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Short Communication

Identification of potential chilli (*Capsicum annuum* L.) accession as a rootstock for managing bacterial wilt disease in bell pepper

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Bacterial wilt caused due to *Ralstonia pseudosolanacearum* is pervasive across the major pepper growing parts of world and impede production. It is reported that 30-100% yield losses in crops was due to bacterial wilt (James et al. 2003). *Ralstonia pseudosolanacearum* is highly heterogeneous bacterial pathogen cause wilting of important crops (Smith et al. 1995). These pathogen propagules remain viable and active in soil for several years making the control almost impossible through any means of cultural management like crop rotation, involvement of biological agents as well as chemical treatments especially in the regions where repeated and protected cultivation is undertaken (Thakur et al. 2014). Bell pepper is highly susceptible to bacterial wilt, which is a major constraint for growing bell pepper in bacterial wilt endemic areas and protected cultivation. Chemical management often leads to the presence of toxic residues in the fruits, thus raises the concern of food safety and reduces the export potential. Incorporation of bacterial wilt resistance genes into bell pepper background takes longer time, as selection must be made towards bell pepper fruit type without pungency. Thus, grafting is an environment-friendly substitute to minimize disease occurrence due to soil-borne pathogens and to elevate the tolerance of susceptible cultivars against the biotic stresses (Rouphael et al. 2018). Numerous rootstocks such as PR-920, PR-921, PR-922 accessions for cv. Nokkwang, PP0237-7065 rootstock for V202230 and PI-201232 rootstock for Indra Hybrid bell peppers have been identified by different workers to manage bacterial wilt in pepper (Jang et al. 2012; Wu et al. 2012; Rana et al. 2015). Nonetheless, grafting of hot and bell peppers is still in infancy in India due to lack of knowledge, awareness, and non-availability of resistant rootstocks. Eastern coastal parts of India are hot spot for bacterial wilt disease in solanaceous vegetable crops, mainly due to prevalence of highly acidic soils, hot and humid tropical climate. This study is the continuation of our previous study, in which one highly bacterial wilt resistant pungent chilli accession 'IIHR-B-HP-130' was identified after sick plot screening for three seasons (Naveena et al. 2020). In this study, this resistant accession was evaluated as rootstock for different commercial bell peppers to manage bacterial wilt in eastern coastal regions of India.

The present investigation was conducted at central Horticultural Experiment Station (ICAR-IIHR) Aiginia, Bhubaneswar, Odisha during October 2019 to February 2020. It belongs to the eastern coastal plain of India and is known as sub-humid characterized by warm moist climate with mild winter, which provides congenial environment for growth and multiplication of bacterial wilt causing pathogen. For the current study, IIHR-B-HP-130 was used as rootstock for four bell pepper commercial hybrids like Inspiration, Pasarella, Bachata and open pollinated variety Arka Mohini. Chilli accession IIHR-B-HP-130 rootstock seeds were sown 5-6 days prior to sowing of bell pepper scions to synchronize germination. After 35-40 days of sowing, grafting was done by adopting cleft method using sterilized blade and specialized grafting clips. Wedge grafting was followed. Rootstock was cut at 5-6 cm from ground level and scion of same thickness was inserted into the rootstock, so that the cambium layer will come in contact for better compatibility and survival percentage. All the leaves of scion except one are removed. After grafting, grafted plants were kept in cool and humid place for healing process covered with black polyethylene sheet. For bacterial wilt screening of the grafted bell peppers, *Ralstonia pseudosolanacearum* isolate was collected from bacterial wilt infected chilli plants from CHES

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(ICAR-IIHR), Bhubaneswar fields. Standard available protocols for culture preparation and artificial root dip inoculation were followed according to Rashmi et al. (2012). Culture was grown on TZC medium containing TTC (2, 3, 5 Triphenyl Tetrazolium Chloride) by spreading method (Kelman 1954). Within 1-2 days, bacterial colonies with creamy white and pointed pink color at the center were observed. Further, sub-culturing of *Ralstonia* from the mother culture was done. For preparation of 150 ml of nutrient broth solution, 2 g of nutrient broth powder was added in 150ml of distilled water and then autoclaved.5ml of 1% TTC solution was added later for staining. Sub cultured pure *Ralstonia* colonies were inoculated into broth solution by using sterile loop and kept in shaking incubator at room temperature @ 150 rpm. After 24 hours, broth solution containing bacteria were centrifuged at 4000 rpm for 15 min at 4 ^{0}C and after centrifugation white pellet was suspended in distilled water of required quantity. Bacterial concentration was made up to 10^{7-} CFU/ml by the use of spectrophotometer [OD =0.3 at A_{650}] (Singh *et al*. 2018). Prior to planting in sick plot, the root system of one month old seedlings were trimmed by using sharp sterilized knife to make primary injury to roots for easy entry of pathogen (bacteria) into plant vascular system. Wounded plants were dipped in solution of bacteria (10^7cfu/ml) for one to two minutes followed by transplanting in the sick plot (Artal *et al*., 2012). After artificial inoculation, grafted and non-grafted plants have been planted at 45 cm x 30 cm spacing in the bacterial wilt sick plot at CHES (ICAR-IIHR), Bhubaneswar. The field was laid out in Randomized Complete Block Design (RCBD) with 3 replications. All the required production technologies were adopted for raising the crop. Plants were monitored for bacterial wilt symptoms at every three days interval. Percentage disease incidence (PDI) was recorded at 30 days post inoculation as per the formulae: [PDI = (Number of plants wilted/ Total number of plants) x 100]. The fruit yield obtained from individual plants of each genotype was recorded and mean fruit yield was worked out. Other parameters such as fruit length (cm), fruit width (cm), and average fruit weight (g) were recorded in grafted plants. All statistical analyses were carried out through WASP- Web Agri Stat Package 2.0 available at ICAR-Research Complex, Goa website.

The identified resistant parent IIHR-B-HP-130 accession has been explored as resistant root stock for bell pepper varieties (Arka Mohini, Inspiration, Bachata & Pasarella) through grafting. About 95% grafting success was observed in all the root stock and scion combinations, indicating good graft compatibility. The grafts were evaluated through artificial inoculation and under sick soil conditions in insect proof net house. Significant differences were observed among the rootstock- scion combinations for bacterial wilt resistance in *Ralstonia pseudosolanacearum* sick plot (Table 1). Arka Mohini grafted onto the IIHR-B-HP-130 rootstock exhibited less % of bacterial wilt (Figure 1). Inspiration grafted onto the IIHR-B-HP-130 rootstock showed negligible wilt symptoms (1.66%), non-grafted Inspiration recorded 73.33% wilt incidence. Non -grafted Pasarella recorded maximum % bacterial wilt incidence (93.3%). Overall, it was found that grafted capsicum showed very less percentage of wilting (0 to 8.33%) whereas non-grafted showed severe wilting (73.33 to 93.33%). Grafted bell pepper cultivation can be explored for commercial cultivation in bacterial wilt prone areas. The low incidence of bacterial wilt in grafted bell peppers is mainly exhibited by resistant rootstock, as resistant genotype roots acts as mechanical barriers and limit the diffusion of bacterial population (Wu et al. 2012). Further, Jang et al. (2012), Kumar et al. (2017), and Ramesh et al. (2016) observed similar results for disease resistance in grafted bell peppers as opposed to non-grafted plants.

Figure 1: Reaction of grafted and non-grafted Capsicum plants to for bacterial wilt disease in sick plot

In the grafted bell peppers, there is a less incidence of bacterial wilt like in grafted Inspiration (1.66%), in grafted Bachata (6.66%), and in grafted Pasarella (8.33%). This incidence was observed due to development of adventitious roots from the scion touching the soil, thereby making entry for the pathogen (Figure 2). The adventitious roots developed in plants where graft union touched the soil. This indicated that there is need to standardize the grafting height and create awareness about use of mulch and reducing intercultural operation, which avoids the development of adventitious roots and avoid this problem. Considerable differences were observed for yield per plant between the grafted and non-grafted bell pepper plants used. All the grafted bell pepper plants produced significantly higher yield per plant when compared to non-grafted plants, which were totally collapsed due to bacterial wilt disease. This

Figure 2: Suicidal roots from Scion when graft union touches the soil causing entry for pathogen

indicated that grafting can be explored on commercial scale for bell pepper cultivation in bacterial wilt prone areas using the resistant accession IIHR-B-HP-130 of chilli as root stock.

Statistical analysis confirmed all the treatments differed significantly from each other for yield per plant (Table 1). Grafting Capsicum on to bacterial wilt resistance rootstock reduced bacterial wilt incidence there by produced yield. Highest yield per plant was recorded in grafted Arka Mohini (1225.00 g), followed by grafted Inspiration (1078 g) and grafted Bachata (1043.67 g) and grafted Pasarella (974.40 g). Whereas in non-grafted treatments maximum incidence of bacterial wilt is recorded there by the yield per plant was very significantly reduced to 22.67 g to 59.46 g yield per plant in non-grafted Bachata and non-grafted Arka Mohini, as most of the plants died at very initial stage of fruiting. Arka Mohini being open pollinated variety and comes up well in open conditions and it has recorded good yield. Other three are commercial F_1 hybrids which are best suited for protected cultivation with good horticultural practices like training, as in our case as we evaluated them under open net house conditions that might be reason for normal/low yields in these hybrids.

Grafted bell pepper exhibited good fruit traits across all the combinations. In the grafted bell peppers, all the varieties maintained the fruit traits of scions and Grafted Arka Mohini showed good blocky fruits average fruit size of 147 g followed by grafted Pasarella (115.66 g),

Table 1: Bacterial wilt incidence in grafted and non-grafted bell pepper under sick plot conditions

Scion	Treatment	Bacterial wilt (%)	Yield per plant (g)
Pasarella	Grafted	8.33	974.40
	Non-grafted	93.33	28.33
Bachata	Grafted	6.66	1043.46
	Non-grafted	90.0	22.67
Inspiration	Grafted	1.66	1078.00
	Non grafted	73.33	143.33
Arka Mohini	Grafted	0.00	1225.00
	Non-grafted	83.33	59.46
Resistant rootstock	IIHR-B-HP-130	0.00	-
Susceptible scion	CM334	100	-
	CD (0.01)	24.72	240.65

grafted Bachata (110.00 g) and grafted Inspiration (106.33 g). The average fruit of non-grafted was not recorded as the fruits were unmarketable and initial stage of fruit development. The fruit length in case of grafted Arka Mohini found to be maximum with 8.12 cm followed by grafted Pasarella (7.06 cm), grafted Bachata (6.10 cm) and grafted Inspiration (5.46 cm). The average fruit length non-grafted treatments were not recorded as the fruits were unmarketable and initial stage of fruit development. Maximum fruit width was exhibited by grafted Arka Mohini (7.99 cm) followed by grafted Bachata (7.73 cm), grafted Pasarella (6.49 cm) and grafted inspiration (6.44 cm). Non-grafted treatments data could not be recorded as fruits were non-marketable initial fruiting stage and plants showed wilting. All the scions (Arka Mohini, Bachata, Pasarella and Inspiration) maintained fruit traits.

Evaluation under sick soil conditions showed that grafted bell peppers showed very less percentage of wilting (0-8.33%) whereas non-grafted showed severe wilting (73.3-93.3%) indicating that grafted capsicum cultivation can be explored for commercial cultivation in bacterial wilt prone areas through use of identified resistant accession as a root stock. Grafted bell peppers showed good yield in sick plot conditions and all the varieties maintained the fruit traits of scions. Grafting through use of this resistant accession will help in eco-friendly management of bacterial wilt disease. In conclusion, the identified bacterial wilt resistant IIHR-B-HP-130 accession of chilli has been explored as potential resistant root stock for bell pepper/capsicum varieties, and grafted capsicum cultivation can be explored for commercial cultivation in bacterial wilt prone areas.

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