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EXPLORATION OF RESISTANCE IN CHILLI VARIETIES/ADVANCED LINES AGAINST FUSARIUM WILT CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *CAPSICI*

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ABSTRACT

Chilli (*Capsicum annum* L.) is a popular edible spice crop grown in tropical areas around the world due to its high nutrient content, which includes carotenoids, fibers, mineral components, oils, proteins, and vitamins. Several biotic and abiotic factors are challenging devastatingly the successful production of chilli. Among these, fusarium wilt is caused by *Fusarium oxysporum* f.sp. *capsici* (*Foc.*) is a potential risk of declining its yield every year. The use of resistant varieties is considered the best option to cope *Foc*. For this purpose, twenty-five varieties/advanced lines of chilli were evaluated against Fusarium wilt under natural field conditions for two years consecutive years 2017-18 and 2018-19 under randomized complete block design (RCBD). Results exhibited that none of the varieties/advanced lines expressed immune response against the disease. Only one variety (BPVLC 14-1) was resistant with 18.76% disease incidence. Uttal, Fengaio, Glaxy-2, Big daddy, GHHP 01, PH-275, Super sky AB, HPO33, and Super king were found moderately resistant (MR) with 21-40% disease incidence. Four varieties/advanced lines viz. Hot-701, Hotshot, Omega, and Silkey Red showed a moderately susceptible response (MS) with 41-50% and Four (Super hot, Patiala F1, Angel F1, and Green King) were susceptible (S) with 51-70% incidence of Fusarium wilt. Seven (Tejal, BSS-410, Big Red AB, SB 6864-HM, Glory F1, Revival, and Amber F1) varieties / advanced lines exhibited highly susceptible (HS) response.

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INTRODUCTION

Chilli or red pepper (*C. annum* L.) is an important vegetable crop of Pakistan that belongs to the family *Solanaceae.* It is native to Central and Southern America. It has a great culinary and nutritional value which leads to potential industrialization; due to such importance, chilli is cultivated in both dry and wet seasons (Castillo-Téllez *et al.*, 2017). Now its trade is dominated in the world through South Asian countries (India, China, and Pakistan) to Morocco, Mexico, Spain, and Turkey (Bosland *et al.*, 2012; Ziino *et al.*, 2009). It is a rich source of capsaicin, vitamin C and A, carotenoids, diterpene glycosides, and polyphenols (Materska, 2014; Asnin and Park, 2015), and is used in traditional medicines for the cure of various ailments including gastric ulcers, diabetes, rheumatism, toothache and alopecia (Tolan *et al.*, 2004). These secondary metabolites confer antioxidants, anti-inflammatory, anti-diabetic, antihypertensive, and anti-mutagenic properties (Chen and Kang, 2013; Tundis *et al.*, 2013). Worldwide production of chilli was estimated to be 7 million tons from an area of 1.5 million ha (Geetha and Selvarani, 2017), while in Pakistan, it is cultivated over

38.4 thousand hectares with the production of 53.7 thousand tones, with the average yield of 1.7 tons/ha (Hussain and Abid, 2011). About 99,699 tonnes of chilli production comes from the province of Sindh, while the contribution of other provinces is 15% only in Pakistan (Khan, 2017).

Chillies are challenged by various biotic (microbial contaminations of fungi, bacteria, nematodes, and viruses) and abiotic (pesticide residues, environmental stress, deteriorated capsaicin, and damaged color values) factors. In Pakistan, its yield declined in the previous decade due to poor cultural practices, low seed quality, and other production constraints including diseases and pest attacks (Khan, 2017). Among all the diseases, wilt disease caused by *FOC* is the most destructive one. Its pathogen is a soil born that prevails almost in all chill producing areas. Worldwide losses due to Fusarium wilt (FW) of chilli were estimated at $65300.00 million. Moreover, its incidence has been reported up to 98% with a yield loss of 100kg/ha (Alegbejo *et al.*, 2006). In Pakistan, the incidence of FW was reported up to 21.9% in farm vegetables from which 16 to 20% is reported only in hot pepper (Shafique *et al.*, 2015). A previous estimate about the yield losses due to wilts in Pakistan recorded 90-115 thousand tones during 1999-2005 (Hussain and Abid, 2011).

Development of yellow leaves, stunted growth of the plant, and continuous decaying until the whole plant wilts, stem discoloration, and girdling in infected plants at the base are the characteristic symptoms of FW, which can be easily seen on infected plants (Suryanto *et al.*, 2010). The infection causes vessel clogging in the plants which are closely resembled to nutritional deficiency, hence the translocation of minerals is reduced to its minimum extent (El-Kazzaz *et al.*, 2008). The development of *Fusarium* is largely dependent upon its microclimate in the soil, presence of moisture favors the successful establishment of FW and its growth in chilli, while in comparison, clayey loam soil is more efficient for the spread of disease because of its high water holding capacity (Latiffah *et al.*, 2009). Similarly, temperature influences the proliferation of pathogen below the soil, but high temperature cast a negative effect on the growth of pathogens (Suárez-Estrella *et al.*, 2003).

Fusarium infection cannot be easily restricted in soil because of its high inoculum level and longer survival ability. It can be quickly controlled with the application of fungicides but this approach is not eco-friendly and adversely affects human beings' livestock and the environment with its toxic residues (Sundaramoorthy *et al.*, 2012). Chemical control is always associated with its harmful effects, it destroys the insect fauna of soil and also develops fungicide resistance due to its frequent application (Hahn, 2014). The use of antagonists in a developing country, like Pakistan, is not practicable in large fields due to its non availability and higher costs (Tanyolaç and Akkale, 2010). The microorganisms isolated from suppressive soils can protect the plants against the FW disease, but this method is time consuming (Veloso and Díaz, 2012). Hence, there is a dire need to find an environment friendly solution for the management of FW in chilli crop. The use of resistant cultivars is a superlative approach for early control measures that can minimize yield losses and is a vital part of integrated disease management (Shahzaman *et al.*, 2015; Rahoo *et al.*, 2017). It is long term and environment friendly which takes less time than variety development. So, it is essential to conduct frequent field trials to search out resistant cultivars with a better yield (Kassi *et al.*, 2018). Thus, in the current research, the objective was to find the source of resistance among indigenous and exotic germplasm against *Foc*.

MATERIALS AND METHODS

Isolation of pathogen *Foc*

Symptomatic plants of Fusarium wilt were collected randomly from the field area of the Department of Plant Pathology, University of Agriculture, Faisalabad (UAF), and brought to the laboratory. Roots of infected plants were rinsed with distilled water, surface sterilized by using 1% NaOCl solution, chopped into small pieces, folded on filter paper to remove excess moisture, and placed in Petri dishes (9 Cm, 100 mm× 15mm) containing Potato Dextrose Agar (PDA) medium. These Petri plates were incubated at 25 ℃ ±2 until the fungal growth appeared (Sarwar *et al.*, 2005). Purified fungal colonies were identified under a stereomicroscope (Frederiksen-30B) and their characteristics were matched with literature (Soesanto *et al.*, 2011). Colony color was white to pale violet, macroconidia were abundant, medium to short length, and thin walled with mostly three septations, while microconidia were with no septation and oval to elliptical (Leslie and Summerell, 2008). After confirmation, the culture of *Foc.* was multiplied by shifting the fungus on pure PDA media plates, incubated at 25 ℃ ±2, and then preserved in the refrigerator (Hotpack corporation, Philadelphia, PA. 19154 USA, model no. 552620) at 4 °Cuntil further use (Ignjatov *et al.*, 2015).

Establishment of sick field

Two susceptible chilli varieties, namely Desi and Maxi were transplanted in an experimental field under an area of 300m². Before transplantation, the experimental plot was sprayed with inoculum by two consecutive applications of *Foc* with an interval of 10 days. The fungal inoculum was prepared by using a 7days old culture of *Foc* and the spore density was adjusted to1×10⁶ spores/mL of water by using a hemocytometer (Neubauer, Precicolor HBG, Germany). After transplantation, plants were inoculated at the root zone through injection (5cc, 21G×1.5) to ensure the establishment of inoculum in the field. After the onset of disease symptoms on the inoculated plants, the samples were taken and Koch's postulates were fulfilled for the confirmation of *Foc* (Naik *et al.*, 2008). After confirmation, the diseased plants were ploughed into the soil and irrigated to create favorable conditions for fungal growth.

Collection of chilli germplasm for the establishment of screening nursery against *Foc.*

Seeds of twenty five chilli varieties/advanced lines, i.e., Amber F1, Glory F1, Revival, Tejal, Big Red AB, Patiala F1, SB 6864-HM, BSS-410, Super hot, Silky Red, Green King, BPVLC 14-1, HOT 701, Omega, Hotshot, Angel F1, Super king, PH-275, Super Sky, Uttal, GHHP 01, Galaxy-2, Big Daddy, HPO33 and Fengaio were taken from the VRI, AARI, Faisalabad, Pakistan. Seeds were sown in the beds of 3m². At three leaf stage, it was shifted to the sick field for identifying the source of resistance against *Foc* under randomized complete block design (RCBD), where plant to plant (P×P) distance was 20 cm and row to row (R×R) distance was maintained at 60 cm.

Status of chilli germplasm for relative resistance and susceptibility to *Foc.*

After the successful establishment of thirty days old chilli nursery and its transplantation in the sick field area having sandy loam soil, data regarding disease incidence was regularly recorded for the development of disease symptoms by using a disease rating scale from 0 to 5 given by Abdel-Monaim and Ismail (2010), where 0= 0% disease incidence is immune and indicated with the symbol of (I); 1= 1-20% resistant (R); 2= 21-40% Moderately resistant (MR); 3=41-50% Moderately susceptible (MS); 4=51-70% Susceptible (S); and 5=71-100% highly susceptible (HS). Disease incidence was calculated by the following formula;

$$\text{Disease incidence (\%)} = \frac{\text{Total number of infected plants}}{\text{Total number of plants}} \times 100$$

Statistical analysis

All the statistical tests were performed using SAS/STAT statistical software (SAS Institute, 1990). The data were subjected to analysis of variance (ANOVA) at the 5 % level of significance. Fisher's least significant difference (LSD) test was used for statistical comparison among treatments (Steel *et al.*, 1997).

RESULTS

Status of chilli advanced lines against Fusarium wilt disease in 2017-18 and 2018-19

During the first year, no variety was found immune but one advanced line (BPVLC 14-1) was found resistant with 1-20% disease incidence, eight varieties/advanced lines (HPO33, Super sky AB, PH-275, GHHP 01, Galaxy-2, Big Daddy, Fengaio and Uttal) expressed moderately resistant response with disease incidence 21-40%, while five varieties/advanced lines (Hot 701, Hot Shot, Omega, Silkey Red and Super king) showed moderately susceptible response with 41-50% disease incidence, four advanced lines (Super hot, Patiala F1, Angel F1, and Green King) exhibited susceptible response with 51-70% disease incidence at the scale, seven varieties/advanced lines (Tejal, BSS-410, Glory F1, Big Red AB, Amber F1, Revival, and SB6864-HM) were found highly susceptible with 71-100% disease incidence (Table 1).

The results of 2nd year regarding disease incidence exhibited significant differences among the varieties/advanced lines (Table 2). Minimum disease incidence was recorded in advanced line BPVCL 14-1 and was categorized as resistant with disease incidence 1-20% at the scale, nine varieties/advanced lines (Fengaio, Super hot, Glaxy-2, Uttal, Super Sky AB, HPO33, PH-275, Big Daddy and Super king were found moderately resistant with disease incidence 21-40%. Four advanced lines (Big Red AB, Hot 701, Omega and Silky Red) exhibited moderately susceptible response with disease incidence 41-50%. Susceptible response was observed in four advanced lines (Glory F1, Angle F1, Hot Shot and GHHP 01) with disease incidence 51-70% at the scale. Maximum disease incidence 71-100% was exhibited by seven advanced lines (Green king, Tejal, BSS 410, Patiala, Amber F1, Revival, and SB 6864-HM) (Table 2).

Table.1 Response of chilli varieties/advanced lines against Fusarium wilt during 2017-18.

Sr. #	Varieties/lines	Disease incidence (%)	Disease rating	Response
1	Tejal	97.767 a	5	HS
2	BSS-410	83.100 b	5	HS
3	Glory F1	78.900 bc	5	HS
4	Big red AB	76.267 cd	5	HS
5	Amber F1	74.067 cd	5	HS
6	Revival	71.500 d	5	HS
7	SB 6864-HM	71.400 d	5	HS
8	Super hot	63.433 e	4	S
9	Patiala F1	63.367 e	4	S
10	Angel F1	54.333 f	4	S
11	Green king	52.967 f	4	S
12	Hot 701	45.333 g	3	MS
13	Omega	44.533 g	3	MS
14	Hot shot	44.533 g	3	MS
15	Silky red	43.467 g	3	MS
16	Super king	40.733 gh	3	MS
17	HPO33	37.333 hi	2	MR
18	Super sky AB	35.500 hi	2	MR
19	PH-275	34.933 i	2	MR
20	GHHP 01	34.433 i	2	MR
21	Galaxy – 2	28.167 j	2	MR
22	Big daddy	25.567 jk	2	MR
23	Fengaio	24.700 jk	2	MR
24	Uttal	22.500 kl	2	MR
25	BPVLC 14-1	18.767 l	1	R

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD = 2.011, where test value ($P \leq 0.05$).

Table 2 Response of chilli varieties/advanced lines against Fusarium wilt during 2018-19.

Sr. #	Varieties/lines	Disease incidence (%)	Disease rating	Response
1	Tejal	94.200 a	5	HS
2	BSS-410	85.433 b	5	HS
3	Big red AB	76.400 c	5	HS
4	SB 6864-HM	76.333 c	5	HS
5	Glory F1	75.233 c	5	HS
6	Revival	75.167 c	5	HS
7	Amber F1	74.300 c	4	HS
8	Patyala F1	65.500 d	4	S
9	Super hot	64.767 d	4	S
10	Angel F1	55.333 e	4	S
11	Green king	53.333 e	4	S
12	Silky Red	45.500 f	3	MS
13	Hot 701	45.100 f	3	MS
14	Hot shot	44.867 f	3	MS
15	Omega	43.667 fg	3	MS
16	Super king	39.833 gh	2	MR
17	HPO33	36.100 hi	2	MR
18	PH-275	33.500 i	2	MR

19	GHHP 01	33.433 i	2	MR
20	Super sky	33.433 i	2	MR
21	Glaxy – 2	27.167 j	2	MR
22	Big daddy	26.500 jk	2	MR
23	Uttal	22.300 kl	2	MR
24	Fengaio	21.333 l	1	MR
25	BPVLC 14-1	16.033 m	1	R

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD = 2.011 test (P ≤ 0.05).

DISCUSSION

Fusarium wilt of chilli caused by *Foc* is a critical soil born disease which causes complete failure of crops in susceptible varieties during favorable temperature and humidity. There are several management practices adopted by the farmers to overcome the disease caused by *Foc*, but obviously, these practices are costly and have other constraints like harmful effects on human health and are non-eco-friendly on long term basis. Hence, the only instant way is screening of available gene pool to identify the sources of resistance to combat *Foc.* of chilli crop.

Results of previously carried out experiments showed that resistance exists in some genotypes of chilli; so there is a need to screen and identify such sources of resistance. Bajwa *et al.* (2004) screened 33 chilli pepper varieties/advance lines against Fusarium wilt where they observed only two advanced lines (10549 and 10553) showed resistant behavior. Similarly, thirty varieties of chilli pepper were evaluated by Joshi *et al.* (2012) against Fusarium wilt and only two varieties/advanced lines namely (CO- 4 and DLC- 352) exhibited resistant response. They also observed the growth parameters of germplasm and found maximum root length and shoot length in moderately resistant advanced line named G_4 Hot pepper which justifies the results of the current study.

In resistant plants, the activity of enzymes such as cytochrome oxidase, polyphenol oxidase, and peroxidase is boosted up to a certain level as compared to susceptible plants. These enzymes give rise to certain biochemical reactions which impede the establishment and multiplication of pathogen (Ochoa-Alejo and Gómez-Peralta, 1993). Shafique *et al.* (2018) investigated the biochemical profile of most susceptible (Ghotki) and resistant (Dandicut) varieties of chilli to identify the markers of resistance. Results concluded that tannins, phenolic compounds, flavonoids, saponins, and coumarins were higher in quantity in resistant variety as compared to susceptible. Similarly, defense related enzymes including peroxidize, phenyl ammonia lyase, polyphenols oxidase, and superoxide dismutase were also in an elevated amount in resistant variety in comparison (Shafique *et al.*, 2018).

Inheritance characteristics (Yield, size and shape of fruit, plant height, root length, and shoot length) are desirable characteristics of resistant varieties and also play a very important role in the selection for breeding material. The yield of resistant varieties is not always higher than susceptible varieties. Manu *et al.* (2014) experimented to observe the characteristics of resistant breeding material. One resistant parent P_3 was crossed with three susceptible parents namely SNK, RAJPOOT, and KA_2. All the hybrids (P_3×SNK, P_3×Rajpoot, and P_3×KA_2) showed resistant behavior with pendant fruit direction and vigorous shoot health which also justifies the results of the current study (Manu *et al.*, 2014). Plants have R genes (resistance genes) which trigger resistance in plants to a specific pathogen, i.e., bacteria, viruses, fungus, or nematode. These genes produce proteins that recognize the pathogen effectors. R genes PR2a, Osmotin-like PR5, acidic glucanase, Chitinase 3 and Metallothionein have higher expressional rates as compared to susceptible varieties (Shafique *et al.*, 2018). Repeated cultivation of resistant variety in the same geographical area sometimes also gives rise to aggressive strains of the pathogen. Screening of different gene pools to identify the sources of resistance is helpful to incorporate them into breeding programs and to improve the cultivars to the desired level. So the evaluation of resistant germplasm against *Foc* is the finest solution to combat the disease.

CONCLUSION

One advanced line (BPVLC 14-1) was found resistant sand nine advanced lines/varieties (Uttal, Fengaio, Galaxy-2, Big daddy, GHHP 01, PH-275, Super sky AB, HPO33, and Super king) exhibited moderately resistant response against wilt disease caused by *Foc.* Therefore, these advanced lines/varieties are recommended to include in further breeding programs for the development of resistant and high yielding cultivars.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.