Combining ability and heterosis of tobacco (*Nicotiana tabacum*) resistance to bacterial wilt

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Abstract. Bacterial wilt is one of the main diseases of tobacco, caused by *Ralstonia solanacearum*. One of the best approaches to control this pathogen is developing tobacco-resistant varieties. Combining ability analysis can be used to preliminarily identify the advantages and disadvantages of cross combinations and parents in earlier generations. The study aimed to estimate combining ability and heterosis of tobacco lines to bacterial wilt. Six tobacco genotypes were crossed in diallelic mating design to produce 15 F1’s. These F1’s and their parents were inoculated with the pathogen in the glasshouse condition based on Huang and Allen's method. The experiment used Random Block Design with three replications. The analysis of combining ability was estimated following Griffing's method-II and model-I, which is as described by Singh and Chaudhary. The analysis revealed that both additive and dominant genes controlled the resistance to *R. solanacearum*. The cross between NtJU and NtSP1 genotypes could generate F1 hybrid varieties that have more resistance to bacterial wilt. Tobacco genotype Nt_U2 showed a significant and negative value of general combining ability. Therefore, it can be used as a donor parent in the tobacco breeding program to generate resistant wilt tobacco varieties.

Keywords: Diallelic crossing, GCA, SCA, *Ralstonia solanacearum*

1. Introduction

Tobacco (*Nicotiana tabacum* L.) is an agricultural commodity that has high economic value. In Indonesia, tobacco leaves are generally processed as sliced tobacco which is used as raw material for the kretek cigarette industry. Information from various tobacco areas in Indonesia shows that the commodity can contribute 40%-65% to farmers' total income. One of the obstacles in tobacco cultivation that has not been solved so far is bacterial wilt disease attack. The tobacco-growing areas in Indonesia that are infected by bacterial wilt disease increase every year in which the economic losses due to the disease infection is ranging from 40% - 60% [1].

*Ralstonia solanacearum* is a bacterium that causes bacterial wilt and is one of the most damaging phytopathogens of solanaceous plants, including tobacco. The pathogen exists worldwide, primarily in tropical and subtropical regions [2]. *R. solanacearum* is soil-borne that penetrates through plant roots, invades xylem tissue, and develops irreversible wilting rapidly, leading to the plant's death [3, 4]. The age, nutritional status of the host, environmental factors, and the antagonism of the pathogen will determine the speed and severity of the development of wilt symptoms [2, 5].

Many researchers have conducted studies on disease control but with limited success. Controlling the disease is challenging because the pathogen has a broad host range, widespread distribution, and...
vast genetic mutation [6, 7]. Due to the limited strategies in efficiently exterminating *R. solanacearum* from infected soils, using resistant cultivars presumably the best disease control strategy [8]. In the process of developing varieties, genetic information about the desired character will increase the achievement of producing varieties with the desired traits, including resistance to disease [9]. The genetic information that is essential for practical breeding and selection [8, 9] could be achieved by a diallelic crossing approach.

A diallel crossing is a cross made between all pairs of parents so that the potential for general and specific combining ability is known. Meanwhile, combining ability analysis helps identify superior parents or promising cross combinations for cultivar development, i.e., potentially high yielding and disease resistance. The available combinations can be divided into three groups: (1) p pure lines alone, (2) a set of p(p-1) F1, and (3) a set of p(p-1) reciprocal F1 [10]. Griffing [11] divided diallelic crosses into four (4) methods and two (2) models based on the consideration of including a group or not. Singh stated that diallel cross analysis is used to estimate combining ability value in hybrids and assist breeders in increasing selecting segregated populations. Furthermore, the advantage of using diallelic analysis is that the breeding material can be selected from an early stage. So, it is possible to estimate hybrid performance using specific combining ability and parental performance using general combining ability [10, 12].

The study focused on estimating the combining ability of the tobacco genotypes tested and obtaining information on the phenomenon of heterosis on the resistance character of tobacco genotypes tested to bacterial wilt disease caused by *R. solanacearum*. The study results will be used to develop resistant tobacco varieties to bacterial wilt in which any high yielding variety or hybrid developed should have a resistance to bacterial wilt.

2. Materials and Methods

2.1 Parents and crosses

The six parents viz (1) NtKA20, (2) NtGKm, (3) NtSP1, (4) NtU2, (5) NtJU2, and (6) NtGKn were crossed in all combinations (6 x 6) without reciprocals to produce 15 F1. Crosses to obtain F1 seeds were carried out in the Plant Breeding's greenhouse of the Indonesian Sweetener and Fiber Crops Research Institute (ISFRI) in 2018. Parents, F1 progenies were then evaluated in the Phytopathology greenhouse of ISFRI in 2019.

The experiment was arranged in a randomized complete block design with three replications. Experimental units included teen (10) plants of each parent and twenty (20) plants of each cross. Tobacco seedlings were planted in polybags with a volume of 10 kg soil. The potting soil mixture of sand: soil: compost (1:3:1) was pasteurized with steam before use. The treatments were evaluated for bacterial wilt reactions in the greenhouse.

General combining ability (GCA) and specific combining ability (SCA) variances and their effects were estimated according to Griffing Method-II and Model-I [11] as described by Singh and Chaudhary [10].

2.2 Inoculum preparation and root inoculation

The inoculum was taken from tobacco plants infected with bacterial wilt disease. Isolation, purification, and inoculum propagation were carried out in the Phytopathology Laboratory of ISFRI. The inoculum was grown on CPG (Casein Peptone Glucose) agar medium [13] until pure cultures without contaminants were obtained (Figure 1). Pure cultures were pre-tested on susceptible plants for getting symptoms expressions. The results of these tests were isolated and reproduced as inoculum material at the time of inoculation [14].
Figure 1. (a) Virulent colonies of *R. Solanacearum* on CPG agar medium; (b) Bacterial streaming in clear water from tobacco stem cross-section of plant infected by *R. solanacearum*

*R. solanacearum* strain isolated locally from infected tobacco plants. The isolate was grown on casein-peptone-glucose (CPG) agar and stored at 28°C for 48 h. Several fluidal colonies were transferred to plates containing the agar media for multiplication at 28°C for 24 h. The inoculum was prepared by flooding plates with sterile water and resuspending bacterial cells to an optical density at 600 nm = 0.3 (1 × 10^8 CFU/ml) [15]. The inoculum was further diluted to 1 × 10^6 CFU/ml immediately prior to inoculation. Four-week-old seedlings, approximately in the five-leaf stage, were inoculated by pouring 10 ml of 1 × 10^6 inoculums on the soil surface at the base of each plant. Plant roots were wounded before inoculation. Observations began two days after inoculation with an interval of two days until the plants were 60 days old (Figure 2). Observations using the modified Winstead and Kelman method [16, 17], by measuring bacterial wilt disease index (BWDI), which is as follows:

$$BWDI = \frac{\sum_{i=1}^{n} a_i n_i}{A x N} \times 100\%$$

BWDI = bacterial wilt disease index  
\(a\) = scale value of each attack category  
\(n\) = number of plants at a certain attack scale value  
\(A\) = highest attack scale value  
\(N\) = total number of plants observed

The attack scale values used are:

- 0 = healthy  
- 1 = 1 - 10% wilting leaves  
- 2 = 10.1 - 25% wilting leaves  
- 3 = 25.1 - 50% wilting leaves  
- 4 = 50.1 - 75% wilting leaves  
- 5 = 75.1 - 100% wilting leaves or plant death

The data obtained were grouped using resistance criteria as described by Wernsman *et.al* [16] and Csinos *et.al.* [18] which is divided into:

- 0 - 17% = Resistant  
- 17.1 - 42% = Moderate resistant  
- 42.1 - 73% = Susceptible  
- > 73% = Very susceptible
Figure 2. (a) Symptom of bacterial wilt of tobacco caused by *R. solanacearum* showing wilting of leaves; (b) Brown discoloration of tobacco stem tissues caused by *R. solanacearum*

2.3 Diallelic analysis

Combining ability effects and variances were calculated following Griffing's [11] method 2, model 1 (all possible combinations excluding reciprocals) as described by Singh dan Chaudhary [10]:

\[ g_i = \frac{1}{(n + 2)} \{ \sum (Y_i + Yii) - 2/(n) \ Y \} \]
\[ s_{ij} = Yij - \frac{1}{(n + 2)} \{ (Y + Yii + Yj + Yij) + 2/(n + 1) (n + j) \ Y \} \]

\( g_i \) = general combining ability; \( s_{ij} \) = specific combining ability

To test the standard error value of \( g_i \) and \( s_{ij} \) are used:

\[ S_e g_i = \{ n - 1 \ \sigma_e / (n)(n + 2) \}^{1/2} \]
\[ S_e s_{ij} = \{ 2(n - 1) \ \sigma^2 e / (n + 1)(n + 2) \}^{1/2} \]

Meanwhile, gene action analysis was used:

\[ \sigma^2 \text{additive} = \frac{\text{MS}_{gj} - \sigma^2 \ e}{p + 2} \]
\[ \sigma^2 \text{non additive} = \frac{\text{MS}_{sij} - \sigma^2 \ e}{2} \]

Heritability value as described by Sprague [10]

\[ H(1) = \frac{\sigma^2 \ g}{\sigma^2 \ p} \]
\[ H(s) = \frac{\sigma^2 \text{additive}}{\sigma^2 \ p} \]

\( H (1) \) = \( H \) in broad sense
\( H (s) \) = \( H \) in narrow sense
\( \sigma^2 g \) = genetic variance
\( \sigma^2 p \) = phenotype variance

Heterosis standard (HS) value as described by Fehr [10]

\[ \text{HS} = \frac{F1 - \text{MP}}{\text{MP}} \times 100\% ; \quad \text{Se HS} = \left( 3\times \sigma^2 e / 2r \right)^{1/2} \]

\( \text{MP} \) = Mid parent value
3. Results and discussion

The analysis of variance for combining ability shows that both general combining ability (GCA) and specific combining ability (SCA) is very significant for BWDI value (Table 1). The significantly mean square value for BWDI indicates the resistant character is controlled by additive and dominant variance, which demonstrated the important role of both components in the inheritance of the resistance to bacterial wilt in tobacco. Griffing [11] stated that GCA and SCA are composed of various forms of gene roles. The variance of GCA consists mainly of additive genetic variance, while the variance of SCA consists mainly of dominant genetic variance [19]. Furthermore, Kumari suggests that the variance of additive, dominant, and additive x additive interactions are essential to determine the inheritance pattern of the studied characters [20].

| Character | σ²A | σ²D | σ²A/(σ²A + σ²D) | H₁ | Hₛ |
|-----------|-----|-----|-----------------|----|----|
| BWDI      | 0.0151 | 0.0376 | 0.2865 | 72.39 | 20.74 |

Further observation on the genetic parameter for the BWDI value shows that the role of the dominant variance is +2.5 times greater than that of the additive variance. In addition, the calculation of the value of the relative role of additive genes to the total genetic variance is less than 0.5. Collectively these data demonstrated that the environment influences the resistance to bacterial wilt in tobacco. The result agrees with the Monma's research [21] results, which suggests that the resistance to *R. solanacearum* in tomato is partially dominant towards vulnerability depending on the level of resistance of the parents used. Huet [22], in his review, stated that the availability of resistance to bacterial wilt in Solanaceous crops expressed differently under changing environmental conditions. Moreover, *R. solanacearum* strains have been found to be under an extensive genetic diversity worldwide.

The data from Table 2 shows that the lower additive variance value compared to that of the non-additive variance result in the heritability value included in a narrow sense (20.74%). The low heritability value indicates that the resistance trait has high environmental effects, which may be also in less gene interaction [23]; hence, it is recommended the selection of the resistance character be carried out with a loose selection intensity [23, 24].

Improvement of bacterial wilt resistant is the major goal of the tobacco breeding program. Combining ability was found essential in governing disease resistance in Solanaceous crops, including tobacco. GCA effects for parents and SCA effects for crosses were estimated, which are presented in Table 3.
Furthermore, negative heterosis value were NtJU and NtSP1. Both genotypes also give the highest negative heterosis value were NtJU and NtSP1. Both genotypes also give the highest resistance to bacterial wilt.

Table 3. General combining ability value (diagonal) and specific combining ability value (above diagonal) for BWDI in 6x6 partial diallelic crosses.

| Parents  | NtGKm  | NtKA20 | NtSP1  | NtU2  | NtJU2 | NtGKn |
|----------|--------|--------|--------|-------|-------|-------|
| NtGKm    | -0.028 | -0.065 | 0.0585 | 0.1561 | 0.0923| 0.0111|
| NtKA20   | 0.035  | -0.0052| 0.0323 | 0.0486| 0.1073|
| NtSP1    | 0.081* | 0.0561 | -0.2376** | 0.0510|
| NtU2     | -0.086*| -0.010 | 0.0179**|
| NtJU2    | -0.0238| 0.0048 |
| NtGKn    | 0.019  |        |        |

General combining ability is due to genes, which are largely additive in their effects, and specific combining ability is due to the genes with dominance or epistatic effect. Both additive and nonadditive effects are essential in governing disease resistance in commercial crops [25]. Furthermore, negative GCA and SCA effects are preferable for disease resistance on the basis of a scale where the highest value is associated with more disease attacks [26]. In this study, the negative GCA for BWDI value indicated that the parents used have resistance to R. solanacearum. Among the six parents used, the NtU2 genotype exhibited a significantly negative GCA effect; hence, the NtU2 proved to be a good combiner and can be considered as a donor parent to improve the tobacco resistance to R. solanacearum. Although the SCA value was significant (Table 1), only 2 of the 15 hybrids had a significantly negative value (Table 3). The two crosses are (NtSP1 x NtU2), with an SCA value of -0.0238 (significantly negative); and (NtU2xNtGKn) with an SCA value of -0.0179 (significantly negative). Based on the SCA value that consistently delivers a negative value on the resistance character; hence, the two crosses have the potential to produce hybrids lines that are resistant to bacterial wilt disease. According to Callaway, hybrids having high GCA values are not always obtained from a cross between parents which have high GCA values but can also be obtained from a cross between respective parents with high and low GCA values [27]. In such circumstances, complementary genes work, or the resistance character was governed by non-additive gene action [23].

The percentage of a hybrid character from the mid-parent is expressed by the heterosis standard. The high heterosis negative value indicates that the improvement of resistance to R. solanacearum will be more successful if the goal is a hybrid [28]. In the condition, the influence of dominant and epistatic genes in the heterozygote formation. Estimation of standard heterosis is presented in Table 4.

Table 4. The mean BWDI value of parents, parental median value, and standard heterosis (%) in 6x6 partial diallelic crosses

| Parents  | NtGKm  | NtKA20 | NtSP1  | NtU2  | NtJU2 | NtGKn |
|----------|--------|--------|--------|-------|-------|-------|
| NtGKm    | 1.63*  | 0.64%  | 4.18%  | 22.45%* | -9.14% | 16.25%* |
| NtKA20   | 1.70   | 1.78   | 0.27%  | 12.99% | 0.55% | 14.97%* |
| NtSP1    | 1.795  | 1.865  | 1.96   | 10.70% | -17.49% | 8.22% |
| NtU2     | 1.47   | 1.54   | 1.635  | 1.38  | 3.14% | 29.86%** |
| NtJU2    | 1.75   | 1.82   | 1.915  | 1.59  | 1.87  | 2.33% |
| NtGKn    | 1.6    | 1.67   | 1.765  | 1.44  | 1.72  | 1.57   |

*) Significant at P < 0.05
**) Significant at P < 0.01
#) The mean BWDI value of parents (diagonal), parental median value (below diagonal), and standard heterosis (above diagonal)

Heterosis which has a negative value is more desirable in this study, because the variable used in estimating heterosis value is BWDI. Thus, the lower the BWDI value, the more resistant the genotype is to the bacterial wilt. Our study result shows that the genotypes of the cross that gave the highest-negative heterosis value were NtJU and NtSP1. Both genotypes also give the highest-negative SCA
value (Table 3), which means that they contribute to the resistance trait. Hence, the cross between NtJU and NtSP1 are potential to produce F1 hybrid lines that are resistant to \textit{R. solanacearum}.

4. Conclusions

The present results reveal that the additive and dominant genes contribute in resistance trait to \textit{R. solanacearum} in the tobacco genotypes tested. Among the six parents used, the NtU2 genotype proved to be a relevant source for generating resistance varieties to bacterial wilt. Considering the high negative SCA effect, the cross between NtJU and NtSP1 could be utilized for obtaining F1 tobacco hybrid varieties that have more resistance to bacterial wilt.

Acknowledgment

The authors would like to thank the reviewers for their valuable suggestions. The current work was supported by the Indonesian Agency for Agricultural Research and Development through DIPA/2018-2019 of the Indonesian Sweeteners and Fiber Crops Research Institute.

References

[1] Titik Y 2009 Perspektif \textbf{8} 11 – 16
[2] Hayward AC 1991 \textit{Annu. Rev. Phytopathol.} \textbf{29} 65–87. doi: 10.1146/annurev.phyto.29.090191.000433
[3] Genin S 2010 \textit{New Phytol.} \textbf{187} 920–928 doi: 10.1111/j.1469-8137.2010.03397.x
[4] Grimault V, Anais G, and Prior P 1994 \textit{Plant Pathol.} \textbf{43} 663-668.
[5] Denny T 2000 \textit{Trends Microbiol} \textbf{11} 486–489
[6] Caldwell D et al 2017 \textit{Phytopathol} \textbf{107} 528–536 http://dx.doi.org/10.1094/PHYTO09-16-0353-R
[7] Wenneker M, Verdel, MSW, Beuningen ARV, Derks JH, and Janse JD 1999 \textit{Eur J of Plant Pathol} \textbf{105} 307-315.
[8] Agrios G N 2005 5th Edn. Amsterdam Elsevier Academic Press
[9] Huet G 2014 \textit{Front. Plant Sci.} \textbf{5} 715.
[10] Singh RH, and BD Chaudhary 1985 Kalyani Publishers, Ludhiana, India. 318 p.
[11] Griffith B 1956 \textit{Aust J of Biol Sci} \textbf{9} 463-493.
[12] Arwiyanto T and Sudarmadi 1996 \textit{J Perlindungan Tanaman Indonesia} \textbf{2} 60 – 65.
[13] Acquaah, G 2007 Oxford: Wiley Blackwell.
[14] French EB, L Gutarra, P Aley, and J Elphinstone 1995 \textit{Fitopatol} \textbf{30} 126–130
[15] Huang Q and Allen C 2000 \textit{Physiol and Mol Plant Pathol} \textbf{57} 77-83. http://dx.doi.org/10.1006/pmpp.2000.0283
[16] Winstead NN, Kelman A 1952 \textit{Phytopathol} \textbf{42} 628–634.
[17] Arwiyanto T, M Goto, S Tsuyumu, and Y Takikawa 1994 \textit{Phytopath Soc Japan.} \textbf{60} 421-430.
[18] Csinos AS, BA Fortnum, NT Powel, JJ Reilly, and HD Shew 1984 \textit{Tob. Sci.} \textbf{28} 153-155.
[19] Sibiya J, Tongoona P, Derera J, RJ VN 2013 \textit{Euphytica} \textbf{185} 349-362.
[20] Koshiba J, Dikshit HK, Singh B, and Singh D 2015 \textit{Sci Hortic} \textbf{181} 26-33.
[21] Momma N 2008 \textit{JARQ}, \textbf{42} 1 7-12
[22] Huet G 2014 \textit{Frontier in Plant Sci.} \textbf{5} 1-5
[23] Chigeza G, Masingaidze K, Shanahan P 2014 \textit{Euphytica} \textbf{195} 2 183-195
[24] Bouchetat F, and Assat A 2019 Heliyon \textbf{5} e02744
[25] Derera J, Pixley KV, Giga DP \textit{et al.} 2014 \textit{J of Stored Prod Res} \textbf{59} 24-35
[26] Mukankusi C, Derera J, Melis R \textit{et al.} 2011 \textit{Euphytica} \textbf{182} 11-23
[27] Qian Y L \textit{et al} \textit{2013} \textit{Euphytica} \textbf{192} 259–266. doi: 10.1007/s10681-012-0846-2
[28] Longin CFH \textit{et al.} 2013 \textit{Theor Appl Genet} \textbf{126} 2791-2801