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Suppression of bacterial wilt in susceptible scion of tomato (*Solanum lycopersicum*) and brinjal (*Solanum melongena)* using *Solanum torvum* as resistant rootstock

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ABSTRACT

The autografts of tomato (*Lycopersicon esculentum* L.), brinjal (*Solanum melongena* L.) chilli (*Capsicum* sp.), and citrus (*Citrus* spp.), showed 86, 87, 84, and 88 % graft efficiency respectively. Moreover, homograft of tomato, brinjal and citrus showed 83, 81 and 84% graft efficiency respectively. *In vitro* micrografting protocol performed within 8 days after seed germination and had up to 97% success rate without use of any phytohormones. Moreover, tomato and brinlal grafted on *S. torvum* rootstock showed the resistance to bacterial wilt. However, non -grafted tomato and brinjal showed bacterial wilt symptoms. The developed microgarted protocol helps in the production of bacterial wilt resistant tomato and brinjal by the use of *S. torvum* as a rootstock.

Key Words: Bacterial wilt, Brinjal, Micrografting, Rootstock, *S. torvum*

Tomato (*Lycopersicon esculentum* L.) and brinjal (*Solanum melongena* L.) are very popular vegetables; in India (Tomar and Saha, 2018). They suffer from several biotic and abiotic stresses. Among these, bacterial wilt caused by *Ralstonia solanacearum* is most devastating. The several efforts has been taken to overcome these stresss. However, grafting was utilized to manage bacterial wilt in tomato crops worldwide (Ganiyu *et al*., 2018). The use of tolerant rootstocks for grafting brinjalt varieties is most effective approach to control bacterial wilt disease (King *et al*., 2008). Resistance to *R. solanacearum* has been identified in various accessions (Rotino *et al*., 2014). There are some reports of improving production and productivity of guava (*Psidium guajava* L.) and pomegranate (*Punica granatum* L.) (Nowrozy, 2017; Kholia *et al*., 2022). However, there is no report on *in vitro* micrografting of tomato, brinjal, chilli, and citrus. Therefore, an experiment was conducted.

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MATERIALS AND METHODS

The seeds of brinjal (Shirish and *Solanum torvum*; resistance to bacterial wilt), tomato [(Arka Meghali; sensitive to bacterial wilt (Konappaa *et al*., 2016)], chilli (selection) and citrus (Sai Sarbati and Jambheri, mostly used as rootstock in citrus breeding) were collected form UHS, Banglore, and Mahatma Phule Krishi Vidyapeeth, Rahuri. Bacterial wilt pathogen (*R. solanacearum*) was collected from commercial biofertilizer unit, Vidhya Pratishthan, Baramati.

The 50 seeds of tomato, brinjal, chilli, and citrus, were surface sterilized as per Dsapute *et al* (2019) in 70% (v/v) ethanol for 5 min, followed by 1% sodium hypochlorite for 5 min and rinse the seeds five times with 1to 50 ml sterile water. Seeds were transferred on ½ Murashige and Skoog (MS) plates/bottels with the help of sterilized forceps in a laminar chamber. Plates/bottels were placed in a growth chamber in vertical position at 26±2°C, light/dark of 16h/8h photoperiod and 1600 lux intensity light and relative humidity of 60-70% for 7 - 10 days until cotyledons were fully expanded. The micrografting protocol was followed as per the Gebhardt and Goldbach (1988) with minor modification.

Briefly, 40 seedlings of each plant were micrografted under sterile conditions in a laminar chamber, stem of rootstock and scion were cut very close to true leaves. The scion and rootstock were placed very close to each other in ½ MS medium without any phytohormons and keep the plates in vertical at all times. The autografting of tomato [Arka Meghali/Arka Meghali; brinjal (Shirish/Shirish), chilli (chilli1/chilli1) and citrus (Sai Sharbati/ Sai Sharbati) performed between the scion and rootstock of the same plants of same species (here after autograft plants). Also, homografting of tomato (Arka Meghali/*S.torvum*), brinjal (Shirish/*S.torvum*) and citrus (Sai Sharbati/ Jambheri) performed between scion and rootstock of different plants of same species (here after homograft plants).

Auto- and homo- grafts were grown for 5 - 10 days at 23°C under light and dark condition of 16 / 8 h, until roots were well developed. After 10 days, grafting efficiency (%) of each plant was calculated based on the formula given below (Fig. 1). The 15 days old grafted plants transferred to pot containing cocopeat and utilized for further purpose. The graft (%) efficiency= (Final Graft No. /Initial Graft No.) × 100

A single colony of *R. solanacearum* was inoculated in conical flask containing 500 ml luria broth and incubated at at 28 ^{O}C for 48 hrs on rotary shaker at 150 rpm. Culture broth was centrifuged at 10,000 rpm for 12 min at 10 ^{O}C. The pellet was resuspended in distilled water and bacterial suspensions were spectrophotometrically adjusted to O.D 600 nm = 0.1 [approximately1 × 108 colony forming unit (cfu) ml^{-1}] (Bhavana *et al.,* 2016).

After 15 days of growth, tomato and brinjal grafts were transplanted into pots containing cocopeat and soil in 1:1 proportion. When grafted plants exhibited 3–4 true leaves, *R. solanacearum* inoculums was applied using root damage infused inoculum method as described by Namisy *et al.* (2019) and Bhavana *et al*., 2016. Briefly, Roots of tomato grafts were injured with the help of a knife by cutting through soil 1–2 cm away from the stem base before inoculation. The 20 ml of *R. solanacearum* suspension was poured into each pot and the inoculated grafts were kept in greenhouse chamber for 45 days. Non-inoculated graft plants were included with water and used as the control. Grafts were watered in excess two times a day after inoculation to maintain the soil moisture high till the end of the experiment, approximately 2 month. All grafts were kept in a greenhouse after inoculation and bacterial wilt symptoms were observed once a week for three weeks and percentage (%) of wilt incidence was calculated by using the formula.

% wilt incidence = (No. of plants wilted / total no. of plants used for inoculation) × 100.

RESULTS AND DISCUSSION

Out of 50 seedlings, 40 equal size of seedling of each plant were used as scion and rootstock for micrografting. The grafts were observed after every 2 days up to 10 days and graft efficiency was calculated. After 2 days of grafting the formation of callus tissue was observed at grafting site. The micrograft efficiency was 97- 68% in auto and homo grafts. The very high micrograft efficiency was recorded in autograft of citrus (88%), brinjal (87%) tomato (86%) and chilli (84%). However, homo grafts of tomato brinjal and citrus showed 83, 81 and 84 percent grafting efficiency respectively (Fig.1). These results showed that developed protocol has very good grafting efficiency without use of any phytomormons and can be applicable for broad range of plant species.

The autograft of tomato (Arka Meghali/Arka Meghali) showed symptoms of plants wilted and died rapidly, 2 weeks after inoculation of *R. solanacearum*. The autografted plants showed 100% wilt incidence and indicated susceptibility to bacterial wilt. However, homograft of tomato (Arka Meghali/*S. torvum*) could not show any symptoms of bacterial wilt, 2 weeks after inoculation of *R. solanacearum*. The homograft plants of tomato showed only 0% wilt incidence and indicated resistance to bacterial wilt. The control auto and homo grafts observed as healthy plants. Similarly, autograft of brinjal (Shirish/ Shirish) showed 95% wilt incidence with the symptoms of plants wilted and died rapidly after inoculation. However, only 5% of brinjal homograft (Shirish/*S.torvum*) showed symptoms of bacterial wilt. The control auto and homo grafts showed healthy plants. These results suggested that use of rootstock in homograft of wild brinjal are resistance to *R. solanacearum,* and tomato and brinjal inoculated with *R. solanacearum* could not show any symptoms of bacterial wilt.

The resulting union often results in a more productive plant (Tamilselvi and Pugalendhi, 2015). Grafting has primarily been used to reduce the occurrence of soil-borne disease in non-native fruit vegetable plants, primarily tomato, brinjal, pepper

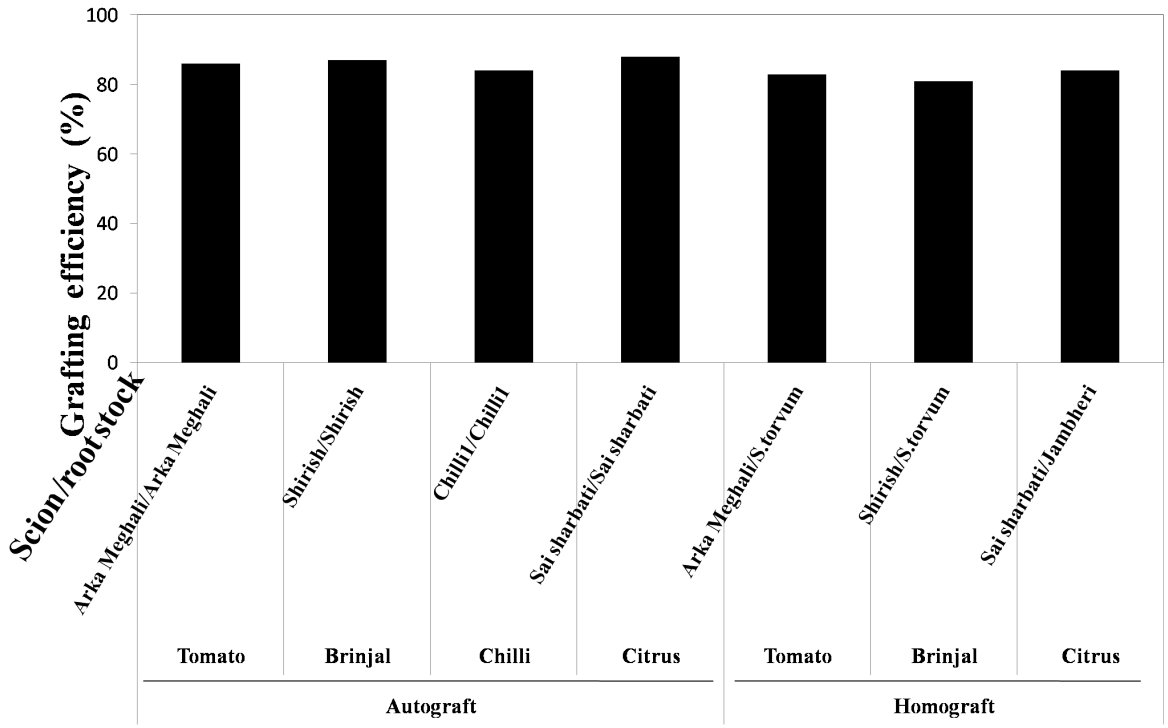

Fig. 1. Grafting efficiency of auto- and homo- grafts of various plants. The grafting efficiency was calculated by using successfully grafts and total number (#40) of graft performed.

and production of virus-free plants in citrus (Singh *et al.,* 2019). Although a disease tolerant rootstock can generally improve tolerance of a susceptible scion. The grafting of tomato and citrus has been utilized for protein movement, response to salinity and phosphate homeostasis (Spiegelman *et al.,* 2015). We developed first time *in vitro* micrografting protocol for tomato, chili, brinjal and citrus without use of any phytohormones.

We observed that formation of callus like tissues 2 days after grafting and this is responsible for joining of grafts. The wound callus is formed by division of parenchyma cells from cambium, phloem, xylem, and pith near the wounded surface. In herbaceous plants, callus cells are first formed at vascular bundles and cortex, with only a few in the pith. Mixing of wound callus cells of both graft partners can lead to further connection between scion and rootstock. The secondary plasmodesmata are able to connect cells between rootstock and scion to form a continuous symplast. Thus, substance exchange between adjacent cells and cell communication can be achieved. There was very high rate of microgafting efficiency (70-90 %) were observed in used plants (Fig.1). Singh *et al.* (2019) found the effect of various phytohormones such as kinetin, N6-benzylaminopurine (BAP) indole-3-acetic acid (IAA) on efficiency of citrus micrograting and observed maximum 56.8% graft efficiency.

Tomato and brinjal homografts grafted on rootstock of *S. torvum* showed resistance to *R. solanacearum.* The *Solanum melongena* complex has three species, namely, *S. melongena*, *S. incanum* and *S. insanum*. Wild relatives of Solanum, viz. *Solanum torvum*, *S. indicum*, *S. insanum*, *S. surattense*, *S. pubescens*, *S. gilo*, and *S. khasianum* are widely distributed in South India, Shivalik hills and North-eastern region (Reddy *et al*., 2022). Rootstock of *S. torvum* showed highly resistance to bacterial wilt and resulted in a good fruit yield in scion (Ramesh *et al.,* 2016). The *S. torvum* as resistant to bacterial wilt was recomanded (Bainsla *et al.,* 2016). Besides, *S. torvum* has been also found compatible with tomato and displayed high level of waterlogging tolerance (Bahadur *et al.,* 2015). The *S. torvum* rootstock can be used in grating of tomato and brinjal to develop bacterial wilt resistance. This micrografting protocol can be used in the production of tomato and brinjal against the bacterial wilt.

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