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Original Research Article

Studies on Challenge Inoculation for Combined Resistance to Tomato Leaf Curl Virus and Root Knot Nematode in Tomato (*Solanum lycopersicum* L.)

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A B S T R A C T

The objective of this study was to screen the two newly synthesized tomato F_1 hybrids *viz.*, CLN 2123A X HN2 and HN2 X CLN 2123A to find out the combined resistance to leaf curl virus and root knot nematode along with their parents and check varieties/hybrids. The experiment was conducted under glass house condition through artificial inoculation. The experiment was laid out in a randomized block design and replicated thrice. The results revealed that the newly synthesized hybrids CLN 2123A X HN2 and HN2 X CLN 2123A registered low level of percent of disease infection and co efficient of infection and these hybrids showed high values of defense enzymes *viz.*, peroxidase and poly phenol oxidase after graft inoculation indicated that these hybrids had resistance to tomato leaf curl virus disease. These two newly synthesized hybrids were on par with tomato leaf curl virus resistant check hybrid Lakshmi for tomato leaf curl virus disease incidence. The hybrid HN2 X CLN 2123A showed low incidence of root knot nematode by registering lower values of number of root knot nematode females (9.66), number of egg mass (5), root knot index (2) and high acid phosphatase enzyme activity (92.34*m* moles of *p*-nitrophenol) and higher root length after nematode inoculation indicated that this hybrid had resistance to root knot nematode. The hybrid HN2 X CLN 2123A was on par with nematode resistant parent HN2 and nematode resistant variety Hisar Lalit for the nematode incident traits. These results suggested that the hybrid HN2 X CLN 2123A have tolerance to both tomato leaf curl virus and root knot nematode incidence. Further the isozyme study also proved that the hybrid HN2 X CLN 2123A showed combined resistance to leaf curl virus and root knot nematode by exhibiting more PO isoform.

Keywords

Tomato, TLCVD, Root knot nematode, Tolerance, Peroxidase, Ploy phenol oxidase, Acid posphatase, Isozyme

Introduction

Tomato (*Solanum lycopersicum* L.) is a solanaceous vegetable with native of Peru-Ecuador of South America. Tomato is very popularly grown throughout India and it ranks the largest crop after potato and sweet potato. In India, Andhra Pradesh is the largest grower followed by Bihar, Karnataka, Maharastra and Orissa. Tomato occupies prime position among vegetables. Owing to its ease in cultivation, short duration and high yield it is considered as important cash crop and is largely cultivated throughout India. Health benefits of tomato particularly free radical scavenging activities that prevent from several types of cancer, increasing the importance of this crop. However biotic and abiotic stress limits its profitable cultivation (Butani, 1977). Several pests and diseases affect tomato crop during the cropping period. Virus diseases are the

most devastating that may cause total loss of crop. Leaf curl virus disease is one such important virus disease caused by tomato leaf curl virus, which belongs to Gemini virus group (Loanous, 1985). In India, the disease is severe in all most all parts of the country with the highest severity in Punjab, Haryana, Uttar Pradesh, Tamil Nadu, Karnataka, Kerala, Gujarat and Andhra Pradesh. Similarly root knot nematode (*Meloidogyne incognita*) a polyphagous nematode pest causes severe yield loss in tomato (Barberena *et al.,* 1991). To manage TLCV and root knot nematode incidence various control measures have been suggested by several workers (Sikora and Grew, 1993). However, indiscriminative use of pesticides and withdrawal of many nematicides and soil fumigants, which are expensive, labour intensive and associated with many ecological hazards limits this option of control measures and emphasized the need for alternative strategies to control the leaf curl virus and nematode infestation (Sethi and Gaur, 1986). Development of resistant/tolerant cultivars would have proven commercially successful. Instead of developing a hybrid / variety resistant to one disease, multiple disease resistant variety/hybrids would be a highly appropriate strategy in organic farming. With this view the newly synthesized F1 hybrids *viz*., CLN 2123A X HN2 and HN2 X CLN 2123A were screened by challenge inoculation under screen house for tomato leaf curl virus and root knot nematode disease.

Materials and Methods

The present investigation "studies on challenge inoculation for combined resistance to tomato leaf curl virus and root knot nematode in tomato (*Solanum lycopersicum* L.) was carried out under screen house at the College Orchard, Department of Vegetable Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore – 3 during 2006 to 2007. The experimental material consisted of three F_1 hybrids *viz*., CLN 2123A X HN_2, HN_2 X CLN 2123A and LCR $_2$ X CLN 2123A and their parents namely CLN 2123A, HN_2 and LCR_2 along with resistant check hybrid Lakshmi (leaf curl resistant) and variety Hisar Lalit (Nematode resistant) and susceptible check variety CO 3. Graft inoculation has been used to inoculate TYLCV. The experiment was laid out in randomized block design and replicated thrice. For leaf curl virus transmission graft inoculation method was adopted.

Graft inoculation (TYLCV)

Twenty five days old healthy plants of each test plant were planted in the 5 kg pot. Twenty pots were maintained in each treatment and replicated thrice in a Randomized Block Design. The pots filled with steam sterilized soil mixture with an equal amount of coarse sand. The pots were arranged in the green house and the plants were grown under greenhouse conditions at a temperature of 22-30^{o}C. The disease was transmitted by grafting diseased scion to healthy stocks of tomato plants at 30 DAP. A portion of stem tissue of (scion) having severe disease symptoms was side grafted and tied air tightly with polythene film to help transmission of the disease within three weeks. The plants were kept for 3 months in green house conditions in order to observe symptom development (Vasudeva and Sam Raj, 1948).

Inoculation (Nematode)

Infected roots from pure culture were cut into small pieces about 2cm long and placed in 0.5% sodium hypochlorite (NaoCl)

solution. The container is shaken for about 3 minutes to dissolve the gelatinous matrix and freeing the eggs from the egg mass and incubated for 48 hours under laboratory condition. The inoculation concentration was adjusted to a known number by addition of water. The nematode inoculum was pipetted in to 2cm deep depth near to the rhizosphere of the plants and then covered with sterile sand. Each pot was inoculated with *Meloidogyne incognita* larva at second juvenile stage (J_2). Each pot was inoculated at the rate of 2 larvae (J_2)/g of soil two weeks after planting.

The observations like per cent of disease infection**,** Co-efficient of infection, number of root knot nematode females per gram of roots, number of egg mass per gram of roots, number of eggs per egg mass, root knot index, root length and fruit weight, were made. The data were subjected to statistical analysis (Panse and Sukhatme, 1976) and the results are presented in the table 1.

Assessment of nematode resistance

The degree of resistance is indicative of the nematode to develop on a host plant. The roots were removed after 45 days of inoculation and washed free of soil. The number of galls and egg masses were assessed replication wise. The genotypes were indexed based on the method suggested by Heald *et al.*, (1989) as follows.

Host enzyme analysis

Host enzymes *viz.*, peroxidase (PO), poly phenol oxidase (PPO) and Acid Phosphatase were assayed. For the study of peroxidase (PO), poly phenol oxidase (PPO) activities recently matured physiologically active leaves (fifth leaf from the top) of 10 randomly selected plants collected at 0, 24, 48, 72, 96 and 120 hour after grafting were used. Whereas for acid phosphatase activity roots of inoculated test plants collected at 0, 24, 48, 72, 96 and 120 hour were used. Plants with no inoculation were kept as control. The peroxidase and poly phenol oxidase activities were assayed by the method prescribed by (Srivastava, 1987) and expressed as change in absorbance per minute per g of fresh weight of tissue. Acid phosphatase activity was assayed as per the procedure suggested by Ferreira *et al.*, (1998) expressed as moles of *p*-nitrophenol released per minute per mg protein.

Results and Discussion

Significant differences were observed between parents, hybrids and check variety/hybrids for the traits per cent of disease infection and co-efficient of infection at 60 DAP *i.e.* one month after graft inoculation (Table 1). Graft inoculation for leaf curl virus transmission was also reported by Tomlinson (1987). Mean values of parents, hybrids and check variety/hybrids for per cent of disease infection and Co-efficient of infection after graft inoculation showed that the lowest values of 13.25 and 4.26 for per cent of disease infection and Co-efficient of infection were registered by the parent CLN 2123 A when compared to other parent HN_2 (56.69 and 76.66). Among the hybrids evaluated the direct cross CLN 2123 A X HN_2 and reciprocal cross HN_2 X CLN 2123 A recorded the lowest per cent of disease infection (9.84 and 8.66) and co-efficient of infection (15.33 and 8.66) values after graft inoculation. In case of check variety/hybrids the TLCV resistant check Lakshmi recorded the lowest per cent of disease infection (15.66) and co-efficient of infection (8.89). The results indicated that these two hybrids were on par with the leaf curl resistant check Lakshmi for leaf curl virus disease incidence and fell under resistant category. The

another hybrid LCR_2 X CLN 2123 A registered a percent of disease infection value of 22.66 and co-efficient of infection value of 18.75 and fell under moderately resistant category. While the highest per cent of disease infection and co-efficient of infection was observed by susceptible check CO 3 (58.33 and 79.33). These results are in accordance with the findings of Pico *et al.*, (2001).

Challenged inoculation of tomato genotypes for root knot nematode resistance under pot culture was previously done by Ehlers *et al.*, (2000), Wu *et al.*, (2009) and Abd-Elgawad and Kabeil (2010). For root knot nematode the hybrid HN_2 X CLN 2123A registered lower values for number of root knot nematode females per g. of roots (8.33), number of egg mass per g. of roots (7.66), number of eggs per egg mass (193.00) and root knot index (2.33) indicated that this hybrid coming under the category of moderately resistant to root knot nematode. Similarly the parent HN_2 and resistant check Hisar Lalit registered lower values for number of root knot nematode females per g. of roots (5.54 and 5.02), number of egg mass per g. of roots (4.33 and 4.66), number of eggs per egg mass (175.33 and 187.33) and root knot index (2.22 and 2.10) indicated that they are coming under the category of moderately resistant to root knot nematode incidence. While the remaining genotypes *viz.*, CLN 2123A, CLN 2123A X HN_2, LCR_2, LCR_2 X CLN 2123A, Lakshmi and CO 3 registered higher values for number of root knot nematode females per g. of roots, number of egg mass per g. of roots, number of eggs per egg mass and root knot index indicated that they are coming under susceptible category to root knot nematode incidence (Table 1).

Mean values of parents, hybrids and check variety/hybrids for root length showed that the parent HN_2 recorded the highest value (39.50 cm) than the other parent CLN 2123A (24.71). Among the hybrids evaluated the reciprocal cross HN_2 X CLN 2123 A recorded the highest root length value as 43.53 cm. With regard to check variety/hybrids the nematode resistant check HisarLalit registered the highest root length (40.03 cm) while the susceptible check CO 3 recorded the lowest value for this trait (23.53 cm). The genotypes HN_2, HN_2 X CLN 2123A and HisarLalit recorded higher root length might have due to nematode resistant nature. The results indicated that the nematode resistant/tolerant genotypes produced higher root length when compared to susceptible genotypes.

Evaluation of parents for fruit weight revealed that the highest fruit weight (57.90 g) was observed by HN_2 than other parent CLN 2123 A (45.70g). Among the hybrids the reciprocal cross HN_2 X CLN 2123 A recorded the highest fruit weight (56.10 g) and it was on par with the direct cross CLN 2123A X HN2 (54.60 g). Mean values of check variety/hybrids showed that the TLCV resistant check Lakshmi recorded the highest value of 61.30 g. as fruit weight.

Biochemical basis of resistance

The results revealed that there were clear cut differences were observed among the genotypes evaluated for peroxidase and polyphenol oxidase activity. The results of peroxidase activity and polyphenol oxidase activity in the graft inoculated plants of parents, hybrids and check varieties/hybrids showed that peroxidase and polyphenol oxidase activity increased after grafting and continued to show increase in its activity till 96 hours. After that there was a slight decrease in the activity of peroxidase and polyphenol oxidase. Between the two hybrids tested the reciprocal cross HN_2 X

CLN 2123 A recorded the highest peroxidase (0.690 changes in OD per minute per g. of leaves) and polyphenol oxidase (0.395 changes in OD per minute per g. of leaves) activity in the pooled mean. It was closely followed by the direct cross CLN 2123A X HN_2 (0.665 changes in OD per minute per g. of leaves and changes in OD per minute per g. of leaves). Among the parents CLN 2123A registered the highest peroxidase activity (0.507 changes in OD per minute per g. of leaves) and polyphenol oxidase activity (0.328changes in OD per minute per g. of leaves) than other parent HN2 (0.465 changes in OD per minute per g. of leaves and 0.262 changes in OD per minute per g. of leaves). With respect to check varieties/hybrids the TLCV resistant check Lakshmi showed the highest peroxidase activity of 0.694 changes in OD per minute per g. of leaves and polyphenol oxidase activity of 0.396 changes in OD per minute per g. of leaves. While the susceptible check CO 3 registered the lowest peroxidase activity of 0.354 changes in OD per minute per g. of leaves and polyphenol oxidase activity of 0.142 changes in OD per minute per g. of leaves (Table 2). The enzyme estimated in the present investigation showed that resistant genotypes possessed higher PO and PPO activities than susceptible genotypes. Higher peroxidase and polyphenol oxidase activity registered by the two newly synthesized hybrids indicates the tolerance nature to leaf curl virus disease (Table 3). The tolerance/resistance nature of these two hybrids might be due to the involvement of the resistant parent CLN 2123A which one is a multiple cross derivative having the blood of *Lycopersicon hirsutum* a wild species resistant to leaf curl virus. It is also interesting to note that the peroxidase and polyphenol oxidase activities of both the hybrids were on par with the peroxidase and polyphenol oxidase activities of leaf curl virus resistant check Lakshmi. Increased activity of peroxidase and poly phenol oxidase enzymes in resistant genotypes was also reported by Sundar *et al.*, (1998) and Kalaiarasan (2009).

Similar trend was noticed in acid phosphatase activity. Estimation of acid phosphatase activity in the roots of parents, hybrids and check variety/hybrids showed that the highest acid phosphatase activity was registered by the reciprocal cross HN_2 X CLN 2123 A as 80.03*m* moles of *p*-nitrophenol released per minute per mg protein for hybrids. Among the parents the nematode resistant parent HN_2 registered the highest value of acid phosphatase activity as 82.83*m* moles of *p*-nitrophenol released per minute per mg protein. When compared check variety/hybrids for this trait the nematode resistant check HisarLalit recorded the highest acid phosphatase activity value of 83.58*m*moles of *p*-nitrophenol released per minute per mg protein whereas the susceptible check CO 3 recorded the lowest acid phosphatase activity of 39.79*m* moles of *p*-nitrophenol released per minute per mg protein (Table 4). The resistant/tolerant character of the newly bred hybrid HN_2 X CLN 2123A might be due to the involvement of the resistant parent HN_2. Earlier findings on tomato acid phosphatase were made by Erion *et al.*, (1992).

Isozyme analysis (Fig. 1)

Acrylamide gel electrphorosis was done to study the isozyme variation among the genotypes. Peroxidase isozyme analysis was done for seven genotypes of study. The leaves of two synthesized hybrids *viz*., CLN 2123 A X HN2 and HN2 X CLN 2123 A and their parents *viz*., CLN 2123 A and HN_2 along with TLCV resistant check, nematode resistant check HisarLalit and susceptible

check CO 3 were taken at 96 hours after grafting. The results of the peroxidase isozyme analysis revealed that, there was uniform appearance PO1 isoform in CLN 2123A, CLN 2123 A X HN_2, HN_2 X CLN 2123 A and Lakshmi and the isoform PO1 was not appeared in the other genotypes *viz*., CO 3, $HN_2$and HisarLalit revealed that the newly synthesized hybrids CLN 2123 A X HN_2, HN_2 X CLN 2123 A were resistant to leaf curl virus but the intensity of band was varying among the genotypes. More number of isoforms was observed in both direct as well as reciprocal cross. The susceptible check CO 3 exhibited faint bands and a total of only two isoforms were noticed at 96 hours after grafting. Similarly, the isoform PO2 appeared only in the nematode resistant check HisarLalit, nematode resistant parent HN_2. The same isoform PO 2 also appeared in the newly bred hybrid HN_2 X CLN 2123 A suggested that the hybrid HN_2 X CLN 2123 A coming under the category of nematode resistant. It was also interesting to note that the hybrid HN_2 X CLN 2123 A produced both the isoform PO1 and PO2 indicated that this hybrid HN_2 X CLN 2123 A have combined resistance to both leaf curl virus and root knot nematode.

From the result it was inferred the two synthesized hybrids *viz.,* CLN 2123A X HN_2 and HN_2X CLN 2123A showed tolerance to tomato leaf curl virus as they registered lower values for per cent of disease infection and co-efficient of infection. However the reciprocal cross HN_2 X CLN 2123A recorded the lowest values of number of root knot nematode females per gram of roots, number of egg mass per gram of roots, number of eggs per egg mass and root knot index and higher values for root length and fruit weight when compare to their parents and check varieties indicating that this hybrids exhibits tolerance to nematode also. It was also well supported by higher host enzyme activities *viz.,* peroxidase polyphenol oxidase and acid phosphatase and isozyme study.

Table.1 *Per se* performance of tomato genotypes for TLCV under challenge inoculation

Varieties	Per cent of disease infection	Co-efficient of infection	No. RKN females/	No. of egg mass/g	No. eggs / egg mass	Root knot	Root length (cm)	Fruit weight
CLN 2123A	9.84	4.26	39.98	29.82	256.32	5.00	24.71	45.70
HN2	56.69	76.66	5.54	4.33	175.33	2.22	39.50	57.90
CLN 2123A X HN2	13.25	8.66	28.00	27.33	220.66	3.75	33.44	54.60
HN2 X CLN 2123A	15.33	8.86	8.33	7.66	193.00	2.33	45.53	56.10
LCR2	55.66	78.30	46.00	30.66	286.00	5.00	28.20	58.60
COTH2	22.66	18.75	45.33	38.66	275.00	5.00	29.53	52.50
HisarLalit	53.33	77.54	5.02	4.66	187.33	2.10	40.03	46.40
Lakshmi	15.66	8.89	44.66	34.66	253.66	5.00	32.20	61.30
CO3	58.33	79.33	48.00	38.00	270.33	5.00	23.53	53.00
SED	0.86	1.46	0.88	0.66	2.91	0.10	0.28	0.25
CD (P=0.05)	1.82	3.10	1.87	1.40	6.18	0.22	0.59	0.55

Table.2 Peroxidase activity (changes in OD per minutes per gram of leaves)

Genotypes	Inoculated							
	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours	Mean	Control
CLN 2123A	0.342	0.489	0.525	0.567	0.576	0.540	0.507	0.312
HN2	0.234	0.443	0.521	0.531	0.536	0.522	0.465	0.210
CLN 2123A X HN2	0.392	0.550	0.682	0.767	0.808	0.793	0.665	0.340
HN2 X CLN 2123A	0.454	0.596	0.688	0.780	0.821	0.801	0.690	0.410
LCR2	0.236	0.360	0.452	0.531	0.587	0.510	0.446	0.220
COTH2	0.393	0.530	0.620	0.767	0.821	0.793	0.654	0.356
HisarLalit	0.287	0.410	0.530	0.587	0.618	0.601	0.506	0.243
Lakshmi	0.455	0.590	0.710	0.501	0.808	0.802	0.694	0.420
CO3	0.200	0.320	0.352	0.412	0.431	0.411	0.354	0.190
SED	0.0055	0.005	0.0059	0.006	0.006	0.0057	0.0055	0.0049
CD (P=0.05)	0.0111	0.011	0.012	0.012	0.012	0.011	0.0111	0.009

Table.3 Polyphenol oxidase activity (changes in OD per minutes per gram of leaves)

Genotypes	Inoculated							
	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours	Mean	Control
CLN 2123 A	0.062	0.100	0.313	0.456	0.529	0.510	0.328	0.058
HN2	0.043	0.060	0.210	0.352	0.489	0.420	0.262	0.031
CLN 2123 A X HN2	0.092	0.130	0.386	0.532	0.610	0.600	0.392	0.085
HN2 X CLN 2123 A	0.091	0.120	0.398	0.552	0.611	0.600	0.395	0.087
LCR2	0.031	0.061	0.137	0.312	0.413	0.398	0.225	0.020
COTH2	0.058	0.110	0.386	0.529	0.532	0.502	0.486	0.043
HisarLalit	0.048	0.053	0.229	0.341	0.430	0.410	0.252	0.030
Lakshmi	0.063	0.140	0.397	0.568	0.607	0.602	0.396	0.058
CO3	0.038	0.050	0.143	0.210	0.221	0.167	0.142	0.020
SED	0.0014	0.0015	0.0017	0.005	0.0056	0.0054	0.0016	0.0014
CD (P=0.05)	0.0029	0.0031	0.0035	0.011	0.012	0.012	0.003	0.0028

Table.4 Acid phosphatase activity (*m* moles of *p*-nitrophenol released per minute per mg protein)

Genotypes	Inoculated							
	0 hours	24 hours	48 hours	72 hours	96 hours	120 hours	Mean	Control
CLN 2123A	46.20	53.06	55.21	60.01	62.11	58.17	55.79	44.69
HN2	85.11	92.11	101.03	120.21	126.06	120.60	107.52	82.83
CLN 2123A X HN2	73.06	84.22	90.11	100.01	112.92	110.00	95.05	70.60
HN2 X CLN 2123A	82.30	92.01	98.99	117.61	120.60	118.19	104.95	80.03
LCR2	44.30	52.80	55.09	61.31	64.10	60.90	56.42	40.62
COTH2	50.90	60.10	71.06	77.66	80.32	78.40	69.74	46.71
HisarLalit	86.66	94.70	110.20	123.12	130.60	128.06	112.22	83.58
Lakshmi	52.83	60.80	68.19	72.73	82.79	78.90	69.37	50.40
CO3	42.90	50.94	54.18	62.11	66.13	60.37	56.11	39.79
SED	0.9250	0.9550	0.9551	1.002	1.002	0.9551	0.9551	0.9249
CD (P=0.05)	1.9610	1.9810	1.9812	2.004	2.004	1.9812	1.9812	1.96

Assessment of nematode resistance

Percentage of galls	Gall Index	Plant Response
No galls	1	Highly resistant
1-25	2	Resistant
>25-50	3	Moderately resistant
>50-75	4	Susceptible
>75	5	Highly susceptible

Expression of TLCV symptoms one month after graft inoculation in susceptible genotype

Fig.1 Induction of defense enzyme (Peroxidase) in tomato genotypes

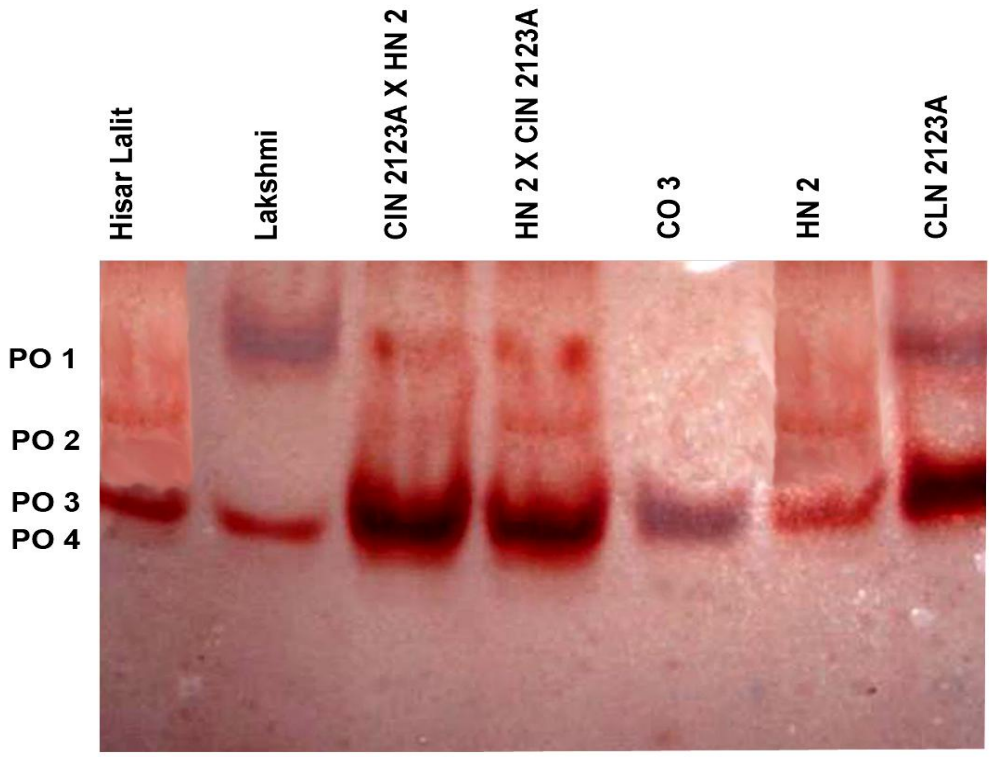
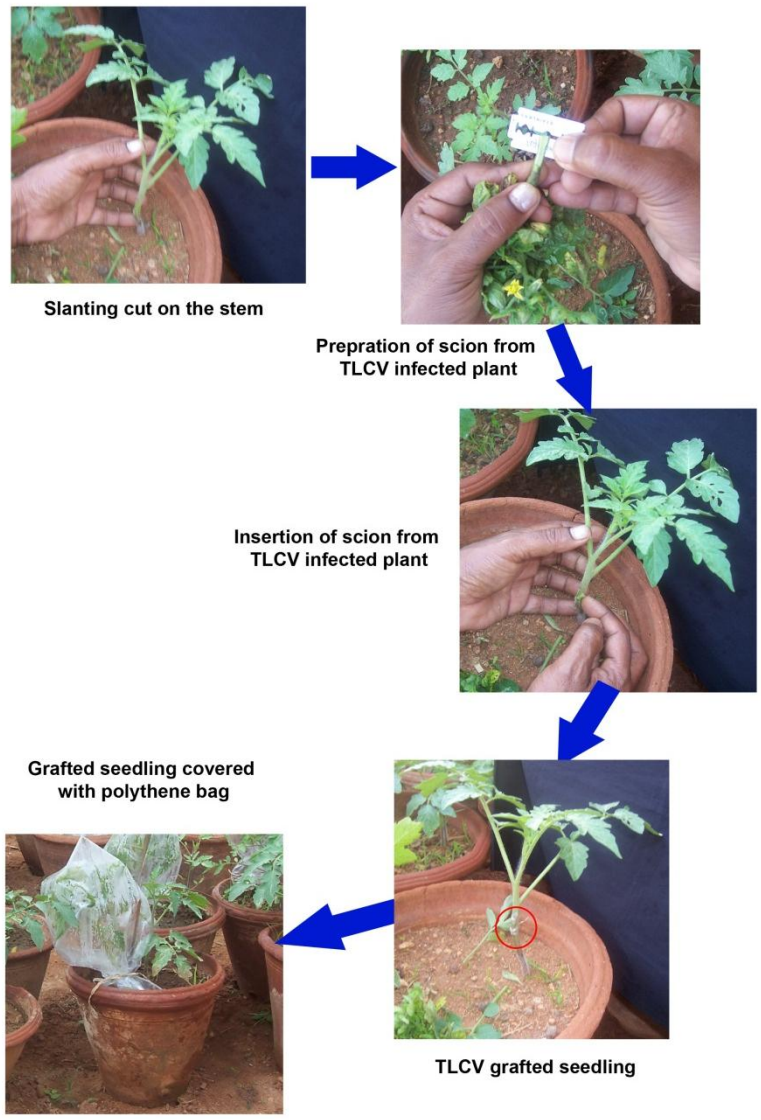


Plate.1 Sequence steps in graft inoculation



References

Abd-Elgawad, M.M.M. and S.S.A. Kabeil, 2010. Management of the root knot nematode *Meloidogyne incognita* in tomato. Egypt. J. Am. Sci., 6: 256-262.

Butani, D.K. 1977. Insect pests of vegetable – Tomato pesticides, 11: 33-36.

Ehlers, J.D., W.C. Matthews, A.E. Hall and P.A. Roberts, 2000. Inheritance of a broad-based form of root-knot nematode resistance in cowpea Crop Sci., 40: 611-618.

Erion, J.L., B. Ballo, T.W. Fox and L.A. May, 1992. Tomato acid phosphatase-1 gene from a nematode resistant cultivar. Plant Physiol., 98: 1535-1537.

Ferreira, C.V., J.M. Granjeiro, E.M. Taga and H. Aoyama, 1998. Purification and characterization of multiple forms

of soybean seed acid phosphatase. Plant Physiol. Biochem., 36: 487-494.

Heald, C.M., B.D. Bruton and R.H. Davis, 1989. Influence of *Glomus intracidices* and soil phosphorus on *Meloidogyne incognita* infecting *Cucumismelo*. J. Nematol., 21: 69-73.

Jarquin-Barberena, H., A. Dalmasso, G. de Guiran and M.C. Cardin, 1991. Acquired virulence in the plant parasitic nematode *Meloidogyne incognita*. I. Biological analysis of the phenomenon. Rev Nématol 14: 299–303.

Kalaiarasan, P., 2009. Biochemical markers for identification of root knot nematode (*Meloidogyne incognit*a) resistance in tomato. Karnataka J. Agric. Sci., 22: 471-475.

Loannous, N. 1985. Yellow leaf curl and other virus disease of tomatoes in Cyprus. Plant Pathology. 34: 428-434.

Panse, V.G. and Sukhatme, P.V., 1976. Statistical methods for agricultural workers. ICAR, New Delhi.

Pico, B., M. Ferrriol, M.J. Diez and F.N. Vinals, 2001. Agro inoculation methods to screen *Lycopersicon* for resistance to tomato yellow leaf curl virus. J. Plant Pathol, 83: 215-220.

Sethi, C.L. and Gaur, H.S., 1986. Nematode management: An overview. In: plant Parasitic nematodes of India: Problem and Progress. Ed. G. Swarup and D.R. Dasgupta, IARI, New Delhi. pp. 424-445.

Sikora, R.A. and Grew, N., 1993. Nematode parasites of food legumes. In plant parasitic nematodes in sub-tropical and tropical Agriculture. Ed. M. Luc. R.A., Sikora and J. Bridge, J. CAB International Institute of Parasitology, Wallingford, UK. P – 629.

Srivastava, S.K. 1987. Peroxidase and polyphenol oxidase in *Brassica juncea* plants infected with *Macrophomina phaseolina* (Tassi) Goid and their implication in disease resistance. J. Phytopathol., 120: 249-254.

Sundar, A.R., R. Viswanathan, D. Mohanraj and P. Padmanaban, 1998. Role of oxidative enzymes in sugarcane and *Colletotrichum falcatum* interaction. Acta Phytopathol. Entomol. Hungarica, 38: 297-304.

Tomlinson, J. 1987. Epidemiology and control of virus diseases of vegetables. Annals of Applied Biology, 110, 661–681.

Vasudeva, R.S. and J. Samraj. 1948. A Leaf curl disease of tomato. Phytopath, 38: 364-369.

Wu, W.W., H.L. Shen and W.C. Yang, 2009. Sources for heat stable-resistance to Southern root-knot nematode (*Meloidogyne incognita*) in *Solanum lycopersicum*. Agric. Sci. China, 8: 697-702.