



## Response of gladiolus genotypes to *in vivo* inoculation of *Fusarium oxysporum* f. sp. *gladioli* on growth, flowering and disease incidence

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### ABSTRACT

In the present study 30 gladiolus genotypes were evaluated for different traits and reaction to two virulent strains of *Fusarium*, viz. IARI isolate (*F. oxysporum* f. sp. *gladioli* – Fog isolate 1) and Pantnagar isolate (*F. oxysporum* f. sp. *gladioli* - Fog isolate 2). The two healthy uniform sized corms were planted in individual pots (12”) filled with potting mixture (soil: FYM: sand; 2:1:1) in five replications. Before planting, the corms were treated with suspension of *Fusarium* ( $3 \times 10^6$ /ml) for 24 hr. Among the genotypes, Dhanwantri (14.59%), Pusa Unnati (14.59%), Swarnima (16.54%), and hybrid Smokey Lady  $\times$  Oscar (22.92%) were found tolerant to both the *Fusarium* isolates. The maximum plant height, spike length, rachis length and mean number of florets/spike were noted in Pusa Unnati, but the earliest spike emergence took place in Jyotsana (87.37 days), earliest opening of first floret was noted in Melody (103.79 days). The maximum mean corm weight (87.58 g) was noted in Jyotsana, while maximum diameter was found in Dhanwantri (6.56 cm). Leaves and corms were sampled at 60 days of planting for estimation of different biochemical parameters, viz., total chlorophyll, total phenol, malondialdehyde (MDA) content and H<sub>2</sub>O<sub>2</sub> peroxidation. The tolerant genotypes had higher levels of leaf total chlorophylls and total phenols. The H<sub>2</sub>O<sub>2</sub> evolution and malondialdehyde (MDA) content showed marked increment due to *in vivo* inoculation of the pathogen. Correlation studies with plant mortality index (PMI) showed a positive correlation for DSI (0.899), while negative correlations were estimated for MDA content (0.879\*\*) followed by H<sub>2</sub>O<sub>2</sub> formation (0.862\*\*), total phenol ( $r = 0.810^{**}$ ) and total chlorophyll ( $r = -0.754^{**}$ ). Results showed that genotypes, namely, Dhanwantri, Pusa Unnati, Swarnima, and hybrid Smokey Lady  $\times$  Oscar were *Fusarium* wilt tolerant along with desirable agronomic traits, thus can be recommended for commercial cultivation.

**Key words:** Biochemical changes, *Fusarium* wilt resistance, Gladiolus, *In vivo* screening

Gladiolus is known as queen of bulbous flowering crops, because of its fascinating and dazzling coloured spikes and having good keeping quality. It ranks first in cut flower production in bulbous group due to its adaptability, relatively easy to grow also suitable for bedding and exhibition. Despite having large area under production, gladiolus is much hindered due to incidence of various diseases, viz. *Fusarium* wilt, dry or neck rot, core or spongy rot, bacterial scab, grey mold (Goo *et al.* 2015). Among the diseases, *Fusarium* wilt is the most devastating, causing wilt or yellows in gladiolus leading to heavy economic loss to the farmers, by reducing production of quality spikes and corm & cormel production. *Fusarium oxysporum*

f. sp. *gladioli* has a world-wide distribution. In India, Singh (1969) was first to report gladiolus wilt caused by *Fusarium oxysporum* f. sp. *gladioli* [(Massey) Snyder and Hansen] from Lucknow, Uttar Pradesh. Being a soil borne disease it spreads through infected parent corms or from the infested soil. The fungus gets entry through the fibrous roots, contractile roots and leaf bases near or below the soil surface. For *Fusarium* wilt tolerance, screening of resistant host germplasm and application of biotechnological tools are being adopted for its meaningful management. Due to the high cost and toxicity associated with application of chemical fungicide, developing resistant genotypes may be the most efficient, economical, and environment friendly strategy to reduce the threat of this malady. Hence, there is an urgent need to screen germplasm for wilt tolerance, besides desired vegetative and reproductive traits that can be further used in breeding programme. Hence, the present study was undertaken.

### MATERIALS AND METHODS

Thirty diverse gladiolus genotypes, viz. Shabnam,

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Gulal, Mohini, Dhanwantri, Berlew Open, Little Fawn Open, Pusa Kiran, Pusa Shubham, Pusa Manmohak, The Barton, Urmi, Pink Parasol Open, Urvashi, Hunting Song, Melody, Surya Kiran, hybrid (Melody × Mayur) × Heady Wine, hybrid Green Willow × Oscar, Smokey Lady × Oscar, hybrid Suchitra × Melody, Friendship, Green Lilac Open, Pusa Vidushi, Swarnima, Pusa Red Valentine, Pusa Srijana, Gunjan, Jyotsana, Suchitra and Pusa Unnati procured from different sources were evaluated for their reaction to *Fusarium* wilt and performance post artificial inoculation. These genotypes were assessed for their resistance to two geographically distinct isolates of *F. oxysporum* f. sp. *gladioli*, viz. IARI culture (Isolate 1) and Pantnagar culture (Isolate 2). Screening of the germplasm was undertaken in pot (12") during 2015-16 and 2016-17 in polyhouse of the Division of Floriculture and Landscaping, ICAR-IARI, New Delhi. Experiment was laid down in a complete randomized design. Sterilized potting mixture comprising of soil, sand and well decomposed Farm Yard Manure (FYM) in the ratio of 2:1:1 was used. Sixteen uniform sized healthy corms of all the genotypes were planted in replicated trials. Two corms were planted in each pot. For pathogen inoculation, the corms were soaked for 24 hr in two *Fog* inoculum (cfu  $3 \times 10^6$  spores/ml) a day (24 hr) before planting followed by watering with sterile tap water. Corms potted in the sterile potting mixture without any fungal inoculation were taken as control. Each treatment was replicated four times.

Infected corms having white mycelial growth collected from dying plants showing wilt symptoms were procured from the fields of IARI, New Delhi (isolate 1) and Pantnagar, Uttarakhand (isolate 2). The surface-sterilized corm pieces were placed on Potato Dextrose Agar (PDA) medium in petriplates and were incubated at 25°C for 7 days with regular observation for the growth of pure colonies. For growth of pure *Fusarium*, single spores were transferred onto petriplates having PDA medium (Cappelli 1994). Furthermore, purification of the fungus was done and the pure cultures were incubated in PDA slants for identification of pathogen. The pathogen was mass multiplied on potato dextrose broth in flask and incubated at 25°C. Spore concentration was calculated by hemocytometer and for treatment; a density of  $3 \times 10^6$ , was prepared and corms were treated. Data regarding disease incidence and mortality was recorded using standard procedures.

After *in vivo* fungal inoculation in pots, different parameters were recorded at specific intervals. The different parameters studied were vegetative growth, i.e. corm sprouting percentage, days to sprouting, plant height, leaf length and number; and flowering traits like days to spiking, days to opening of first floret, No. of florets, floret diameter, and field-life of spike. Further, corm traits like weight and diameter were also recorded following standard methods. Different biochemical parameters like total chlorophyll content in leaf, total phenols, MDA content and H<sub>2</sub>O<sub>2</sub> peroxidation assay in corm tissue were estimated in healthy and diseased plants for each genotype. Total chlorophyll was estimated as per the method suggested by Hiscox and

Israelstam (1979), total phenol content was estimated as per the method described by Malick and Singh (1980), and malondialdehyde (MDA) level was assayed following thiobarbituric acid (TBA) method (Hagage 1990). The determination of H<sub>2</sub>O<sub>2</sub> content was performed following method described by Mandal *et al.* (2008).

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

### *Vegetative growth parameters*

Vegetative growth of gladiolus genotypes was recorded in terms of sprouting of corms, days to sprouting, plant height, leaf length and number and root length. There was 100% corm sprouting in control and treated plants (Table 1). Irrespective of *Fog* isolates, gladiolus genotypes Gulal (24.70 days) and hybrid Green Willow × Oscar (24.69 days) took maximum duration for corm sprouting, while Swarnima (14.50 days) followed nonsignificantly by Pusa Kiran (16.94 days) were early. Irrespective of genotypes, the un-inoculated control (18.39 days) gave early sprouting in comparison to the both isolates. The interaction effect was found to be non-significant for two *Fog* isolates. Genotypes, Swarnima (13.50 days) took the minimum days for bud sprouting, while hybrid GW × Oscar (25.17 days) was most delayed.

There was highly significant difference for plant height in the control and plants inoculated with *F. oxysporum* f. sp. *gladioli* IARI or Pantnagar isolates (Table 2). Among the genotypes, irrespective of *Fog* isolate, the maximum plant height was recorded in Pusa Unnati (122.16 cm), which was significantly followed by Suryakiran (107.21 cm), while minimum was recorded in Urmi (65.58 cm). Irrespective of genotypes, the plants were longer in control (103.08 cm), while it was shorter in *Fog* isolate 1 (79.98 cm) inoculated plants. The interaction effect of two factors was found significant. Maximum plant height was recorded in Pusa Unnati in control (125.08 cm), whereas it was minimum (52.37 cm) in Urmi with *Fog* isolate 1.

Leaf parameters are important for disease evaluation, since infection affect the foliage growth and decline in the photosynthetic pigments. The most significant visual symptom was yellowing of leaf progressing from tip to downward. Irrespective of the *Fog* isolates, the maximum leaf length was observed in Dhanwantri (48.78 cm), followed nonsignificantly by Pusa Swarnima (45.54 cm), while minimum length was observed in Suchitra (28.74 cm). Irrespective of genotype, the leaf length was general longer in control (40.76 cm), while it was minimum due to inoculation of *Fog* isolate 1 (30.89 cm). The interaction effect of two factors was found significant. Genotype Dhanwantri (55.12 cm) recorded longest leaves in un-inoculated control, while it was found minimum (23.50

Table 1 Response of genotype and *Fusarium* isolate on days to corm sprout in gladiolus genotypes due to *in vivo* infection (pooled data of 2016 and 2017)

Genotype	Days to corm sprout (cm)			
	Control	Isolate 1	Isolate 2	Mean
Shabnam	20.67	24.33	21.83	22.28
Gulal	21.67	27.43	25.00	24.70
Mohini	18.00	21.04	20.33	19.79
Dhanwantri	17.33	19.33	18.00	18.22
Berlew Open	17.83	21.00	19.00	19.28
Little Fawn Open	19.50	23.17	21.33	21.33
Pusa Kiran	15.33	18.67	16.83	16.94
Pusa Shubham	21.83	26.67	23.00	23.83
Pusa Manmohak	17.00	22.00	18.50	19.17
The Barton	19.83	23.33	20.50	21.22
Urmi	20.33	27.17	24.33	23.94
Pink Parasol Open	22.67	25.00	22.67	23.45
Urvashi	16.17	19.17	17.00	17.45
Hunting Song	19.67	23.00	20.67	21.11
Melody	18.33	20.00	19.17	19.17
Suryakiran	18.00	20.17	19.33	19.17
(Melody × Mayur) × H. Wine	18.50	24.83	20.50	21.28
Green Willow × Oscar	24.50	24.39	25.17	24.69
Smokey Lady × Oscar	17.67	19.17	18.33	18.39
Suchitra × Melody	14.50	18.00	15.17	15.89
White Friendship	19.50	24.17	22.33	22.00
Green Lilac Open	16.33	19.33	17.83	17.83
Pusa Vidushi	18.33	19.33	18.83	18.83
Swarnima	13.50	15.50	14.50	14.50
Pusa Red Valentine	17.83	21.17	18.83	19.28
Pusa Srijana	13.33	14.50	13.67	13.83
Gunjan	20.50	25.83	21.33	22.55
Jyotsana	17.17	20.67	19.50	19.11
Suchitra	15.67	20.67	17.17	17.84
Pusa Unnati	20.17	21.00	20.67	20.61
Mean	18.39	21.67	19.71	
CD ( $P=0.05$ )				
Genotype (G)				3.90
Isolate (I)				2.14
G × I				4.06

cm) in Urmi with *Fog* isolate 1.

The maximum leaf number was observed in Pusa Unnati (8.83) followed by Dhanwantri (8.70), while minimum was in Pink Parasol (6.16) followed by Pusa Manmohak (6.45). There was no significant difference for leaf number in the control and plants inoculated with both the *Fusarium* isolates. The interaction effect of genotype and *Fog* isolate was found to be significant. The maximum leaf number was

registered in un-inoculated Urvashi in control (9.37), while minimum was recorded in Pink Parasol with *Fog* isolate 1.

#### Floral parameters

There was no significant difference observed for both the *Fog* isolates with regard to genotypes for days to spike emergence (Table 3). Among the genotypes, irrespective of *Fog* isolate, the earliest spike emergence (87.37 days) was recorded in Jyotsana non-significantly followed by Dhanwantri (88.37 days) and Melody (88.58 days), while it was most delayed in Pink Parasol (116.29 days) followed nonsignificantly by Pusa Unnati (113.83 days). Irrespective of genotype, the earliest spike emergence was observed in control as compared to inoculated ones. The interaction effect of genotype and *Fog* isolate was found significant. The earliest spike emergence was noted in White Friendship (80.00 days), while it was most delayed in Pink Parasol (122.25 days). Long spike length is an important trait to catch the market owing to consumer preference. Irrespective of *Fog* isolates, the maximum mean spike length was recorded in Pusa Unnati (81.03 cm) significantly followed by Melody-OS (73.16 cm) and Jyotsana (70.41 cm), whereas minimum was observed in Urmi (40.29 cm) nonsignificantly followed by hybrid (Melody × Mayur) × H. Wine (42.09 cm). In general, longer spike length was recorded in control followed by *Fog* isolate 2. The interaction effect of the two factors was found significant. Maximum spike length was recorded in Hunting Song in control (86.50 cm), whereas it was minimum (30.00 cm) was in hybrid (Melody × Mayur) × Heady Wine with *Fog* isolate 1.

Irrespective of *Fog* isolates, rachis length was recorded maximum in Pusa Unnati (60.66 cm) significantly followed by Swarnima (53.03 cm), while minimum was in Urmi (26.49 cm) nonsignificantly followed by Urvashi (32.50 cm). Irrespective of genotypes, *Fog* isolate 1 had more pronounced effect on rachis length as compared to isolate 2. The interaction effect of genotype and *Fog* isolate was found significant. Genotype Pusa Unnati had the maximum rachis length (65.12 cm), while Urmi had the shortest (22.12 cm) showing the detrimental effect of *Fog* inoculation. Both the *Fog* isolates had marked negative effect on floral parameters in all the gladiolus genotypes.

There was significant difference among the different genotypes for trait days to opening of first floret due to *Fog* inoculation (Table 5). The earliest opening of first florets was recorded in Melody (103.79 days) non-significantly followed by Suryakiran (104.59 days), while it was most delayed in Pink Parasol (132.49 days). Irrespective of genotypes, the two *Fog* isolates had pronounced effect on opening of first floret. The earliest opening of florets was recorded in control (108.89 days) followed by isolates 2 (115.13 days) followed by *Fog* isolate 1 (120.83 days). The interaction effect was also found to be significant. The earliest floret opening was observed in White Friendship (98.75 days) in control, while it was most delayed in Pink Parasol (137.38 days) with *Fog* isolate 1. The maximum mean number of florets per spike was recorded in Pusa Unnati (15.33) followed by

Table 2 Response of genotype and *Fusarium* isolate on plant height and leaf parameters in gladiolus genotypes due to *in vivo* infection (pooled data of 2016 and 2017)

Genotype	Plant height (cm)				Leaf length (cm)				Leaves / plant			
	Control	Isolate 1	Isolate 2	Mean	Control	Isolate 1	Isolate 2	Mean	Control	Isolate 1	Isolate 2	Mean
Shabnam	105.50	92.25	98.00	98.58	45.37	32.87	40.50	39.58				Mean
Gulal	90.25	72.25	80.12	80.87	39.62	31.75	36.00	35.79	8.25	6.37	7.99	7.53
Mohini	98.37	83.50	87.00	89.62	43.87	31.62	36.62	37.37	7.50	7.15	7.30	7.31
Dhanwantri	112.63	98.37	98.25	103.08	55.12	44.37	46.87	48.78	9.37	7.62	8.75	8.58
Berlew Open	126.00	87.36	94.13	102.49	52.62	32.5	43.00	42.71	9.25	8.25	8.62	8.70
Little Fawn Open	94.37	65.87	73.75	77.99	37.50	28.87	33.75	33.37	8.25	6.37	7.37	7.33
Pusa Kiran	94.12	59.75	81.50	78.46	37.37	29.25	33.50	33.37	8.50	6.75	7.75	7.66
Pusa Shubham	88.625	68.75	70.87	76.08	34.0	26.25	32.25	30.83	7.37	6.75	7.25	7.13
Pusa Manmohak	110.12	72.00	82.75	88.29	42.5	30.75	38.37	37.20	7.25	5.62	6.50	6.45
The Barton	97.37	71.75	80.12	83.081	40.12	27.87	36.00	34.66	8.00	6.00	7.00	7.00
Urmi	86.5	52.37	57.87	65.58	47.00	23.50	33.50	34.66	7.87	6.37	6.75	7.00
Pink Parasol Open	118.37	80.87	93.62	97.62	46.87	27.75	34.50	36.37	7.13	5.12	6.25	6.16
Urvashi	92.25	70.87	82.12	81.74	44.12	34.62	41.12	39.95	9.37	6.87	7.75	8.00
Hunting Song	113.00	83.00	74.87	90.29	41.75	33.87	38.75	38.12	7.62	7.00	7.50	7.37
Melody	112.875	87.25	93.62	97.92	34.87	28.62	34.12	32.53	8.13	7.87	7.50	7.83
Suryakiran	116.00	99.75	105.87	107.21	35.62	31.37	32.87	33.28	7.50	6.75	7.37	7.20
(Melody × Mayur) × H. Wine	89.00	69.75	81.12	79.95	32.62	26.12	27.87	28.87	7.63	7.62	7.37	7.54
Green Willow × Oscar	82.125	67.00	68.75	72.62	31.50	26.00	31.75	29.75	7.63	6.62	6.62	6.95
Smokey Lady × Oscar	99.25	84.50	91.25	91.66	40.12	33.87	36.12	36.71	7.13	6.75	6.75	6.87
Suchitra × Melody	106.37	78.37	86.75	90.49	45.62	27.12	34.87	35.87	8.13	7.75	7.87	7.91
White Friendship	106.12	73.50	77.37	85.66	43.12	25.00	36.00	34.71	8.00	7.62	7.87	7.83
Green Lilac Open	99.75	82.75	88.50	90.33	34.87	26.00	34.00	31.62	7.37	6.25	7.12	6.91
Pusa Vidushi	105.25	88.62	91.00	94.95	36.25	34.37	36.50	35.70	7.50	7.62	7.37	7.50
Swarnima	96.125	90.00	92.75	92.95	49.50	42.62	44.50	45.54	8.00	7.37	7.50	7.62
Pusa Red Valentine	123.00	93.00	98.25	104.75	43.87	34.87	40.37	39.70	8.13	8.12	7.87	8.04
Pusa Srijana	94.125	82.50	84.87	87.16	33.62	27.25	28.75	29.87	7.63	7.50	7.37	7.50
Gunjan	98.375	81.12	84.12	87.87	38.62	27.50	36.12	34.08	7.87	7.25	7.50	7.54
Jyotsana	104.62	76.13	86.00	88.91	40.75	29.62	38.50	36.29	7.87	7.25	7.25	7.45
Suchitra	106.63	68.37	82.37	85.78	29.75	29.37	27.12	28.74	8.00	8.25	8.25	8.16
Pusa Unnati	125.37	117.87	123.25	122.16	44.5	41.37	43.25	43.04	7.63	7.37	7.25	7.41
Mean	103.08	79.98	86.36		40.76	30.89	36.25		9.00	8.75	8.75	8.83
<i>CD (P=0.05)</i>												
Genotype (G)				7.71				4.58				1.67
Isolate (I)				4.92				3.22				n s
G × I				8.76				5.76				2.11

Dhanwantri (14.21), whereas minimum was in Urmi (6.29). There was no significant difference in the isolates under control. Pusa Unnati had recorded the maximum florets in control as well after inoculation with the two *Fog* isolates. The mean floret diameter was found maximum in Pusa Unnati (8.92 cm), which was at par with Shabnam (8.75 cm) followed by Jyotsana (8.49 cm), whereas minimum

was in hybrid (Melody × Mayur) × Heady Wine (6.97 cm). There was no significant difference for floret diameter of genotypes in control and *Fog* isolate inoculation.

#### *Corm and cormel production*

Results showed that maximum mean corm weight (87.58 g) was recorded in Jyotsana, while it was minimum

Table 3 Response of genotype and *Fusarium* isolate on reproductive traits in gladiolus genotypes due to *in vivo* infection (pooled data of 2016 and 2017)

Genotype	Spike emergence (days)				Spike length (cm)				Rachis length (cm)			
	Control	Isolate 1	Isolate 2	Mean	Control	Isolate 1	Isolate 2	Mean	Control	Isolate 1	Isolate 2	Mean
Shabnam	94.75	110.75	100.25	101.92	69.87	54.25	58.87	60.99	43.87	31.87	35.37	37.04
Gulal	94.00	102.63	95.87	97.50	48.50	37.75	46.00	44.08	38.00	27.85	32.75	32.86
Mohini	93.87	96.87	95.75	95.50	72.00	68.00	69.25	69.75	44.87	38.00	40.62	41.16
Dhanwantri	86.00	91.62	87.5	88.37	63.50	55.25	60.12	59.62	48.37	41.75	45.37	45.16
Berlew Open	91.13	99.75	95.00	95.29	73.25	47.87	56.37	59.16	59.25	30.75	37.12	42.37
Little Fawn Open	100.37	114.37	107.50	107.41	62.37	38.12	43.00	47.83	50.37	29.25	35.87	38.49
Pusa Kiran	96.50	108.63	99.87	101.66	54.12	35.13	49.37	46.21	41.12	26.25	33.62	33.66
Pusa Shubham	97.75	108.37	99.37	101.83	57.37	46.37	50.00	51.25	37.00	29.75	33.25	33.33
Pusa Manmohak	98.87	114.00	112.37	108.42	72.50	45.12	52.12	56.58	52.00	29.50	34.50	38.66
The Barton	86.25	98.37	95.50	93.37	63.37	49.25	54.00	55.54	46.25	33.37	36.62	38.74
Urmi	93.25	110.37	108.75	104.13	49.75	33.37	37.75	40.29	26.87	22.12	30.50	26.49
Pink Parasol Open	108.50	122.25	118.12	116.29	82.50	49.25	66.37	66.04	49.87	30.50	40.75	40.37
Urvashi	88.37	97.375	92.12	92.62	56.25	40.75	46.75	47.92	40.25	27.00	30.25	32.50
Hunting Song	91.50	103.00	94.62	96.37	86.50	66.75	57.00	70.08	61.50	38.75	33.37	44.54
Melody	83.25	94.62	87.87	88.58	76.25	70.50	72.75	73.16	45.62	39.37	44.25	43.08
Suryakiran	87.75	91.87	91.87	90.50	74.12	62.37	68.62	68.37	53.00	40.25	43.62	45.62
(Melody × Mayur) × H. Wine	83.25	107.75	104.75	98.58	52.12	30.00	44.15	42.09	42.00	26.62	32.37	33.66
Green Willow × Oscar	91.62	102.50	98.00	97.37	48.62	34.25	44.25	42.37	35.25	25.00	31.87	30.70
Smokey Lady × Oscar	95.25	102.62	98.37	98.75	57.50	57.50	62.50	59.16	45.12	40.75	45.50	43.79
Suchitra × Melody	94.75	108.00	104.87	102.54	71.62	54.37	62.12	62.70	48.87	29.87	37.12	38.62
White Friendship	80.00	102.87	101.25	94.71	67.62	37.37	39.15	48.04	58.37	25.85	27.12	37.11
Green Lilac Open	86.62	100.62	92.25	93.16	53.37	41.62	50.87	48.62	39.87	33.50	38.37	37.24
Pusa Vidushi	92.87	96.50	94.75	94.71	71.75	64.62	71.75	69.37	45.50	36.75	42.50	41.58
Swarnima	102.25	104.12	103.00	103.13	73.12	66.50	71.12	70.24	55.00	51.35	52.75	53.03
Pusa Red Valentine	90.87	100.37	97.12	96.13	75.87	52.37	59.75	62.66	62.87	37.75	44.25	48.29
Pusa Srijana	95.00	106.75	98.50	100.08	57.87	51.62	53.37	54.28	41.62	34.12	38.12	37.95
Gunjan	95.00	102.25	104.62	100.62	66.37	56.25	61.37	61.33	50.62	38.75	42.87	44.08
Jyotsana	84.25	90.00	87.87	87.37	79.00	61.50	70.75	70.41	56.00	43.75	49.25	49.66
Suchitra	89.75	102.12	100.62	97.50	73.25	38.87	53.37	55.16	45.50	35.50	41.87	40.95
Pusa Unnati	110.87	116.00	114.62	113.83	82.12	80.87	80.12	81.03	65.12	59.00	57.87	60.66
Mean	92.81	103.57	103.6		66.41	50.93	57.09		47.66	34.49	38.98	
CD ( $P=0.05$ )												
Genotype (G)				5.27				6.54				5.99
Isolate (I)				3.53				4.03				3.72
G × I				6.67				7.40				6.72

in hybrid Green Willow × Oscar (40.16 g) irrespective of *Fog* isolates (Table 4). Irrespective of genotype, maximum corm weight was found in control followed by *Fog* isolates 2 and 1. The interaction effect of genotype and *Fog* isolate was found significant. The maximum corm weight was found in hybrid Suchitra × Melody (107.6 g) in control, whereas minimum was registered in Urmi (25.37 g). Lower

difference was noted for corm diameter in the genotypes and also for *Fog* isolates. The maximum mean corm diameter was found in Dhanwantri (6.19 cm), while minimum was in Urmi (3.91 cm). The two *Fog* isolates had negligible effect on corm diameter. However, the maximum corm diameter was registered in Dhanwantri and Pink Parasol (6.56 cm) in un-inoculated control, whereas minimum was in Urmi

Table 4 Response of genotype and *Fusarium* isolate on corm and cormel production in gladiolus genotypes due to *in vivo* *Fusarium* infection (pooled data of 2016 and 2017)

Genotype	Corm wt. (g)				Corm dia. (cm)				Cormels / plant			
	Control	Isolate 1	Isolate 2	Mean	Control	Isolate 1	Isolate 2	Mean	Control	Isolate 1	Isolate 2	Mean
Shabnam	78.50	56.12	73.00	69.20	5.87	5.17	5.95	5.66	11.87	7.37	13.75	10.99
Gulal	69.87	50.85	63.75	61.49	5.93	4.52	5.28	5.24	31.00	11.25	19.62	20.63
Mohini	68.12	53.35	57.25	59.57	5.60	4.91	5.03	5.18	31.63	19.75	28.37	26.58
Dhanwantri	82.87	77.87	81.25	80.66	6.56	5.85	6.18	6.19	23.00	16.87	26.62	22.16
Berlew Open	77.00	58.50	71.75	69.08	5.92	4.33	5.55	5.26	22.12	14.25	17.50	17.95
Little Fawn Open	50.37	43.00	48.15	47.17	5.08	3.97	4.21	4.42	14.00	7.37	15.13	12.16
Pusa Kiran	61.87	48.25	56.15	55.42	6.10	4.05	4.76	4.97	14.12	11.75	13.25	13.04
Pusa Shubham	47.62	36.62	49.12	44.45	4.47	3.91	4.22	4.20	10.00	9.13	10.25	9.79
Pusa Manmohak	57.25	43.87	51.75	50.95	5.86	4.48	5.27	5.20	24.12	12.13	17.25	17.83
The Barton	48.12	42.5	50.50	47.04	6.51	5.02	5.56	5.69	13.12	5.50	6.13	8.25
Urmi	45.62	25.37	36.25	35.74	5.00	2.83	3.91	3.91	8.37	2.87	4.00	5.08
Pink Parasol Open	72.87	58.50	67.37	66.25	6.56	4.38	5.10	5.34	28.50	10.13	15.75	18.12
Urvashi	47.00	35.00	44.25	42.08	5.35	3.75	4.61	4.57	23.87	14.12	20.12	19.37
Hunting Song	75.75	48.87	63.37	62.66	6.08	5.18	5.51	5.59	32.00	13.87	15.87	20.58
Melody	46.25	36.75	43.87	42.29	4.42	3.33	4.38	4.04	16.37	9.87	13.25	13.16
Suryakiran	46.87	40.37	42.12	43.12	5.12	4.28	4.85	4.75	16.00	8.37	12.00	12.12
(Melody × Mayur) × H. Wine	52.50	38.75	44.75	45.33	5.25	3.54	4.51	4.43	28.25	10.87	13.00	17.37
Green Willow × Oscar	45.50	30.63	44.37	40.16	4.46	4.76	4.4	4.54	21.75	9.37	11.50	14.21
Smokey Lady × Oscar	43.50	38.75	43.25	41.83	4.925	4.23	4.77	4.64	15.00	11.12	14.25	13.45
Suchitra × Melody	107.60	46.87	80.12	78.20	6.03	4.68	5.17	5.29	36.87	14.62	20.00	23.83
White Friendship	94.87	34.00	57.37	62.08	5.62	3.5	3.87	4.33	40.12	6.75	10.50	19.12
Green Lilac Open	80.87	59.37	66.25	68.83	5.91	5.1	5.68	5.56	27.50	14.87	19.12	20.5
Pusa Vidushi	76.75	70.25	64.87	70.63	4.61	4.1	4.42	4.37	28.25	16.00	19.75	21.33
Swarnima	51.13	48.12	52.50	50.58	5.65	4.9	5.32	5.29	31.75	23.50	28.50	27.92
Pusa Red Valentine	73.62	54.25	70.87	66.25	5.96	5.03	5.66	5.55	32.62	18.50	27.12	26.08
Pusa Srijana	66.12	50.12	65.87	60.71	5.21	4.87	5.12	5.06	27.62	16.50	27.25	23.79
Gunjan	90.37	72.50	79.25	80.70	5.41	4.56	4.61	4.86	35.37	18.12	22.87	25.45
Jyotsana	95.62	78.75	88.37	87.58	5.48	5.23	5.51	5.40	29.75	23.50	30.00	27.75
Suchitra	94.25	71.25	79.75	81.75	5.25	4.38	4.58	4.73	17.00	7.12	12.50	12.21
Pusa Unnati	57.25	50.00	50.5	52.58	5.18	5.21	5.28	5.22	30.00	26.25	27.00	27.75
Mean	66.86	49.98	59.60		5.51	4.468	4.97		24.06	13.05	17.74	
<i>CD (P=0.05)</i>												
Genotype (G)				7.38				1.54				3.37
Isolate (I)				4.21				1.04				3.40
G × I				9.29				1.98				6.27

(2.83 cm) with *Fog* isolate 1. Similar findings were earlier reported by Balam et al. (2009) and Riaz et al. (2010) on different genotypes.

Production of cormel was greatly affected by *Fusarium* inoculation. The maximum number of cormels per plant was recorded in Swarnima (27.92) non-significantly followed by Jyotsana and Pusa Unnati (27.75), whereas minimum was

noted in Urmi (5.08) significantly followed by Pusa Shubham (9.79). Irrespective of genotype, control plants recorded the maximum mean number of cormels per plant (24.06) followed by *Fog* isolate 1 (17.74) and isolate 2 (13.05). The interaction effect of genotype and *Fog* isolate was found to be significant. The maximum number of cormels per plant was found in hybrid Suchitra × Melody (36.87)

Table 5 Response of genotype and *Fusarium* isolate on flowering parameter in gladiolus genotypes due to *in vivo* infection (pooled data of 2016 and 2017)

Genotype	Day to open first floret (days)				Florets / spike				Floret dia. (cm)			
	Control	Isolate 1	Isolate 2	Mean	Control	Isolate 1	Isolate 2	Mean	Control	Isolate 1	Isolate 2	Mean
Shabnam	110.5	128.13	122.87	120.50	9.87	9.37	10.50	9.91	9.58	7.58	9.11	8.75
Gulal	108.62	120.25	110.00	112.95	10.12	7.37	10.00	9.16	8.26	7.42	8.33	8.00
Mohini	106.87	114.13	109.37	110.12	12.00	10.75	10.62	11.12	9.12	7.92	8.93	8.65
Dhanwantri	105.00	107.87	106.37	106.41	14.37	13.75	14.50	14.21	8.57	8.72	8.73	8.67
Berlew Open	103.12	112.50	109.25	108.29	11.50	7.87	11.25	10.21	8.75	7.37	8.66	8.26
Little Fawn Open	114.65	128.75	120.87	121.42	12.00	9.87	13.25	11.72	8.15	6.88	7.96	7.66
Pusa Kiran	113.75	127.13	118.37	119.75	11.25	8.75	9.87	9.95	8.76	7.31	8.23	8.10
Pusa Shubham	115.62	129.62	118.12	121.12	9.00	8.00	9.50	8.83	7.66	6.46	7.42	7.18
Pusa Manmohak	118.37	133.00	127.25	126.21	12.25	8.37	9.50	10.04	8.92	8.07	8.33	8.44
The Barton	102.75	117.37	111.75	110.62	11.25	8.37	9.62	9.74	9.02	7.08	7.47	7.85
Urmi	106.00	129.63	128.12	121.25	6.62	4.75	7.50	6.29	8.03	6.35	7.3	7.22
Pink Parasol Open	127.37	137.37	132.75	132.49	10.25	7.00	8.62	8.62	7.98	6.61	7.07	7.22
Urvashi	106.50	115.00	108.25	109.92	10.50	8.37	9.62	9.49	9.06	7.53	7.90	8.16
Hunting Song	108.87	121.13	114.50	114.83	10.87	8.00	9.65	9.50	8.87	6.87	8.30	8.01
Melody	100.62	109.25	101.50	103.79	10.87	9.12	10.75	10.24	8.58	7.12	8.16	7.95
Suryakiran	102.62	105.62	105.50	104.58	11.35	9.75	11.75	10.95	7.73	7.23	7.50	7.48
(Melody × Mayur) × H. Wine	100.12	127.00	115.25	114.12	7.37	6.50	8.37	7.41	7.45	6.57	6.90	6.97
Green Willow × Oscar	110.00	122.37	114.50	115.62	7.50	7.50	8.25	7.75	8.35	7.08	7.87	7.76
Smokey Lady × Oscar	104.50	114.75	106.37	108.54	12.12	11.50	13.00	12.20	7.13	7.07	7.42	7.20
Suchitra × Melody	115.62	131.37	122.00	122.99	7.87	6.37	8.50	7.58	8.06	7.31	7.70	7.69
White Friendship	98.75	127.75	124.87	117.13	10.37	6.12	7.37	7.95	8.38	6.83	7.47	7.56
Green Lilac Open	104.62	115.75	106.25	108.87	8.00	8.00	10.12	8.70	7.40	7.73	7.63	7.58
Pusa Vidushi	110.00	113.25	108.50	110.58	10.50	9.37	12.12	10.66	8.33	7.72	8.20	8.08
Swarnima	121.25	124.75	120.12	122.04	10.87	10.87	11.50	11.08	8.75	7.78	8.28	8.27
Pusa Red Valentine	105.25	114.50	109.37	109.71	12.75	11.37	12.87	12.33	8.65	7.63	8.48	8.25
Pusa Srijana	106.87	119.37	114.37	113.54	10.37	9.37	11.75	10.49	7.68	7.01	7.88	7.52
Gunjan	110.25	121.12	116.50	115.95	11.25	9.12	10.75	10.37	8.52	7.75	8.20	8.15
Jyotsana	100.12	109.375	106.62	105.37	12.37	10.50	12.87	11.91	8.40	8.57	8.52	8.49
Suchitra	100.12	116.5	114.75	110.45	9.25	7.87	9.50	8.87	8.75	7.06	7.97	7.92
Pusa Unnati	128.25	130.5	129.375	129.37	15.12	15.25	15.62	15.33	8.93	8.88	8.97	8.92
Mean	108.89	120.83	115.13		10.65	8.97	10.63		8.39	7.38	8.029	
<i>CD (P=0.05)</i>												
Genotype (G)				5.18				3.08				1.36
Isolate (I)				3.54				1.32				1.03
G × I				6.37				3.16				1.84

non-significantly followed by Gunjan (35.37), whereas minimum was recorded in Urmi (2.87) non-significantly followed by The Barton (5.5).

#### Disease incidence

The data on disease severity and plant mortality due to artificial *in vivo* inoculation of the two *Fog* isolates are

presented in Table 6. There was highly significant difference (14.59-55.21%) with regard to disease severity among the genotypes treated with both the *Fog* isolates. Irrespective of *Fog* isolate, maximum disease severity was recorded in Urmi (55.21%) which was at par with Melody (47.92%) and White Friendship (46.88%), while Dhanwantri and Pusa Unnati (14.59%) had the least disease incidence non-

Table 6 Response of genotype and *Fusarium* isolate on disease severity index and plant mortality index in gladiolus genotypes due to *in vivo* infection (pooled data of 2016 and 2017)

Genotype	Disease severity index (%)			Plant mortality index (%)		
	Isolate 1	Isolate 2	Mean	Isolate 1	Isolate 2	Mean
Shabnam	41.67	27.08	34.38	18.75	10.42	14.59
Gulal	31.25	22.92	27.09	10.42	8.33	9.38
Mohini	29.17	18.75	23.96	12.50	2.08	7.29
Dhanwantri	16.67	12.50	14.59	6.25	0.00	3.13
Berlew Open	47.92	29.17	38.55	20.83	8.33	14.58
Little Fawn Open	37.50	22.92	30.21	14.58	8.33	11.46
Pusa Kiran	33.33	22.92	28.13	14.58	4.17	9.38
Pusa Shubham	47.92	25.00	36.46	20.83	2.08	11.46
Pusa Manmohak	47.92	25.00	36.46	22.92	12.50	17.71
The Barton	50.00	22.92	36.46	22.92	12.50	17.71
Urmi	62.50	47.92	55.21	35.42	25.00	30.21
Pink Parasol Open	47.92	22.92	35.42	18.75	8.33	13.54
Urvashi	45.83	31.25	38.54	14.58	12.50	13.54
Hunting Song	39.58	25.00	32.29	18.75	8.33	13.54
Melody	56.25	39.58	47.92	22.92	20.83	21.88
Suryakiran	41.67	37.50	39.59	12.50	6.25	9.38
(Melody × Mayur) × H. Wine	47.92	31.25	39.59	25.00	12.50	18.75
Green Willow × Oscar	43.75	22.92	33.34	20.83	10.42	15.63
Smokey lady × Oscar	25.00	20.83	22.92	8.33	2.08	5.21
Suchitra × Melody	33.33	14.58	23.96	10.42	6.25	8.34
White Friendship	56.25	37.50	46.88	25.00	18.75	21.88
Green Lilac Open	45.83	29.17	37.50	25.00	18.75	21.88
Pusa Vidushi	29.17	22.92	26.05	14.58	8.33	11.46
Swarnima	20.90	12.18	16.54	10.42	4.17	7.30
Pusa Red Valentine	35.42	27.08	31.25	14.58	12.50	13.54
Pusa Srijana	20.83	12.50	16.67	8.33	0.00	4.17
Gunjan	31.25	22.92	27.09	16.67	10.42	13.55
Jyotsana	16.67	14.58	15.63	10.42	6.25	8.34
Suchitra	45.83	39.58	42.71	20.83	16.67	18.75
Pusa Unnati	18.75	10.42	14.59	8.33	0.00	4.17
Mean	38.47	25.28		16.88	9.24	
<i>CD (P=0.05)</i>						
Genotype (G)			6.16			5.03
Isolate (I)			7.34			4.86
G × I			9.36			7.04

significantly followed by Swarnima (16.54%) and hybrid Smokey Lady × Oscar (22.92%). In the present study, our results showed that plant mortality index ranged from 3.13-21.88%, irrespective of *Fog* isolates. The maximum plant mortality was recorded in Urmi (30.21%) significantly followed by White Friendship and Green Lilac Open (21.8825%). *Fusarium* isolate 1 treated genotypes showed the higher disease incidence in comparison isolate 2, which suggest its higher virulence. Based on disease incidence, genotypes Dhanwantri, Pusa Srijana, Swarnima and Pusa

Srijana were found tolerant to wilt. While, genotypes Urmi and White Friendship were found susceptible to wilt. The other genotypes were intermediate for their disease susceptibility. Pathogenicity of fungus might be location-specific and has been reported by several workers (Taj *et al.* 2016, Shanmugam *et al.* 2009).

#### *Biochemical changes and correlation with disease incidence*

*Fusarium* wilt infection causes general leaf yellowing in

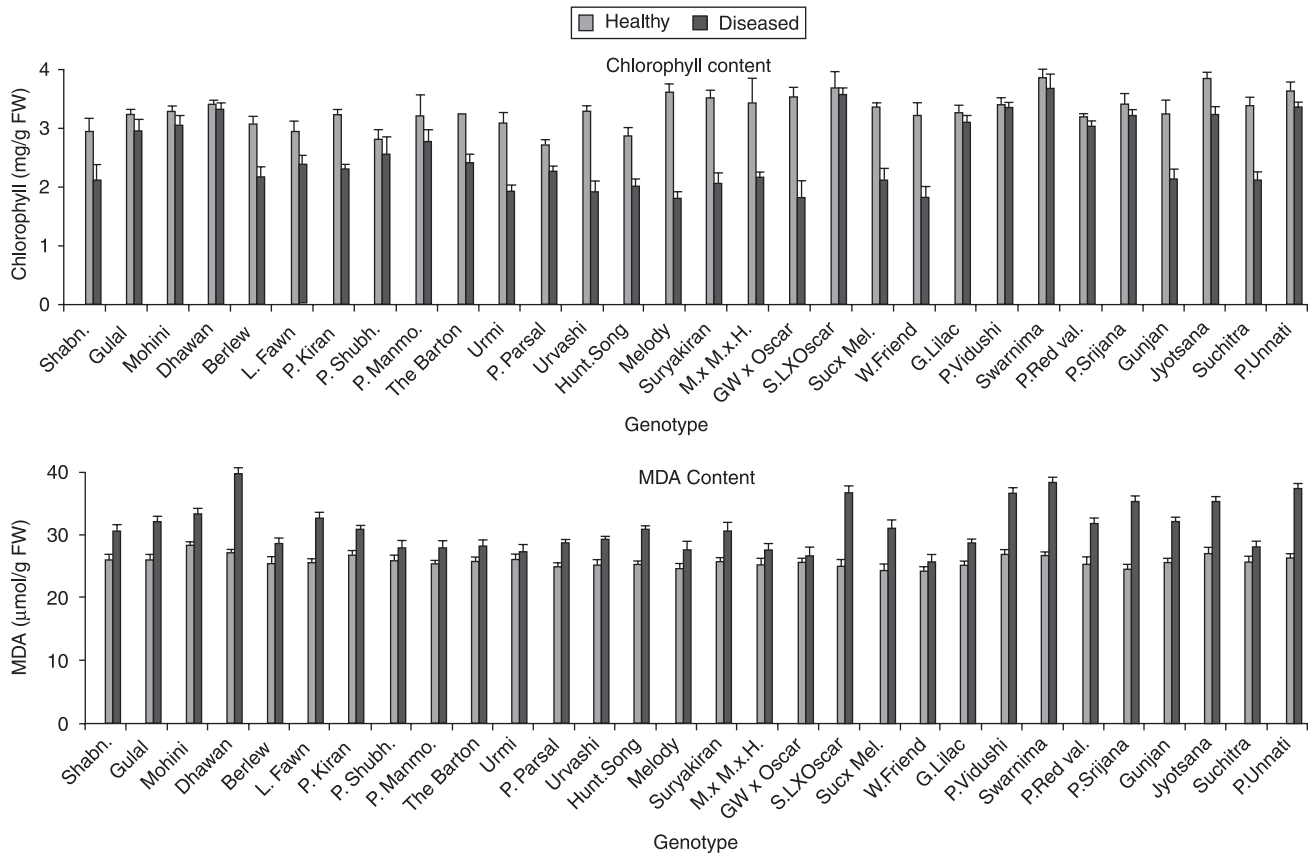


Fig 1 Variation in total chlorophylls content in leaf and MDA content in corm tissue of un-inoculated and inoculated (*Fusarium* sp.) gladiolus genotypes.

gladiolus, which starts from the base near to the mother corm. Yellowing of leaf is directly related with rapid degradation of chlorophyll. As evident, there was a significant difference in total chlorophyll content in all the genotypes post *Fusarium* inoculation compared to non-inoculated control (Fig 1). Chlorophyll content was higher in non-inoculated control plants irrespective of the genotypes, while it was lower in *Fog* inoculated counterparts. The maximum chlorophyll degradation (43.67%) was observed in White Friendship followed by Urvashi (42.09%), while minimum decrease was observed in Pusa Vidushi (1.76%) followed by hybrid Smokey Lady x Oscar (2.80%) and Dhanwantri (2.92%). Degradation of chlorophyll was negatively correlated with disease severity index ( $r = -0.754^{**}$ ).

The extent of disease infection caused by *Fusarium*

was measured in term of cell damage due to production of reactive radicals that oxidized the lipid present in cell membrane. Lipid peroxidation, which is measured in terms of malondialdehyde (MDA) content was found maximum in Mohini (27.99  $\mu\text{mol/g FW}$ ) nonsignificantly followed by Dhanwantri (27.22  $\mu\text{mol/g FW}$ ), Pusa Vidushi (26.91  $\mu\text{mol/g FW}$ ) and Swarnima (26.56  $\mu\text{mol/g FW}$ ), while it was lowest in hybrid Suchitra x Melody (23.85  $\mu\text{mol/g FW}$ ) nonsignificantly followed by White Friendship (24.18  $\mu\text{mol/g FW}$ ), Pusa Srijana (24.48  $\mu\text{mol/g FW}$ ) and Melody (24.66  $\mu\text{mol/g FW}$ ) with control. There was significant difference noted due to upsurge in MDA content in all the genotypes after pathogen inoculation. The maximum MDA content was found in Dhanwantri (39.89  $\mu\text{mol/g FW}$ ) significantly followed by Swarnima (38.25  $\mu\text{mol/g FW}$ ) and hybrid S.

Table 7 Correlation among different parameters with respect to *Fusarium* wilt incidence in gladiolus.

Parameter	Chlorophyll	MDA	Total phenols	H <sub>2</sub> O <sub>2</sub>	DSI	PMI
Chlorophyll	1.000					
MDA	0.785**	1.000				
Total phenols	0.699**	0.848**	1.000			
H <sub>2</sub> O <sub>2</sub>	0.708**	0.852**	0.874**	1.000		
DSI	0.754**	-0.879**	-0.810**	-0.862**	1.000	
PMI	-0.609**	-0.803**	-0.718**	-0.815**	0.899**	1.000

\*\*Correlation is significant at the 0.01 level (2-tailed).

Lady × Oscar (36.67 μmol/g FW). The maximum increment in MDA content was estimated in hybrid S. Lady × Oscar (47.09%) followed by Dhanwantri (46.53%) and Swarnima (44%). Minimum increase in MDA content was observed in Urmi (5.98%) followed by White Friendship (7.54%) and Pusa Shubham (7.93%). The MDA content in corm tissue was negatively correlated with disease severity index ( $r = -0.879^{**}$ ). The peroxidation of unsaturated lipids of cell membranes is the prominent symptoms of oxidative stress in plants. In our study, marked increase in MDA content as product of lipid peroxidation was observed in some genotypes that might be proven as tolerant genotypes. Phenolic compounds enhance the mechanical strength of host cells, having role in lignin and suberin synthesis that prevents the spread of infection (Singh *et al.* 2014). In the present study, the amount of total phenol was higher in corms of non-inoculated and inoculated resistant genotypes, viz. Dhanwantri, hybrid Smokey Lady × Oscar, Pusa Vidushi, Swarnima, Pusa Srijana and Pusa Unnati. Total phenolic content increased sharply in all the genotypes inoculated with *Fusarium* isolates as compared to non-inoculated. The maximum total phenol content was observed in Dhanwantri (1.32 mg/g FW) nonsignificantly followed by Pusa Swarnima (1.30 mg/g FW) and Pusa Srijana (1.27 mg/g FW), while it was lowest in Pusa Manmohak and White Friendship (0.81 mg/g FW). In all the un-inoculated (control) plants, maximum phenol content was estimated in

Dhanwantri (1.17 mg/g FW), while it was lowest in Pink Parasol open (0.71 mg/g FW). Phenol contents was also correlated with disease severity index ( $r = -0.879^{**}$ ) and plant mortality index ( $r = -0.718^{**}$ ). Our findings are in agreement with the results obtained on plant-pathogenic fungal infection. It has been reported that phenols and their components are toxic to the pathogen, while their higher endogenous levels suggest resistant genotypes (Agrios 1997, Ray *et al.* 2015, Singh *et al.* 2017).

The level of H<sub>2</sub>O<sub>2</sub> generation was tremendously increased in *Fusarium* inoculated gladiolus genotypes as compared to non-inoculated. The maximum up-surge of hydrogen peroxide generation was found in Dhanwantri (33.03 μmol/g FW) significantly followed by Pusa Swarnima (32.14 μmol/g FW), hybrid S. Lady × Oscar (31.98 μmol/g FW), Pusa Srijana (31.66 μmol/g FW) challenged with *Fusarium*. While lowest increase was found in White Friendship (21.75 μmol/g FW). The maximum increment in H<sub>2</sub>O<sub>2</sub> generation was recorded in Dhanwantri (79.95%), hybrid S. Lady × Oscar (77.40%), Swarnima (76.05%) and Pusa Unnati (75.41%) upon *Fusarium* inoculation (Fig 2). The above results are in conformity with the earlier findings of Mandal *et al.* (2008) in tomato. Increase in H<sub>2</sub>O<sub>2</sub> production was negatively correlated with disease severity index ( $r = -0.862^{**}$ ) and PMI ( $r = -0.815^{**}$ ). Our results suggest that H<sub>2</sub>O<sub>2</sub> generation in *Fog* inoculated gladiolus genotypes may be one of the strategies of their defense

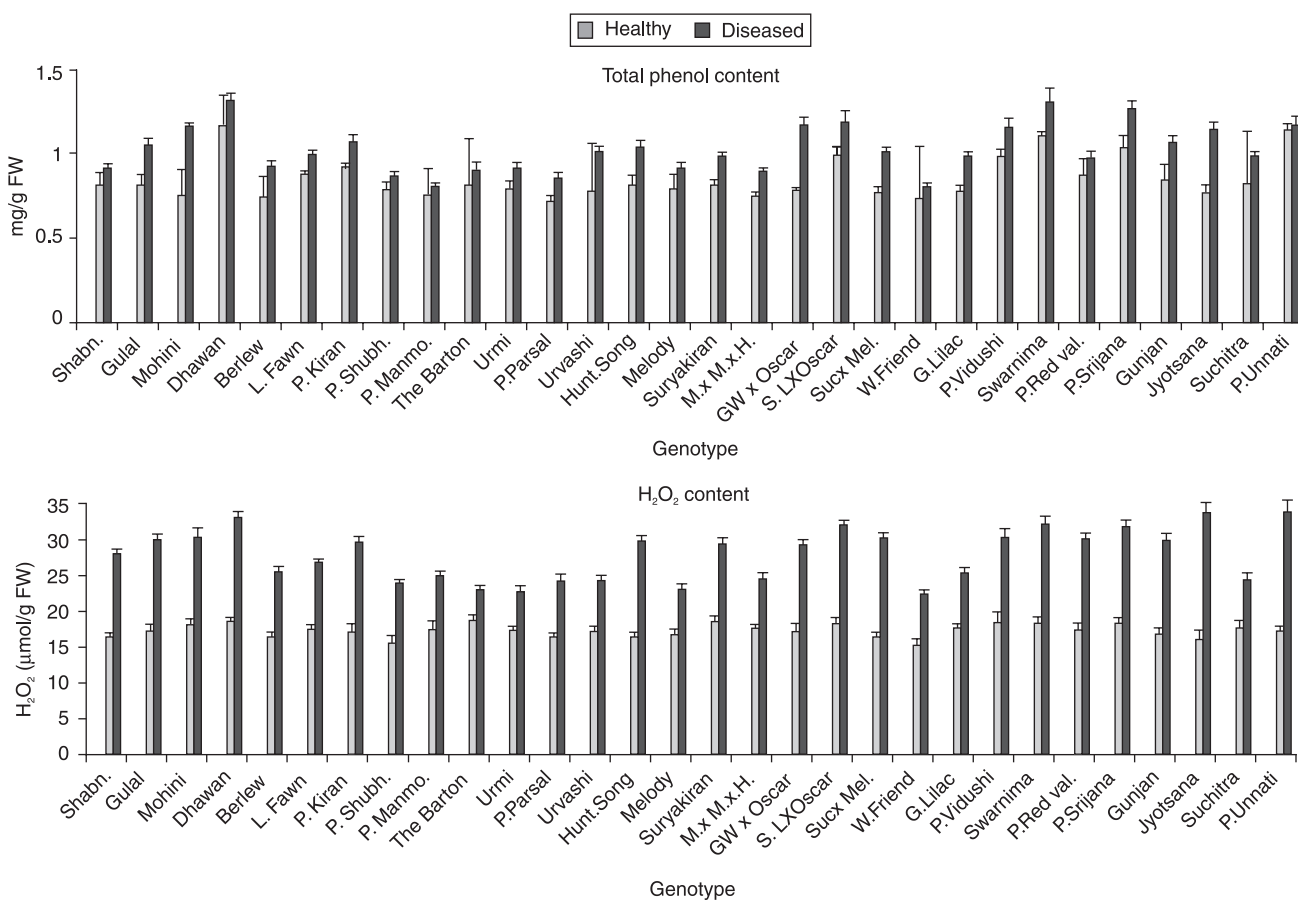


Fig 2 Variation in total phenols and H<sub>2</sub>O<sub>2</sub> evolution in corm tissue of un-inoculated and inoculated (*Fusarium*) gladiolus genotypes

post infection of pathogen. Generation of H<sub>2</sub>O<sub>2</sub> above the threshold level is sufficient to trigger the cell death that requires rapid formation of reactive oxygen species. Thus, cell death is only observed after inoculation with the pathogen, which causes a massive, prolonged oxidative burst and is not induced in the absence of an oxidative burst in control cells.

The results of the present study clearly indicated that among the 30 genotypes screened for *Fusarium* tolerance and field performance performance, four genotypes, viz. Dhanwantri, Pusa Unnati, Swarnima, and hybrid Smokey Lady × Oscar were found more tolerant. Pathogenicity of fungus depends upon place from which it is already isolated/acclimatized. Amongst the two *Fog* isolates, isolate 1 (IARI) showed more virulence than isolate 2 (Pantnagar) arrange alphabe.

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