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

V.M. KAKADE, S.S. KARANDE, D.A. DHOKANE, R.M. JADHAV AND R.WADEKAR

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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.

Correspondence to:
S.S. KARANDE, Department of Plant Biotechnology, College of Agricultural Biotechnology, LONI (M.S.) INDIA
Authors' affiliations:
V.M. KAKADE, D.A. DHOKANE, R.M. JADHAV AND R. WADEKAR, Department of Plant Biotechnology, College of Agricultural Biotechnology, LONI (M.S.) INDIA