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Lateral Root from Scion Rescued the Susceptibility to Bacterial Wilt in Grafted Tomato on Wilt Resistant Brinjal Root Stock

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ABSTRACT: *Arka Meghali*, tomato variety developed by IIHR, Bangalore was released for *kharif* season cultivation. The North Bengal specific local brinjal having proven performance in all seasons was used as stock for grafting with *Arka Meghali* to heighten bacterial wilt resistance. The grafting process was optimized and transplanted into the field. The field performance, it was revealed that grafted *Arka Meghali* showed 88.26±6.44% wilt symptom as compared to non-grafted tomato (100% wilt), *Arka Meghali*. In the sick plot, non-grafted brinjal showed 11.11±2.77% wilt symptom. Further research revealed that upper portion above grafted point in scion showed higher OD at 600nm as compared to below portion of the grafted *Arka Meghali* (i.e. brinjal root stock). The non-grafted brinjal showed negligible concentration of bacterial titer in both below and above ground portion. Very interestingly, it was revealed that lateral root from the scion in the grafted tomato showed high titer of bacteria which contributed to the higher titer in scion but bacterial titer was not enriched into root stock. The lateral root from root stock maintained lower bacterial titer as compared to lateral root from scion in grafted tomato. Therefore, rescue of the wilt susceptibility in grafted tomato was contributed by the lateral root from scion in grafted tomato in the field condition.

Keywords: *Arka Meghali; Solanum lycopersicum* L.; *Ralstoniasolanacearum*; Grafting; Lateral root; Susceptibility.

INTRODUCTION

Ralstonia solanacearum, an economically devastating soil borne pathogen affected tomato production (Ji *et al.,* 2007; McAvoy *et al.,* 2012) globally. Soil fumigation with chloropicrin and methyl bromide proved ineffective in the management of this pathogen (Chellemi *et al.,* 1997; Driver and Louws, 2002; Enfinger *et al.,* 1979) although methyl bromide was recognized as banned chemical. Grafting of susceptible tomato as scion onto resistant rootstocks was an effective technique for bacterial wilt (BW) management (McAvoy *et al.,* 2012; Rivard and Louws, 2008) which was commonly followed in Asian and Mediterranean countries for the greenhouse production (Besri, 2001; Cohen *et al.,* 2007; Lee, 2003; Lee *et al.,* 1998). According to USDA record, 65 tomato rootstocks have been identified for commercial breeders (USDA, 2014) for resistance packages. About 20 of the 65 rootstocks were assigned as resistant to BW. Furthermore, the elevated level of BW resistance was evidenced among the rootstocks (McAvoy *et al.,* 2012). Moreover, McAvoy *et al.* (2012) identified that rootstocks may modulate yield performance as compared to the non-grafted plants. The grafted tomato grown in greenhouses and open field conditions could potentially increase fruit size, weight, and yield as compared with non-grafted controls (Kyriacou *et al.,* 2017).

Grafting was evidenced as a promising and an alternative tool in vegetable crops like tomato as a good alternative to conventional breeding methods for enhancing tolerance to biotic, abiotic stresses and soil pathogens. The well-known vegetables watermelon, squash, cucumber, bitter gourd, tomato and eggplant, etc. were used as scion and protocol are already developed onto different rootstocks. The grafted vegetables were more popular for effectively curtailing from infections by soil-borne pathogens as well as heightened tolerance to abiotic stresses such as soil salinity, toxicity of heavy metals, drought and waterlogging. In unfavorable weather situation, plants often faced transient or permanent waterlogging which modulated physico-chemical properties of soil like pH, redox potential and available oxygen level etc. For example, waterlogging consequence hypoxia (deficiency of O_2) or anoxia (absence of O_2) in soil environment. Tomatoes were sensitive to hot-wet season and highly sensitive to waterlogged conditions (Ezin *et al.,* 2010; Bhatt *et al.,* 2015). In northern part of West Bengal, tomato was not able to withstand in rainy season due to frequent and heavy rainfall record. But at the same time, selected wild brinjal, having medicinal value according to local farmers was withstand successfully and fruiting in the same environment. Therefore, grafting of tomato was performed exploring local brinjal root stock. Tomato variety '*Arka Meghali'* developed from IIHR, Bangalore was used for this study as it was recommended in rainfed condition but did not withstand in the field condition. This variety is developed for rainfed cultivation suitable for *kharif* season having duration of 125 days with yield potential of 18t/ha. Moreover, it was not wilt resistance variety. Thus, the resistance root stock of local brinjal having resistance against bacterial wilt infection was explored for grafting with susceptible tomato as scion to evaluate the above aground bacterial wilt symptom on leaf foliage. Hence, *Arka* Meghali' was used as scion over local brinjal root for the present study to prove the hypothesis.

MATERIAL AND METHODS

Tomato – *Arka Meghali*: Tomato variety 'Arka Meghali' developed from IIHR, Bangalore was used for this study as it was recommended in rainfed condition. The terai zone was experienced with frequent rainfall. A pedigree selection (F_8) was followed from the cross 'Arka Vikas' × 'IIHR 554' for developing this variety. The plant is semi-determinate with narrow dark green leaves with good canopy. Fruits are medium (65g) in mass, having oblate with light green shoulder and deep red fruits. This tomato is suitable for fresh market. This variety is developed for rainfed cultivation suitable for *kharif* season having duration of 125 days with yield potential of 18t/ha (https://www.iihr.res.in/tomato-arka-meghali-0).

Grafting environment: The controlled environment having with 6000 LUX of light intensity, 14-hour light condition per day, and 70% of Relative Humidity was maintained in plant growth room (PGR). The grafted tomato was further maintained in the PGR with initial transparent and closed plastic chamber for high humidity a well as light pass.

Grafting process: The local brinjal root stock was used for the experiment. The root stock was 7-10 days older than tomato. First of all, 21 days old brinjal was cut in the top portion and 14 days scion was transferred into stock. The scion was further clipped for maintaining constant touch of the scion to the stock. After 7 days later, the graft clip was removed. The grafting environment was performed in controlled environment maintaining high humidity.

Establishment of grafting: The initial period was very crucial for establishment the grafted tomato. Immediately after clipped, the grafted tomato was put in closed transparent chamber for high humidity as well as light pass. The whole plastic chamber was gain maintained in PGR.

Transplanting of grafted tomato: The grafted tomato on local brinjal was transplanted in March. The date of experiment was performed in May when severe wilt was evidenced at field condition in the grafted tomato.

Observation of infected grafted tomato: Regular field visit showed that non grafted tomato showed rapid wilting initially while grafted tomato also showed delayed wilting. It was interesting that grafted tomato shoed high wilting in the later stage but local brinjal did not show wilt symptoms.

Bacterial ooze out experiment: The root was harvested in grafted tomato, non-grafted tomato and local brinjal. The below ground root portion and just above portion shoot from the grafted point were considered for ooze out experiment. In case of grafted tomato, above portion and below portion of grafted place were considered for ooze out experiment.

Temporal scale data recording: The ooze out experiment was followed in temporal scale from different grafted tomato having wilt disease symptom. The three time points like in 30min, 60min and 12h were considered to evaluate the bacterial ooze out which was measured at 600nm OD.

Statistical software: One-way ANOVA and Tukey's HSD Calculator was used for calculation (https://www.icalcu.com/stat/anova-tukey-hsd-calculator.html) to calculate p values at 0.05% level of significance to see any significant difference was present or not among the different time points for a particular aphid inoculum. The MedCalc statistical software (https://www.medcalc.org/calc/comparison_of_means.php) was also explored to calculate the difference between the observed means in two independent samples. Analysis of Variance (ANOVA) was calculated from online available software (https://www.danielsoper.com/statcalc/calculator.aspx?id=43).

RESULT AND DISCUSSION

Successful grafting of tomato on brinjal: A number of successful grafted tomato was evidence in the controlled environment for field transplantation (Fig. 1). After successful grafting, the grafted tomato was maintained in the controlled environment conditioned with 6000 LUX of light intensity, 14-hour light condition per day, and 70% of Relative Humidity for 15 days more (Fig. 1). The grafted tomato along with non-grafted tomato and brinjal were also transplanted in the same day in the field condition in valley.

Infestation rate in the field condition: The grafted tomato along with non-grafted tomato and brinjal were transplanted in valley. The plant-to-plant distance was 60cm and valley to valley distance was 90cm. After transplantation, standard practice of weeding, watering, etc. were followed. At 2 months' time point, 100% non-grafted *Arka Meghali* showed wilt symptom and died whereas grafted *Arka Meghali* showed 88.259±6.44% wilt disease (Fig. 2.A). The local brinjal showed only 11.111±2.778% wilt symptom (Fig. 2 A). Therefore, grafted tomato did not show expected level of performance at field condition when root stock showed unexpected level of wilt resistance.

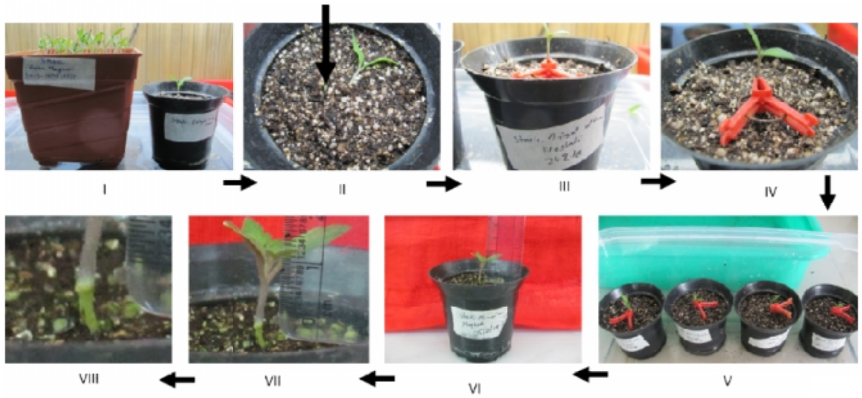


Fig. 1. Process of grafting in tomato on brinjal stock. Both plant ready for grafting. Brinjal on single and small pot (I). The top portion of brinjal was cut (II). The scion was attached and hold by grafting clip (III). The close-up view on grafting clip which hold tomato as scion (IV). The grafted tomato was ready for incubation on transparent and closed plastic container (V). After 7 days, the grafting clip was removed (VI). The close-up view of grafted tomato (VII). The zoomed view in the region of grafted region between tomato (scion) and brinjal (stock) (VIII).

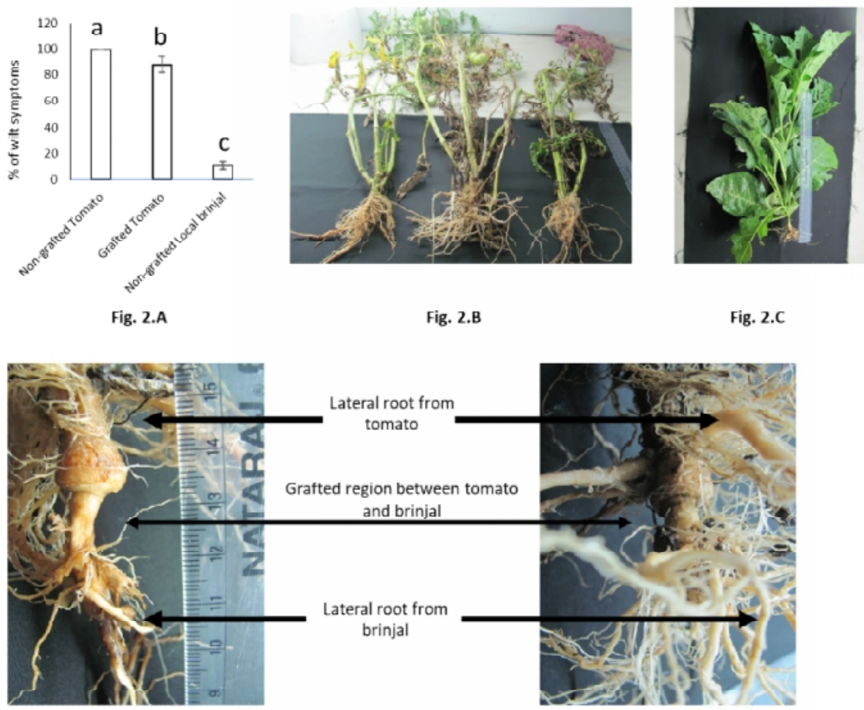


Fig. 2. Field performance of grafted tomato at 2 months after transplantation. **A.** After transplantation into main field, 100% ArkaMeghali showed wilt symptoms whereas grafted Arka Meghali showed 88.26±6.44% wilt symptom. Interestingly, brinjal (UBB-8) showed 11.11±2.77% wilt symptoms at 2 months after transplantation.

Ooze out experiment in grafted *Arka Meghali*, non-grafted local brinjal and *Arka Meghali*: The grafted tomato was uprooted and documented the infected plant for further experiment (Fig. 2.B). At the same time, healthy brinjal plant was also uprooted (Fig. 2.C) for ooze out experiment. The main root as well as lateral root from grafted tomato were focused for the study (Fig. 2.D). First, the grafted region was identified and accordingly classified the tomato lateral root as well as brinjal lateral root in the grafted *ArkaMeghali* (Fig. 2.D).

Ooze out experiment in grafted *Arka Meghali*, non-grafted brinjal and *Arka Meghali* at 30 and 60 minutes: Bacterial ooze out experiments in 30 and 60 minutes from grafted tomato was monitored focusing on the main root and shoot (Fig. 3). The result showed that upper portion from the grafted point showed significantly higher concentrated oozed out bacterial concentration (0.658±0.022) as compared to lower portion (0.061±0.008) in grafted tomato (Fig. 3.A). It was interesting finding was that lower portion (brinjal root) maintained significantly lower bacterial colony as compared to upper portion (tomato part) from the grafted region in both 30 and 60 minutes (Fig. 3.A and B). It was basic question how the higher concentration was maintained in the upper portion? In the non-grafted tomato, interestingly lower and upper portion showed higher concentrated oozed out bacterial concentration

(0.335±026 in upper and 0.355±0.022 in lower) in both 30- and 60-minutes time point ooze out duration (Fig. 3.A and B). In the non-grafted brinjal, the lower concentrated oozed out bacterial concentration was *evidenced*.

Ooze out experiment in grafted *Arka*Meghali, non-grafted brinjal and *Arka*Meghali at 12h: Independent experimental set was maintained for 12h experiment (Fig. 4.A). The result showed that higher concentrated bacteria was maintained in upper portion from the grafted point in grafted tomato (2.129±031) as compared to lower portion (0.354±0.181). In the non-grafted tomato, interestingly lower and upper portion showed higher concentrated oozed out bacterial concentration (1.259±0.037 in upper and 1.368±0.098 in lower).

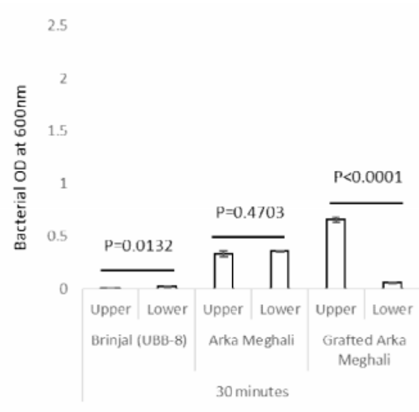


Fig. 3.A

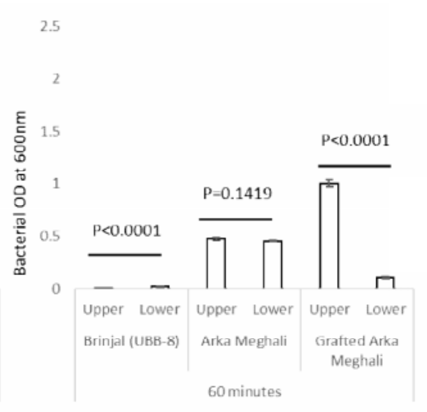


Fig. 3.B

Fig. 3. Bacterial ooze out experiments in 30 and 60 minutes from grafted tomato. Fig. 3.A. The result showed that upper portion from the grafted point in grafted tomato showed significantly higher concentrated oozed out bacterial concentration (0.658±0.022) as compared to lower portion (0.061±0.008). In the normal tomato, interestingly lower and upper portion showed higher concentrated oozed out bacterial concentration (0.335±026 in upper and 0.355±0.022 in lower). In the brinjal, the lower concentrated oozed out bacterial concentration was evidenced. Fig. 3.A. The same trend was evidenced in 60 minutes.

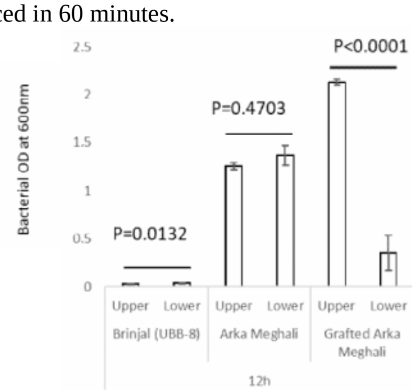


Fig. 4.A

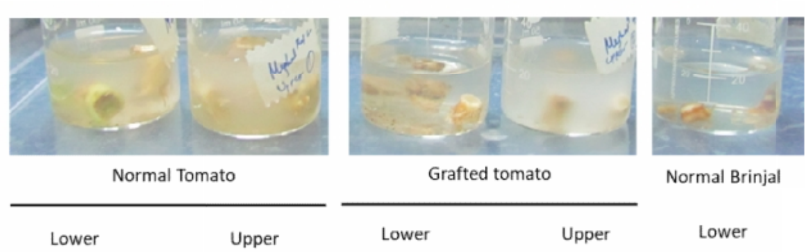
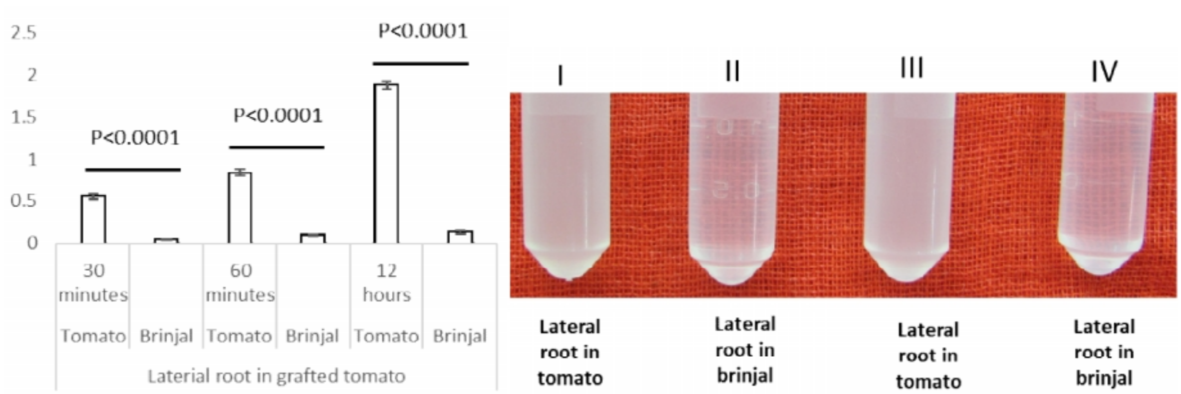


Fig. 4.B

Fig. 4. Bacterial ooze out experiment recorded in 12 hours. **4.A.** The result showed that higher concentrated bacteria was maintained in upper portion from the grafted point in grafted tomato (2.129±031) as compared to lower portion (0.354±0.181). In the normal tomato, interestingly lower and upper portion showed higher concentrated oozed out bacterial concentration (1.259±0.037 in upper and 1.368±0.098 in lower). In the normal brinjal, the lower concentrated oozed out bacterial concentration was evidenced. **4. B.** The pictorial view of oozed out bacteria from grafted tomato, normal tomato and normal brinjal.

In the non-grafted brinjal, the lower concentrated oozed out bacterial concentration was evidenced. Before taking record at 600nm, the oozed-out solution was snapshotted at 12h from grafted tomato, non-grafted tomato and non-grafted brinjal (Fig. 4.B) to see the concentrated oozed out bacterial concentrated suspension.

Ooze out experiment in lateral root from above and below portion from the grafted tomato: Bacterial concentration in lateral root in grafted tomato in temporal scale (30min, 60min and 12h) were documented (Fig. 5). In the grafted tomato, the lateral root from scion (tomato) showed significantly higher concentrated bacteria as compared to lateral root from stock (brinjal) in all time points' ooze out experiment. The oozed-out bacteria from tomato lateral root (tube I and III) attenuated transparency as red background was not visible as compared to lateral root from brinjal in grafted tomato (tube II and IV; Fig. 5.B).



A. **B.**

Fig. 5. Bacterial concentration in lateral root in grafted tomato in temporal scale. **Fig. 5 A.** In the grafted tomato, the lateral root from scion (tomato) showed significantly higher concentrated bacteria as compared to lateral root from stock (brinjal) in all time points' ooze out experiment. **B.** The oozed-out bacteria enriched tube from tomato lateral root attenuated transparency as red background was not visible as compared to lateral root from brinjal in grafted tomato.

Grafting was well known practice mainly in woody plants in agriculture for centuries. The grafting technology to non-woody plants like tomatoes is more recent (Kubota *et al.,* 2008). Grafted tomatoes contributed a substantial proportion of total tomato production in Europe and Asia. As for example, ~ 50 to 70 million grafted plants per year was explored in Spain and contributed about 40% of the country's tomato production (Raymond 2013). Grafted tomatoes typically consist of an improved variety as scion (the top part of the plant that produces the fruit) and a rootstock that may be resistant to one or more soil borne pathogens (Oztekin *et al.,* 2009; Masterson *et al.,* 2016). It was reported that tomato scions can be successfully grafted to rootstocks of tobacco (Yasinok *et al.,* 2009a), eggplant (Oda *et al.,* 2005), potato (Peres *et al.,* 2005), or wild *Solanum* species (Cortez-Madrigal 2012; Lopes and Mendonça 2016). Different rootstocks showed differential advantages, such as increased tolerance to abiotic stressors including drought (Cantero Navarro *et al.,* 2016a), salinity (Rao *et al.,* 2013; Albacete *et al.,* 2015a), or low temperatures (Venema *et al.,* 2008a). Many studies showed the enhanced yield of tomatoes from grafted plants when compared to the non-grafted scion cultivars (Barrett *et al.,* 2012a; Djidonou *et al.,* 2013a; Rivard *et al.,* 2010a; Rysin and Louws 2015). Therefore, the local brinjal root stock which was wild brinjal and recognized as medical merit by local people was used as stock as it was very resistant to all type of disease specially wilt and performed any climatic condition in North Bengal.

The benefit from grafting largely depends on the selection of the rootstock and the grafting technique employed (Ginoux and Laterrot, 1991). Rootstock-scion combinations need to be tested in order to optimize crop performance in the field condition for promotion of commercial acceptance of the technique. The high yielding varieties of tomato, *Arka Meghali* was released by IIHR (ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka 560089) particularly for rainfed condition. Therefore, it was chosen for experiment in terai zone in North Bengal zone. The wilt resistant rootstocks of eggplant which was recorded as medicinal value was chosen from the local acceptance (Bhatt *et al.,* 2015). Our mission was to evaluate the enrichment of merit of medicinal value in the tomato as well. The vascular system was evolved in the higher plant for efficient re-allocation of photosynthate and signaling molecules within plant system. SE Element sap, enriched with simple sugars, peptide, and amino acids was an attractive niche area to a number of vascular specific pathogens including bacteria. Bacterial wilt (bacterium: *Ralstonia solanacearum*) recognized as a devastating disease of both field and greenhouse tomatoes. High soil temperatures and high moisture levels favor disease development. Leaves first appear dull green, wilt during the day and recover at night. Leaves eventually yellow and brown at the margins, completely wither and die. Wilt progression varies by crop.

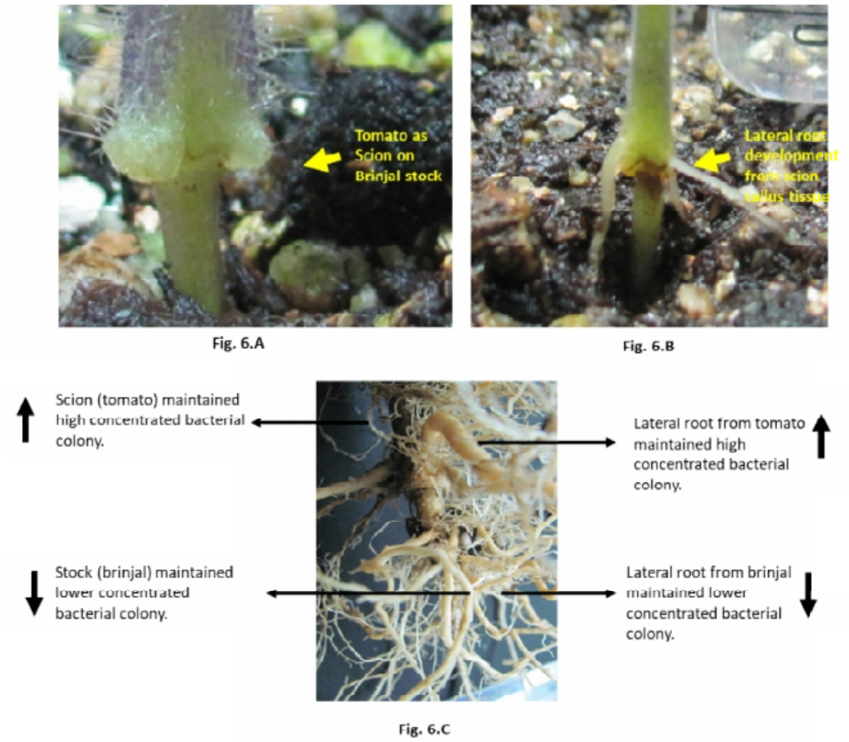


Fig. 6. Summary of the present finding. **A.** The scion developed callus which was pointed. **B.** The developed callus induced lateral root which was arrowed. **C.** The lateral root developed from scion (tomato) maintained high concentrated bacterial colony resulting in enrichment of bacterial concentration in above ground shoot. The lateral root developed from stock (brinjal) attenuated low concentrated bacterial colony in the same soil environment condition as well as lower concentrated bacterial population.

Characteristically, a callus tissue was developed at the bottom of the scion which was documented in the Fig. 6A. This callus tissue induced lateral root formation without any physical sensing to soil (Fig. 6B). But no wilt symptom was recorded in the controlled environment as the soil was sterilized artificial soil. The situation of experiencing wilt disease was evidenced in the field condition at 2 months after transplantation when all non-grafted tomato was died but grafted tomato showed delayed wilting which was documented in Fig. 2B. The present study identified that bacteria entered through lateral root in grafted tomato and caused wilt disease but brinjal root inhibited the entry of causal organism from soil as well as from scion in grafted tomato. The present study identified that wilt causing bacteria entered through lateral root from tomato scion and enhanced higher titer value in the scion portion whereas the lateral root from root stock did not contribute to the higher titer value in the scion. Therefore, it was recommended that selection of proper tomato cultivar or inhibiting the growth of lateral root from scion should be restricted for achieving the benefit from grated tomato. Therefore, it was recommended that tomato variety having lateral branchless may be explored for bacterial resistance on local brinjal root stock or restricted the lateral root from tomato scion by cultural practices.

Authors Contribution. BP, KS and MM performed experiments and assisting in analysis and checking the manuscript. NK contributed in assisting in writing manuscript, analysis, and discussion. HAM conceptualized the idea, navigated all experiments and wrote the manuscript.

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Compliance with ethical standards (e.g., Conflict of interest). The communicating author (HAM) declared that there is no conflict of interest.

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