



Varietal evaluation and biochemical changes due to field incidence of *Fusarium* wilt in gladiolus genotypes

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ABSTRACT

Thirty gladiolus genotypes were evaluated for agronomic traits and natural screening to *Fusarium* wilt during 2015 and 2016. Among the genotypes, Suchitra × Melody took the minimum days (11.24) to bud sprout post planting, while Tha Barton registered 72 days for spike emergence. Pusa Unnati recorded the maximum spike and rachis length, while Pusa Manmohak, Pusa Red Valentine and Pusa Unnati recorded maximum number of florets per spike. Maximum field-life of spikes was recorded in Pusa Unnati followed by Pusa Red Valentine. After about 45 days of planting, different genotypes showed varied disease incidence (3.47-45.13%) and plant mortality index (0-35.47%). Genotypes, Swarnima, Pusa Unnati and Suryakiran were found more resistant to fusarium wilt, while Urmi and white Friendship were found highly susceptible. Leaves and corms were sampled at 75 days of planting for estimation of different biochemical parameters, namely, total chlorophylls, total sugars, total phenols, and malondialdehyde (MDA) content. The resistant genotypes had higher contents of total chlorophylls; higher phenols but lower TSS and MDA contents in corms. Negative correlations were estimated for DSI and biochemical parameters like total chlorophyll ($r = -0.974^{**}$), total phenol ($r = 0.925^{**}$). Positive correlations were reported among DSI and total sugars ($r = 0.938^{**}$) and MDA content ($r = 0.918^{**}$). Hence, these parameters can be employed as biochemical markers for screening of gladiolus genotypes for *Fusarium* tolerance.

Key words: Agronomic traits, biochemical changes, gladiolus, *Fusarium* wilt.

INTRODUCTION

Gladiolus (*Gladiolus hybridus* Hort.) is one of the most popular ornamental bulbous flowering crop grown for cut flower and garden display and it belongs to the family Iridaceae and sub family Ixioideae. It is native to South Africa and has been cultivated globally. Gladiolus is becoming very popular among the bulbous flowering crops due to its beautiful dazzling coloured spikes, varied range of spike length, florets size and number as well as long vase-life. Despite of having good ornamental characters, this crop is very susceptible to different *Fusarium* species that leads to spike loss upto 40%. *Fusarium* is soil borne fungus belongs to Deuteromycetes class. It is very difficult to control, since it survives in soil and corms as both spores and mycelium in latent form for prolonged period. There is genetic variation in resistance levels among the gladiolus genotypes and also variation in virulence may present in *Fusarium oxysporum* pv. *gladioli*. There are many races found in *F. oxysporum* pv. *gladioli* that means not all resistance hosts are resistance/susceptible to all isolates of *fusarium*. Hence, there is need of continuous screening of genotypes that might be used further in improvement programme.

MATERIALS AND METHODS

Thirty gladiolus genotypes, namely, Shabnam,

Gulal, Mohini, Dhanwantri, Berlew-OS, Little Fawn, Pusa Kiran, Pusa Shubham, Pusa Manmohak, Tha Barton, Urmi, Pink Parsal, Urvashi, Hunting Song, Melody-OS, Surya Kiran (Melody × Mayur) × H. Heady, GW × Oscar, S. Lady × Oscar, Suchitra × Melody, White Friendship, Green Lilac-OS, Pusa Vidhushi, Swarnima, Pusa Red Valentine, Pusa Srijana, Gunjan, Jyotsana, Suchitra and Pusa Unnati were planted in field in October 2015 and 2016. Experiments were conducted in a randomized block design. Ninety six corms of each genotype were planted in four replications. Different vegetative traits, i.e. days to sprouting, plant heights, leaf No., leaf length and width, corm weight and diameter; and reproductive traits, namely, spike length, rachis length, No. of florets per spike were recorded.

The natural incidence of *Fusarium* wilt and plant mortality was calculated using the method suggested by Riaz *et al.* (14). The disease severity was determined by the adopting the suggested disease rating scale, i.e. 0 = No disease symptoms, 1 = Yellowing of leaves, 2 = Wilting, and 3 = Dead and were accordingly grouped as highly resistant (0-5%), tolerant (6-10%), moderately tolerant (11-20%), moderately susceptible (21-30%) and susceptible (31-50%).

Different biochemicals were estimated following standard procedures. Total leaf chlorophyll content

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was measured by DMSO method (Hiscox and Israelstam, 10), total soluble sugars by the anthrone reagent method (Hedge and Hofreiter, 8), total phenols (Malick and Singh, 11) and malondialdehyde level by thiobarbituric acid (TBA) method (Hagage, 7) at 45 days after planting. Analysis of variance (ANOVA) was carried out for different parameters following Completely Randomized Design. Pearson's correlation coefficient (r) among DSI and biochemical

traits was computed using statistical analysis system software (SPSS version 2.1).

RESULTS AND DISCUSSION

There was a marked variation recorded in vegetative growth, flowering and *Fusarium* wilt incidence in the 30 gladiolus genotypes when evaluated under natural field conditions (Tables 1-4). As evident, the days to corm sprout ranged from 11.67-

Table 1. Variation in vegetative growth and corm parameters in gladiolus genotypes under field conditions (pooled data).

Genotype	Days to 50% corm sprouting	Plant height (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Corm wt. (g)	Corm dia. (cm)
Shabnam	15.66	106.87	9.00	42.44	1.94	22.75	77.25	6.47
Gulal	17.67	85.50	8.50	35.69	3.24	15.63	67.5	6.73
Mohini	15.67	91.62	9.50	41.00	3.13	33.88	68.63	5.93
Dhanwantri	12.67	107.87	10.25	51.37	3.37	15.37	85.13	6.70
Berlew-OS	13.67	117.75	8.62	50.12	3.21	16.13	74.50	5.87
Little Fawn-OS	16.00	92.62	9.00	36.12	3.25	15.75	53.50	5.81
Pusa Kiran	13.00	86.37	8.13	33.75	2.38	14.93	60.63	5.91
Pusa Shubham	23.67	76.50	7.25	35.12	3.33	20.25	53.00	5.41
Pusa Manmohak	21.34	97.63	8.37	42.75	3.98	10.37	53.63	6.08
Tha Barton	16.34	92.25	8.00	34.50	2.93	15.75	51.13	6.28
Urmi	17.67	81.50	7.00	46.00	3.27	13.00	49.63	5.43
Pink Parsal-OS	21.67	108.00	10.12	51.62	2.73	17.75	66.37	6.58
Urvashi	14.80	87.50	7.87	46.25	4.03	16.37	52.37	5.66
Hunting Song	16.00	100.25	8.25	36.37	2.76	20.13	76.25	6.51
Melody-OS	17.00	95.50	7.37	36.50	3.30	17.87	45.87	4.85
Suryakiran	15.00	111.37	8.13	38.00	2.45	17.25	44.63	4.81
(Melody × Mayur) × H. Wine	17.34	94.37	7.13	32.37	2.88	15.75	49.00	5.25
Green Willow × Oscar	23.84	77.25	7.00	34.62	3.61	19.25	44.37	4.21
Smokey Lady × Oscar	15.67	88.62	8.37	35.75	2.50	14.37	47.13	4.90
Suchitra × Melody	11.67	98.50	8.87	49.87	4.81	16.25	101.75	6.51
White Friendship	18.33	114.00	7.75	37.87	3.78	22.63	81.63	6.53
Green Lilac-OS	16.00	95.12	7.75	31.25	2.78	16.50	86.25	6.01
Pusa Vidushi	12.50	94.87	7.62	34.37	3.91	9.25	56.50	4.95
Swarnima	13.17	91.62	8.87	47.50	3.76	14.13	44.37	5.03
Pusa Red Valentine	14.33	119.12	7.87	39.87	4.5	16.75	70.63	6.10
Pusa Srijana	11.89	87.50	7.62	29.75	3.30	17.37	61.88	4.94
Gunjan	19.17	91.75	7.87	33.25	3.41	18.25	93.63	5.08
Jyotsana	17.50	91.00	8.75	34.12	3.55	15.75	96.38	5.38
Suchitra	15.34	102.25	8.12	31.00	3.20	18.87	89.75	5.40
Pusa Unnati	18.34	129.75	9.00	39.38	4.05	24.88	52.75	5.25
Range	11.67-23.84	129.75-81.50	7.00-10.25	31.00-51.37	4.81-1.94	33.88-9.25	101.75-44.37	6.73-4.22
CD _{0.05}	1.73	1.98	0.77	3.21	0.34	3.12	13.65	0.75
CV (%)	6.40	1.45	6.67	5.86	7.36	12.76	14.94	9.36

Table 2. Variation in floral traits in gladiolus genotypes under field conditions (pooled data).

Genotype	Spike emergence (days)	Spike length (cm)	Rachis length (cm)	Florets No./ spike	Spike field-life (days)
Shabnam	86.37	70.00	41.37	16.87	15.13
Gulal	80.25	53.63	38.12	13.25	14.87
Mohini	87.12	73.25	46.25	13.87	15.37
Dhanwantri	80.25	65.13	45.00	14.50	12.87
Berlew-OS	79.00	78.87	54.87	18.00	16.50
Little Fawn-OS	91.75	61.75	45.62	17.75	16.37
Pusa Kiran	86.87	54.00	36.62	12.87	15.62
Pusa Shubham	86.25	55.00	38.25	12.50	18.50
Pusa Manmohak	100.62	69.63	49.37	18.62	13.13
Tha Barton	72.25	65.75	45.75	13.37	15.00
Urmi	86.50	52.00	27.00	9.75	12.75
Pink Parsal-OS	109.62	79.13	46.75	14.00	14.25
Urvashi	84.75	55.50	38.37	13.37	15.37
Hunting Song	84.50	80.63	58.87	14.37	16.25
Melody-OS	74.62	73.12	45.37	15.87	18.00
Suryakiran	81.25	75.00	52.62	16.87	16.63
(Melody × Mayur) × H. Wine	78.37	56.12	41.87	10.00	11.50
Green Willow × Oscar	88.37	52.87	41.25	10.50	11.50
Smokey Lady × Oscar	96.00	58.50	45.00	11.75	13.87
Suchitra × Melody	87.25	75.38	53.12	13.62	20.75
White Friendship	80.75	74.37	58.50	17.37	16.62
Green Lilac-OS	86.50	54.50	40.00	8.87	17.50
Pusa Vidushi	87.00	73.62	42.75	14.00	16.37
Swarnima	100.25	68.63	50.87	13.37	13.87
Pusa Red Valentine	84.5	80.50	61.75	18.50	20.37
Pusa Srijana	91.375	57.37	42.37	15.87	15.75
Gunjan	91.375	69.62	49.12	16.00	13.87
Jyotsana	79.125	75.75	53.50	17.26	16.37
Suchitra	85.75	76.50	46.12	14.12	14.62
Pusa Unnati	104.75	92.37	62.75	18.37	21.25
Range	109.62-72.25	92.37-52.00	62.75-27	18.62-8.87	21.25-11.50
CD _{0.05}	2.98	1.877	1.627	1.47	1.93
CV (%)	2.43	1.971	2.478	7.23	8.75

23.84 days. The earliest corm sprouting was recorded in Suchitra × Melody (11.27 days), followed by Pusa Srijana (11.89 days), while it was most delayed in Green willow W × Oscar (23.84 days). Data revealed in genotypes Dhanwantri, Pusa Kiran, Suchitra × Melody, Pusa Srijana and Pusa Vidhushi sprouted in 11-13 days could be rated as early sprouting types, while Green Willow × Oscar, Pusa Shubham, Pusa Manmohak and Pink Parsal-OS had late sprout (21-23 days). Most of the genotypes exhibited intermediate

sprouting duration (14-20 days). Plant height at induction complete emergence of spike was noted maximum in Pusa Unnati (129.75 cm) significantly followed by Pusa Red Valentine (119.12 cm). Shortest plants were seen in Urmi (81.50 cm). Most of the genotypes had plant height over 1 m. The leaf length varied significantly, *i.e.* from 31.00 cm in Suchitra to 51.37 cm in Dhanwantri. The leaf number ranged from 7.00 to 10.25 per plant. Minimum number of leaves were found in Urmi and Green willow × Oscar

Table 3. Variation in biochemical status of gladiolus genotypes after field screening for *Fusarium* wilt incidence.

Genotype	Leaf chlorophyll (mg /g FW)	Corm		
		Total soluble sugars (mg/g FW)	Total phenols (mg/g FW)	MDA ($\mu\text{mol g}^{-1}$ /FW)
Shabnam	2.32	20.77	0.77	26.12
Gulal	2.47	18.78	0.87	24.40
Mohini	2.39	19.64	0.75	25.08
Dhanwantri	2.52	18.38	0.94	23.54
Berlew-OS	2.17	21.09	0.74	26.65
Little Fawn-OS	2.13	21.47	0.75	26.85
Pusa Kiran	2.31	20.57	0.78	26.39
Pusa Shubham	2.08	21.64	0.66	26.89
Pusa Manmohak	2.25	20.93	0.73	25.79
Tha Barton	2.15	21.25	0.69	26.56
Urmi	2.00	22.28	0.65	27.61
Pink Parsal-OS	2.15	21.50	0.69	26.01
Urvashi	2.22	21.26	0.71	25.96
Hunting Song	2.53	19.43	0.88	23.67
Melody-OS	2.02	22.16	0.66	26.85
Suryakiran	2.72	18.29	1.11	22.28
(Melody \times Mayur) \times Heady Wine	2.16	21.57	0.68	26.82
Green Willow \times Oscar	2.16	21.54	0.71	26.04
Smokey Lady \times Oscar	2.79	18.32	0.94	22.86
Suchitra \times Melody	2.58	19.37	0.92	23.47
White Friendship	2.06	21.91	0.65	27.13
Green Lilac-OS	2.36	21.31	0.75	26.12
Pusa Vidushi	2.57	18.51	0.92	23.25
Swarnima	2.89	18.18	1.17	22.19
Pusa Red Valentine	2.34	20.57	0.77	26.28
Pusa Srijana	2.64	18.74	0.94	23.32
Gunjan	2.32	20.52	0.80	25.58
Jyotsana	2.36	21.30	0.77	26.33
Suchitra	2.15	21.67	0.68	26.16
Pusa Unnati	2.81	18.25	1.13	21.89
Range	2.89-2.00	22.28-18.18	1.17-0.65	27.61-21.89
CD _{0.05}	0.103	0.816	0.082	0.712
CV (%)	2.64	2.443	6.22	1.72

(7.00) compared to maximum noted in Dhanwantri and Mohini (10.24). Longest leaf was recorded in Pink Parsal (51.62 cm) compared to shortest in Pusa Srijana (29.75 cm). The widest leaves were observed in Suchitra \times Melody (4.81 cm) significantly followed by Urvashi (4.03 cm), whereas it was most narrow in Shabnam (1.94 cm). There was significant variation found in corm parameters (Table 1). Average corm weight varied significantly among the genotype and was found maximum in Suchitra \times Melody (101.75 g) non-significantly followed by Jyotsana (96.38 g) and Gunjan (93.63 g), whereas lowest was in genotype Green willow \times Oscar and Swarnima (44.37 g). Maximum corm size was observed in Gulal (6.73 cm) non-significantly followed by Pink Parsal-OS (6.58 cm), while smallest was in Smokey Lady \times Oscar (4.21 cm).

Significant variations were recorded for various characters studied among the 30 genotypes. These differential expressions of traits are the direct result of genotype and the physio-biochemical status of the corms. Similar finds have been studied by several workers in gladiolus (Kadam *et al.*, 9). Plant height at flowering stage and spike fully opened and number of leaves at flowering stage were important traits.

Similarly, root length varied considerably, *i.e.* from 9.25 cm in Pusa Vidushi to 33.88 cm (Mohini) (Table 1). Field incidence of *Fusarium* wilt was noted at 75 days of planting. Genotypes Urmi and Pusa Manmohak showing symptoms of fusarium wilt had root length of 13.00 and 10.37 cm, respectively. However, Pusa Vidushi having smaller roots was categorized as moderately tolerant. Most of tolerant genotypes had longer roots, suggesting that for penetration of fungus mycelium it is succulence of root and not length, which is important for susceptibility (Chandra *et al.*, 4).

Different genotypes exhibited significant variation for flowering characters, *i.e.* days required to spike initiation and floret opening (Table 2). Tha Barton had early spike initiation (72.25 days), whereas Pink Parsal-OS was late (109.62 days). Maximum spike length was found in Pusa Unnati (92.37 cm) significantly followed by Hunting Song (80.63 cm) and Pusa Red Valentine (80.50 cm), whereas shortest spikes were noted in Urmi (52.00 cm). Rachis length was maximum in Pusa Unnati (62.75 cm), which was at par with Pusa Red Valentine (61.75 cm), while the shortest length was recorded in Urmi (27.00 cm) significantly followed by Pusa Kiran (36.62 cm). Floret number per spike was maximum in Pusa Manmohak (18.62) followed by Pusa Red Valentine (18.50), Pusa Unnati (18.37) and Berlew-OS (18.00). Minimum number of florets per spike was found in Green Lilac-OS (8.87) followed by Urmi (9.75). The variation in

Table 4. Correlation among different parameters with respect to *Fusarium* wilt incidence in gladiolus.

Parameter	Chlorophyll	TSS	Phenols	MDA	DSI	PMI
Chlorophyll	1.000					
TSS	-0.931**	1.000				
Phenols	0.958**	-0.916**	1.000			
MDA	0.873**	-0.688**	0.857**	1.000		
DSI	-0.974**	0.938**	-0.971**	-0.918**	1.000	
PMI	-0.598**	0.633**	-0.587**	-0.730**	0.618**	1.000

**Significant at the 0.01 level (2-tailed).

different floral traits expressed under field conditions by the genotypes may be due to their unique genetic makeup and partially due to the effect of prevailing environmental conditions. Maximum field spike-life was recorded in Pusa Unnati (21.25 days), which was at par with Suchitra × Melody (20.75 days) and Pusa Red Valentine (20.37 days). In gladiolus, ideal cut flower genotypes should produce big sized daughter corms and good number of corms and cormels, which are the genotypic traits owing unique recombinant of individual germplasm and their pedigree (Balaram and Janakiram, 2; Poon *et al.*, 12). Owing to unique genotype, the gladiolus germplasm varied considerably for flowering traits, too.

Based on Disease Severity Index (DSI) noted for the 30 genotypes, Swarnima was rated highly resistant (0-55); Pusa Unnati tolerant (6-10%); Suryakiran, S. Lady × Oscar, Pusa Srijana, Dhanwantri and Pusa Vidushi were moderately tolerant (11-20%), Shabnam, Gulal, Mohini, Pusa Kiran, Hunting Song, Suchitra × Melody, Green Lilac, Pusa Red Valentine, Gunjan and Jyotsana were found moderately susceptible (21-30), BL Little, Little Fawn, Pusa Shubham, Pusa Manmohak, Tha Barton, Urmi, Pink Parsol, Urvashi, Melody, Melody × Mayur × H. Wine, GW × Oscar, White Friendship, Suchitra (31-50%) were found susceptible to disease in field (Table 5). Among the gladiolus genotypes, Urmi and White Friendship recorded the maximum disease

severity index (45.14 and 44.45) with maximum number of plant died. While, Gulal, Smokey Lady × Oscar, Swarnima, Pusa Red Valentine, Pusa Unnati exhibited only symptoms of *Fusarium* wilt, but with no mortality of plants (Fig. 1).

Disease reaction of different genotypes against *Fusarium* wilt has previously been examined under different growing conditions (Riaz *et al.*, 14; Shanmugam, 15). Natural variations in disease susceptible and tolerant genotypes due to different rot causing pathogens have also been earlier noticed in gladiolus (Riaz *et al.*, 14; Amrutha *et al.*, 1). Earlier reports have suggested that fungal infection induces increase in antioxidative abilities, such as SOD, guaiacol peroxidase, catalase, APX, and flavonoid content, suggesting that biotic stress lead to their enhanced level. The importance of PPO, POD, chlorogenic acid, and total soluble phenols in defense mechanisms against pathogen cannot be ruled out (Ray *et al.*, 13). Resistant genotypes showed higher content of phenols in corm tissue compared to susceptible ones.

There was a sharp reduction in total chlorophyll contents in disease affected leaves of susceptible genotypes as compared with resistant genotypes. Pusa Swarnima and Pusa Unnati recorded 44 and 40% higher chlorophyll content as compared to susceptible genotype Urmi. Total chlorophylls showed highly negative correlation ($r = -0.974^{**}$) and moderate

Table 5. *In vivo* reaction of gladiolus genotypes to *Fusarium* wilt disease.

Category	Genotype(s)
Highly resistant (0-5)	Swarnima
Tolerant (6-10)	Pusa Unnati
Moderately tolerant (11-20)	Suryakiran, Smokey Lady × Oscar, Pusa Srijana, Dhanwantri, Pusa Vidushi
Moderately susceptible (21-30)	Shabnam, Gulal, Mohini, Pusa Kiran, Hunting Song, Suchitra × Melody, Green Lilac-OS, Pusa Red Valentine, Gunjan, Jyotsana
Susceptible (31-50)	Berlew-OS, Little Fawn-OS, Pusa Shubham, Pusa Manmohak, Tha Barton, Urmi, Pink Parsal-OS, Urvashi, Melody, (Melody × Mayur) × Heady Wine, Green Willow × Oscar, White Friendship, Suchitra

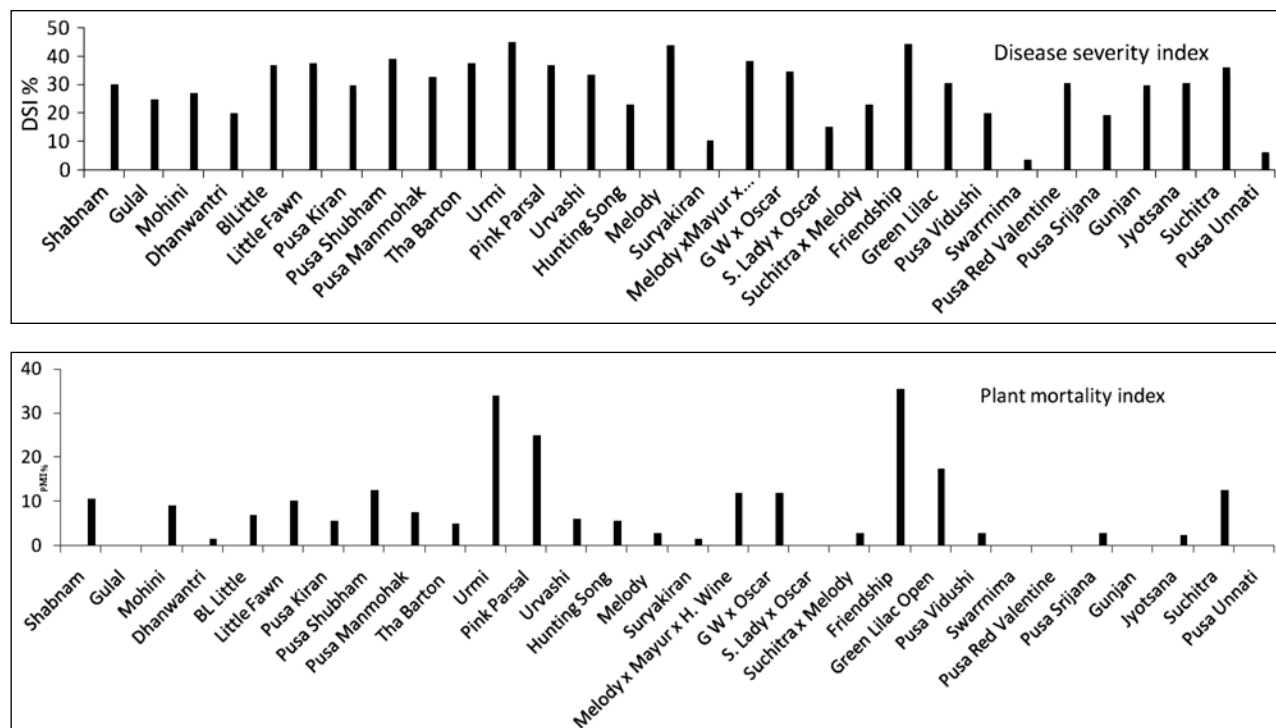


Fig. 1. Plant mortality and disease severity index (%) due to *Fusarium* wilt incidence in gladiolus genotypes (at 75 days of planting).

negative correlation ($r = 0.598^{**}$) with DSI and PMI, respectively. The results are in agreement with Gurjar *et al.* (2015), where susceptible genotypes were identified with lower levels. Corms of susceptible genotypes had higher total soluble sugars compared to resistant. Corms of Urmi (22.28 mg/g FW) non-significantly followed by Friendship (21.91 mg/g FW) and Suchitra (21.67 mg/g FW) had highest content, and these genotypes showed susceptibility to disease. Lowest content found in Swarnnima (18.18 mg/g FW) was non-significantly followed by Pusa Unnati (18.25 mg/g FW), that was resistant to disease. The correlation studies revealed that total sugars were positively correlated ($r = 0.938^{**}$) with DSI. Higher total soluble sugars in the tissue facilitate the fungus invasion and growth by dissolving cell wall and plasma membrane. Similar results were reported by Gurjar *et al.* (6) and Sharma *et al.* (16). The highly resistant genotype was Swarnnima (1.17 mg/g FW) followed by Pusa Unnati (1.13 mg/g FW) and Suryakiran (1.11 mg/g FW) as compared to Urmi and White Friendship (0.65 mg/g FW). As evident from the resistant genotypes had higher total phenols than susceptible genotypes. Total phenols content showed high negative correlation ($r = -0.971^{**}$) with DSI. The highest level of MDA was observed in Urmi (27.61 $\mu\text{mol/g FW}$), whereas, Pusa Unnati showed

the lowest (21.89 $\mu\text{mol/g FW}$), in diseased leaves, which was significantly different (Table 3). The MDA levels were higher in susceptible as compared to resistant genotypes. Total MDA content in corms showed high negative correlation ($r = -0.918^{**}$) with DSI and ($r = -0.730^{**}$) with PMI (Table 4). Our results showed increased phenolic compounds facilitating resistance in genotypes to counter fungal infection and growth. These findings are in agreement with those of Goswami *et al.* (5). The phenolic limits the pathogen invasion during plant defense responses. Peroxidase enzyme oxidizes and polymerizes phenolics into quinones, which possess ability to inactivate enzymes. These oxidized phenolic compounds possess increased antimicrobial activity and could play role in inhibiting pathogen development. The enzyme also polymerizes phenolic acids to form lignin, thereby further enhancing resistance to pathogens. Fungus infection enhances loss of cell membrane integrity, increased the production of reactive oxygen species and cause antioxidative enzymes inefficient leading to cellular disruption. Increased ROS acted toxic agents for lipid peroxidation and membrane damage (Bao *et al.*, 3).

Based on above results, it may be concluded that among the 30 gladiolus genotypes, the agronomic performance of cvs Pusa Unnati, Pusa Red Valentine,

Suryakiran, Swarnima and Dhanwantri were found most promising. Based on biochemical parameters Pusa Unnati, Swarnima and Dhanwantri were found tolerant to Fusarium infection owing to high phenolic compounds, high leaf chlorophyll and low MDA and sugar contents in corm tissue. These biochemical parameters can be used as markers for screening genotypes for *Fusarium* wilt.

ACKNOWLEDGEMENT

Senior author acknowledges the Union Grant Commission, Ministry of Human Resources Development for Providing National Fellowship.

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Received : April, 2017; Revised : August, 2017;

Accepted : November, 2017