African Journal of Basic & Applied Sciences 3 (5): 219-222, 2011
ISSN 2079-2034

PCR Based Method for Testing Fusarium Wilt Resistance of Tomato

Pritesh Parmar and R.B. Subramanian

BRD School of Biosciences, Sardar Patel University, Vallabh Vidyanagar-(Gujarat) India

Abstarct: Identification of the resistance variety amongst the population in minimum time limits is crucial. Resistance to Fusarium wilt of twenty five tomato varieties was compared between conventional (*in vitro* and *in vivo* assay) and the newly developed PCR based method. The PCR method used in the present study identifies the resistant entity in a day, comparison to conventional methods which may show false positive response due to environmental conditions in few days to month. This method is reproducible and shows a high correlation with the conventional method. It will be very useful to plant breeders to select wilt resistance in tomato.

Key words: Tomato · Fusarium wilt · Screening · Simple sequence repeats

INTRODUCTION

Tomato is considered as one of the world's most important and popular vegetables. Many constraints affect the productivity and quality of tomato among which disease play a salient role. Soil-borne fungal pathogens cause severe diseases of many important crops. Their control is mainly based on the application of toxic chemicals to the soil, thus representing a serious problem for the environment. *Fusarium oxysporum lycopersici*, the cause of the wilt disease of plants both in the field and in the greenhouse, is a well-studied pathogen of tomato plants. The identification and utilization of tomato plant varieties resistant to the disease represents a valid alternative to the use of chemicals.

Conventional method of breeding and selection for a high yielding disease resistant variety is a long process which requires many years. To speed up the screening process tools like *in vitro* and *in vivo* assay, molecular markers are required. Of course methods like *in vitro* and *in vivo* are being practiced since decades but that are time consuming, cumbersome, laborious and requires confirmation with an efficient methods like use of molecular markers. With this objective, the research aimed to develop a molecular method for the early screening of plants resistant to wilt to be used in breeding programs.

Race 1 type of *F. oxysporum f. sp. lycopersici* is normally being observed in different parts of the country in India. I-1 or I gene which is located on chromosome 11 is responsible for the resistance against race 1 type of fungus but still it is not being characterized and sequenced. Immense work has been carried out against the races of 2 & 3 all over the world because it is commonly being observed. RFLP and RAPD markers were developed against race 2 and 3 but no marker is available against race 1 except RFLP probe TG 194 but it requires application of extensive tool like southern hybridization. SSR markers are becoming the preferred molecular markers in crop breeding and genetic studies because of their properties of genetic co-dominance, high reproducibility and multiallelic variation. Thus microsatellite marker developed as per [1] was employed in the present study for the identification of resistant varieties.

MATERIALS AND METHODS

Plant Material: The plant material for the study consisted of Twenty five varieties of tomato (*Solanum lycopersicon*) Table 1. Plants were raised in an open field (Anand agriculture university, Anand) around 50 seeds per entity with drip-irrigation and necessary care.

***In vitro* Bioassay:** In vitro bioassay was performed as per [2]. Leaves positioned laterally at the second node were collected from the apex of the sixty days old plants grown in open field washed under running water and

Corresponding Author: R.B. Subramanian, BRD School of Biosciences, Sardar Patel University, Post Box no. 39, Vallabh Vidyanagar-388120 (Gujarat) India. Tel:+91-2692-234402/234412. Fax: +91-2692-236475/237258, E-mail: rb_subramanian@spuvvn.edu.

Table 1: Screening and grouping of the tomato varieties by molecular marker, *in vitro* and *in vivo* analysis

No.	Code in this study	Name	Growth habit	Fruit	Origin	Evaluation
1	NDT-96	Narendra Dutta tomato	D	Medium, round, slightly pointed stigmatic end	Faizabad, UP	R
2	AND-1	Anand-1	D	Medium, round	Unknown	S
3	GT-1	Gujarat-1	D	Medium, Round	JAU, Junagadh	P
4	GT-2	Gujarat-2	D	Medium, Heart shaped, Dark red	AAU, Anand	P
5	PR	Pusa ruby	I	Medium, Flat	IARI, New Delhi	S
6	JR	Junagadh ruby	D	Medium, round	JAU, Junagadh	S
7	Heamsona	Heamsona	I	Medium, oblate, firm	Novartis seed, Pune	R
8	CO-3	Marutham	D	Round, capsicum red	NBPGR, New Delhi	S
9	ARTH-3	ARTH-3	D	Oval round, deep red	Ankur seeds, Nagpur, Maharashtra	P
10	DVRT-2	Kashi Anupam	D	Large, flatish round, slightly indented at blossom end of fruit	IIVR, Varanasi	P
11	Feb-4	Feb-4	D	Medium, round	Unknown	P
12	Sel-18	Hissar Lalima	D	Round, Large, Fleshy	HAU, Hissar	P
13	KS-118	KS-118	D	Medium, round	NBPGR, New Delhi	P
14	SL-120	SL-120	Semi-D	Large, round, fleshy, less acidic, less seeded	IARI, New Delhi	P
15	Sel-7	Hissar Arun	D	Round, deep red, Medium- large	HAU, Hissar	P
16	BT-20	Bacterial wilt resistant	I	Medium, round	OUA&T, Bhubaneswar	P
17	Avinash-2	Avinash-2	Semi-D	Medium, square, round	Novartis seed, Pune	P
18	Arka Vikas	Arka Vikas	Semi-D	Medium, oblate	IIHR, Bangalore	P
19	ATL-4/53	ATL-4/53	D	Medium, round	Unknown	S
20	Maha-2	Maha-2	D	Medium, round	Unknown	S
21	Namdhari-2535	Namdhari-2535	D	Medium, Oval	Namdhari seeds pvt. ltd. Bangalore	P
22	Cherry	Cherry	D	Small, round	New Delhi	P
23	KS-17	KS-17	D	Medium, round	NBPGR, New Delhi	P
24	TBK-2/39	TBK-2/39	D	Medium, round	Unknown	R
25	Wild	LA-3042/3475/ GP-10	I	Medium, oval	U.C. Davis, USA	R

I=Indeterminate, D=Determinate, S= Susceptible, P=Partial resistant, R=Resistant

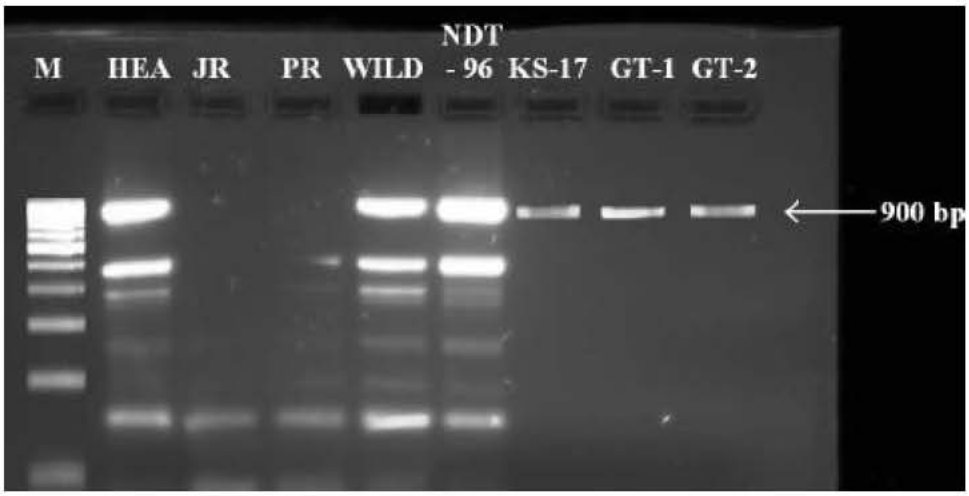

Fig. 1: Highly polymorphic profile obtained with use of SSR-67 primer. Resistant cultivars like Wild (GP-10), NDT-96 and Heamsona showed alleles of size 900 +900 in comparison to tolerant cultivars KS-17, GT-1and GT-2 and Null allele with susceptible cultivars JR and PR

surface sterilized by dipping in to 1% sodium hypochlorite solution for 3 minutes, followed by sterile distilled water rinse. Leaves of all the varieties were then punched to a 1 cm disc and transferred to petri dishes containing 1% basal agar medium with the help of sterile forceps under aseptic conditions. For each varieties two set of Petri

dishes were kept, the experimental set of Petri dishes were flooded with 15X Fungal culture filtrate solution and control set were flooded with potato dextrose broth. Plates were incubated at room temperature for 48 hrs.

In Vivo Bioassay: Field raised varieties were challenged with pathogenic material as per [3] and the symptoms were measured from 0-4 scale [4].

Genomic DNA Extraction: Genomic DNA was extracted in bulk from young fresh leaves of each variety, using the phenol - chloroform method [5] and quantified on a spectrophotometer (Unichem ά).

SSR Marker and PCR: SSR 67 a marker found to be linked to the fusarium wilt resistance according to Parmar *et al*., 2009 was used for the identification of resistant varieties among the tomato population.

PCR Reaction: Amplification was carried out in 12.5µl of reaction mixture, containing 7µl distilled water, 1.25µl of 10x assay buffer with 15mM $MgCl_2$, 2µl of 100ng template DNA, 1.5µl of primer, 1µl dNTP mix and 0.25µl Taq DNA polymerase. PCR was performed in a thermal cycler (Applied biosystem). The PCR profile starts with initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 30 sec. annealing as 54°C the primers for 45 sec. extension at 72°C for 45 sec. and finally extension at 72°C for 5 min. The products were size-separated on a 2.5% Agarose (High resolution Type I, Sigma Make) gel in 1×TAE buffer at 50Volt, stained with ethidium bromide (1 $\mu g \cdot ml^{-1}$) and visualized on a GelDoc (ά innotech).

RESULTS AND DISCUSSION

PCR based marker developed [1] was used for the screening of tomato varieties available at Anand Agriculture University Anand. On amplification of twenty five varieties with molecular marker only six varieties showed the presence of 900+900 alleles and fifteen varieties showed the presence of 900 allele. The varieties that showed the presence of 900+900 alleles and 900 allele are listed as resistant(R) and partial resistant (P) in Table 1 respectively. During evaluation six varieties could not showed the presence of 900+900 and 900 alleles so it is inferred that the varieties are null for the particular alleles and it is grouped in to the class susceptible(S).

RFLP probe TG-194 [6] for the detection of resistance variety against race 1 type fungus among the tomato population by southern hybridization and autoradiography. The method requires extensive tools and professional experts where as proposed method is simple and can be achieved without professional in minimum time period.

On classifications of tomato varieties in to three classes the varieties that were grouped in to particular class were analyzed by *in vitro* assay [2]. Analysis through conventional *in vitro* bioassay confirmed the findings obtained by the present method. Zhu *et al.* classified the tomato varieties in to susceptible and resistance against the late blight disease through *in vitro* studies after five days of incubation [7] where as Parmar *et al.* classified the tomato varieties after two days of incubation through *in vitro* assay [2]. In comparison to *in vitro* method the present method requires only four hrs.

Nelson *et al.* detected small difference in resistance of tomato to late blight according to leaf age, leaf & leaf let position and plant age through bioassay [8]. The proposed method does not affected by the environmental and physiological condition and the output is obtained with in a day.

The screening was also carried out by another conventional method that is *in vivo* assay to see the response of varieties when challenged with fungus under open environmental condition where it actually faces the problem. The response by each variety inferred that the varieties were correctly evaluated at lab conditioned but it took three months. The susceptible varieties showed the scale 4 that represents the severity of disease to 80-90%, resistant showed zero scale reading and partial resistant showed scale between 1-3 representing the severity of disease between 10-80% (Parmar *et al*., 2011). Zhu et al 2006 classified the tomato varieties against late blight after three months as the nature infection required plant to be under matured condition. In comparison to *in vivo* the present method require minimum time period to discriminate the resistant variety.

Both *in vitro* and *in vivo* methods were found to be efficient for the grouping of different varieties but it has limitation that it is tedious, time consuming and uneconomical. These methods are valuable when a small fraction of samples to be evaluated but it is not the case when comes to breeder where lots of varieties to be processed. Comparisons of molecular to *in vitro* and *in vivo* data produced generally similar conclusions of relatedness among accessions, confirming the utility of

molecular analysis. DNA produced useful marker patterns, given sufficient care and analysis gave strong evidence of major genetic differences among different tomato accessions in minimum time period with ease that would have not been achieved by *in vitro* and *in vivo* bioassay with ease, minimum labor, minimum budget and in minimum period of time.

In conclusion this method will be useful in screening of resistant varieties as well as evaluation of breeding lines in relation to fusarium wilt.

ACKNOWLEDGEMENTS

This work was supported by grants for scholarship to meritorious students from University Grants commission (UGC), Government of India. We would like to thank Professor. K. B. Kathiria and Dr. A. D. Patel from Anand Agricultural University, Anand for providing the different tomato varieties.

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