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# Effect of watermelon bud necrosis orthotospovirus (WBNV) on plant survival and vine length of watermelon progenies derived from resistant prebred line

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**Abstract**
A study was conducted to understand the effect of watermelon bud necrosis orthotospovirus (WBNV) on plant survival and vine growth in watermelon segregating progenies derived by crossing a watermelon bud necrosis virus resistant prebred line i.e., DWM-45 with Arka Manik which is a commercially popular but WBNV susceptible variety to produce $F_1$, $F_2$, $BC_1P_1$ and $BC_1P_2$ generations. Here, DWM-45, a resistant prebred line was derived by selection and contentious selfing of citron (*Citrullus lanatus* var. *citroid*), a wild species closely associated with cultivated sweet watermelon and reported to possess WBNV resistance. We performed natural epiphytic screening of parental lines i.e., DWM-45 and Arka Manik along with $F_1$, $F_2$, $BC_1P_1$ and $BC_1P_2$ populations against WBNV infection under field conditions. The results revealed a clear trend: generations with high Percent disease index (PDI) i.e., $P_2$ and $BC_1P_2$ exhibited lower plant survival rates and shorter vine length, while those with lower PDI (%) values i.e., $P_1$, $F_1$ and $BC_1P_1$ had a greater chance of survival and higher vine growth.

**Keywords:** WBNV, plant survival, vine growth, *Citrullus lanatus* var. *citroid*

## Introduction
Watermelon (*Citrullus lanatus*) is one of the important fruit vegetable crops covering about seven per cent of vegetable cropping area in the world. The area and production of watermelon in the world is about 4.44 million hectares and 162 million tonnes. In India, watermelon is a major cucurbitaceous vegetable being cultivated in an area of 119 thousand hectares with a production of 3.25 million tonnes (FAO, 2021) [3]. This crop is being popular due to its lower calorific content and considered as a heart healthy food due to absence of cholesterol and negligible fat and sodium. It is also known as a mood food associated with the happiness.

Watermelon bud necrosis disease has emerged as one of the major diseases which limit the watermelon production significantly. Watermelon bud necrosis orthotospovirus (WBNV) (Family: *Tospoviridae*, *Bunyavirales*) belongs to serogroup-IV (Adams *et al*., 2017; Jain *et al*., 2014, Jain *et al*., 1998) [1, 6, 5] which is transmitted through thrips. It was first recorded during 1991 infecting watermelon at Indian Institute of Horticultural Research (IIHR), Bengaluru (Singh & Krishnareddy, 1995) [16]. Disease incidence reported due to WBNV ranged from 39% to 100% (Krishnareddy & Singh, 1993) [9] with a yield loss up to 100% (Kunkalikar *et al*., 2011; Jain *et al*., 1998, 2007; Singh & Krishnareddy, 1995) [16, 11, 6]. The field symptoms of WBNV in watermelon initially develop as shortened internodes and necrosis on apical bud, stem, petiole and fruit stalk. Infected plants produce unmarketable small, deformed fruits with uneven surface and necrotic or chlorotic rings, depending on the cultivar (Mandal *et al*., 2012) [12].

Although several control measures (cultural, chemical and biological) have been suggested for vector management, practically no effective control has been achieved so far for the management of WBNV (Kumar *et al*., 2006) [10]. Hence, host plant resistance has been suggested as the most feasible management option for the control of this disease (Riley & Pappu, 2000) [15]. However, not many efforts have been made in this direction. In case of host resistance, plants health, survival and growth are directly correlated with the final yield of the plant, therefore it is important to understand how disease affect these traits. There is only one

report (Nagesh *et al*., 2018) [13], to study the effect of WBNV on plant survival and vine growth in the progenies of watermelon derived by crossing WBNV resistant genotype with susceptible genotype. Therefore, we studied the effect of watermelon bud necrosis disease on plant survival and vine length in different progenies ($F_1$, $F_2$, $BC_1P_1$ & $BC_1P_2$) of watermelon derived by crossing resistant prebred line 'DWM-45' with WBNV susceptible variety (Arka Manik).

## Material and methods

### Plant material

The experimental materials for the present study comprised of one WBNV resistant genotype, viz., DWM-45 (pre-bred line) derived by selection and continues selfing of citron (*Citrullus lanatus* var. *citroid*), a wild species closely associated with cultivated sweet watermelon and reported to possess resistance genes to several diseases of watermelon. This resistant genotype (DWM-45) crossed to commercially popular WBNV-susceptible variety Arka Manik to generate $F_1$, $F_2$ and backcross ($BC_1P_1$ & $BC_1P_2$) populations. The $F_1$, $F_2$, $BC_1P_1$ & $BC_1P_2$ progenies along with parental lines involving DWM-45 and Arka Manik were evaluated under natural epiphytic conditions for disease reaction during summer of 2022 in the main field of Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi (Fig. 1).

### Experimental design and layout

The evaluation trial was laid out in a randomized complete block design with three replications. Each replication of $P_1$, $P_2$ and $F_1$ consisted of 11 plants; $BC_1P_1$ and $BC_2P_2$ consisted of 16 plants; and $F_2$ consisted of 51 plants. All package of practices except insecticidal sprays were followed to raise the crop. Data were recorded for disease severity at 10 days interval, starting from 30 to 60 days after sowing (DAS).

### Disease screening

Natural epiphytotic screening of different population ($F_1$, $F_2$, $BC_1P_1$ & $BC_1P_2$) along with parental lines against WBNV infection was carried out during summer season, when natural vector population was high favoring natural disease occurrence. Hill planting technique were followed to grow different progenies ($F_1$, $F_2$, $BC_1P_1$ & $BC_1P_2$) under unmulched condition along with infector genotype were used to attract thrips (vector of WBNV). Popular commercial variety, viz., 'Arka Muthu' that is highly susceptible to WBNV was used as infector line. The disease severity scored visually for symptoms on a scale of 0–3 as suggested by Sugiyama *et al*. (2009) [17] with slight modifications. Disease score: 0 = no symptom, 1 = slight crinkling of leaves, 2 = crinkling with yellowing or silver mottling of leaves and 3 = dieback or severe bud necrosis.

The disease severity scores of individual plants thus recorded were used to calculate per cent disease index (PDI) using the following formula:

$$PDI = \frac{\text{Total sum of numerical ratings}}{\text{Number of observations x Maximum disease rating}} \times 100$$

**Fig 1:** Field based natural epiphytic screening of different watermelon progenies against WBNV.

The disease reaction of individual plants in $F_2$ and backcross ($BC_1P_1$ & $BC_1P_2$) progeny were classified based on the PDI ± SE of resistant and susceptible parents into two major phenotypic classes, *viz*., resistant and susceptible as suggested by Thirthamallappa and Lohithaswa (2000) [18].

### Serological & molecular validation to validate WBNV infection

To confirm WBNV infection in the field during natural epiphytic screening, direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA) and reverse transcription–polymerase chain reaction (RT-PCR) assay was performed using the leaf samples of plants showing bud necrosis symptoms. DAC-ELISA assay was performed using the WBNV-NSs protein specific antiserum (Basavaraj *et al*., unpublished) as per the protocol described by Clark and Bar-Joseph (1984) [2]. Besides this, RT-PCR were performed using primers specific to 'nucleocapsid' (N) gene of WBNV as described by Holkar *et al*. (2017) [4].

## Results

### Symptoms of WBNV in watermelon during screening

In field condition the initial symptoms of the disease on leaves appeared as chlorotic spots, mild mosaic mottling, shortened internodes and necrosis on apical bud, petiole, stem and fruit stalk. Infected plants produce deformed, unmarketable small fruits with uneven surface and necrotic or chlorotic rings. Further mid-veins and lateral veins of leaves turned black and became thick and distorted (Fig. 2).

### Confirmation of WBNV infection in the diseased watermelon plants in the field

Samples were collected randomly from the 15 plants showing necrosis symptoms for DAC-ELISA using WBNV-NSs

protein specific antiserum to confirm presence of WBNV in the experimental field. The DAC-ELISA absorbance values ($A_{405}$) varied between 0.72 to 1.17 in the disease sample values, which were higher as that of healthy plant sample/negative control (0.35) and buffer (0.31) (Table 1). RT-PCR assay was performed using WBNV nucleocapsid (N) gene specific primers for further confirmation of WBNV infection. The RNA was isolated from healthy and diseased watermelon plants samples to perform RT-PCR. The diseased plant samples resulted the amplicon of about 750 bp size, while it was absent in healthy plant samples.

**Performance of different generations of cross (DWM-45 × Arka Manik) against bud necrosis disease**

In field, to create epiphytic condition and to avoid escapes in natural epiphytic screening the infector rows were planted 10 days in advance the test. Check variety (Arka Muthu) which is highly susceptible to WBNV served as a reference check to decide the escapes, in which 88.88% PDI was recorded by 60 DAS confirming that escapes were less than 15%. Analysis of variance revealed significant differences among the different generations ($F_1$, $F_2$, $BC_1P_1$ & $BC_1P_2$) in this screening experiment. The means for different generations of the cross primarily provided an idea of their disease response under natural epiphytic screening conditions (Table 2). At 60 days after sowing (DAS) when checks attained 70% threshold PDI, were used for analysis, and interpretation of PDI. Among different generation maximum PDI was observed in susceptible check i.e., Arka Muthu followed by WBNV susceptible parent (Arka Manik) with 81.11% PDI. Less PDI were observed in PDI resistant parent i.e., DWM-45 (3.33%), $F_1$ (5.55%) and $BC_1P_1$ (16.02%) at 60 DAS. In $F_2$ generation also PDI was less as compared to susceptible parent but slightly higher than resistant parent ($P_1$). Based on PDI (%) values of plants in $F_2$, $BC_1P_1$ and $BC_1P_2$ populations, we observed 149 resistant and 53 susceptible plants in $F_2$, 52 resistant and 0 susceptible and 29 resistant and 23 susceptible resistant and susceptible plants in $BC_1P_1$ and $BC_1P_2$ populations respectively.

**Effect of WBNV on plant survival and vine length in different generations**

Data in Table 2 suggest that among the tested generations, susceptible check (Arka Muthu) and Arka Manik ($P_2$) exhibited the highest PDI (%) values, with 88.88% and 81.11% respectively. These varieties also demonstrated the lowest plant survival percentages (34.99% and 39.99% respectively) and the shortest vine lengths (144.50 cm and 193 cm respectively) among the different generations screened against WBNV. In contrast, the resistant parent variety, DWM-45, as well as the $F_1$ and $BC_1P_1$ generations, exhibited the low PDI (%) values (3.33%, 5.55%, and 16.02% respectively). These generations displayed higher rates of survival and vine growth, with measurements of 268 cm, 341 cm, and 320 cm respectively at 60 DAS (Days after sowing).

**Fig 2:** WBNV symptoms on watermelon plant (a), leaf (b) and fruit (c).

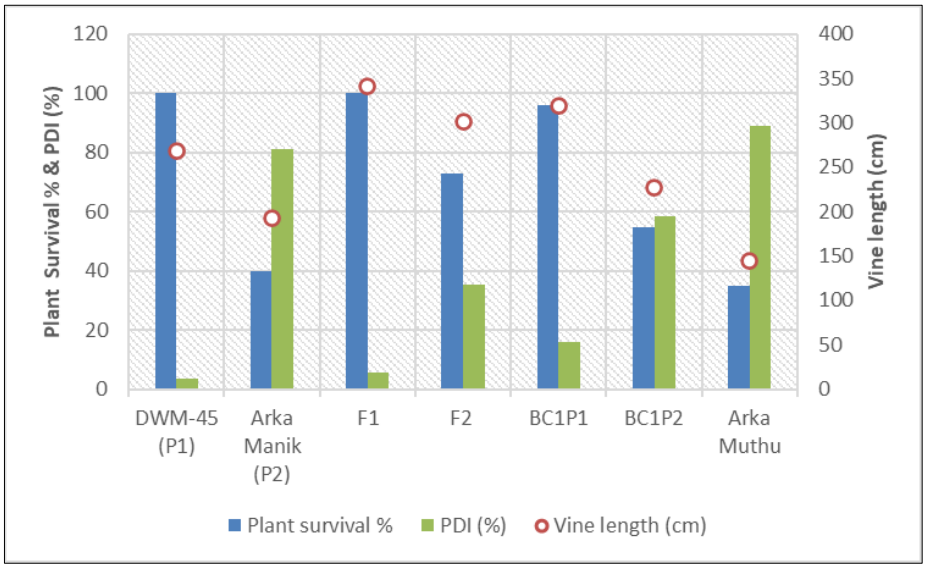


**Fig 3:** Plant survival%, vine length (cm) and PDI (%) in different generations at 60 DAS.

**Table 1:** DAC-ELISA absorbance ($A_{405}$) values of 15 randomly selected bud necrosis symptom plants leaf samples from experimental field to confirm the presence of WBNV for disease screening under field condition.

| Sample | ELISA ($A_{405}$) | Disease score | | | |
|---|---|---|---|---|---|
| | | 30 DAS | 40 DAS | 50 DAS | 60 DAS |
| Plant-1 | 1.10 | 1 | 2 | 2 | 3 |
| Plant-2 | 0.72 | 1 | 2 | 2 | 3 |
| Plant-3 | 0.94 | 0 | 1 | 1 | 2 |
| Plant-4 | 1.13 | 0 | 1 | 1 | 2 |
| Plant-5 | 1.04 | 1 | 1 | 2 | 2 |
| Plant-6 | 1.17 | 0 | 1 | 1 | 2 |
| Plant-7 | 0.79 | 0 | 0 | 1 | 2 |
| Plant-8 | 1.00 | 0 | 0 | 1 | 2 |
| Plant-9 | 1.17 | 1 | 2 | 3 | 3 |
| Plant-10 | 1.05 | 0 | 0 | 1 | 2 |
| Plant-11 | 0.88 | 1 | 2 | 3 | 3 |
| Plant-12 | 1.05 | 0 | 1 | 1 | 2 |
| Plant-13 | 0.98 | 1 | 2 | 3 | 3 |
| Plant-14 | 1.03 | 1 | 2 | 2 | 3 |
| Plant-15 | 0.83 | 0 | 0 | 1 | 2 |
| Positive control | 1.27 | | | | |
| Negative control | 0.35 | | | | |
| Buffer | 0.31 | | | | |

Sample with $A_{405}$ value double than that of negative control are considered as susceptible; DAS-Days after sowing.

**Table 2:** Plant survival%, vine length (cm) and per cent disease index (PDI) values in different generations of the cross DWM-45 × Arka Manik at 60 DAS

| Generations | Plant survival (%) | Vine length (cm) | PDI (%) |
|---|---|---|---|
| DWM-45 ($P_1$) | 100±0 | 268.50±8.5 | 3.33±1.11 |
| Arka Manik ($P_2$) | 39.99±3.33 | 193.0±7.0 | 81.11±1.11 |
| $F_1$ | 100±0 | 341±9.0 | 5.55±3.33 |
| $F_2$ | 72.87±1.87 | 301±9.0 | 35.14±4.12 |
| $BC_1P_1$ | 96.11±1.88 | 320±10.0 | 16.02±5.76 |
| $BC_1P2$ | 54.80±2.88 | 226.50±8.5 | 58.33±1.93 |
| Arka Muthu | 34.99±1.66 | 144.50±5.5 | 88.88±2.22 |
| C.D. | 7.39 | 31.54 | 9.79 |
| SE(m) | 2.095 | 8.94 | 3.05 |
| C.V. | 4.15 | 4.93 | 9.50 |
| p Value | 0*** | 0.00004*** | 0*** |

DAS=Days after sowing.

**Table 3:** Pearson correlation coefficient analysis between plant survival percentage, vine length (cm) and PDI (%) in different generations

| | Vine length (cm) | PDI (%) |
|---|---|---|
| Plant survival percentage | 0.899** | -0.993** |
| Vine length (cm) | | -0.907** |

Interestingly, the $F_2$ generation had a higher PDI value (35.14%) compared to the resistant parent i.e., DWM-45 (3.30%). However, despite this, the $F_2$ generation still demonstrated a higher survival rate i.e., 72.87% and vine growth (301 cm) as compared to the susceptible parent i.e., Arka Manik (39.99% & 193 cm) and $BC_1P_2$ (54.80% & 226.50 cm).

**Correlation between plant survival percentage, vine length (cm) and PDI (%)**

Table 3 illustrates the correlation between PDI (%), plant survival percentage, and vine length (cm) in different generations resulting from the cross between a watermelon bud necrosis disease-resistant prebred line, namely DWM-45, and Arka Manik, which is highly susceptible to watermelon bud necrosis disease. These findings suggested that percent disease index (PDI) had negative correlation with that of plant survival percentage (-0.993) and vine length (-0.907). Furthermore, there was a positive correlation between plant survival percentage and vine length (0.899) of plants in different generations of this cross.

## Discussion

After conducting DAC-ELISA and RT-PCR tests on leaf samples collected from plants exhibiting bud necrosis symptoms in an experimental field, it was conclusively determined that the disease was caused by Watermelon bud necrosis virus (WBNV). The variation in absorbance values obtained from the DAC-ELISA tests across different samples could be attributed to differences in virus concentrations, indicating varying stages of infection.

By evaluating the response of both parental lines and their progenies through natural screening for watermelon bud necrosis disease resistance, it was inferred that the trait is inherited monogenetically. Disease ratings in the $F_1$, $F_2$, $BC_1P_1$, and $BC_1P_2$ generations, when observed under field conditions, fell within the range exhibited by the parental lines. In the $F_2$ population, the PDI slightly increased but remained lower than that of the susceptible parent.

In subsequent backcross generations, the PDI of $BC_1P_1$ approached that of the resistant parent, while $BC_1P_2$ exhibited a PDI closer to that of the susceptible parent i.e., Arka Manik. DWM-45 ($P_1$), $F_1$, $BC_1P_1$, and $F_2$ generations demonstrated slower disease progression, lower PDI, and higher plant survival rates compared to Arka Manik ($P_2$), the $BC_1P_2$ population, and the susceptible check variety, Arka Muthu. The reduced incidence of the disease and the trait of slower disease progression proved valuable in minimizing yield losses and reducing the need for additional disease control measures. In such cases, plants can withstand epidemics for extended periods without being significantly affected by the disease. Similar observations of slower disease progress have been reported in other crops. For example, Kesmala *et al*. (2004) [8] found slow disease progression in peanuts infected with Groundnut bud necrosis orthotospovirus (GBNV), while Nascimento *et al*. (2006) [14] noted the same phenomenon in cucumbers infected with cucumber mosaic virus (CMV).

## Conclusions

Overall, these observations highlight the inverse relationship between PDI values and plant performance. Lower PDI values corresponded to higher survival rates and increased vine growth, while higher PDI values were associated with reduced survival and stunted vine growth. The results revealed a clear relationship, indicating that generations with higher PDI values exhibited lower plant survival percentages and shorter vine lengths at 60 days after sowing (DAS). Conversely, generations with lower PDI values demonstrated higher plant survival rates and greater vine growth.

In conclusion, this study confirms that the Watermelon bud necrosis disease caused by Watermelon bud necrosis orthotospovirus (WBNV). The $F_1$ and $BC_1P_1$ progenies exhibited disease resistance traits similar to the resistant parent (DWM-45), resulting in reduced disease occurrence, slower disease progress, and higher plant survival rates. These findings have significant implications in minimizing yield

losses and reducing the need for excessive disease management interventions. The observed slower disease progression in watermelon, as well as in other crops, highlights the potential for developing strategies to combat viral diseases through breeding and genetic improvement programs.

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**References**

1. Adams MJ, Lefkowitz EJ, King AM, Harrach B, Harrison RL, Knowles NJ, *et al*. Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses. Archives of virology. 2017;162(8):2505-2538.
2. Clark MF, Bar-Joseph M. Enzyme immunosorbent assays in plant virology. In Methods in virology. 1984;7:51-85.
3. FAO. Food and agricultural organization, Rome, Italy. 2021; Retrieved from [http://www.faostat.fao.org]. [Visited on 05 June, 2023].
4. Holkar SK, Kumar R, Yogita M, Katiyar A, Jain RK, Mandal B. Diagnostic assays for two closely related tospovirus species, Watermelon bud necrosis virus and Groundnut bud necrosis virus and identification of new natural hosts. Journal of Plant Biochemistry and Biotechnology. 2017;26(1):43-51.
5. Jain RK, Bag S, Umamaheswaran K, Mandal B. Natural infection by tospoviruses of cucurbitaceous and fabaceous vegetable crops in India. Journal of Phytopathology. 2007;155(1):22-25.
6. Jain RK, Mandal B, Pappu HR, Holkar SK. ICTV taxonomic proposal.005 aV. A. v2. 2014; *Tospovirus_sp*. Create 1 new species in the genus Tospovirus, family *Bunyaviridae*.
7. Jain RK, Pappu HR, Pappu SS, Reddy MK, Vani A. Watermelon bud necrosis tospovirus is a distinct virus species belonging to serogroup IV. Archives of virology. 1998;143(8):1637-1644.
8. Kesmala T, Jogloy S, Wongkaew S, Akkasaeng C, Vorasoot N, Patanothai A. Heritability and phenotypic correlation of resistance to Peanut bud necrosis virus (PBNV) and agronomic traits in peanut. Songklanakarin J. Sci. Technol. 2004;26(2):129-138.
9. Krishnareddy M, Singh SJ. Immunology and molecular based diagnosis of tospovirus infecting watermelon. In Golden jubilee symposium on horticultural research: changing scenario. 1993; Bangalore, India (Vol. 247).
10. Kumar NK, Venkatesh N, Kalleshwaraswamy CM, Ranganath HR. Seasonal incidence of thrips and bud necrosis virus on watermelon. Pest Management in Horticultural Ecosystems. 2006;12(2):85-92.
11. Kunkalikar SR, Poojari S, Arun BM, Rajagopalan PA, Chen TC, Yeh SD, Ravi KS. Importance and genetic diversity of vegetable-infecting tospoviruses in India. Phytopathology. 2001;101(3):367-376.
12. Mandal B, Jain RK, Krishnareddy M, Krishna Kumar NK, Ravi KS, Pappu HR. Emerging problems of tospoviruses (*Bunyaviridae*) and their management in the Indian subcontinent. Plant Disease. 2012;96(4):468-479.
13. Nagesh GC, Rao ES, Pitchaimuthu M, Varalakshmi B, Lakshmana Reddy DC, Samuel DK, Krishna Reddy M. Genetic analysis of resistance to watermelon bud necrosis orthotospovirus in watermelon ((*Citrullus lanatus* (Thunb.) Matsum & Nakai). Plant breeding. 2018;137(5):814-822.
14. Nascimento LCD, Pensuk V, Costa NPD, Assis Filho FMD, Pio-Ribeiro G, Deom CM, Sherwood J. Evaluation of peanut genotypes for resistance to Tomato spotted wilt virus by mechanical and thrips inoculation. Pesquisa Agropecuária Brasileira. 2006;41:937-942.
15. Riley DG, Pappu HR. Evaluation of tactics for management of thrips-vectored tomato spotted wilt virus in tomato. Plant disease. 2000;84(8):847-852.
16. Singh SJ, Krishnareddy M. Watermelon bud necrosis: a new tospovirus disease. Tospoviruses and Thrips of Floral and Vegetable Crops. 1995;431:68-77.
17. Sugiyama M, Okuda M, Sakata Y. Evaluation of resistance to melon yellow spot virus in a cucumber germplasm collection. Plant Breeding. 2009;128:696-700.
18. Thirthamallappa, Lohithaswa HC. Genetics of resistance to early blight in tomato. Euphytica. 2000;113:187-193.