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Morphological changes in the compatible grafts of tomato cv. PKM 1 with different solanaceous rootstocks

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Abstract
Tomato (*Solanum lycopersicon*) is a most popular and widely grown vegetable crop in the world. The varied climatic conditions leading to increased biotic and abiotic stresses will affect the normal vegetative, flowering and reproductive stages and hence the yield of the crop. Grafting is a promising tool practiced in several vegetables like tomato, brinjal, watermelon to control the predominance of biotic and abiotic stresses. As tomato is widely grown vegetable, a study was proposed to understand the morphology of the compatible graft of tomato cv. PKM 1 with different solanaceous rootstocks. The different solanaceous rootstocks used for the graft were tomato genotypes of *Solanum lycopersicum* (LE 523, LE 828 & LE 102), *Solanum torvum, Solanum aculeatissimum and Solanum sisymbriifolium.* The cleft grafting method was followed and the parameters like graft success percentage, number of leaves, leaf area, shoot length and shoot diameter were recorded after 30 days of grafting. The compatible rootstock and scion can be assessed based on the survival rate of grafted plants and vegetative growth of the scion. The tomato grafts of *Solanum torvum* * PKM 1 showed high graft success percentage of 95% whereas LE 102 * PKM 1 had least graft success percentage of 9.34%. The graft, LE 828 * PKM 1 showed increased shoot length (25.62cm), leaf area (65.51cm^2), number of leaves (4.6) and shoot diameter (3.56mm) compared to other successful grafts. The successful grafts with increased growth may due to regeneration of vascular bundles across the graft interface leading to increased water and nutrient flow through the graft union.

Keywords: Rootstock, scion, cleft grafting, compatibility, tomato

Introduction
Tomato (*Solanum lycopersicon* L.) is one of the most significant vegetable and it is typically cultivated for its edible purpose. It is an important source for carbohydrates, minerals, essential amino acids and vitamins (Draie, 2017) [6]. It comes under the category of protective foods because it contains antioxidant like lycopene and carotene. It is widely produced for fresh produce market and processed into various forms such as dried tomato fruits, candies, ketchup, powdered, paste, and canned tomato fruits. India is the second leading producer of tomato in area and production was about 2.65 MT after china in the world. Madhya Pradesh ranks first in total area and production of tomato followed by Karnataka and Andhra Pradesh whiles a Tamil Nadu rank eleventh in position was about 840.21 thousand MT (Horticulutural Statistics at a Glance, 2017). Despite its prominence, several factors can limit tomato production, such as biotic and abiotic stress (Venema *et al*., 2008) [27]. To overcome some of these obstacles, farmers rely on grafting technique, which binds rootstock and scion by means of tissue regeneration and developing into a single plant (Zeist *et al*., 2017) [29]. Such tool makes use of root systems (rootstocks) that are resistant to biotic and abiotic stresses (Zeist *et al*., 2017) [29].
Grafting was initially carried out to limit the infection by soil-borne diseases like Fusarium wilt (King *et al*., 2010) [13] and recently it was used to enhance nutrient uptake (Davis *et al*., 2008) [4], water-use efficiency (Rouphael *et al*., 2008) [22], fruit quality (Turhan *et al*., 2011) [26], yield (Petran, 2013) [20] and to induce resistance to abiotic stress (Schwarz *et al*., 2010) [23]. Bletsos and Olympios, (2008) [3] and Petran (2013) [20] reported that *Solanum torvum is a* promising eggplant rootstock for tomato interspecific grafting for its resistance to a wide range of soil borne pathogens, including *Verticillium dahlia*, *Ralstonia solanacearum*, *Fusarium oxysporum* and root-knot nematodes. *Solanum aculeatissimum and Solanum sisymbriifolium* are also resistance to fungal disease like *Verticillium dahlia* and hence can be recommended as rootstock for grafting (Bletsos and Olympios, 2008) [3]. The grafted plants with rootstock as *Solanum torvum* are not only resistance to soil borne diseases and also provide resistance to flood and drought conditions (Petran. 2013) [20]. Bletsos and Olympios, (2008) [3] reported that the eggplants grafted on *Solanum torvum* having good compatibility and strong root system and healthy plants.

Owing to their utilization of rootstocks having vigorous root system, grafted plants usually show increased uptake of water and minerals like phosphorus and nitrogen when compared with self-rooted plants (Lee *et al*., 2010 [15]; Leonardi and Giuffrida, 2006) [16]. Increased uptake of water and nutrients results in increased fruit size, yield and profit, which can be of value to farmers (Pogonyi *et al*. 2005) [21].

Better performance of grafts is the result of compatible graft union with active rootstock/scion interactions. The compatibility and interaction of rootstock/scion depends on selection of rootstock and scion, grafting technique and healing of the graft union (Bletsos and Olympios, 2008 [3]; Lee *et al*., 2010 [15]). The incompatibility between tomato scion and *Solanum intergrifolium* rootstock with a smaller diameter of rootstock than scion was observed by Bletsos and Olympios (2008) [3]. Thus stem diameters of a rootstock and a scion seedling must be of comparable size at the time of grafting that played an important role in compatibility of grafts (Black *et al*., 2003[2]; Tamilselvi and Pugalendhi, 2017 [25]).

Tissue affinity between rootstock and scion comprises morphological, physiological and chemical aspects of the plants (Sirtoli *et al*., 2008) [24]. Failure of graft union can be caused by mismatching of the scion and rootstock, lack of skill and expertise, adverse environmental conditions, diseases and incompatibility (Hartman *et al*., 2002 [11]). The most evident is rootstock/scion incompatibility, is the undergrowth and/or overgrowth of the scion, leading to decreased water and nutrient flow through the graft union, ultimately causing wilting (Davis *et al.*, 2008 [4]). Characterization of incompatibility is not a simple process because graft combinations can unite initially with apparent success, but gradually develop incompatibility symptoms with time, due to failure at the union or development of abnormal growth patterns. Thus compatible grafts should be identified for the tolerant rootstocks screened for the biotic and abiotic stresses. Hence, this study was conducted to find out the graft compatibility screened solanaceous rootstocks for flood tolerance with PKM 1 cv. as scion based on the morphology of the success grafts.

Materials and Methods

The present study was carried out to select healthy and compatible grafts of commercial cv. PKM 1 tomato on solanaceous rootstocks. The pot culture experiment was carried out at the Department of Crop physiology (11^o N latitude, 77^o E longitude; 426.7 MSL), Tamil Nadu Agricultural University, Coimbatore and the experimental period was from 9th November 2015 to 20th April 2016.

Seedlings of commercial tomato cv. PKM 1 were used as scion and the solanaceous spp were used as rootstocks. *Solanum lycopersicum* (LE 523, LE 828, and LE 102) and *Solanum torvum*, *Solanum aculeatissimum* and *Solanum sisymbriifolium* were used as rootstocks. The seeds were treated with Gibberellic acid @ 500ppm for enhancing germination. The seeds were sown uniformly in the well prepared portrays maintaining a thin film of water. Fifteen days after sowing of solanum rootstocks and scion (PKM 1) were uniformly transplanted to 5x7 inch polybags with 2:1:1 proportion of red soil: FYM: sand. Placing one plants per bag, aiming to provide a larger amount of substrate, larger area for root growth to increase growth, development.

The method of grafting was cleft grafting and the scion and rootstock with three to four true leaf stages, having similar stem diameter was taken for grafting. The materials used for grafting are carbon steel blades for tissue cutting, grafting clips for stem fixing and polythene covers. Grafting was carried out in greenhouse, in a shady place protected from the wind, and to avoid wilting of the grafted plants. The grafts were maintained in healing chamber for 15 days to enhance the survival rate and after 15 days of grafting, the side shoots of rootstocks were removed. Foliar spray of water was effective in controlling wilt and improving survival of the grafts.

After 15 days of grafting, the graft union was identified by counting of the number of healed grafts in percentage. The compatible plants were transplanted into a pot containing mixture of soil: FYM: sand in the ratio of 3:2:1. The transplanted pots were kept in greenhouse for avoiding direct sunlight for 5 to 7 days.

The morphological parameters were recorded at 30 DAG (days after grafting) by selecting 3 samples randomly from each treatment. Shoot length was measured from the base of the shoot to the apical portion of the plant and the mean was worked out and expressed in cm and the number of expanded true leaves was counted from the randomly selected tagged plant. Leaf area per plant was measured using a Leaf Area Meter (LICOR, Model LI 3000) and expressed as cm^2. The stem diameter was measured 5cm above the ground level (Eugene Ofori, 2015 [7]) with an electronic digital calliper and expressed in mm.

Results and Discussion

Grafting is a complex structural and biochemical process that connects the rootstock and scion. Graft union consists of the healing process after joining scion and rootstock and this process is triggered by cambial regeneration, from which a callus fills the gap area between rootstock and scion tissues, making a continuous connection between vascular elements of both sides at graft point (Martinez-Ballesta *et al*., 2010) [17]. Plants form callus at the graft interface, which enables water to flow from the rootstock to the scion when the callus develops vascular bundles (Moore, 1984) [19], which indicates the compatibility of the rootstock and scion.

In the present study the compatibility of rootstock and scion was identified by graft success percentage for the cleft grafting method and significant differences were observed in graft success percentage (Table 1). Among the six rootstocks and scion combination, *Solanum torvum* * PKM 1 recorded significantly maximum number of graft success percentage (95%) compared to other grafts. The cleft grafting was successful for the TNAU Tomato hybrid CO-3 scion and *Solanum torvum* as rootstock registered highest success percentage (90.67%) (Dhivya., 2014) [5]. The formation of callus tissue bridges the grafted region by spreading into gaps between grafted regions and fusing with the proliferation layer. This adhesion of callus allows the interconnection of opposing plasmodesmata, and allows the flow of xylem exudates between rootstock and scion tissues thereby increasing survival in graft combinations (Tamilselvi and Pugalendhi, 2017) [25]. But LE 102 * PKM 1 graft showed significantly less number of graft success percentage (9.34%) compared to other grafts which might be due to vigour, irrespective of the genetic proximity of tomatoes to eggplants (Gisbert *et al.*, 2011) [10]. Kawaguchi *et al*. (2008) [13] stated that lower affinity between scion and rootstock of genotypes occurs because of its poor vascular connection. In addition to this fact, rootstocks that differed from scion on the feature had less morpho-physiological affinity. It is not always possible to make connections between both vascular tissues for healing process, because of the incompatibility of the species (Zeist *et al*., 2017) [29]. Improper connection of vascular bundles between the scion and the rootstock decreases the water flow and water absorption by roots. It results in suppressed stomatal conductance and scion growth (Atkinson and Else, 2001) [1].

The commercial cv. PKM 1 recorded significantly greater plant height (29.94 cm) (Table 2), leaf area (70.29 cm^2) (Table 3), number of leaves (5.6) (Table 4) and shoot diameter (3.84 mm) (Table 5) than the grafted plants as the time taken for the graft union increased the growth period. Among the grafted plants, LE 828 * PKM 1 had significantly greater plant height (25.62 cm), leaf area (65.51cm^2), leaf number (4.6) and shoot diameter (3.56 mm) followed by LE 523 * PKM 1 had significantly greater plant height (24.70cm) and shoot diameter (3.48mm) followed by *Solanum sisymbrifolium* * PKM 1 with the plant height of 21.22cm, leaf area - 54.43 cm^2, number of leaves - 3.8 and shoot diameter - 3.44 mm next to *Solanum sisymbrifolium* * PKM 1 the better performed graft was *Solanum aculeatissimum* * PKM that had significantly greater leaf area - 59.50 cm^2 and number of leaves – 4.0 but recorded lowest shoot length (18.64cm) and shoot diameter (3.28mm). The growth of *Solanum torvum* * PKM 1 was poor with lowest plant height (17.40 cm), leaf area (52.38 cm^2) and shoot diameter (3.14 mm) compared to other grafted plants because of its shrub for growth habit.

As per the previous reports (Fernandez-Garcia *et al*., 2004b) [9] the formation of xylem and phloem vessels through the graft union takes 8 days after grafting in tomato plants. The root hydraulic conductance was also good which supports the success of the graft union at 8 days after grafting (Martinez-Ballesta *et al*., 2010) [17] the formation of xylem and phloem in the grafted plants resulted in less morphological growth as compared to the nongrafted plant i.e., PKM 1. The graft combination LE 102 * PKM 1 had least graft success percentage (9.34%) which might be due to poor regeneration of vascular bundles at the graft interface. These variations in the growth of grafted plants were influenced by the different rootstocks used and also the nature of their growth habit (Xu *et al*., 2015) [28]. The herbaceous rootstocks like LE 828, LE 523 and *Solanum aculeatissimum*) are of fast growing nature, than the *Solanum sisymbriifolium* which is a subshrub. The growth was very slow in the *Solanum torvum* * PKM 1 graft as the rootstock *Solanum torvum* was a shrub (Jaiswal. 2012) [12].

Thus the graft success percentage and growth rate reveal that *Solanum torvum and* LE 828 were the best rootstocks followed by LE 523, *Solanum sisymbriifolium and Solanum aculeatissimum.* The LE 102 cannot be used as a rootstock for grafting though it has the character for flood tolerance because of the poor regeneration of vascular bundles at the graft interface.

Table 1: Graft success percentage (%) of tomato cv. PKM 1 grafted with different solanaceous rootstocks

Treatments (Tomato grafts)	Graft Success (%)
T2- *Solanum torvum* * PKM 1	95.00
T3 - *Solanum aculeatissimum* * PKM 1	64.00
T4 - *Solanum sisymbriifolium* * PKM 1	69.40
T5 - LE 828 * PKM 1	57.33
T6 - LE 523 * PKM 1	13.33
T7 - LE 102 * PKM 1	9.34
Mean	51.40
SEd	3.67
CD(P=0.05)	7.58

Table 2: Shoot length (cm) of tomato cv. PKM 1 grafted with different solanaceous rootstocks

Treatments (Tomato grafts)	Shoot length (cm)
T1 - Control (PKM 1)	29.94
T2- *Solanum torvum* * PKM 1	17.40
T3 - *Solanum aculeatissimum* * PKM 1	18.64
T4 - *Solanum sisymbriifolium* * PKM 1	21.22
T5 - LE 828 * PKM 1	25.62
T6 - LE 523 * PKM 1	24.70
Mean	22.92
SEd	0.27
CD(P=0.05)	0.56

Table 3: Leaf area (cm^2) of tomato cv. PKM 1 grafted with different solanaceous rootstocks

Treatments (Tomato grafts)	Leaf area (cm^2)
T1 - Control (PKM 1)	70.29
T2- *Solanum torvum* * PKM 1	52.38
T3 - *Solanum aculeatissimum* * PKM 1	59.50
T4 - *Solanum sisymbriifolium* * PKM 1	54.43
T5 - LE 828 * PKM 1	65.51
T6 - LE 523 * PKM 1	52.26
Mean	59.06
SEd	0.48
CD(P=0.05)	0.99

Table 4: Leaf number of tomato cv. PKM 1 grafted with different solanaceous rootstocks

Treatments (Tomato grafts)	Number of leaves
T1 - Control (PKM 1)	5.6
T2- *Solanum torvum* * PKM 1	3.6
T3 - *Solanum aculeatissimum* * PKM 1	4.0
T4 - *Solanum sisymbriifolium* * PKM 1	3.8
T5 - LE 828 * PKM 1	4.6
T6 - LE 523 * PKM 1	3.4
Mean	4.17
SEd	0.36
CD(P=0.05)	0.73

Table 5: Shoot diameter (mm) of tomato cv. PKM 1 grafted with different solanaceous rootstocks

Treatments (Tomato grafts)	Shoot diameter (mm)
T1 - Control (PKM 1)	3.84
T2- *Solanum torvum* * PKM 1	3.14
T3 - *Solanum aculeatissimum* * PKM 1	3.28
T4 - *Solanum sisymbriifolium* * PKM 1	3.44
T5 - LE 828 * PKM 1	3.56
T6 - LE 523 * PKM 1	3.48
Mean	3.46
SEd	0.12
CD(P=0.05)	0.24

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