Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 879-884

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-7 pp. 879-884**
Journal homepage: http://www.ijcmas.com

Original Research Article

Screening of M_4 Mutants of Chilli (*Capsicum annuum* L.) against *Fusarium* wilt (*Fusarium solani*) Resistance

Shobha[1*], B. V. Tembhurne[1], M. K. Naik[2], Hasan Khan[1] and B. V. Patil[3]

[1]Department of Genetics and Plant Breeding, College of Agriculture, UAS, Raichur, India
[2]Department of Plant Pathology, University of Agricultural and Horticultural Sciences Shivamogga, India
[3]Department of Entomology, College of Agriculture, UAS, Raichur, India
**Corresponding author*

A B S T R A C T

Chilli (*Capsicum annuum* L) is an important spice crop, it is being used as a source of vegetables, spice, colourant and for some medicinal applications. India is the largest exporter of chilli in the world and contributes one-fourth of the total quantity of chilli exported in the world. The crop suffers from many pests and diseases. Among them wilt, anthracnose or fruit rot or die back, murda complex, leaf spot, and powdery mildew are major threat in its production. Chilli wilt caused by *Fusarium solani* is becoming more serious in chilli growing tracts of India and also in Karnataka particularly in black cotton soils leading up to 25 per cent yield loss. The incidence of wilt varied from 0 to 75 per cent in different states of India. To identify the resistant genotypes against *Fusarium* wilt disease, several genotypes have been undertaken for mutant studies, among 216 chilli (*Capsicum annuum* L.) mutants 20 genotypes were selected in M3 generation along with 5 checks namely P3 as resistant and Byadagi dabbi, Byadagi kaddi, Namdhari and G-4 as susceptible in green house condition. The rapid root-dip-transplanting technique has been employed in screening of resistant sources. The selected 20 genotypes were selfed in M3 generation along with 5 checks and screened for *Fusarium* wilt resistance in M4 generation. Among them 8 genotypes showed resistant reaction, 6 genotypes were found to be moderately resistant and 7 genotypes registered susceptible reaction. However, 4 genotypes recorded the highly susceptible reaction. None of the genotypes were found to be immune among the tested M4 population.

Keywords

Chilli, Mutagenic population, *Fusarium* wilt, resistance

Introduction

Chilli (*Capsicum annuum* L.) is a spice cum vegetable crop belongs to the family Solanaceae. The genus is native to Central and South America (Pickersgill, 1991) and includes the species *C. chinense, C. baccatum, C. frutescens, C. pubescens and C. annuum.* Among these five species, *C. annuum* is the most important one because it is cultivated in both tropical and temperate area in the world and it is the most versatile of the five species. In contrast, the other four species are cultivated in limited regions in the world or only in tropical areas and they are mainly used as spices. Chilli is grown in almost all the countries covering an area of 1.9 m ha with a production of 34.5 MT with a productivity of 17.5 tonnes per hectare (Anonymous, 2014). India is the major and highly erratic producer, consumer and exporter of chilli, covering an area of 0.76

million hectare with a production of 1.61 MT averaging a productivity of 2.1tonnes per ha (Anonymous, 2015).

India is the largest exporter of chilli in the world and contributes one-fourth of the total quantity of chilli exported in the world. But China is not far behind and it has been posing a severe competition to the Indian exporters due to India's variable supply and high domestic consumption**.** In India, chillies are grown in almost all the states. Among all the states Andhra Pradesh is the largest producer of chilli in India covering an area about 0.13 million hectare and production of 0.73 Mt followed by Maharashtra and Karnataka and covering an area of 0.09 and 0.08 m ha with a production of 0.04 and 0.11 MT respectively. Similarly the production and productivity of other states are like Odisha (0.07 million hectare and 0.07 million hectare), Madhya Pradhesh (0.05 million hectare and 0.13 MT), and Tamil Nadu (0.05 million hectare and 0.01 MT) (Anonymous, 2015). India has the potentiality to increase the production in order to promote export in meeting its domestic requirements. However, despite of continuous efforts at various levels, the chilli productivity did not gain momentum.

This could be attributed to a number of limiting factors of which, lack of superior genotypes/varieties for further development of superior high yielding cultivars (or) hybrids. Further, the crop suffers from many pests and diseases. Among chilli diseases wilt, anthracnose or fruit rot or die back, murda complex, leaf spot, and powdery mildew are major problems. Chilli wilt caused by *Fusarium solani* is becoming more serious in chilli growing tracts of India (Singh *et al.,* 1998) and also in Karnataka particularly in black cotton soils leading up to 25 per cent reduction in yield (Madhukar and Naik, 2004).

Host plant resistance has been a choice in all crop improvement programmes and is perhaps the best method available to tackle the soil borne diseases in particular. *Fusarium* wilt is a typical soil borne disease which can be mitigated appropriately by use of disease resistant cultivars. Further the use of resistant variety will go a long way not only in reducing loss due to disease but also in avoiding fungicidal toxicity likely to occur due to their application to soil, and some of the morphological characters helps in yield improvement. Hence, mutagenic studies on *Fusarium* wilt (*Fusarium solani*) resistance is very much useful for further breeding programme in chilli.

The pathogen being typically soil-borne and is very difficult to achieve economic control of the disease through other means. Literature pertaining to this indicated that there is a need to take up the resistance breeding programme. History of success in various soil-borne diseases indicated that the host plant resistance or developments of resistant hybrids/varieties are the appropriate strategies to overcome the wilt of chilli. Keeping these points in view, the investigations were carried out to identify *Fusarium* wilt disease resistant genotypes among the mutated generation.

Materials and Methods

Two hundred sixteen mutants of M_3 generation along with genotype P3 as resistant and Byadagi dabbi, Byadagi kaddi, Namdhari and G-4 as susceptible checks were used to carry out investigations. Experiment was carried out at College of Agriculture, Raichur, by using Completely Randomised Design (CRD) design. Seeds of chilli resistant to *Fusarium* wilt *i.e* P3 was treated using 3 different doses of Ethyl Methyl Sulphonate (EMS) with concentration of 0.5 %, 1.0% and 1.5 % with

control during 2013-14. M3 population has been generated by repeated selfing and screening. Two sixteen plants from M3 population were selected and raised in sick plot (cement ring of size 90cm diameter and 30cm height) along with P3 (resistant parent) and susceptible checks *viz.*, Byadgi dabbi, Byadagi kaddi, Namdhari and G-4. Among 216 plants, 20 plants shown resistance based on superior parameter and were selected for *Fusarium* wilt screening. The causal pathogen was isolated from the diseased root samples of chilli, after surface sterilization and were aseptically transferred to potato dextrose agar (PDA) medium in Petri-plates and incubated at 25 ± 2 ^{o}C for 7 days. The isolates were purified by singal spore and hypal tip isolation methods. The pathogen was identified with the help of morphological/cultural characteristics.

The fungus *F. solani* isolations were sub cultured on PDA (fig.1) petriplates and allowed to grow at 28 ± 1 ^{o}C for fifteen days. Such petriplates were preserved in refrigerator at 5 ^{o}C and maintained by sub culturing once in a month and culture was used for future study. The fungus was mass multiplied on potato dextrose broth (PDB). 100 ml of PDB was taken into 250 ml Borosil conical flask and autoclaved at 1.1 kg/cm^2 (121.6 ^{o}C) 15 lb pressure for 20 minutes. The mycelial disc cut from the margin of a week old culture grown on petri dish was inoculated into PDB under aseptic conditions. The flasks were incubated at 28 ±1 ^{o}C for 15 days. The mycelial mat were collected after 15 days by filtering with Whatman No. 42 filter paper disc of 12.5 cm diameter and washed with sterile water and used for further experiments. The spore suspension was prepared using waring blender to disturb the spores in sterile water and filtered through cheesecloth before use and spore load ml^{-1} was computed by using a Haemocytometer.

Rapid root dip transplanting technique method was developed by Naik *et al.*, (1996) was followed during the study. Chilli seedlings were raised in a plastic trays containing sterilized sand in a nylon net house and protected with two insecticidal sprays of Melathion (0.1%) and Monocrotophos (0.05 %) to prevent the viral disease.

Three weeks old seedlings were uprooted carefully without damaging the roots after watering the pots, roots thoroughly washed in running tap water and three mm tip of roots were cut and immersed in spore suspension of *F. solani* (fig. 2) for 45 min and planted in a plastic cups containing sterilized soil (fig.3). About 15 days after transplanting again, *F. solani* spore suspension was poured to the soil to enrich the inoculum level. In the cases where isolates produced typical wilting symptoms, the fungus was successfully re-isolated and Koch's postulates were proved.

Results and Discussion

Host plant resistance has been an apt choice in all the crop improvement programmes and is essential to recommend the cultivars directly for cultivation in endemic area, infested with soil borne fungus, although the resistant variety is always one of the best way and will go a long way in reducing loss due to wilt.

In order to identify the resistant genotypes against *Fusarium* wilt, 20 genotypes were selected in M3 generation along with 5 checks namely P3 as resistant and Byadagi dabbi, Byadagi kaddi, Namdhari and G-4 as susceptible in green house condition. The method employed for screening resistant sources was rapid root-dip-transplanting technique as described in material and methods.

Fig.1 Culture of *Fusarium solani* on PDA Culture of *F. solani* on PDA

Fig.2 Root dipping in *Fusarium solani* inoculum

Fig.3 Transplanting of pre inoculated seedlings

Fig.4 Susceptible genotype showing wilting symptom

Fig.5 Resistant genotype

Table.1 List of chilli mutants/genotypes showing resistance reaction against *Fusarium* wilt

Sl. No.	Per cent infection	Mutants/Genotypes	Total number of Mutants/genotypes	Remarks
1	0	Nil	0	Immune
2	1-10	P3 (resistant check) M -379, M -250, M -362, M -395, M -441, M -296, M -319	8	Resistant
3	11-25	G-4, M -281, M -315, M -371, M -370, M -262	6	Moderately Resistant
4	26-50	M -270, M -294, M -309, M -351, M -384, M -298, M -305	7	Moderately Susceptible
5	51-100	Byadagi dubbi, Byadagi kaddi, Namdhari, (susceptible checks) M-357	4	Susceptible

Within 5-7 days of inoculation a typical wilting symptom was noticed. Disease symptoms were characterized by an initial slight yellowing of the foliage and wilting of the upper leaves that progress in a few days into a permanent wilt with the leaves still attached. On the young seedlings, initially water soaked areas developed at the collar region and a brown sunken lesion which soon appeared as girdled resulting in seedling collapse where underground stems were found dry, brown but the roots were soft and the vascular system of the lower stem was discoloured and the infected plant ultimately wilted.

The selected 20 genotypes were selfed in M3 generation along with 5 checks and screened for *Fusarium* wilt resistance in M4 generation. Among them 8 genotypes showed resistant (fig.5) reaction *viz.*, P3, M -379, M-250, M-362, M-395, M-441, M-296 and M- 319, 6 genotypes *viz*., G-4, M-281, M -315, M-371, M-370 and M-262 were found to be moderately resistant and 7 genotypes *viz*., M-270, M-294, M-309, M-351, M-384, M-298 and M-305 registered susceptible reaction. However, Bydagi dabbi, Byadagi kaddi, Namdhari and M-357 recorded the highly susceptible reaction (fig.4). None of the genotypes were found to be immune among the tested M4 population which is shown in Table1. Such resistant responses of chilli genotypes against *Fusarium* wilt disease was earlier observed by Ahmed *et al.*, (1994), Nayeema *et al.*, (1995), Singh *et al.*, (1998), Joshi *et al.*, (2012), Devika Rani *et al.*, (2008) and Akram *et al.*, (2014). Similar type of response were observed by several workers (Singh *et al.*, 1996; Singh *et al.*, 1997 and Silme and Ilhan, 2010) in different crops such as watermelon, pigeon pea, chickpea, cumin, potato and linseed *etc*.

From the investigations carried out during study, mutation breeding is an important aspect in disease management practice when compared to all other means. So the resistance genotypes can be further used for selection and stabilizing suitable genotypes against *Fusarium* wilt resistance breeding programme in chilli (C*apsicum annuum* L.).

Acknowledgement

All the authors acknowledge their heartfelt gratitude to UAS, Raichur for the financial support extended for conducting the present investigation.

References

Ahmed, N., Tanki, M. I. and Mir, N. M., 1994. Screening of advance breeding lines of chilli and sweet and hot pepper cultivars against *Fusarium* wilt. *Pl. Dis. Res.,* 9(2): 153-154.

Akram, W., Tehmina, A. and Aqeel, A., 2014. Basal susceptibility of tomato varieties against different isolates of *Fusarium oxysporum* f. sp. *lycopersici. Int. J. Agric. Biol.,* 16(1): 171-176.

Anonymous, 2005. *Annu. Rep. Network project on wilt of crops* submitted to ICAR, New Delhi, p. 7.

Anonymous, 2014. www.FAO.com

Anonymous, 2015. www.Indiastat.com

Devika Rani, G. S., Naik, M. K., Patil, M. B. and Patil, M. G., 2008. Screening of chilli genotypes against *Fusarium* wilt caused by *Fusarium solani* (mart.) sacc. *J. Veg. Sci*., 35(1): 49-54.

Joshi, M., Srivastava, R., Sharma, A. and Anil, P., 2012. Screening of resistant varieties and antagonistic *Fusarium oxysporum* for biocontrol of *Fusarium* wilt of chilli. *J. Pl. Pathol. Microbiol*, 3(5): 134.

Madhukar, H. M. and Naik, M. K., 2004. Evaluation of bioagents against *Fusarium* wilt of chilli *Capsicum annuum*). In: Proc. 15th International Plant Protection Towards 21st Century held in Beijing, China. pp. 540.

Naik, M. K., Pramanick, K., Deshpande, A. H. and Sinha, Poonam, 1996. Standardization of screening technique against *Fusarium* wilt of chilli. National Symposium of *Indian Soc. Mycol. Pl. Pathol*. held at Shantiniketan, West Bengal., pp. 113-114.

Nayeema, J., Ahmed, N., Tanki, M. I. and Das, G. M., 1995. Screening of hot pepper germplasm for resistance to *Fusarium* wilt [*F. pallidoroseum* (Cook) Sacc.]. *Capsicum and eggplant Newsletter,* 14: 68-71.

Pickersgill, B. 1991. Cytogenetics and evolution of *Capsicum* L. In: Tsuchiya, T. and P.K. Gupta (eds.) Chromosome Engineering in Plants: Genetics, Breeding, Evolution. Part B, Elsevier, Amsterdam, pp. 139–160.

Silme, R. S. and Ilhan, M. C., 2010. Screening for resistance to *Fusarium* wilt in induced mutants and world collection of sesame under intensive management. *Turkish J. Field Crops,* 15(1): 89-93.

Singh, A., Singh, A. K. and Singh, A., 1998. Screening of chilli germplasms against *Fusarium* wilt. *Crop Res.,* 15: 132-133.

Singh, D. K., Jha, D. K. and Haque, M. F., 1997. Field screening chickpea cultivar against *Fusarium* wilt. *J. Res.,* 9: 201-202.

Singh, R. P., Harichand. S. and Chand, H., 1996. Identification of multiple disease resistance in chickpea at Hissar, Haryana. Int. *Chickpea Pigeonpea Newsletter,* 3: 32-33.