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Short Communication

Genetic analysis of bacterial wilt resistance in hot pepper (*Capsicum annuum* L.)

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Chilli or hot pepper (*Capsicum annuum* L.) is economically important vegetable cum spice crop. It belongs to nightshade Solanaceae family. Owing to its high cash value, it is widely cultivated in India. During 2017-18, India produced 2.40 million tons of chilli from an area of 859790 ha and exported 0.44 million tons (Spice board 2017-18). However, the productivity in India is very low (1.9 t/ha) due to many biotic and abiotic constraints. Among biotic category, bacterial wilt caused by soil-borne pathogen *Ralastonia solanacearum is* pervasive across the hot and humid tropics of world. This pathogen has very wide host range of 54 families plant species (Wicker et al. 2007) and considered as second most economically important pathogen among bacterial pathogens (Mansfield et al. 2012). *Ralstonia solanacearum* biovars 1, 3, 3a, 3b and 4 of race 1 are predominantly causes bacterial wilt in hot or sweet pepper (Lopes et al. 2004, Liao et al. 2005). In India, biovar 3 of race 1 is predominant mainly in humid areas (Markose et al. 1996). The bacteria enter into the plants through wounds or secondary root initiation points (Pradhanang et al. 2005) and blocks vascular parenchyma by colonizing inside it. Blockage of water translocation channels followed by sudden wilting of plants occurs in plant, owing to rapid multiplication of *R. solanacearum* colonies in xylem, ultimately causing the death of plant. Host plant resistance to bacterial wilt is most appropriate strategy. Bacterial wilt is more prevalent in tropical coastal belt of India, and there is need to develop varieties/hybrids having resistance as wilt outbreak leads to death of plants. In this connection at Central Horticultural Experiment Station (CHES) (ICAR-IIHR), Bhubaneswar (hot spot region), several germplasms have been screened through sick plot evaluation and artificial inoculation and we identified IIHR-B-HP 130 as highly resistant accession against bacterial wilt (IIHR Newsletter April to June 2019). Further understanding of the inheritance pattern of resistance is very important and foremost step in planning breeding strategy. However, there is very scarce information on genetics of bacterial wilt resistance in chilli. Earlier studies reported digenic recessive (Thakur 1990), two to five genes with additive effect (Lafortune et al. 2005), incomplete dominance (Sharma et al. 2005), monogenic recessive (Thakur et al. 2014), monogenic dominant and inhibitory gene action (13:3) (Devi et al. 2015). In the present study, we developed populations for bacterial wilt resistance using contrast parents and to further advance breeding programme the current experiment was conducted to understand the inheritance of resistance for adopting the best breeding strategy.

Two accessions IIHR-B-HP-130 (resistant parent) and CM334 (susceptible parent) for bacterial wilt were crossed to develop F_1 hybrid. F_1 was selfed to produce F_2 population and backcrossed with both the parents to develop B_1 (F_1 × IIHR-B-HP-130) and B_2 (F_1 × CM334) populations, respectively. To study the inheritance of bacterial wilt disease resistance, 45 plants of resistant parent, 23 plants of susceptible parent, 18 plants of F_1 population, 211 plants of F_2 segregating population, 59 plants of $B_1$and 52 plants of B_2 backcross population were screened for bacterial wilt resistance.

Bacterial isolate maintenance and inoculation preparation: The bacterial ooze was collected from the infected plants from the sick plot and cultured. Inoculum preparation and inoculation were carried as per the standard procedure (Du et al., 2017, Artal et al., 2012 and Gopalakrishnan et al. 2005) with minor modifications. The bacterial ooze extracted from the stem portion of infected plant was

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streaked on to the nutrient agar petri plates containing TTC (2, 3, 5 Triphenyl Tetrazolium Chloride) solution. Petri Plates were incubated at room temperature (26±2°C) and examined daily for the growth of the pathogen. Within 24-48 hours the bacterial colonies were observed as creamy white, fludal growth with pointed pink colour at the center of the colony. Single colonies were picked and sub cultured. The freshly grown R. solanacearum colonies were added to 150 ml of nutrient broth with a sterile loop and allowed to grow on a shaking incubator maintained at 28°C and150 rpm. After 24 hours, grown bacterial cultures were centrifuged at 4000 rpm for 15 min at 4°C and bacterial pellets were re-suspended in required volume of sterile distilled water to obtain a concentration of 10^6 CFU/ml by adjusting the O.D value of solution to 0.3 with the help of spectrophotometer (Gopalakrishnan et al. 2005).

Artificial inoculation of plants: Seedlings were grown in pro-trays of 50 wells filled with cocopeat media and proper nursery management practices were followed. Prior to artificial inoculation of seedlings, plants were withheld with irrigation to create temporary wilting and one third of the root system of each plant was slightly injured by inserting sharp sterilized knife about 2 cm away from the stem to facilitate penetration of bacteria to plant vascular system. Inoculation was carried out on one month old seedlings in the morning. Each plant was inoculated with a 5ml of bacterial suspension by the help of micropipette (Artal et al. 2012). Further after inoculation to create more disease pressure, the artificially challenged seedlings were planted at a spacing of 50cm x 50 cm in sick plot and other recommended agronomical practices were followed, frequent irrigations were given to maintain enough moisture in field.

Bacterial wilt scoring: Experimental plants were monitored for wilting at every 3 days interval up to75 days after inoculation of transplanting the inoculated plants in sick plot. Wilted plants were checked for vascular discoloration and confirmed through ooze test. Individual plants in F2, B1 and B2 populations were recorded as resistant/ susceptible.

The percent plant survival rate was calculated by the following formula based on the observations made.

Plant survival (%) =

$$\frac{\text{Number of healthy plants in the last recording}}{\text{Total number of plants}} \times 100$$

Chi square test: The disease reaction of individual plants in different segregating generations (F2 and backcross populations) were classified into two major phenotypic classes viz., resistant and susceptible based on symptoms of wilting, irreversible death and ooze test. The data on resistant and susceptible class were subjected to analysis of goodness of fit for various gene ratios of classical Mendelian genetics (Snedecor and Cochran 1967).

Bacterial wilt symptoms and mean performance of different generations: Initial symptoms of leaf dropping followed by permanent wilting of plants appeared 29 days after inoculation and within 10 to15 days entire susceptible parent (CM334) showed complete wilting. To confirm the bacterial wilting, vascular discoloration and ooze test were performed. The susceptible parent (CM 334) showed wilting after 29 days of inoculation and in F2 progeny the wilting started at 33DAI and gradually increased and continued up to 75DAI. The parent IIHR-B-HP130 exhibited highly resistant reaction while CM334 showed a highly susceptible reaction. The bacterial wilt incidence was mainly observed during flowering to fruiting stages, and there was very less incidence during first 35 days after inoculation i.e. pre-flowering stage. Indicating that best stage to record the observations is during flowering to fruiting stage. The F_1 hybrid between resistant and susceptible parent showed a high level of susceptibility indicate susceptibility is dominant over the resistance. The resistant parent IIHR-B-HP130 and susceptible parent CM334 had 95.65 percent and 4.34 percent plant survival rate, respectively. Survival rate recorded in F1, F2, B1 and B2 generation populations were 11.11%, 38.38%, 88.13% and 13.46%, respectively.

Inheritance of bacterial wilt resistance: There is very scarce information on inheritance studies of bacterial wilt resistance in chilli. Oligogenic with additive effect

Table 1: Segregation analysis bacterial wilt disease resistance in populations of IIHR-B-HP130 × CM334 cross

Population	Total	Susceptible	Resistant	Percent survival (%)	Digenic model		
					Ratio	Chi square value	Probability
IIHR-B-HP130	45	2	43	95.55			
CM334	23	22	1	4.34			
F1	18	16	2	11.11			
F2	211	130	81	38.38	9 (S): 7(R)	2.44^{NS}	0.116
B1(R)	59	7	52	88.13	3 (S): 1(R)	5.42*	0.019
B2 (S)	52	45	7	13.46	1 (S): 0(R)	0.94^{NS}	0.33

conferring resistance in doubled haploid (DH) population of PM-687 (resistant) and Yolo Wonder (susceptible) (Lafortune et al. 2005). In PBC-631/California Wonder, PBC-631/Yolo Wonder and IHR-546/California Wonder, Sharma (2007) reported monogenic dominant nature of inheritance of bacterial wilt resistance. Devi et al. (2015) reported monogenic dominant nature in the EC 464107/Sweet Happy I cross, whereas two genes with dominant and recessive epistasis in the EC 464107/ Kandaghat Selection cross and EC 464115/Kandaghat Selection crosses. Recently Du *et al*., 2019 reported partial dominant gene action. The varying genetics of bacterial wilt resistance is due to use of different parental material and the prevailing *Ralastonia* isolates used in for screening in different studies. The present study was undertaken at Bhubaneswar (hot and humid tropical region of India) which is hot spot for bacterial wilt. From earlier studies of germplasm evaluation, we identified IIHR-B-HP 130 as highly resistant accession against bacterial wilt. Reaction (resistance/susceptibility) of individual plants is observed and subjected to chi square analysis. The chi-square values were non-significant for 9:7 digenic ratio model ($\div^2$ = 2.46) indicating that the susceptible parent had two dominant alleles ($Bwr_1Bwr_1Bwr_2Bwr_2$) and resistant parent had two recessive alleles ($bwr_1bwr_1bwr_2bwr_2$) for both loci. Complementary gene action (9:7) is probably due to segregation of Bwr_1 and Bwr_2 genes. Bacterial wilt resistance in F_2 plants is expressed when one of the genes is in homozygous recessive condition ($Bwr_1\_bwr_2bwr_2$ *or* $bwr_1bwr_1Bwr_2\_$) or both the genes in homozygous recessive condition ($bwr_1bwr_1bwr_2bwr_2$). On contrary the plants having both the dominant genes ($Bwr_1\_Bwr_2\_$) were susceptible. F1 which is heterozygous at both the loci ($Bwr_1bwr_1Bwr_2bwr_2$) found to be highly susceptible with only 11.11 % survival rate.

B2 population (backcross with susceptible parent) showed high frequency of susceptibility (45 plants wilted out of 52 plants screened) and found to nearest to Digenic ratio of 1 (susceptible):0 (resistant) ($\div^2$ = 0.94). However, B_1 population (backcross with resistant parent) but the segregation pattern didn't follow expected 3 (susceptible): 1 (resistant) as per digenic ratio and frequency of resistant plants were high (52 plants out of 59 plants were resistant ($\div^2$ = 5.42). The deviation in B_1 population might be due to the accumulation of minor genes conferring resistance, as the backcross was done with resistant parent. This shows that backcrossing with one more generation will result in good accumulation of minor genes along with the two major recessive genes. Overall this inheritance study shows that the bacterial wilt resistance in IIHR-B-HP-130 is controlled by digenic with complementary gene action with other minor genes conferring the resistance. IIHR-B-HP-130 (resistant parent) can also be directly utilized as root stock for hot and belle pepper cultivation to combate bacterial wilt disease. Further characterization and molecular mapping will accelerate the resistance breeding programme.

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