



Breeding Interspecific Hybrid Lines of Tomato (*Solanum lycopersicon* L.) Resistance to Peanut bud Necrosis Virus (PBNV)

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Abstract

Field screening of F₃ to F₆ generation interspecific tomato hybrids derived lines for resistance to Peanut bud necrosis virus (PBNV) was carried out in the Department of Horticulture, Agricultural College and Research Institute, Madurai, Tamil Nadu Agricultural University, Tamil Nadu, during 2019-2021. PBNV is the most destructive and economically important plant virus in tomato which causes yield loss up to 80 to 100 per cent. The present study was formulated to develop PBNV resistant lines in tomato suitable for commercial cultivation. In this present study, three lines, 10 wild species were crossed and developed 30 interspecific hybrids. Among the thirty interspecific hybrids evaluated, four interspecific crosses viz., H4 - Arka Vikas x PSR11668 (*S. pimpinellifolium*), H6 - Arka Vikas x EC519809 (*S. peruvianum*), H17 - CO3 x EC519791 (*S. habrochaites*), and H27 - PKM1 x EC519791 (*S. hirsutum*) were screened in advanced generations under natural field condition. No insecticide spray was given during entire crop production period. The disease incidence was assessed on 30th, 45th, 60th and 90th day after transplanting based on Per cent Disease Incidence (PDI %) and disease reaction scale. Further in F₄ generation, totally seventeen lines from the above four crosses were evaluated. In F₅ and F₆ generation 10 lines and 3 lines respectively from two crosses viz., H6 - Arka Vikas x EC519809 (*S. peruvianum*) and H27 - PKM-1 x EC519791 were evaluated. The results revealed that, PBNV disease infection was seen in all the stages of crop growth. Among the four interspecific hybrids, maximum degree of field resistance was observed in 2 lines in, H6 - Arka Vikas x EC519809 (*S. peruvianum*) followed by 1 line in H27 - PKM-1 x EC519791. The plants showing resistance will be released as PBNV resistant variety or it may be used as a source for PBNV resistance in tomato breeding programmes.

Keywords: Tomato; Interspecific Hybrids; Field Screening; Resistance to PBNV

Abbreviations

PBNV: Peanut Bud Necrosis Virus; AVRDC: Asian Vegetable Research and Development Center; GBNV: Groundnut bud necrosis; TSWV: Tomato spotted wilt virus; IIHR: Indian Institute of Horticultural Research; NBPGR: National Bureau of Plant Genetic Resource; TNAU: Tamil Nadu Agricultural University; ELISA: Enzyme-linked immunosorbent assay; MSL: Mean Sea Level; PDI:

Percentage of Disease Incidence; EC: Exotic collection; Co: Coimbatore; PKM: Periyakulam; Kg: Kilogram; G: Gram

Introduction

India is the second largest producer of vegetables in the world, next to china. Among the vegetable crops, tomato (*Solanum lycopersicum* L.) is the third largest cultivated vegetable crop in tropical

and sub-tropical regions of our country. Fresh tomatoes and tomato products are rich source of bioactive compounds, including carotenes (lycopene, β - carotene), ascorbic acid, tocopherol, and phenolic compounds. In India tomato is cultivated in an area of 8.41 lakh hectares, with the total production of 19.7 million tonnes in 2017-18. India is one of the largest producers of tomatoes in the world, second only to China. Around 11 % of the total world production of tomatoes is cultivated in India. There are more than 15000 varieties of tomato in the world. There are more than 1000 varieties of tomatoes cultivated in India. However, only a few are commercially available and others are consumed locally. Several seed companies have bred the tomatoes further and have brought out new varieties with specific attributes. Tomato is susceptible to over 40 different groups of viruses [1]. Of which, *Peanut bud necrosis virus* (PBNV) belongs to the member of genus *Tospovirus* sero group IV, family *Bunyaviridae*, is a most economical and destructive plant virus affecting tomato. The incidence of tospovirus on vegetable crops especially tomato is increasing year by year [12]. The first report on the occurrence of tospovirus in tomato was recorded at Australia in 1915, where as in India the occurrence was first noticed in 1964 at Nilgiris, Tamil Nadu [18,21]. The disease is exclusively vectored by *Thrips palmi* Karny, in a circulative and propagative manner and transmitted in natural condition [17]. The host range of PBNV comprises of agricultural crops, horticultural crops and ornamental crop. In Horticultural crops, it infects five different family of vegetables crops namely Solanaceae, Fabaceae, Cucurbitaceae, Amaranthaceae and Chenopoidaceae [4,23]. The characteristic symptom of tospovirus on tomato are necrosis of young growing bud, bronzing of leaves with brown necrotic lesions, followed by wilting of the plant in severe cases and the ripened fruit exhibit circular markings as concentric band of red and yellow broken rings of about one cm in diameter [7]. In tomato, the PBNV disease occurrence takes place throughout the year but the severity depends on the stage of the crop, season, and location and can cause yield loss up to 80 to 100 per cent [16]. The management of PBNV by adopting proper cultural practices is less effective and difficult due to wide host range for virus and vector. Besides, prophylactic spray schedule of pesticide for controlling thrips is ineffective due to continues migration of thrips into the host plant from the surrounding area. Generally wild relatives are renowned for their useful genes towards resistance to TSWV identified the source of resistance for TSWV for the first time in *S. pimpinellifo-*

lium and several other sources of resistance was recorded in *S. peruvianum*, and *S. hirsutum* [2]. By utilizing these resistance sources in tomato breeding programs, it is possible to develop resistant hybrids against PBNV which is an effective way to overcome PBNV infection. With the above background the study was undertaken to develop resistance in tomato against PBNV through interspecific hybridization programme [14,19].

Materials and Methods

The materials used in this study were initially collected from NBPGR, New Delhi and IIHR, Bangalore. Totally ten parents were used for the crossing programme, which is present in table 1. In F_1 generation, thirty crosses were screened for PBNV resistance under natural field condition. Out of thirty crosses, four hybrids showed resistance to PBNV in both field screening and artificial screening through ELISA at Horticulture College and Research Institute, TNAU Coimbatore, these four crosses were further advanced to F_2 generation for field screening. The 35 single plant progenies selected in F_3 from four crosses were advanced to F_4, F_5, F_6 generations as detailed in Table 2. The present research was carried out at the Department of Horticulture, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India during August 2019 to September 2021. The experimental site comprises of warm tropical climate with sand loamy soil, which is located at an altitude of 158 m above MSL between 09°58'30.5'N latitude and 078° 12'27.4E longitudes. Tomato seedlings were sown in protrays under insect proof net and standard nursery practices were followed without any application of pesticides. When the tomato seedlings attained 25 days old, they were transplanted in the main field with a spacing of 30 X 30 cm. For every two rows of tomato, one row of cowpea var. Co (CP) - 7 was planted which acted as a host crop and create favorable environment for PBNV. Recommended package of practices were followed as given in crop production guide, TNAU and no pesticide was sprayed during the entire crop production period to facilitate the natural field infection of PBNV. The disease incidence was assessed on 30, 45, 60, 90 days after planting based on Per-cent disease incidence, PDI (%) = Number of plants affected with PBNV / Total number of plants X 100 and disease scoring was categorized into six different disease rating grade (11, 22) The data on per-cent disease incidence was statistically analyzed (Table 2).

Thirty F_1 hybrids have been evaluated along with parents and check variety to study their performance. Among the thirty hybrids evaluated, four hybrids were selected and forwarded for F_2 & F_3 progeny evaluation. Population of each progeny consists of 250 plants. (Table 1)

- H6 ($L_1 \times T_6$) - Arka Vikas x EC519809 (*S. peruvianum*)
- H17 ($L_2 \times T_7$) - CO3 x EC519791 (*S. habrochaites*)
- H4 ($L_1 \times T_4$) - Arka Vikas x PSR11668 (*S. pimpinellifolium*)
- H27 ($L_3 \times T_7$) - PKM1 x EC519791 (*S. hirsutum*)

Disease reaction scale

A disease reaction scale was adapted to assess the resistance of a given strain. Symptom severity grades designated with numerical values of 0-4 were given on the basis of visual observation (5). To quantify the disease severity, calculations were made as shown below (Table 3).

Results and Discussion

Interspecific hybrid progenies were evaluated in F_3 , F_4 , F_5 and F_6 generation for growth, yield and resistance to PBNV.

Performance of interspecific progenies in F_3 generation evaluation

Based on F_2 generation yield and resistance towards Peanut Bud Necrosis Virus (PBNV), thirty-five superior performing progenies were evaluated in F_3 generation. The highest plant height was exhibited in the cross H4-13 (171 cm) followed by H4-10 (169 cm), while the lowest plant height was exhibited in the cross H17- 2 (107 cm). The highest number of fruits per plant was recorded in the cross H4-115 (178) followed by H4 - 10 (160), the highest single fruit weight was exhibited in the cross H6-48 (68.3 g) followed by H6-100 (67.3 g). The highest yield per plant was exhibited in the cross H6-100 (4.40 kg/plant) followed by H6-48 (4.20 kg/plant), while the lowest yield per plant was exhibited in the cross H4-99 (1.22 kg/plant)(Table 4).

Resistance to PBNV

However, from four Interspecific hybrids, the cross H6 - Arka Vikas x EC519809 (*S. peruvianum*) showed maximum field resistance of 3 lines namely H6-48, H6-100, H6-212 and the lines H6-53, H6-71, H6-77, H6-152 are moderately resistant. From the cross H4- Arka Vikas x PSR11668 (*S. pimpinellifolium*) out of 8 lines screened for PBNV resistance, the lines H4-10 and H4-115 showed resistance to PBNV infection and the lines H4-43, H4-105, H4-120 are moderately resistant. From the cross H17 - CO3 x EC519791 (*S.*

habrochaites) out of 8 lines screened for PBNV resistance, the line H17-2 and H17-44 showed resistance to PBNV infection and the lines H17-18, H17-38 show moderately resistant. From the cross H27 - PKM1 x EC519791 (*S. habrochaites*) out of 9 lines screened for PBNV resistance, the line H27-173 showed resistance to PBNV infection and the lines H27-66, H27-91 are moderately resistant.

Performance of interspecific progenies in F_4 generation evaluation

Morphological and yield characters

Based on F_3 generation yield and resistance towards Peanut Bud Necrosis Virus (PBNV), seventeen superior performing progenies were evaluated in F_4 generation. The highest plant height was exhibited in the cross H6-48-44 (152.50 cm) followed by H4-48-61 (147.70 cm), while the lowest plant height was exhibited in the cross H17-2-6 (105.50 cm). The least number of days taken for 50 per cent flowering was observed in the cross H6-48-61 (28.48 days) followed by H6-48-44 (28.56 days). The highest number of fruits per cluster was exhibited in the cross H4-115-18 (5.30) followed by H6-48-61 (4.50), similarly, the highest fruit set per cent was observed in the cross H6-212-91 (78.36) followed by H4-115-18 (74.64). The highest number of fruits per plant was recorded in the cross H4-115-18 (175.20) followed by H27-173-22 (128.60), the highest single fruit weight was exhibited in the cross H6-100-11 (67.98 g) followed by H6-48-44 (67.45 g). The highest yield per plant was exhibited in the cross H6-212-91 (4.78 kg) followed by H6-100-11 (4.67 kg), while the lowest yield per plant was exhibited in the cross H4-115-18 (1.97 kg). The highest titrable acidity was observed in the cross H6-100-23 (0.54 %) followed by H6-148-61 and H27-173-22 (0.49 %), while the lowest titrable acidity was observed in the cross H17-2-23 (0.39 %). The highest ascorbic acid was exhibited in the cross H4-115-18 (25.23 mg 100 g⁻¹) followed by H6-212-91 (25.21 mg 100 g⁻¹), while the lowest ascorbic acid was exhibited in the cross H6-100-3 (23.18 mg 100 g⁻¹). The highest total phenol was recorded in the cross H6-48-44 and H6-212-91 (0.98 µg/g) followed by H6-100-11 (0.97 µg/g), while the lowest total phenol was recorded in the cross H17-2-5 (0.83 µg/g)(Table 5).

Resistance to PBNV

In the cross H6 Arka Vikas x EC519809 (*S. peruvianum*), nine hybrid derived lines were evaluated for *Peanut bud necrosis virus* (PBNV) resistance and the mean PDI per cent ranged from 3.75 to 14.47 per cent. Out of nine lines evaluated, five lines are found to be resistant (R) viz., H6- 48-44, H6-100-11, H6-100-23, H6-100-

36 and H6-212-91. In the cross H17-C03 x EC 519791 (*S. habrochaites*), out of four lines evaluated, one line was found to be resistant (R) viz., H17-2-6. In H27-PKM-1 x EC 519791 (*S. habrochaites*) cross, three hybrid derived lines were evaluated for *Peanut bud necrosis virus* (PBNV) resistance and one line was found to be Resistance (R) viz., H27-173-22.

Performance of interspecific progenies in F₅ generation evaluation

Morphological and yield characters

Based on F₄ generation yield and resistance towards Peanut Bud Necrosis Virus (PBNV), ten superior performing progenies were evaluated in F₅ generation. Among the ten lines evaluated the highest plant height was exhibited in the cross H6-48-44- 17 (172.50 cm) followed by H6-212-91-16 (170.50 cm). Earliness in terms of days taken for 50 per cent flowering was observed in the cross H6-48-44 - 17 (32.50 days) followed by H6-212-91-16 (33.40 days). The highest number of fruits per cluster was exhibited in the cross H6-48-44 - 17 (4.50) followed by H6-212-91-16 and H6-212-91-26 (4.20), similarly, the highest fruit set per cent was observed in the cross H6-212-91 (78.36) followed by H4-115-18 (74.64). The highest number of fruits per plant was recorded in the cross H27-173-22-23 (121.50) followed by (128.60), the highest single fruit weight was exhibited in the cross H6-100-11 (67.98 g) followed by H6-48-44 (67.45 g). The highest yield per plant was exhibited in the cross H6-212-91 (4.78 kg) followed by H6-100-11 (4.67 kg), while the lowest yield per plant was exhibited in the cross H4-115-18 (1.97 kg). The highest titrable acidity was observed in the cross H6-100-23 (0.54 %) followed by H6-148-61 and H27-173-22 (0.49 %), while the lowest titrable acidity was observed in the cross H17-2-23 (0.39 %). The highest ascorbic acid was exhibited in the cross H4-115-18 (25.23 mg 100 g⁻¹) followed by H6-212-91 (25.21 mg 100 g⁻¹), while the lowest ascorbic acid was exhibited in the cross H6-100-3 (23.18 mg 100 g⁻¹). The highest total phenol was recorded in the cross H6-48-44 and H6-212-91 (0.98 µg/g) followed by H6-100-11 (0.97 µg/g), while the lowest total phenol was recorded in the cross H17-2-5 (0.83 µg/g).

Resistance to PBNV

In the cross H6 Arka Vikas x EC519809 (*S. peruvianum*), nine hybrid derived lines were evaluated for *Peanut bud necrosis virus* (PBNV) resistance and the mean PDI per cent ranged from 3.75 to 14.47 per cent. Out of nine lines evaluated, five lines are found to be resistant (R) viz., H6- 48-44, H6-100-11, H6-100-23, H6-100-36 and H6-212-91. In the cross H17-C03 x EC 519791 (*S.*

habrochaites), out of four lines evaluated, one line was found to be resistant (R) viz., H17-2-6. In H27-PKM-1 x EC 519791 (*S. habrochaites*) cross, three hybrid derived lines were evaluated for *Peanut bud necrosis virus* (PBNV) resistance and one line was found to be Resistance (R) viz., H27-173-22 (Table 6).

Performance of interspecific progenies in F₆ generation evaluation

Based on F₅ generation yield and resistance towards Peanut Bud Necrosis Virus (PBNV), three superior performing progenies were evaluated in F₆ generation. Among the three lines evaluated the highest plant height was exhibited in the cross H6-48-44- 17-16 (170.50 cm) followed by H6-212-91-28-21 (162.00 cm). Earliness in terms of days taken for 50 per cent flowering was observed in the cross H6-48-44- 17-16 (30.50 days) followed by H6-212-91-28 - 21 (35.00 days). The highest number of fruits per cluster was exhibited in the cross H6-48-44 - 17 - 16 (4.20) followed by H6-212-91-28-21 (4.10), similarly, the highest fruit set per cent was observed in the cross H27-173-22 - 23-6 (74.00). The highest number of fruits per plant was recorded in the cross H27-173-22-23 - 6 (115.50) followed by (110.0) in the cross H6-48-44 - 17 - 16, the highest single fruit weight was exhibited in the cross H6-48-44-17 - 16 (37.00 g) followed by H6-212-91- 28 - 21 (32.00 g). The highest yield per plant was exhibited in the cross H6-48-44 - 17 - 16 (3.8 kg) followed by H27-173-22 - 23-6 (3.20 kg). In the cross H6 Arka Vikas x EC519809 (*S. peruvianum*), two hybrid derived lines were evaluated for *Peanut bud necrosis virus* (PBNV) resistance. In H27-PKM-1 x EC 519791 (*S. habrochaites*) cross, one hybrid derived lines were evaluated for *Peanut bud necrosis virus* (PBNV) resistance and all the three lines were found to be resistant [10]. These results are depicted in (Table 7). The present results were coincides with some other authors and observed that none of the varieties screened were fully resistant [8,10,17,20,21]. However, they showed varied response to the infection. ARTH-4 among the hybrids and CO-3 among the varieties showed less susceptibility while the rest were highly susceptible. *L. peruvianum* appears to be the best resistance source for TSWV [2,8]. NPH accessions 201 and 374, LA accessions 111372, 385, 4441-1 and 1113-1, PI accessions 126928, 126930, 126, 944, 126946, 128657, 128600 and 129146 have been found resistant or immune in screening trials [9,13,15,17]. In this, screened 63 tomato entries for resistance to PBNV under field condition during Kharif 2003. Among them only one entry EC5888 showed highly resistant reaction while EC 8630 and EC 2652 were resistant. Pusa Uphar, EC 251709, EC 165700, LE 23, IIHR 2187, IIHR 2272, IIHR 2273 and IIHR 2274 were mod-

erately resistant. These field promising genotypes were further tested by sap inoculation for confirmation of resistance. Two genotypes, viz., EC 8630 and EC 5888 were highly resistant, LE 23 and EC 526512 were resistant and EC 165700 displayed a moderately resistant reaction. Field experiments were conducted in 2007 and 2008 at AVRDC in Hyderabad, India to evaluate 30 improved lines of tomato for yield performance and field tolerance against tomato leaf curl virus and peanut bud necrosis virus. Peanut bud necrosis disease severity recorded was minimum in lines DR2-1 and NC 3220 [20,21].

Among nine different species of tomato, only *Solanum chilense*, *Solanum peruvianum* and the advance breeding lines derived from them shown resistance or moderately resistance response to GBNV. The resistant lines identified in the present investigation can be utilized to study the genetics of resistance to PBNV and for the development of molecular markers linked to the trait, which subsequently will help to incorporate the resistant genes into the desired elite backgrounds of tomato [3,6].

Conclusion

Finally concluded that successfully introgressed the resistant genes into cultivated tomato and developed interspecific tomato plant showing good resistance against PBNV and bearing big size, red color and desired shape tomato fruits. The developed lines may be used for commercial cultivation or it may be used in hybridization programme.

Conflict of Interest

There is no conflict of interest exists.

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