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Inheritance of bacterial wilt resistance and performance of horticultural traits in bell pepper (*Capsicum annuum* var. *grossum*)

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ABSTRACT

The genetic nature of bacterial wilt resistance was studied in bell pepper (*Capsicum annuum* L. var. *grossum* Sendt.) plants using the P_1, P_2, F_1, F_2 and backcross generations obtained from four crosses between four parental lines, viz, EC 464107, EC 464115, Kandaghat Selection and Sweet Happy I considered as standards for resistance and/or susceptibility. The evaluation trial was conducted in bacterial wilt sick plots along with two susceptible checks, namely Indira (Hybrid) and California Wonder (Open-pollinated variety) that were included after every 15th row to ensure uniform distribution of inoculum in the experimental fields. Simultaneously, in a separate block, the parents and their F_1,s were also evaluated for twelve different horticultural traits. The findings revealed that bacterial wilt resistance was governed by a single dominant gene in the cross EC 464107 × Sweet Happy I, whereas two genes with dominant and recessive epistasis dictated resistance in the crosses EC 464107 × Kandaghat Selection and EC 464115 × Kandaghat Selection, respectively. Based upon the mean performance, the hybrid EC 464107 × Kandaghat Selection was the best in terms of total and marketable fruit yield, fruits per plant and fruit length, which underscores its importance to be exploited, especially during wet season. In addition, the resistant parents EC 464107 and EC 464115 can be used to develop bacterial wilt resistant F_1 hybrids as they expressed complete or near complete dominance for resistance in combination with the moderately resistant and susceptible varieties Kandaghat Selection and Sweet Happy I. Backcrossing with commercial cultivars and recombinant breeding accompanied by selection would also help to evolve bacterial wilt disease resistant, high yielding and horticulturally desirable pure line varieties.

Key words: Bell pepper, Dominance, Inheritance*, Ralstonia solanacearum*, Resistance

The pepper species are important group of fruit vegetables ranked second in the Solanaceae family after tomato (Bosland *et al*. 1996). Bell pepper (*Capsicum annuum* L. var. *grossum* Sendt.) also known as sweet pepper, green pepper, vegetable paprika or *Shimla mirch* is grown worldwide for its delicate taste, pleasant flavour and colour and is also the most leading crop under protected structures. Its fruits contain appreciable quantities of vitamin C (ascorbic acid), provitamin A (β-carotene) and other carotenoid pigments such as lycopene and zeaxanthin which are beneficial for prevention of cancer and cardiovascular human diseases (Ghasemnezhad *et al*. 2011).

The yield potential and total production of bell pepper is low due to the high incidence of the diseases and pests. Bacterial wilt, caused by *Ralstonia solanacearum*, is one of the devastating diseases in pepper crops raised under warm temperate, subtropical and tropical areas of the world. Although it is difficult to estimate total economic losses from bacterial wilt, the disease ranks as one of the most important plant diseases in the entire world (Jyothi *et al*. 2012) due to 100 per cent yield losses . In India, the disease is prevalent in Himachal Pradesh, Karnataka, Kerala, Maharashtra, Odisha and West Bengal. Considerable yield reduction (12 to 45%) has been reported from Himachal Pradesh, which resulted into switching of farmers to non-solanaceous crops in the major bacterial wilt affected areas of the state. Due to the soil-borne nature of the pathogen, conventional management strategies of bacterial wilt like crop rotation, adjusting the date of planting, cultural methods and soil treatment are ineffective. Recently, biological control has been investigated which, however, is still in primitive stage. Therefore, breeding for resistant varieties appears to be most promising and practical method to control the disease (Lebeau *et al*. 2011). Due to complex nature of disease, it is difficult to sustain resistance of varieties bred for one area, when introduced elsewhere. Besides, strong host × pathogen × environment interaction, are the cause of failure to achieve stable resistance (Osiru *et al*. 2001). Genotypes reported to be resistant at AVRDC, Taiwan and other states of India either lack in desirable horticultural attributes or succumb under new environment. Hence, there

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is an urgent need to develop area specific bacterial wilt resistant strains for commercial cultivation of the crop. With this background, the present investigation was planned and executed by involving the potential parental lines with the objectives to gather information on nature of inheritance of bacterial wilt resistance and to develop breeding material for selecting bacterial wilt resistant and horticulturally desirable progenies in the segregating generations in Himachal Pradesh.

MATERIALS AND METHODS

The present investigation was undertaken at the Experimental Farm of Department of Vegetable Science and Floriculture, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur (1290.8 m amsl, with latitude 32^0 6' N, longitude 76^0 3' E) from February-August 2010 to 2012. Agro-climatically, the location represents a mid-hill zone of Himachal Pradesh and is characterized by humid sub-temperate climate with high rainfall (2500 mm). The mean weekly meteorological data during the crop growing period of location is given in Fig 1. The soil is acidic in nature with pH ranging from 5.0 to 5.6 and soil texture is silty clay loam.

Bacterial wilt resistant lines were introduced from AVRDC, Taiwan during 2003 and were tested and maintained for about six growing seasons under natural field conditions. Finally, two bacterial wilt resistant accessions (EC 464107 and EC 464115) were selected and further tested for resistance under laboratory conditions (hydroponic solution inoculate with pathogen strain) through the method given by Hoagland and Arnon (1950). Two other parents, Kandaghat Selection (Moderately Resistant) and Sweet Happy I (Susceptible) were also involved in the crossing programme to generate the experimental material. The morphological characters of the parents are given in Table 1. The four crosses, viz. EC 464107 × Kandaghat Selection, EC 464115 × Kandaghat Selection, EC 464107 × EC 464115 and EC 464107 × Sweet Happy I were made to produce F_1 hybrid seed in the polyhouse during summer-rainy and autumn-winter, 2010. Each F_1 was raised to obtain F_2 seed and simultaneously backcrossed to both the parents to produce B_1 and B_2 seeds in the polyhouse during summer-rainy and autumn-winter seasons, 2011. All the parents were crossed to develop the hybrid seed using the recommended procedure of hand emasculation and pollination in a low tunnel polyhouse.

Seeds of six generations, viz. P_1, P_2, F_1, F_2, B_1 and B_2 of four crosses were raised in growing media in plastic pro-trays inside the low tunnel polyhouse on 4 February 2012. Seedlings were transplanted in bacterial wilt sick plots on

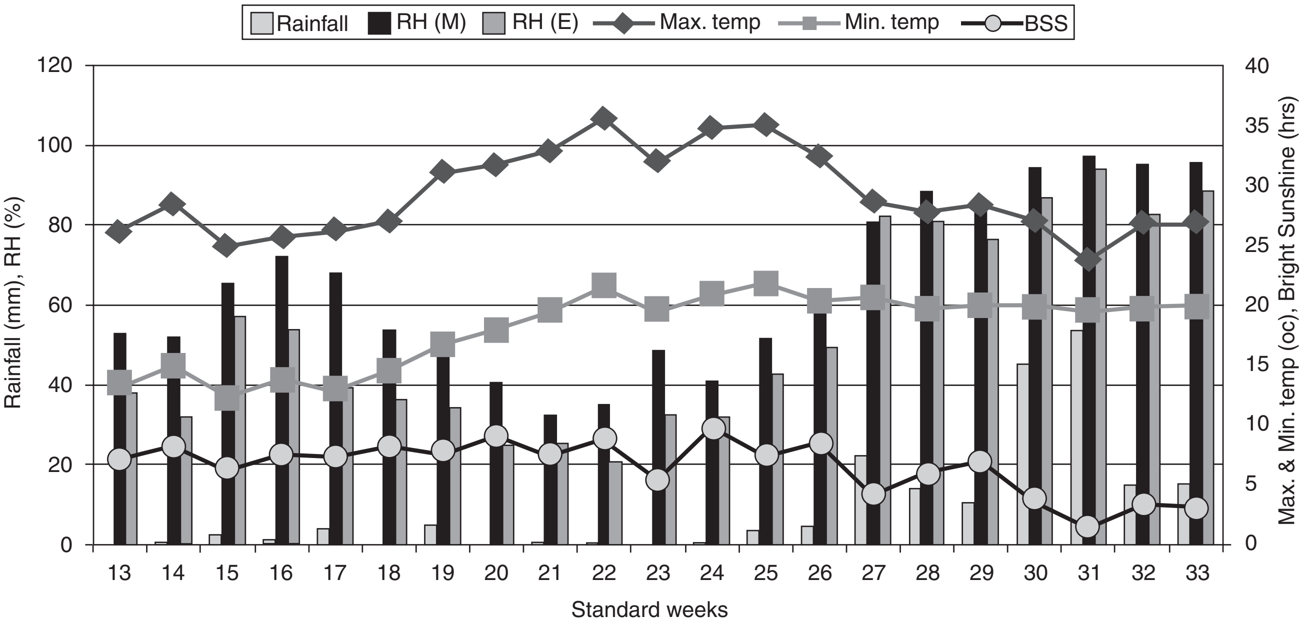


Fig 1 Mean weekly meteorological data for evaluation trial (March to August, 2012).

Table 1 Morphological characteristics of the parents involved in the study

Genotypes	Growth habit	Fruit position	Fruit shape	Fruit shape at pedicel attachment	Blossom end fruit shape
EC 464107	Indeterminate	Pendent	Blocky	Lobate	Sunken
EC 464115	Indeterminate	Pendent	Blocky	Lobate	Sunken
Kandaghat Selection (KS)	Determinate	Semi-pendent	Blocky	Lobate	Sunken
Sweet Happy I (SH-1)	Determinate	Pendent	Blocky	Lobate	Sunken
California Wonder (CW)	Determinate	Semi-pendent	Blocky	Lobate	Sunken
Indira	Determinate	Pendent	Blocky	Lobate	Sunken

10 April 2012, crosswise in a Completely Randomized Block Design (CRBD) with three replications. The plots were also artificially inoculated with *in vitro* multiplied culture of the pathogen to avoid disease escape. The spacing was 30 cm inter-row and 20cm intra-row. Each experimental plot consisted of 1 row of 3m length each for P_1, P_2 and F_1, 4 rows for B_1, B_2 and 7 rows for each F_2. A row of susceptible check varieties Indira (Hybrid) and California Wonder (Open-pollinated variety) was included after every 15th row to ensure uniform distribution of inoculum in the experimental fields. For the mean performance study, same experiment was repeated in separate block with normal spacing of 60 × 45 cm to record horticultural traits.

Data were recorded on randomly tagged 5 plants per replication (excluding border plants) in the non-segregating generations (P_1, P_2 and F_1), 30 plants per replication in the back cross generations (B_1 and B_2) and 60 plants per replication in the segregating generation (F_2). For bacterial wilt incidence, the observations were recorded at weekly interval. To confirm bacterial wilt, all plants showing wilting symptoms were subjected to ooze test up to a final count {90 days after transplanting (DAT)}. The plant survival data as on 90 DAT were utilized to ascertain the genetics of bacterial wilt disease. Plant survival (%) was calculated as:

$$\text{Plant survival (\%)} = \frac{\text{Number of healthy plants in the last recording}}{\text{Number of plants established}} \times 100$$

For mean performance study, five random plants were tagged and observation were recorded on twelve horticultural traits, viz. days to 50 % flowering, days to first picking, lobes per fruit, pericarp thickness (mm), fruit length and width (cm), harvest duration (days), plant height (cm), total and marketable fruits per plant, total and marketable fruit yield/plant (g).

Data were analysed by applying x^2 test of goodness of fit between the expected ratio for the bacterial wilt resistant and susceptible plants.

RESULTS AND DISCUSSION

The plant survival data of parents, F_1's, F_2's and backcrosses (B_1 and B_2) of bell pepper as on 90 days after transplanting (DAT) and the segregation of plants in F_2 and B_2 generations are given in Tables 2, 3 and 4, respectively. The resistant parents, viz. EC 464107 and EC 464115 had 100.0% plant survival under field condition, while complete susceptibility was observed in hybrid Indira and commercial variety California Wonder where not even a single plant could survive till 90 DAT. The results are described crosswise as under:

EC 464107 × KS

In the cross EC 464107 (R) × KS (MR), the survival in F_1 was 91.9%, whereas it was 81.8% in F_2. In B_1 (F_1 × EC 464107), the survival percentage was 84.7, but in B_2 (F_1 × KS), it was only 74.0%. The segregation of F_2 population was in the ratio of 13 (R) : 3 (S).

EC 464115 × KS

In the cross EC 464115 (R) × KS (MR), the survival in

Table 3 Segregation of plants in F_2 population

Crosses	NRP	NSP	Expected ratio (R:S)	χ^2 value (cal)	χ^2 value (tab)	Type of gene action
C1	207	46	13:3	0.05	3.84	Inhibitory
C2	209	35	13:3	3.11	3.84	Inhibitory
C3	204	4	1:0			
C4	117	39	3:1	0.61	3.84	Monogenic dominant

C1, EC 464107 × KS; C2, EC 464115 × KS; C3, EC 464107 × EC 464115; C4, EC 464107 × SH-I; NRP, Number of resistant (R) plants; NSP, Number of susceptible (S) plants

Table 2 Genetics of bacterial wilt resistance in bell pepper crosses

Crosses	G	IPS	RP	SP	PS (%)	Crosses	G	IPS	RP	SP	PS (%)
C1	P_1	43	43	0	100.0	C3	P_1	43	43	0	100.0
	P_2	42	27	15	64.3		P_2	43	43	0	100.0
	F_1	37	34	3	91.9		F_1	42	42	0	100.0
	F_2	253	207	46	81.8		F_2	208	204	4	98.1
	B_1	163	138	25	84.7		B_1	151	147	4	94.7
	B_2	150	111	39	74.0		B_2	157	152	5	94.3
C2	P_1	43	43	0	100.0	C4	P_1	43	43	0	100.0
	P_2	42	27	15	64.3		P_2	40	8	32	55.5
	F_1	44	40	4	90.9		F_1	45	39	6	86.7
	F_2	244	209	35	85.6		F_2	156	117	39	75.0
	B_1	161	143	18	88.8		B_1	183	174	9	93.4
	B_2	166	138	28	83.1		B_2	180	93	87	51.7

G, generation; C1, EC 464107 × KS, C2, EC 464115 × KS, C3, EC 464107 × EC 464115, C4, EC 464107 × SH-I; IPS, Initial plant stand; RP, Resistant plants and SP, Susceptible plants; PS, Plant survival

Table 4 Segregation of plants in B_2 generation

Crosses	NRP	NSP	Expected ratio (R:S)	χ^2 value (cal)	χ^2 value (tab)
F_1 (EC 464107 × KS) × KS	111	39	3:1	0.08	3.84
F_1 (EC 464107 × KS) × EC 464107	138	25	13:3	1.24	3.84
F_1 (EC 464115 × KS) × KS	138	28	13:3	0.38	3.84
F_1 (EC 464115 × KS) × EC 464115	143	18	3:1	0.001	3.84
F_1 (EC 464107 × EC 464115) × EC 464107	147	4	1:0		
F_1 (EC 464107 × EC 464115) × EC 464115	152	5	1:0		
F_1 (EC 464107 × SH-I) × EC 464107	174	9	1:0		
F_1(EC 464107 × SH-I) × SH-I	99	81	1:1	1.80	3.84

NRP, Number of resistant (R) plants; NSP, Number of susceptible (S) plants

F_1 and F_2 were 90.9 and 85.6 per cent, respectively. In B_1 (F_1 × EC 464115), the survival was 88.8%, whereas in B_2 (F_1 × KS) it was 83.1%. The segregation of F_2 population was in the ratio of 13 (R) : 3 (S).

EC 464107 × EC 464115

In the cross EC 464107 (R) × EC 464115 (R), the plant survival in F_1 was 100%, whereas it was 98.1% in F_2. In B_1 (F_1 × EC 464107), the survival percentage was 94.7 and was 94.3% in B_2 (F_1 ×EC 464115). The segregation of F_2 population was in the ratio of 1 (R) : 0 (S).

EC 464107 × SH-I

In the cross EC 464107 (R) × SH-I (S), the plant survival in F_1 was 86.7% whereas in F_2 it was 75.0%. In B_1 (F_1 × EC-464107), the survival was 93.4% whereas in B_2 (F_1 × SH-1) it was only 51.7%. The segregation of F_2 population was in the ratio of 3 (R) : 1 (S) revealing that the resistance was under single monogenic dominant control, which was further confirmed with the expected test cross ratio of B_2 (test cross) in the ratio of 1(R) : 1 (S).

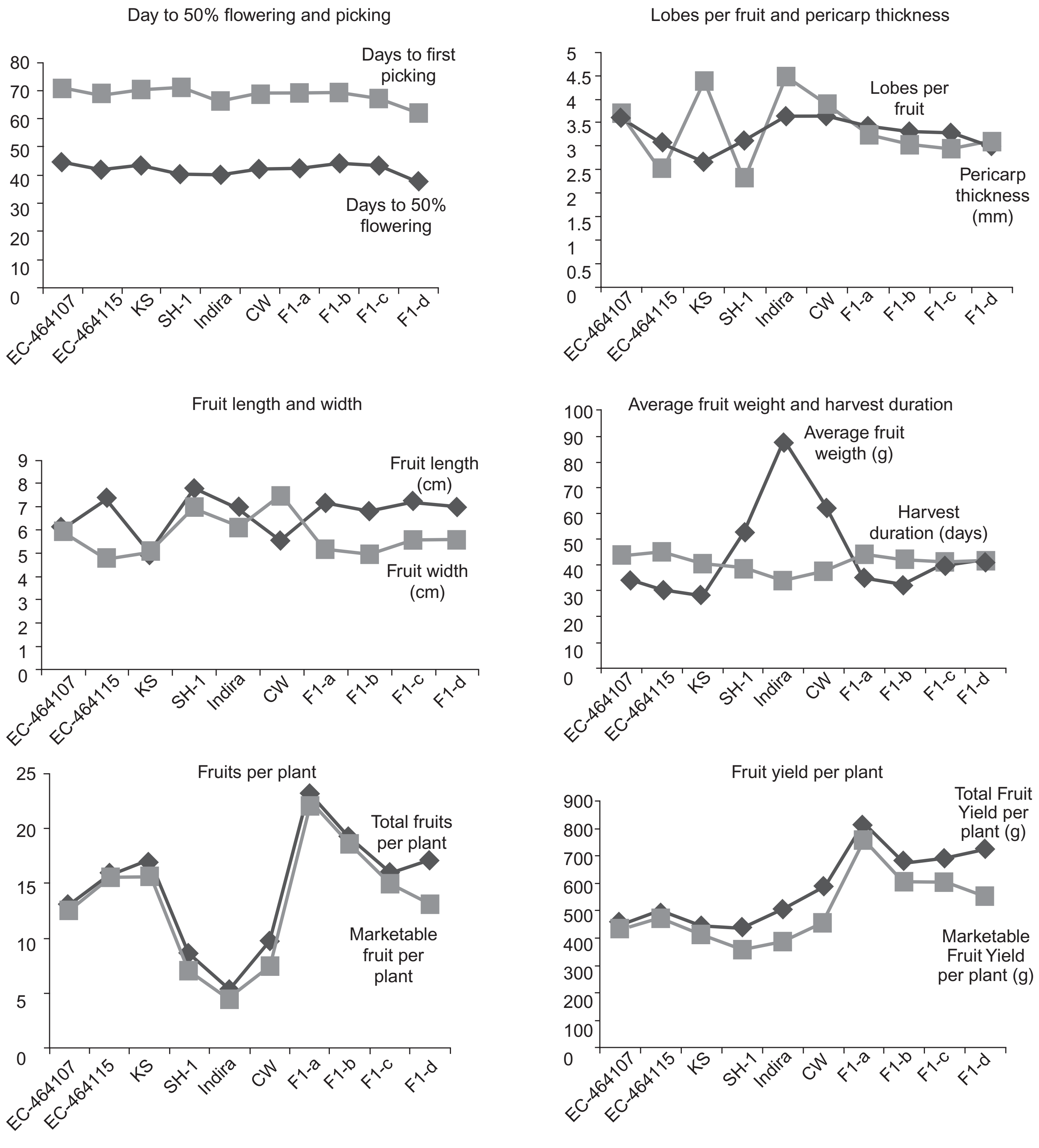
Mew and Ho (1976) have categorized the genotypes to be resistant if the plant survivals are above 80.0%. Thus, this study revealed that crosses between resistant and susceptible parents had produced resistant F_1 plants. In different genetic studies, Rhandhawa (1999) and Sharma *et al.* (2013) also confirmed that resistant × susceptible parents could produce resistant F_1's hybrids. The differences in the plant survivals of resistant (EC 464107 and EC 464115) and moderately resistant (Kandaghat Selection) parents can be accounted on the assumption that probably the number of genes governing resistance in EC 464107 and EC-464115 were greater than in Kandaghat Selection.

Backcrosses in case of resistant × moderately resistant parents [(EC 464107 × KS) × KS, (EC 464107 × KS) × EC 464107, (EC 464115 × KS) × KS and (EC 464115 × KS) × EC 464115] showed deviation from the expected ratio, i.e. 1 (R): 1 (S) that might be due to other genetic reasons. The presence of 'modifier genes' having a minor effect on expression of 'resistant gene' could also have caused a few outlier reactions to occur in otherwise homozygous progenies or due to non uniform distribution of bacteria in sick plots and a few backcross individuals could have resulted from self pollination of F_1's individuals. In disease inheritance studies on cotton leaf curl virus and bacterial blight in Tepary beans, respectively, Haidar (2002) and Urrea *et al.* (1999) also observed deviation from the expected ratio in backcrosses.

In the crosses EC 464107 × KS and EC 464115 × KS, F_2 populations segregated in the ratio of 13 (R): 3 (R) indicating the presence of two major genes with dominant and recessive epistasis or inhibitory type of gene action. In the cross EC-464107 × SH-I, F_2 segregation was in the ratio of 3 (R): 1 (S) revealing that resistance was under simple monogenic dominant control which was further confirmed with the expected test cross ratio of 1 (R): 1(S) in B_2.

In literature, variable reports on the genetics of bacterial wilt resistance in various solanaceous vegetable crops have been reported. Earlier, Tran and ByungSoo (2010) and Sharma *et al.* (2013) in peppers have also reported inheritance of bacterial wilt by utilizing six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2), whereas Lafortune *et al.* (2005) based their findings on F_1 and/or F_2 progenies only. Depending upon the source of resistance and progenies studied in capsicum, inheritance of bacterial wilt resistance was reported as digenic recessive (Thakur 1990), two to five genes with additive effects (Lafortune *et al.* 2005), duplicate epistasis (Tran and ByungSoo 2010) and incomplete dominance to complete dominance (Sharma *et al.* 2013). The parental lines used in this study were different from those of earlier workers and this variation along with differences in the strains of the pathogen and different environmental conditions of study may perhaps be the reason for the discrepancy in results.

Mean performance study showed that the cross combination EC-464107 × SH-I took minimum days to flowering (37.67). This cross also took least days to first picking (62.00) and was at par with the other three crosses, viz. EC 464107 × KS, EC 464115 × KS and EC 464107 × EC 464115. The maximum plant height of 72.10 cm was recorded in the cross EC 464107 × SH-I simulating with the crosses EC 464115 × KS and EC-464107 × EC 464115. The fruits were found available for longer duration in the cross EC 464107 × KS (44.00 days) and were at par with rest of the crosses. Lobes per fruit were more in the cross EC 464107 × KS (3.40) and was similar to the crosses EC 464115 × KS, EC 464107 × EC 464115 and EC 464107 × SH-I. Total fruit yield/plant was more in the cross EC 464107 × KS (810.33g /plant) and was similar to EC-464107 × SH-I. Marketable fruit yield per plant was highest



F_1-a= EC-464107 × KS; F_1-b=EC-464115 × KS; F_1-c =EC-464107 × EC-464115; F_1-d =EC-464107 × SH-I

Fig 2 Mean performances of parents and F_1s for various horticultural traits in bell pepper

in the cross EC 464107 × KS (755.00g/ plant) and no other cross could match the latter for the said trait. However, maximum fruits were produced by the cross EC 464107 × KS having 23.07 and 22.07 total fruits per plant and marketable fruits per plant, respectively, while the cross EC 464115 × KS was at par for both the traits.

Longer fruit were provided by the cross EC 464107 × EC 464115 (7.29 cm) followed by EC 464107 × KS (7.17cm), EC 464107 × SH-I (6.97 cm) and EC 464115 × KS (6.80 cm), respectively. Fruit width was highest in the crosses EC 464107 × EC 464115 (5.57cm) and EC 464107 × SH-I (5.57cm) and was at par with the cross EC 464107 × KS and EC 464115 × KS. The highest average fruit weight was observed in the cross EC 464107 × SH-I (42.27 g) and at was at par with rest of crosses, viz. EC 464107 × KS, EC 464115 × KS and EC-464107 × EC-464115. The maximum pericarp thickness was observed in the cross EC 464107 × EC 464115 (3.27mm) and was at par with rest of the three crosses.

Based upon the mean performance, the hybrid EC 464107 × KS was the best for most of the traits, viz. total fruit yield per plant, total fruits per plant, marketable fruit yield per plant, marketable fruits per plant and fruit length and hence is worth exploiting, especially during wet season as fruits were found available for longer duration in the cross. However, the cross combination EC 464107 × SH-I was best in terms of earliness and plant height.

Nevertheless, the present investigation suggests that the resistant parents EC 464107 and EC 464115 can be used to develop bacterial wilt resistant F_1 hybrids as they expressed complete or near complete dominance for resistance. Since, the fruits of resistant parents and its F_1 hybrid (EC 464107 × EC 464115) were light green in colour and shape was also not fully bell like, backcrossing with commercial cultivars accompanied by selection for bacterial wilt resistance and dark green fruit colour would be useful to improve the fruit colour and shape. These resistant parents can also be used in recombination breeding to evolve bacterial wilt resistant, high yielding and horticulturally desirable pure line varieties or hybrids for wilt prone and high rainfall areas of Himachal Pradesh.

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