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Screening for tomato yellow leaf curl virus (TYLCV) resistance plants using caps marker

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Tomato yellow leaf curl virus resistant plants are screened using different molecular markers, but screening by cleaved amplified polymorphic sequences (CAPS) is most reliable and rapid method. In this DNA is isolated by using CTAB method from leaves of tomato plant. PCR amplification is done by using appropriate primers. After amplification, amplified DNA product are quantified and then restriction digestion is done by using taq-1 restriction enzymes for TY-1 and TY-2 genes. After running the restriction digested mixture on gel electrophoresis, bands for TY-1 resistance gene were seen at 303bp+95bp and TY-2resistance gene at 350bp+100bp.This method is most successful method for screening tomato yellow leaf curls virus resistant plants. The entire process is easy, convenient and reliable for rapid screening of large numbers of plants of tomato. The resistant genotypes procured from this high profile technique are then utilized in breeding programme.

Tomato is the world's largest vegetable crop and known as protective food both because of its special nutritive value and also because of its wide spread production. More than half of the total tomato production was from the six top producing countries: China, USA, Turkey, India, Egypt, and Italy (USDA-FAS 2007). Tomato yellow leaf curl virus (YLCV) causes severe stunting of young leaves and shoots, bushy growth of infected seedlings, stunted and excessively branched, leaves are curled upwards or inwards, flower drop is common, , fruits develop normally. "Markers are identifiable DNA sequences which carries specific gene of interest linked to it." Marker assisted selection (MAS) is indirect selection process where a trait of interest is selected not based on the trait itself but on a marker linked to it. In tomato plants the two genes namely TY1 and TY2 present on the chromosome number 6 and chromosome 11, respectively, provide resistance to tomato plants against tomato yellow leaf curl virus. These two genes present on two different chromosomes provide durable resistance against TYLCV. Cleaved amplified polymorphic sequences are a combination of the PCR and RFLP. The technique involves amplification of target DNA through PCR, followed by digesting with restriction enzymes. Hence CAPS markers rely on differences in restriction enzyme digestion patterns of PCR fragments samples.

The various plant samples of various species of tomato grown in different areas like open field, polyhouse, green houses and lab conditions were provided by the breeders. DNA extraction is done by CTAB method. CTAB method is most effective to remove polysaccharides and breakdown of protein lipid bond during isolation. PCR program perform several parallel reactions, the preparation of a master mix containing water, buffer, dNTPs, primers and Taq DNA Polymerase in a single tube, which can then be aliquoted into individual tubes. $MgCl_2$ and template DNA solutions were then added.

D] PCR and restriction conditions for different CAPS:- [A]TY-1 linked CAPS marker PCR reaction and restriction digestion conditions:.A set of primer for CAPS marker closely linked to with TY-1 gene was procured from Sigma Aldrich. The PCR reaction was carried out in a total volume of 25 ml containing:

Sr. No	Components	25 µl
1.	10X buffer	2.5 µl
2.	$MgCl_2$	1.5 µl
3.	dNTP's	2.5 µl
4.	Primer, F	2 µl
5.	Primer ,R	2 µl
6.	Template DNA	2 µl
7.	Taq DNA pol.	0.2 µl
8.	UPW	12.3 µ l

PCR program:

Initialdenaturation-94^{0}C for 3 mins [94^{0}C for 30secs, 58^{0}C for 1min, 72^{0}C for 2mins} X 35 cy cycles. Final extension-72^0 C for 10mins.

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Expected restriction product size-398bp. :

Sr. No.	Components	25 µl
1.	PCR product	9 µl
2.	Buffer	1.5µl
3.	BSA	0.15µl
4.	Taq 1	0.05µl
5.	UPW	4.3 µl

Restriction digestion condition:

The Restriction digestion is performed by incubating at 65^{0}C.

Expected restriction product size-

RR resistant -303bp+95bp

398bp+303bp+95bp

rr susceptible-398bp

Digestion products were analysed by agarose gel electrophoresis (1.5% agarose w/v with TBE buffer) and visualized by Goldstain view staining. All reagents employed were supplied by Fermentas and New England Biolabs.The gel was documented on gel documentation system (Synoptics) for digital storing.

Ty-2 linked CAPs marker PCR reaction and restriction digestion conditions:

A set of primer for CAPS marker closely with Ty-2 gene was procured from Sigma Aldrich. The PCR reaction was carried out in a total volume of 25 µl containing:

Sr. No.	Components	25 µl
1.	10X buffer	2.5 µl
2.	$MgCl_2$	1.5 µl
3.	dNTP's	2.5 µl
4.	Primer, F	2 µl
5.	Primer, R	2 µl
6.	Template DNA	2 µl
7.	Taq DNA pol.	0.2 µl
8.	UPW	12.3 µ l

PCR program:

Initial denaturation-94^{0}C for 3mins {94^{0}C for 30secs, 65^{0}C for 1min, 72^{0}C for 2mins} X 35 cycles final extension-72^{0}C for 5min.Expected amplification product Size- 450 bp

Restriction digestion conditions:

Sr. No.	Components	15 µl
1.	PCR product	9 µl
2.	Buffer	1.5µl
3.	BSA	0.15µl
4.	Taq 1	0.05µl
5.	UPW	4.3 µl

The Restriction digestion is performed by incubating at 65^{0}C.

Expected restriction product size:

RR Resistant -350bp+100bp

rr susceptible-250bp+100bp

Digestion products were analysed by agarose gel electrophoresis (1.5% agarose w/v with TBE buffer) and visualized by Gold view staining. All reagents employed were supplied by Fermentas and New England Biolabs.

The gel was documented on gel documentation system (Synoptics) for digital storing.

The results obtained from the present investigation are presented below:

Evaluation of digestion pattern of TY-1 linked CAPs primer for identifying homozygous resistant genotypes:

TY-1 linked CAPS primer was used for screening with the genotypes. The amplification product forms a band at 398bp. After restriction digestion with Taq1 enzyme few of the genotypes showed bands at location 303bp and 95bp; some showed at 398bp; some genotypes showed bands at 398bp, 303bp and 95bp (Fig. 1).

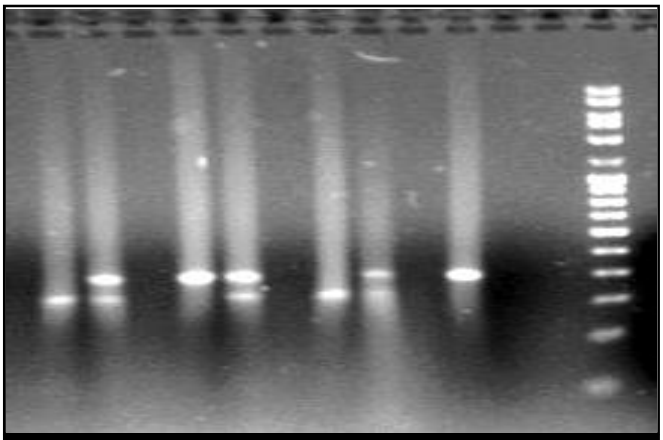

Fig.1 : Band pattern obtained after performing PCR with Ty-1 linked CAPS marker followed by restriction with Taq1(RR- Homozygous Resistant, rr- Homozygous Susceptible, Rr- Heterozygote, M- 100bp ladder)

Evaluation of digestion pattern of TY-2 linked CAPs primer for identifying homozygous resistant genotypes:

TY-2 linked CAPS primer was used for screening with the genotypes. The amplification product forms a band at 450bp. After restriction digestion with Taq 1 enzyme few of the genotypes showed bands at location 350bp and 100bp; some showed at 250bp and 100bp; some

genotypes showed two bands at 350bp and 250bp (Fig. 2).

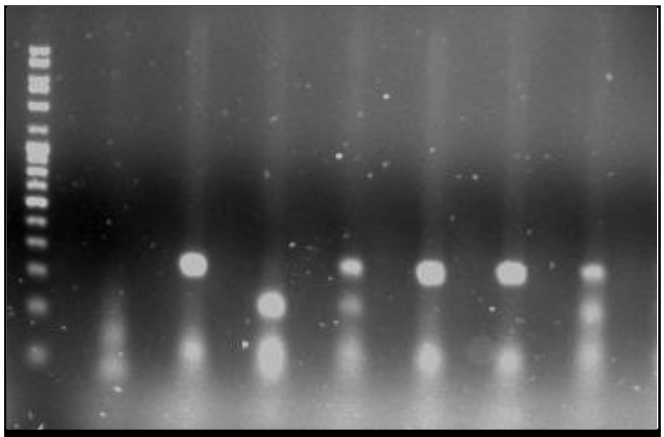

Fig.2 : Band pattern obtained after performing PCR with TY-2 linked CAPS marker followed by restriction with Taq1

Conclusion:

Tomato yellow leaf curl virus causes large scale devastation and losses of tomato crop all around the world and reduces the total production per/hectare and thus reduces the returns to the producer For screening of plant lines having two TYLCV resistance gene TY-1 and TY-2 MAS technique is highly employed. After performing the experiment to detect the presence of TY-1 and TY-2 in provided plant samples, plants having TY-1 and TY-2 are selected for breeding.

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