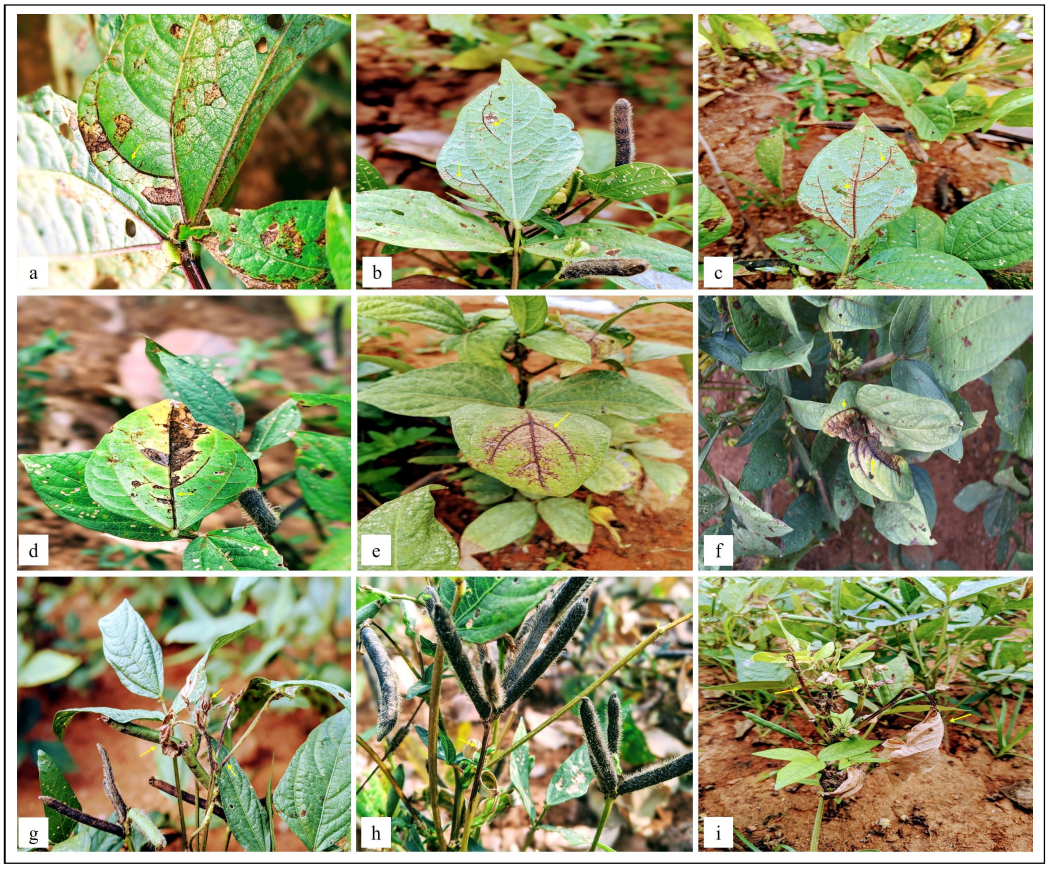

Fig 2: Incidence of stem necrosis on urdbean. Necrotic streaka on adaxial (a, e, f) and abaxial (b, c, d) leaf surfaces, buds (g), stems and petioles (h, i). Drying of the leaves (g, i).

thickness is not a factor in screening the cultivars for their tolerance to DRR disease.

Biochemical parameters

Correlating the disease resistance with biochemical parameters unfolds the mechanisms involved in plant disease resistance. Total free amino acids, total soluble sugars, and phenol content were found to be significantly different among the 39 cultivars (Table 1).

The total free amino acid content of the plants not only influences the colonization of roots by fungi but also impacts the preference of host plants by phloem-feeding *B. tabaci*, the vector of YMD (Buchanan *et al*. 2000; Sood, 2003). The highest amounts of total free amino acids were recorded in cv. LBG 823 (590.33 µg) which is highly resistant to YMD but susceptible to stem necrosis and DRR. Whereas, the lowest amounts of total free amino acids were found in cultivars PBG 32-1 (238.33 µg), VBG 12-111 (238.33 µg), and PU 13-15 (238.43 µg). Barakat and Torky (2017) corroborated the increase of total free amino acids in the leaves of *Lupinus albus* infected by the Bean yellow mosaic virus (BYMV). In a separate study, higher amounts of total free amino acid content were recorded in the *Asparagus* plants colonized by *Glomus sp* after 16 weeks of inoculation (Okada and Matsubara, 2012).

Disease resistance in plants is enhanced by the high availability of total soluble sugars in the plant tissues (Morkunas and Ratajczak, 2014). Total soluble sugars were found to be maximum in cv. SRI (0.237 g) and minimum in YMD resistant cv. PU13-15 (0.012 g) and DKU 99 (0.029 g). The results are comparable to Tamilzharasi *et al* (2018) wherein relatively lower total soluble sugar content was recorded in the YMD resistant urdbean cultivars.

Phenols are synthesized and polymerized in the cell wall of the plants as part of the defense mechanism against various biotic and abiotic stresses (Matern and Kneusel, 1988; Bhattacharya *et al*. 2010). The total phenol content was found to be high in cultivars TJU 258 (16.06 g), ADBG 13-023 (15.38 g), IPU 12-30 (15.04 g). Among which cv. IPU 12-30 is resistant to YMD and susceptible to stem necrosis and DRR. The cultivars namely TJU 258 and ADBG 13-023 were susceptible to all the 3 diseases (Table 1). Comparably, the total phenol content was found to be increased in the leaves of YMD resistant mungbean genotype than those of the susceptible one (Sohal and Bajaj, 1993). The presence of more amount of phenols in the groundnut plants contributed to their resistance to thrips (Kandakoor *et al*. 2014). The results of the present study are in contrast to Mantesh *et al* (2020) wherein the mungbean cultivars with high susceptibility to YMD had shown the least total phenol content.

Correlation analysis

Correlation between morphological and biochemical parameters with the incidence of DRR, YMD, and stem necrosis was analyzed. The data manifested the significant positive correlation of DRR incidence with phenol content, YMD incidence with trichome density and total soluble sugars, and stem necrosis incidence with, trichome density, root thickness, total soluble sugars and phenol content. Whereas, leaf thickness and total free amino acid content exhibited a significant negative correlation with the incidence of all the 3 diseases.

Vector based biochemical characterization of the genotypes is very essential as the deterrence in the feeding nature of the vectors protects particular genotypes from the incidence of viral diseases. Allelochemicals like acyl sugars found to exhibit negative effects on whiteflies. The genotypes with high acyl sugars were least preferred for oviposition and nymph production of whiteflies (Dias *et al*. 2016). Glandular trichomes exudates the compounds like acyl sucroses which deters the whiteflies (Rodriguez-Lopez *et al*. 2020). The acyl sugars and glandular trichomes quantity needs to be explored for each genotype due to their essentiality in viral disease resistance.

CONCLUSION

This study revealed the resistance of cv. GBG 103 to DRR (5%) and YMD (1 score) with the maximum trichome density. No variety was found resistant to stem necrosis disease. Amidst all the morphological and biochemical parameters, the thickness of the leaf and total free amino acid contents were negatively correlated with the incidence of all the 3 diseases. Interestingly, there is a positive correlation between the incidence of DRR and YMD with stem necrosis disease. This indicated that the plant affected by the fungi or virus will get weakened and provides scope for the infection by the other diseases. Thus, the present study divulged the importance of including morphological and biochemical parameters in screening the plants for multiple disease resistance.

Conflict of interest

There is no conflict of interest among the authors.

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