

Solanum torvum as a Compatible Rootstock in Interspecific Tomato Grafting

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Abstract

Two tomato scions ('Celebrity' and 'CLN3212A') were grafted onto *Solanum torvum* and tomato 'Maxifort' rootstock to determine compatibility. Experimental design included self-grafted and non-grafted control rootstocks. Seed vs. vegetative *S. torvum* rootstocks were also compared to further explore grafting options. Average number of days for graft fusion and survival rate was measured for each scion/rootstock combination. Vegetative *S. torvum* cuttings had the poorest grafting success rate as a rootstock (50% for both scions), while all other rootstock genotypes had statistically similar or higher success rates. There was no significant difference in time to graft fusion among any grafted genotypes. High compatibility of seed-derived *S. torvum* suggests its potential use as an interspecific grafting rootstock in areas where access to seed is readily available.

Keywords: Celebrity; Eggplant; Maxifort; Graft combination; Survival rate

Introduction

Plant grafting has been utilized in horticulture since the first millennium BCE [1]. The process involves joining together two parts (a rootstock and scion) from different plants to form a single, living plant. Traditionally, grafting was performed on woody perennials as a method to asexually propagate species that did not root well from vegetative cuttings. Starting in the 20th century, grafting began to be used extensively with annual vegetable crops [2]. Especially popular in East Asia, vegetable grafting allows a grower to combine a scion possessing desirable fruit producing traits with a rootstock that is resistant to a multitude of biotic and abiotic stresses. The resulting union often results in a more productive plant [3,4].

Intraspecific rootstock/scion grafting of vegetables is common because compatibility is higher than with interspecific grafting [5-7]. Intraspecific grafting has been shown to increase resistance to various environmental pressures such as flood, drought, cold, heat and pathogen stressors. However, in some cases the transferred tolerance is not strong enough or a certain desired environmental tolerance does not yet exist within the rootstock germ plasm of that species [8-11]. Vegetables with certain environmental susceptibilities could have grafting-compatible relatives within the same genus that possess a natural resistance to a specific stress. Thus, interspecific grafting could be used to broaden rootstock diversity when environmental pressures surpass the advantages that can be provided by intraspecific grafting alone.

While not as common as intraspecific grafting, the successful use of interspecific grafting in vegetable production is well documented [12]. After grafting eggplant (*Solanum melongena* L.) scions onto a verticillium wilt resistant tomato (*S. lycopersicum*) rootstock 'Lydl', Liu et al. [13] found 0% incidence of the disease on grafted eggplant, compared to 68% incidence on non-grafted controls. Allelopathic chemicals were also found in the tomato rootstock exudates, inhibiting fungal spore germination and mycelium growth. Davis et al. [14] states that watermelon (*Cucumis melo*) grafted onto the rootstock of bottle gourd (*Lagenaria siceraria*) confer significant resistance to *Fusarium* spp.

To identify a useful rootstock for interspecific grafting trials, first a relative with unique environmental resistances must be found, and then

tested for rootstock compatibility. Interspecific grafting compatibility is difficult to predict because the degree of taxonomic affinity necessary for compatibility varies widely across different taxa [1]. Four potential mechanisms of interspecific grafting incompatibility are identified by Andrews and Marquez [15]: cellular recognition, wounding response, plant growth regulators, and incompatibility toxins. Since it is difficult to predict whether an interspecific graft will be successful, individual grafting trials must assess compatibility.

Interspecific grafting may help expand production of tomatoes, a lucrative cash crop with worldwide appeal, but with sensitivity to excessive flooding or drought. This sensitivity limits their production in tropical regions [16,17]. Multiple accessions of tomato rootstock are in use commercially to confer temperature and salinity tolerances, but to date no tomato rootstock has significant resistance to flood conditions [18-20]. Thus, the identification of a flood tolerant rootstock would enable tomato production under flooded conditions. One potential candidate is eggplant, a highly resilient and close relative of tomato with many cultivars whose roots can survive longer in waterlogged soils [21].

Tomato/eggplant interspecific grafting has a history of successfully conferring environmental tolerances to fruit producing scions. Okimura et al. [22] found that egg plants grafted onto *S. integrifolium* x *S. melongena* rootstocks grew better at lower temperatures (18°C to 21°C) than nongrafted plants. Midmore et al. [23] observed that tomato/eggplant interspecific grafts produced acceptable yields during the rainy season in Taiwan. Black et al. [5] recommended using eggplant rootstock for tomatoes when flooding or waterlogged soils are expected, and selecting genotypes resistant to bacterial wilt and other soil borne diseases.

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An especially promising eggplant rootstock for tomato interspecific grafting is *Solanum torvum*, a species native to the western tropics and India that tolerates the climatic pressures of tropical regions [24]. This makes *S. torvum* an ideal candidate for tomato interspecific grafting in equatorial regions, where environmental conditions can make tomato production difficult [17]. There is also an established history of *S. torvum* for use as an intraspecific grafting rootstock in *S. melongena* cultivation for its resistance to a wide range of soil borne pathogens, including *Verticillium dahlia*, *Ralstonia solanacearum*, *Fusarium oxysporum* and *Meloidogyne* spp. root-knot nematodes [25-27]. The compatibility of *S. torvum* as a tomato interspecific grafting rootstock, however, has yet to be determined. If tomato/*S. torvum* compatibility were as high as commercially viable intraspecific combinations, tomato/*S. torvum* interspecific grafting could be used in production areas with high risk of flood and drought stress. Thus, *S. torvum* was selected as the rootstock of interest in this compatibility study for testing against other rootstocks of known high compatibility.

Uniform production of *S. torvum* rootstock seedlings can be challenging as a result of low germination rates leading to poor seedling emergence and slowed early growth [13]. This may be overcome by rooting vegetative cuttings of uniform size for use as rootstock. If there is no difference between the compatibility of seed-derived and vegetatively propagated *S. torvum* in tomato interspecific grafting, then the difficulties of seed production can be eliminated by maintaining *S. torvum* stock plants for rootstock cuttings. Thus, the overall objectives of this study were to assess the compatibility of *S. torvum* as a rootstock for tomato interspecific grafting and to determine any difference in graft compatibility based on the method of rootstock propagation. The tomato rootstock variety Maxifort was chosen as an intraspecific commercial standard rootstock to be compared against *S. torvum* in this study. The tomato varieties Celebrity and 3212 were chosen as scions because they have determinate and indeterminate flowering habits, respectively.

Materials and Methods

The experiment was conducted at the University of Minnesota in Saint Paul, MN, 44.94 N and 93.09 W. In week 18 2013, 48 seeds of *S. torvum* were planted into a plastic seed tray containing the soilless media 'Sunshine Mix #8 LC8' (Sun Gro Horticulture, Vancouver, Canada). All seeds were covered with coarse vermiculite, lightly watered and placed into a greenhouse. Greenhouse conditions were maintained at 21°C day/night and 175 µmol PAR (0700 to 1800 HR). *S. torvum* seeds were acquired from the Virgin Islands Sustainable Farming Institute in St Croix, US Virgin Islands.

Seven days after planting *S. torvum* seeds, 10 cuttings were taken from a *S. torvum* stock plant and rooted in 10 cm tall pots containing

the soilless medium 'Sunshine Mix #8 LC8'. All leaves except meristems were removed from cuttings.

Since *S. torvum* was found to have longer germination times, they were planted earlier to standardize stem diameter during cleft grafting. Twenty days after planting *S. torvum* seeds, all remaining seeds for the experiment were planted using the methods stated above. This included 48 'Maxifort' (*Lycopersicon esculentum* x *Lycopersicon hirsutum*) tomato seeds for rootstock, 72 'CLN 3212A' tomato seeds, and 72 'Celebrity' tomato seeds. 'Maxifort' and 'Celebrity' seeds were acquired from Johnny's Selected Seeds (Winslow, ME), and 'CLN3212A' seeds were acquired from the Asian Vegetable Research and Development Center (AVRDC, Taiwan).

By week 28 2013, all seeds had germinated and seedlings had grown to appropriate grafting size, i.e. the 4-5 true leaf stage [28]. 8 of 10 *S. torvum* cuttings rooted. Cleft grafting was used for all plants; the most commonly used method for solanaceous crops [29]. With a razor blade, rootstocks were cut below the cotyledon and a longitudinal cut was made 1.5 cm deep (termed a "depth cut"), about 75% the depth of the stem. Scions were pruned to 1-3 leaves and the lower stem was cut into a tapered wedge to place inside the depth cut of the rootstock [29]. After insertion, graft unions were wrapped with plastic parafilm (SPI Supplies, West Chester, PA) to improve stability, reduce chance of infection and ensure vascular contact [30]. The scion and rootstock combinations were joined to create 10 different graft combinations (Table 1). Newly grafted plants were immediately placed in a plastic chamber maintained at 21°C day/night at all times [29]. The chamber was constructed by wrapping clear and black plastic around a PVC A-frame skeleton and placed into the greenhouse. Humidity was maintained by sub-irrigating grafted plants on 0.89 cm deep Sure To Grow® capillary mats (Beachwood, OH), which were flushed to saturation with water daily [31].

Each plant was evaluated daily to determine graft fusion and survival in the chamber. Due to inconsistent rooting, only 8 *S. torvum* cuttings were available for grafting. This resulted in "CLN 3212Ax *S. torvum* Veg" and "Celebrity x *S. torvum* Veg" having only four replications each. Evaluation involved observing for wilting symptoms of each plant. When scion turgor pressure was restored in the chamber, the plant was moved outside the chamber. If the plant did not wilt outside the chamber for 24 hours, graft fusion was considered completed, and recorded.

Analysis of variance (ANOVA) was used to compare the differences in survival and average Days To Fusion (DTF) between the 10 'Celebrity' and 'CLN 3212A' graft combinations. If ANOVA determined that treatment means were significantly different than the grand mean ($P < 0.05$), Tukey's Honest Significant Difference (HSD) test was conducted to determine significant differences among means. ANOVA has been used to compare differences in graft compatibility through DTF in perennial and annual crops [27,32]. All statistical analyses were done using R software [33].

Results and Discussion

With 'Celebrity', the *S. torvum* rootstock had the highest average DTF (12.3 days, Table 2) compared to any other graft combination, $P < 0.05$. The 'Celebrity' scion had the lowest survival percentage (50%; Table 2) when grafted onto vegetative *S. torvum* rootstock. Survival was significantly lower than all other rootstock combinations with the exception of the self-grafted Celebrity genotype (70%; Table 2).

In the 'CLN 3212A' scions, there was also no significant difference

Scion	Rootstock	Number of plants successfully grafted
CLN 3212A	none (nongrafted)	10
CLN 3212A	CLN 3212A(self grafted)	10
CLN 3212A	'Maxifort®'	10
CLN 3212A	<i>Solanum torvum</i> seed	10
CLN 3212A	<i>Solanum torvum</i> cutting	4
'Celebrity'	none (nongrafted)	10
'Celebrity'	'Celebrity'(self grafted)	10
'Celebrity'	'Maxifort®'	10
'Celebrity'	<i>Solanum torvum</i> seed	10
'Celebrity'	<i>Solanum torvum</i> cutting	4

Table 1: Scion and rootstock combinations analyzed for grafting compatibility.

'Celebrity' Scion	Rootstock				
	Nongrafted	Celebrity	Maxifort	<i>S. torvum</i>	<i>S. torvum</i> Veg
(n)	10	10	10	10	4
DTF	0a	12.14 b	10.5 b	12.3 b	12 b
Survival (%)	100 a	70 ab	100 a	100 a	50 b

CLN 3212A Scion	Rootstock				
	Nongrafted	3212	Maxifort	<i>S. torvum</i>	<i>S. torvum</i> Veg
(n)	10	10	10	10	4
DTF	0a	11 b	9.9 b	11.2 b	12 b
Survival (%)	100	80	100	80	50

Table 2: Average number of Days to Fusion (DTF) and survival (%) among all 'Celebrity' and 'CLN 3212A' rootstock-scion combinations. Letters denote statistical differences ($P < 0.05$) within rows using Tukey's HSD test.

in DTF among all grafted types (Table 2). Although the CLN 3212A scion also had the lowest survival percentage when grafted onto rooted vegetative cuttings of *S. torvum* (50%; Table 2), the difference was not statistically different from any other rootstock genotype.

When comparing DTF among all rootstock genotypes, there was no significant difference in the amount of time it took for any successfully grafted plant to form a healed graft union (Table 2). *S. torvum* rootstock had the highest average DTF for both Celebrity and CLN 3212A scions, but the difference from other rootstock genotypes was not significant. The lack of significance when analyzing DTF implies that *S. torvum* does not have a greater fusion incompatibility than intraspecific rootstocks.

In both Celebrity and CLN 3212A scions, vegetative *S. torvum* rootstock had a lower survival rate than seed-derived *S. torvum* rootstock, Maxifort, self-grafted and non-grafted rootstocks (Table 2). The reasons for lower compatibility of vegetative compared to seed-derived *S. torvum* rootstock in interspecific grafting may be two fold. Initial adventitious roots formed on cuttings are more adept at oxygen gas exchange but less capable of water uptake than primary root systems [34]. Thus, it is possible that the scions grafted onto vegetative *S. torvum* lost turgor pressure and wilted because of this diminished hydraulic capability. Also, since rootstocks derived from *S. torvum* cuttings would be older in growth (with possible secondary growth) than seedlings, the formation of a vascular cambium in the vegetative rootstock may have inhibited proper graft fusion. More research is needed to determine the exact cause of reduced compatibility in vegetative *S. torvum* rootstock.

Due to the low survival percentage of vegetative *S. torvum* rootstock with both Celebrity and CLN 3212A tomatoes, we do not recommend these combinations for commercial production. Seed-derived *S. torvum* may still be a viable option for interspecific grafting based on the results in this experiment (Table 2). If seed-derived *S. torvum* is shown to be compatible to a wider variety of tomato scions and an effective means of providing strong environmental tolerances to the plant, it could be a valuable tool for growers worldwide. This would be especially true in regions with minimal agricultural infrastructure, such as tropical regions, where access to greenhouses and other environmentally controlled enclosures is declining or unavailable [20,35,36].

As mentioned previously, Maxifort is a commonly used, commercial standard rootstock for tomato grafting, and was utilized as a positive control in this experiment. Statistical pair wise comparisons between Maxifort and seed-derived *S. torvum* rootstock showed no significant difference for DTF or survival percentage on either scion (Table 2). Such results imply that for tomatoes, interspecific *S. torvum* grafting has equal compatibility to intraspecific counterparts.

Previous research documenting the effectiveness of seed-derived *S. torvum* in intraspecific grafting is promising. *S. torvum* rootstock

confers resistance to a wide array of environmental pressures (Singh and Gopalakrishnan [25], Bletsos et al. [26] and Gisbert et al. [27]). Due to the lack of abiotic tolerances in tomato rootstock germplasm, further exploration of *S. torvum* as a flood- and drought-resistant rootstock for tomato scions is merited.

We showed that seed-derived *S. torvum* is a compatible rootstock with the two tested tomato scion cultivars, Celebrity and CLN 3212A. Vegetative *S. torvum* rootstock showed moderate compatibility as an interspecific grafting rootstock, but had a significantly reduced grafting success rate when compared to seed-derived *S. torvum*, Maxifort and self-grafted rootstocks. All grafted plants needed similar number of days for successful graft fusion. Due to its high compatibility, we recommend that the effectiveness of seed-derived *S. torvum* rootstock in providing flood- and drought-tolerances to tomato scions be explored further.

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