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# SCREENING OF *NICOTIANA RUSTICA* TOBACCO GERMPLASM FOR RESISTANCE TO HOLLOW STALK DISEASE IN NORTH BENGAL

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***Rustica* tobacco (*Nicotiana rustica)* germplasm maintained at Central Tobacco Research Institute-Research Station, Dinhata (West Bengal) were screened for resistance to hollow stalk disease under artificial inoculation. A total of 182 *N. rustica* germplasm entries were screened in a phased manner from the year 2007-08 onwards. A scale was considered for disease reaction (soft rot in stem pith) measuring up to 2, 2 - 3 and > 3 cm for resistant, moderately resistant and susceptible types, respectively for cataloguing the entries. In the year 2007-08 a total of 90 entries were screened and 20 were selected based on disease reaction from < 2 cm to 2.05 cm. For confirmation, these 20 entries were again tested in 2008-09 along with 60 new entries. In the year 2009-10, 23 entries were included from the previous year (2008-09) along with 32 new entries. Only accession White Pathar was found to be resistant (soft rot up to 2 cm linear length) consistently for successive three years. Bengthuli, an entry from Assam showed resistant disease reaction for the years 2009 -10 and 2010-11 only.**

## INTRODUCTION

In India, tobacco is an important commercial crop grown in an area of 0.45 M ha which accounts for 0.31% of net cultivable area in the country with 750 M kg production (Krishnamurthy and Narasimha Rao, 2010). India ranks second in tobacco production and exports in the world. In the northern part of West Bengal, the cultivation of *Jati* (*N. tabacum*) and *Motihari* (*N. rustica*) tobacco is mainly restricted to the districts of Cooch Behar and Jalpaiguri with an acreage from 14,000 to 18,000 ha. Major area is occupied by *Motihari* tobacco (12,000 – 14,000 ha). *Motihari* tobacco is a chewing type and finds its end use as *khaini*, *gurakhu*, *gutka*, *khara masala*, *hookah* paste and powder. Hollow stalk disease of *Motihari* tobacco caused by *Erwinia carotovora* sub sp. *carotovora* syn. *Pectobacterium carotovorum* sub sp. *carotovorum*) poses serious threat in *terai* region of North Bengal as the disease is endemic and the loss ranges from 0.5 - 30 %. In case of higher latent infection in plants, the entire crop will be damaged in the event of high rainfall and water logging conditions (CTRI, 2005; 2006).

In FCV tobacco (*N. tabacum*) hollow stalk disease has been reported from U.S.A , Canada (Lucas, 1975) and China (Xia and Mo, 2007) affecting mainly stem (black leg) and cured leaves. From India hollow stalk has been reported on *N. rustica* tobacco only and to a lesser extent in *Jati* tobacco (*N. tabacum)* from *terai* region of North Bengal (CTRI, 2005; 2006; Roy *et al.* 2008). Softarot Erwinia is one of the most important groups of phytopathogens that are destructive to a wide variety of plants both in cultivation and in storage (Perombelon and Kelman, 1980). Members of this group such as *Erwinia carotovora* sub sp. *carotovora* can infect a different plant species under congenial environmental conditions, particularly high humidity (Lucas, 1975; Perombelon and Kelman, 1980; Kotoujansky, 1987). *Erwinia carotovora* sub sp. *carotovora* does not contain a virulence genes nor appear to cause a hypersensitive reaction in plants, nor has any genetically defined resistance to the pathogen is described (Collmer and Keen, 1986; Kotoujansky, 1987; Keen, 1990). Exogenously added salicylic acid (SA) has been found to induce resistance of the tobacco plants to *Erwinia carotovora* sub sp. *carotovora* (Palva *et al.*, 1994). As per the literature, the susceptibility of a given tissue to maceration is not due to enzyme specificity of soft rot pathogen but depends upon factors such as the amount of calcium pectate present, type of

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pectic enzyme inducing maceration, association of non- pectic poloymers, concentration of pectic enzyme inhibitors (phenolic compounds) and degree of tissue hydration (Turner and Bateman,1968). Keeping into consideration the non-occurrence of hollow stalk disease in FCV tobacco (*N. tabacum*) under Indian conditions and very low incidence (0.1 - 1%) of the disease in *Jati* tobacco (*N. tabacum*), it is essential to search worthwhile for source of resistance in accessions of *N. rustica* tobacco.

## MATERIALS AND METHODS

A total of 182 germplasm accessions maintained at Central Tobacco Research Institute- Research Staion, Dinhata were screened for resistance to hollow stalk initiating from the crop season 2007-08. Following standard protocol, seeds were sown in nursery keeping 1 sq m bed under each accession. Forty days old seedlings raised in 9" earthen pots with three replications in RBD design. Recommended package of practices under crop production was followed. The screening was carried out in a phased manner taking 90, 60 and 32 accessions in 2007-08, 2008-09 and 2009-10 respectively.

The culture of *Erwinia carotovora* sub sp. *carotovora* obtained from diseased plants was cultured in crystal violet pectate medium (CVP) (Cuppels and Kelman, 1974) and four days old culture was used for artificial inoculation of plants. Bacterial culture along with media weighing up to 500 mg was blended in a grinder with 20 ml distilled water and used for artificial inoculation. When the plants attained topping stage, a gentle incision was given with a sterilized scalpel at the topped end, middle and bottom portion of the stem and inoculum @ 0.5 ml was point inoculated with the help of a sterilized pipette and the site was immediately covered with moist cotton swab. After four days, the plants were split open and soft rot of pith was measured on a linear scale. A scale was considered to catalogue the accessions as resistant, moderately resistant and susceptible based on soft rot of pith measuring up to 2 cm, 2-3 cm and > 3 cm respectively. An entry found to be within the score limits of resistant reaction (upto 2 cm) were included for repeat screening in the subsequent year. The protocol for final clearance of an entry for resistant disease reaction was based on consistency of results for successive three crop seasons.

## RESULTS AND DISCUSSION

The results of the screening carried out during 2007-08 for resistance to hollow stalk in 90 germplasm entries has been presented in Table 1. The disease reaction of hollow stalk (linear measurement of pith soft rot) in *N. rustica* accessions ranged from 0.62 – 5. 50 cm. A total of 29 accessions were found to be statistically at par with disease reaction score ranging from 0.62 - 2. 40 cm. Only 20 accessions (C-23, C-26, C-302, V-115, SK-267, SK- 393, SK-403, SK-241, HAR-1, HAR-5, HAR-6, HAR-7, Manda, Sikni Manda, White Pathar, Peshwar Local, Chaithar, P-6, T-133 and T-17) exhibiting disease reaction within 2 cm score were selected for repeated screening in the subsequent year.

During 2008-09, new and entries under repeat from previous year have been presented in Table 2. Out of 20 entries under second year screening, four exhibited resistant disease reaction for soft rot (1.8 – 2.0 cm) and were promoted to third year screening (2009-10). Among 60 new entries, based on resistant disease reaction score, 12 were promoted to second year screening (2009-10).

During the year 2009-10, 32 new entries were screened and were presented in Table 3. The results exhibited that only accession White Pathar exhibited resistant disease reaction up to 2 cm (1.55 - 2.0 cm) consistently for three successive years (2007-08, 2008 - 09 and 2009-10). Among 32 new entries, entries screened during 2009-10, the entries Bengthuli Sada (1.5 cm), Rustica (1.85 cm), Bitri 1 (1.90 cm), NP-222 (2 cm) and Bengthuli (2 cm) were showed resistant disease reaction. Five and four entries showing resistant (upto 2 cm) and moderately resistant (2-3 cm) disease reaction respectively were promoted for repeat screening during 2010-11.

During 2010-11, 5 and 4 *N. rustica* accessions showing resistant and moderately resistant respectively to hollow stalk have been presented in Table 4. Only Bengthuli, an indigenous accession from Assam was found to be resistant

**Table 1: Screening of *N. rustica* tobacco germplasm accessions to hollow stalk (2007-08) disease reaction (linear length in cm)**

| S. No. | Accession | DR | S.No. | Accession | DR | S.No. | Accession | DR |
|---|---|---|---|---|---|---|---|---|
| 1 | Black Queen | 2.67 | 31. | Snuff-6 | 3.12 | 61. | SK-404 | 2.15 |
| 2. | Badawn local | 2.65 | 32. | Snuff-7 | 4.70 | 62. | SK-417 | 2.87 |
| 3. | Buland Shahar Collection | 2.65 | 33. | Snuff-8 | 4.32 | 63. | SK-419 | 2.05 |
| 4. | B-41 | 2.45 | 34. | S-4 | 2.76 | 64. | SK-241 | 1.00 |
| 5. | C-5 | 2.25 | 35. | S-7 | 2.47 | 65. | HAR-1 | 1.67 |
| 6. | C-10 | 5.50 | 36. | S-9 | 3.47 | 66. | HAR-5 | 1.27 |
| 7. | C-13 | 3.32 | 37. | S-12 | 2.97 | 67. | HAR-6 | 1.77 |
| 8. | V-116 | 3.47 | 38. | Labu | 2.60 | 68. | HAR-7 | 1.17 |
| 9. | C-19 | 3.05 | 39. | T-238 | 3.00 | 69. | Manda | 1.67 |
| 10. | C-20 | 3.15 | 40. | SK-10 | 4.42 | 70. | Sikni Manda | 1.15 |
| 11. | C-21 | 2.75 | 41. | SK-24 | 2.90 | 71. | Kanpur Local | 4.87 |
| 12. | C-22 | 2.60 | 42. | SK-29 | 3.52 | 72. | Boalmari | 2.52 |
| 13. | C-23 | 1.60 | 43. | SK-48 | 2.65 | 73. | White Pathar | 1.00 |
| 14. | C-24 | 2.20 | 44. | SK-49 | 4.17 | 74. | Peshwar Local | 0.75 |
| 15. | C-25 | 2.75 | 45. | SK-88 | 3.75 | 75. | Hazro | 4.87 |
| 16. | C-26 | 1.87 | 46. | SK-92 | 2.55 | 76. | Calcuttia | 2.40 |
| 17. | C-302 | 1.72 | 47. | SK-101 | 4.12 | 77. | Chaithar | 1.32 |
| 18. | C-304 | 2.82 | 48. | SK-115 | 3.27 | 78. | P-6 | 1.45 |
| 19. | Jaunpur Local | 2.75 | 49. | SK-119(2) | 3.12 | 79. | T-133 | 0.95 |
| 20. | H-26 | 2.10 | 50. | SK-140 | 2.37 | 80. | Tarrale | 2.55 |
| 21. | Muzaffar Nagar Collection | 3.20 | 51. | Sk-141-1 | 3.12 | 81. | T-17 | 1.82 |
| 22. | NP-219 | 2.80 | 52. | Sk-141-2 | 3.62 | 82. | HDM-1 | 3.17 |
| 23. | Dharla | 2.45 | 53. | Sk-196 | 2.02 | 83. | Calcutti-9 | 3.50 |
| 24. | Raja Karampur | 2.57 | 54. | Sk-39 | 2.10 | 84. | K-8 | 4.62 |
| 25. | R-9 | 2.47 | 55. | SK-217 | 3.02 | 85. | Patrahi Local | 5.07 |
| 26. | ST-1 | 2.42 | 56. | V-115 | 0.62 | 86. | Jhuripara | 3.30 |
| 27. | Snuff-2 | 2.55 | 57. | SK-267 | 1.45 | 87. | Rangpur | 1.90 |
| 28. | Snuff-3 | 2.10 | 58. | SK-393 | 1.40 | 88. | GC-1 | 3.87 |
| 29. | Snuff-4 | 2.70 | 59. | Sk-400 | 3.12 | 89. | DD-437 | 3.97 |
| 30. | Snuff-5 | 2.15 | 60. | Sk-403 | 1.80 | 90. | Hemti | 3.82 |
| | **SEm±** | **0.64** | | | | | | |
| | **CD (P=0.05)** | **1.78** | | | | | | |
| | **CV (%)** | **40.14** | | | | | | |

for successive two years of testing (2009-10 and 2010-11). Third year testing for Bengthuli (1.60 cm) and SH-31 (2.15 cm) exhibiting resistant and moderately resistant disease reaction respectively, shall be taken up during 2011-12 for assessing the consistency of results. The line White Pathar as resistant source material shall be incorporated in breeding programme for evolving varieties/lines resistant to hollow stalk disease under North Bengal conditions.

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**Table 2: Screening of *N. rustica* tobacco germplasm accessions to hollow stalk (2008-09) disease reaction (linear length in cm)**

| S. No. | Accession | DR | S.No. | Accession | DR | S.No. | Accession | DR |
|---|---|---|---|---|---|---|---|---|
| 1 | C-23 | 7.90 | 28. | SK-200 | 1.80 | 55. | Chatwana | 5.05 |
| 2. | C-26 | 2.00 | 29. | Bitri | 2.90 | 56. | I-395 | 3.10 |
| 3. | C-302 | 2.00 | 30. | V-166 | 7.15 | 57. | Surya Range | 3.05 |
| 4. | V-115 | 3.05 | 31. | S-22 | 5.60 | 58. | Hirawata | 3.00 |
| 5. | SK-267 | 4.70 | 32. | BVM-5 | 5.15 | 59. | M-2 | 5.70 |
| 6. | SK-393 | 5.08 | 33. | NP-18 | 3.20 | 60. | Gosaigaon -1 | 8.10 |
| 7. | SK-403 | 4.40 | 34. | III-281 | 3.30 | 61. | Gosaigaon-2 | 6.50 |
| 8. | SK-241 | 4.90 | 35. | III-405-1 | 4.70 | 62. | Gosaigaon-3 | 7.25 |
| 9. | HAR-1 | 5.65 | 36. | III-407-2 | 2.40 | 63. | Farukhabad | 5.50 |
| 10. | HAR-5 | 5.15 | 37. | III-445 | 4.15 | 64. | HD-65-40 | 7.90 |
| 11. | HAR-6 | 8.75 | 38. | M- 1 | 4.60 | 65. | Bulk-1 | 6.90 |
| 12. | HAR-7 | 4.10 | 39. | M-3 | 1.90 | 66. | Bulk-2 | 6.50 |
| 13. | Manda | 5.15 | 40. | M-5 | 2.55 | 67. | Tangua Local | 2.30 |
| 14. | Sikni Manda | 3.40 | 41. | M-6 | 3.65 | 68. | Kharagpur | 2.45 |
| 15. | White Pathar | 2.00 | 42. | M-10 | 8.90 | 69. | Motihari | 6.75 |
| 16. | Peshwar Snuff | 7.80 | 43. | M-11 | 6.45 | 70. | Banbag Adampur | 7.60 |
| 17. | Chaithar-1 | 6.70 | 44. | M-8 | 4.15 | 71. | Baisandi | 3.35 |
| 18. | P-6 | 7.35 | 45. | Harpur Sadi | 2.00 | 72. | Chaithar-2 | 6.00 |
| 19. | T-133 | 4.70 | 46. | Kursaila | 2.00 | 73. | Coker-1 | 8.65 |
| 20. | T-17 | 1.80 | 47. | Santinya Parihar Range | 2.60 | 74. | Coker-2 | 2.60 |
| 21. | C-1 | 1.58 | 48. | Khaga Dama Daha Range | 1.90 | 75. | Daiya Kharwar-2 | 5.70 |
| 22. | R-12 | 1.90 | 49. | Daiya 1 Kharwar- | 1.95 | 76. | Damadaha Range | 5.85 |
| 23. | SK-39 | 4.20 | 50. | RT-Bulk | 3.00 | 77. | Goramma | 8.15 |
| 24. | SK-143-2 | 2.40 | 51. | NPS-219 | 1.85 | 78. | K-6 | 2.25 |
| 25. | SK-156 | 1.60 | 52. | I-191 | 1.80 | 79. | Krusaila Small leaf | 4.65 |
| 26. | SK-158-1 | 2.40 | 53. | Aligarh Local | 1.85 | 80. | Maharka | 2.25 |
| 27. | SK-193-2 | 4.50 | 54. | M-9 | 2.15 | | | |
| | **SEm±** | **0.88** | | | | | | |
| | **CD (P=0.05)** | **2.50** | | | | | | |
| | **CV (%)** | **29.10** | | | | | | |

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**Table 3: Screening of *N. rustica* tobacco germplasm accessions to hollow stalk (2009-10) disease reaction (linear length in cm)**

| S. No. | Accession | DR | S.No. | Accession | DR | S.No. | Accession | DR |
|---|---|---|---|---|---|---|---|---|
| 1 | C-26 | 10.53 | 17. | Mainpuri local | 4.30 | 33. | T-131 | 2.50 |
| 2. | C-302 | 5.38 | 18. | Mandhata Khaga | 5.18 | 34. | T-174 | 2.90 |
| 3. | R-12 | 3.86 | 19. | I-104 | 3.00 | 35. | T-192 | 2.00 |
| 4. | SK-156 | 7.90 | 20. | Meerganj Range | 5.70 | 36. | T-128 | 2.50 |
| 5. | SK-200 | 4.70 | 21. | NP-126 | 5.30 | 37. | NP-220 | 4.06 |
| 6. | White Pathar | 1.55 | 22. | P-7 | 4.36 | 38. | NP-222 | 2.00 |
| 7. | T-17 | 4.85 | 23. | Pakur | 3.38 | 39. | Manda | 1.50 |
| 8. | C 1 | 7.23 | 24. | Pandharpuri | 4.55 | 40. | Tangua Manda | 4.73 |
| 9. | M-3 | 7.03 | 25. | Patrahi | 3.38 | 41. | Bombai | 1.90 |
| 10. | Harpursadi | 2.98 | 26. | Rustica-6 | 1.85 | 42. | SH-130 | 5.15 |
| 11. | Kursaila | 3.51 | 27. | Rustica-12 | 3.40 | 43. | SH-31 | 4.06 |
| 12. | Khaga Damadaha | 4.10 | 28. | Rustica local | 4.13 | 44. | Bengthuli | 2.00 |
| 13. | Daiya Kharwar | 4.55 | 29. | Rustica lumitie | 4.95 | 45. | Bengthuli Sada | 1.50 |
| 14. | NPS-219 | 8.00 | 30. | Rustaiya Range | 4.34 | 46. | Tangua-1 | 4.73 |
| 15. | 1-191 | 5.46 | 31. | Sikandra Roa Local | 4.70 | 47. | Bitri-1 | 1.90 |
| 16. | Aligarh Local | 5.46 | 32. | T-26 | 4.48 | 48. | Bombai-1 | 5.15 |
| | **SEm±** | **0.14** | | | | | | |
| | **CD (P=0.05)** | **0.40** | | | | | | |
| | **CV (%)** | **4.63** | | | | | | |

**Table 4: Screening of *N. rustica* tobacco germplasm accessions to hollow stalk (2010-11) disease reaction**

| S. No. | Accessions | Disease reaction – linear soft rot (cm) | Ranking |
|---|---|---|---|
| 1. | I-104 | 5.16 | 6 |
| 2. | Rustica -6 | 4.91 | 5 |
| 3. | NP-222 | 7.0 | 8 |
| 4. | Manda | 5.38 | 7 |
| 5. | SH-30 | 3.70 | 3 |
| 6. | SH-31 | 2.15 | 2 |
| 7. | Bengthuli | 1.60 | 1 |
| 8. | Bengthuli Sada | 8.90 | 9 |
| 9. | Bitri | 4.63 | 4 |
| | **SEm±** | **0.164** | |
| | **CD (P=0.05)** | **0.493** | |
| | **CV (%)** | **5.91** | |

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