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# Grafting tomato on eggplant as a potential tool to improve waterlogging tolerance in hybrid tomato

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## Abstract

This study was conducted at ICAR-IIVR, Varanasi during 2013-14. Four eggplant rootstocks *v.i.z.,* IC-354557, IC-111056, IC-374873 and CHBR-2 were used, while hybrid tomato (Arka Rakshak and Arka Samrat) were used as scion cultivars. Both grafted and non-grafted plants were exposed to waterlogging stress for 72 and 96 hr, respectively during vegetative and reproductive stages. Experimental finding revealed that there was no leaf chlorosis and wilting in the plant, and was less reduction in chlorophyll content (CCI), chlorophyll fluorescence yield (Fv/ Fm) in all plant growth stages, when tomato hybrids were grafted over eggplant rootstocks. In contrast, the non-grafted plants have registered 39.6-41% reduction in Fv/Fm and 41-100 % reduction in CCI at 96 hr after exposing the waterlogging stress. Non-grafted plants wilted and die 4-7 days after relieving from stress; whereas the grafted plants completely recovered from stress shock in 7-10 days after relieving from stress. It may conclude from the present study that grafting tomato onto eggplant rootstocks, particularly on IC-354557 and IC-111056 may improve waterlogging tolerance for 72-96 hr in tomato hybrids.

**Keywords:** Tomato grafting, eggplant rootstocks, waterlogging tolerance, chlorophyll fluorescence

## Introduction

Grafting in vegetable crops, now-a-days has emerged as a promising and an alternative tool to the relatively slow conventional breeding methods aimed at increasing tolerance to abiotic stresses and soil pathogens in fruit vegetables. Scions of vegetable crops like watermelon, squash, cucumber, bitter gourd, tomato and eggplant, etc. can readily be grafted onto different rootstocks prior to being transplanted in the field or in the greenhouses. The main objective of grafting is to reduce infections by soil-borne pathogens and to enhance the tolerance to abiotic stresses such as soil salinity, toxicity of heavy metals, drought and waterlogging. Under natural environmental conditions, plants often get exposed to transient or permanent waterlogging. This induces changes in physico-chemical properties of soil like pH, redox potential and available oxygen level. Therefore, the plants growing on the waterlogged soil face the stressful environment in terms of availability of oxygen; this may be hypoxia (deficiency of $O_2$) or anoxia (absence of $O_2$). Researchers from the Asian Vegetable Research and Development Center (2003) reported that tomatoes are difficult to grow during the hot-wet season, because waterlogged soils can significantly reduce yields. In north Indian plains, tomato is transplanted during S-W monsoon (July-September) where waterlogging is a serious problem for the survival of plants, particularly during the early establishment stage. The tomato is highly sensitive to flooding conditions (Bray *et al.*, 2001; Ezin *et al.*, 2010; Bhatt *et al.*, 2015). The success of 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 optimise crop performance and promote commercial acceptance of the technique. High yielding varieties of tomato can be successfully grafted over waterlogging tolerant rootstocks of eggplant (Bhatt *et al.*, 2015). With the objective to identify suitable waterlogging tolerant eggplant rootstocks, the improved tomato cultivars were grafted on four rootstocks of eggplant (*Solanum melongena* L.) during early vegetative (15-20 days after transplanting, DAT), active vegetative stage (40-45 DAT) and reproductive stages (60-70 DAT).

## Materials and Methods

**Plant materials:** The experiment was conducted using eggplant rootstock (*Solanum melongena* L.) grafted with tomato scion (*Solanum lycopersicum* L.) at ICAR-Indian Institute of Vegetable Research, Varanasi during 2013-14. Rootstock of four eggplant accessions; IC-354557,

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IC-111056, IC-374873 and CHBR-2 were grafted with two hybrid tomato cultivars (Arka Rakshak and Arka Samrat). Non-grafted tomato plants (Arka Rakshak and Arka Samrat) were also used as a control for comparison.

**Preparation of graft:** Seeds of above 4 genotypes of eggplant were sown in the last week of June at 1.5-2.0 cm depth in small plastic glasses (size 7× 9 $cm^2$) filled with soil, sand, vermicompost and cocopit (2:1:1:1). After one week, the seeds of scion cultivars of tomato were also sown in small earthen pots accommodating about 50 seedlings. The pots of rootstocks and scions were irrigated daily for 25 days. Four to five weeks old seedlings of eggplant were used as rootstocks, whereas three to four weeks tomato seedlings as scions. Splice or side grafting was followed wherein 45° slant-cut made on the sides both in the rootstock and scion at matching grafting ensuring aligning of their cambium, and clip together with a silicone grafting clip. Grafted plants were soon transferred in grafting chamber where the maximum temperature was never allowed to above 30°C, and relative humidity was in ranged of 80 to 85% during most of the time. The light interception in grafting chamber was not more than 25% of the total incoming radiation. After 10-12 days of establishment of graft union, the grafted plants were transplanted into bigger pots (30 × 30 $cm^2$ size) filled with mixture of field soil and vermicompost (3:1 ratio), and kept in the greenhouse for another 10-12 days for the establishment of seedlings.

**Waterlogging treatment:** Grafted and non-grafted plants were exposed to waterlogged conditions for 72 and 96 hr during early vegetative (15 DAT), active vegetative growth (45 DAT) and reproductive stages (75 DAT). Pots were placed in the large water tank (LBH= 2.5 × 1.25 × 0.60 $m^3$) with maintaining 8 to 10 cm water above the pot soil surface. The average maximum, minimum temperature and humidity during the experimentation was 24.0/12.1°C, 92/61% in February, and 32.2/16.7°C, 83/45% in March.

**Plant wilting, yellowing and adventitious root formation:** After removing from flooding treatment, individual plants were scored for waterlogging tolerance on the basis of plant wilting, yellowing and development of aerial adventitious roots using a modified scale of 0-5 developed Yeboah *et al.* (2008). Adventitious root formation (ARF) was scored visually on a 0-3 scale following the methods of Yeboah *et al.* (2008). A third flooding tolerance score of individual plants (in terms of leaf yellowing percentage) was determined using a scale of 1-6 (Mohanty and Ong, 2003). The genotypes that exhibit high adventitious root formation above soil surface have the ability to withstand the negative effect of flooding, and also to increase water as well as mineral uptake and compensate for the loss of the original roots. The adventitious roots can obtain oxygen from the air and absorb nutrients; this characteristic may play an important role in its adaptation to flooding conditions.

**Chlorophyll content index (CCI) and chlorophyll fluorescence measurement:** The ratio of flurescence variable (Fv) and flurescence maximum (Fm) is an indicator of plant stress resulting from damage to photosystem II. Chlorophyll fluorescence parameter (Fv/ Fm) was recorded every day upto 10 days after removal from the waterlogging treatment. Measurements were made on fully expanded leaf of the upper canopy after a 30 min dark adaptation. Dark fluorescence (Fo), maximal fluorescence (Fm) and photochemical yield (Fv/Fm) were recorded. CCI was measured every day upto 10 days after removal from the waterlogging treatment by the Chlorophyll Content Meter (CCM 200, Apogee instruments Inc. USA). The average of two readings was taken at third upper expanded leaflet.

## Results and Discussion

***Leaf chlorosis, wilting and development of aerial roots:*** The most important visual symptom of waterlogging stress in the plant is epinasty, leaf yellowing and wilting. In the present experiment, the grafted as well as non-grafted plants were scored for tolerance to waterlogging stress using scale 0-5 (Yeboah *et al.*, 2008). Results indicated that the grafted plant did not show any wilting symptoms, and plant recovered from shock after 10 days of withdrawal from stress. The non-grafted tomato hybrids exhibited wilting symptoms 72 hr after removing from stress, and plant die $10^{th}$ days of its removal from waterlogging (fig. 1a). During active vegetative stage, after 96 hr of waterlogging stress, Arka Samrat grafted on IC-354557 performed well followed by Arka Rakshak on IC-374873. Grafted plants on these eggplant rootstocks did not exhibit symptom of wilting and yellowing, and plant recovered well 10 days after stress (fig. 1b). Experimental finding obtained at reproductive stage revealed that tomato hybrid Arka Rakshak on IC-111056 and Arka Samrat on IC-354557 found the best options for survival under waterlogging situation. It is obvious from fig. 1c that the grafted plant over these eggplant rootstocks did not show any symptoms of leaf yellowing and wilting during 96 hr of waterlogging stress, and 10 days after removal of stress. In contrast to this, the non-grafted tomato hybrids start leaf yellowing and wilting 96h of waterlogging stress, and they wilt and die 10 days after water-logging stress. Leaf chlorosis and wilting is the major indicator of waterlogging stress as reported by Reyna *et al*., (2003) in soybean, Cai *et al.* (1996) and Boru *et al.* (2001) in wheat, and Kuo *et al.* (1982) and Bhatt *et al.* (2015) in tomato. When soil is saturated with excess water, gas diffusion is reduced in the soil and consequently reduces $O_2$ supply to the roots. This reduction in $O_2$ causes the damage to the root system of the plant. During hypoxic and anoxic conditions, the production of ATP declines and consequently decrease root metabolism. The decrease in energy supply in turn decreases ion transport in the root and influences the nutrient use efficiency. In addition, the reduction in available energy could decrease other physiological processes such as gas exchange, chlorophyll kinetics, osmotic adjustment and carbohydrate metabolism of the plant. Similar to our findings, Bhatt *et al.* (2015) also reported that when eggplant was used as rootstock for flooded tomato, there was low reduction in leaf water potentials and greater accumulation of sugar as osmolytes, possibly through osmo-regulation. Increased sugar levels and starch in plant following flooding has also been reported by many workers (Wample and Davis, 1983; Gravatt and Kirby, 1998; Chen *et al*., 2005; Gimeno *et al*., 2012). Magnitude of sugar accumulation is decisive in ascertaining the plant capacity to confer stress tolerance.

**Chlorophyll fluorescence and chlorophyll content index:** The differing response of grafted and non-grafted plants in term of chlorophyll fluorescence and chlorophyll content index (CCI) suggest the possibility of using both parameters to screen for waterlogging tolerance. The most common parameter used for measuring chlorophyll fluorescence is Fv and Fm ratio (photochemistry of PS II). It is an important photosynthetic parameter to evaluate the performance of plants under stress (Maxwell and Johnson, 2000). In present investigation, first to ten days after flooding, the Fv/Fm range from 0.756 to 0.706 for Arka Rakshak on IC-111056; 0.707 to 0.773 for Arka Samrat on CHBR-2; 0.718 to 0.768 for Arka Samrat on IC-354557 at early vegetative stage (fig. 2a). At active vegetative stage, the Fv/Fm values were 0.643 to 0.726 for Arka Rakshak on IC-374873; 0.596 to 0.752 for Arka Samrat on IC-354557 (fig. 3a). Similarly, at reproductive stage, the Fv/Fm values ranged between 0.557 to 0.754 for Arka Samrat on IC-354557; 0.646 to 0.783 for Arka Rakshak on IC-111056; 0.0 to 0.717 for non-grafted Arka Rakshak and 0.0 to 0.678 for non-grafted Arka Samrat (fig. 4a). It was concluded that after exposure of plants for 72h of flooding, Fv/Fm did not vary significantly up to 8 days after flooding, thereafter intense wilting started, and Fv/Fm decreased by 39.6-41% in non-grafted plants, while in grafted plants the reduction was just 9.78-11.5%. Ezin *et al.* (2010) also reported a significant reduction in chlorophyll fluorescence in the susceptible genotypes of tomato under waterlogging because of loss in the yield of PSII photochemistry thereby reduction of photosynthetic rate. Bhatt *et al.* (2015) has also reported 10-14% reduction in Fv/Fm values in non-grafted or self-grafted tomato, whereas it was only 1-7% when eggplant was used as rootstock. The response of Chl fluorescence in non-grafted plants to flooding indicated that during flooding apart from decrease in stomatal conductance,

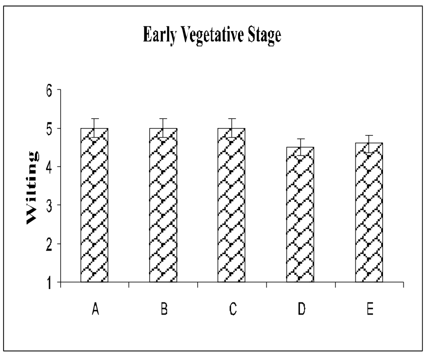



**Fig. 1a**

A- Arka Samrat on CHBR-2
B- Arka Rakshak on IC-111056
C- Arka Samrat on IC-354557
D- Arka Rakshak
E- Arka Samrat

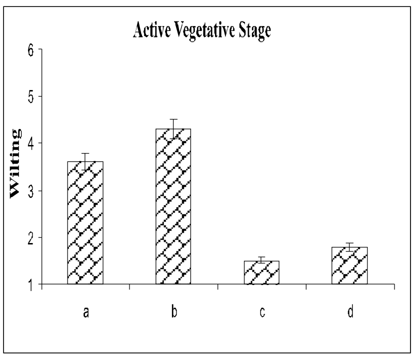



**Fig. 1b**

a- Arka Rakshak on IC-374873
b- Arka Samrat on IC-354557
c- Arka Rakshak
d- Arka Samrat

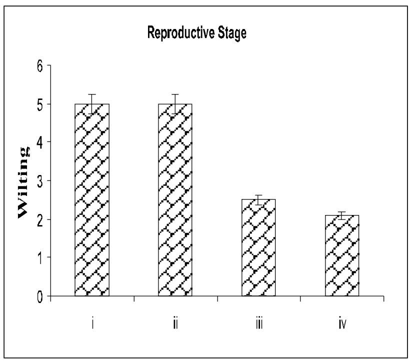
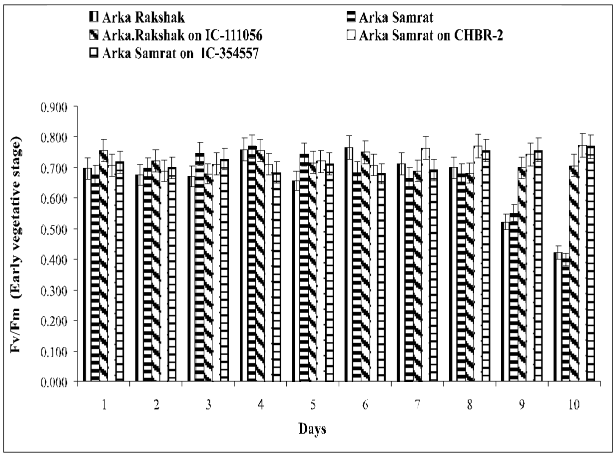
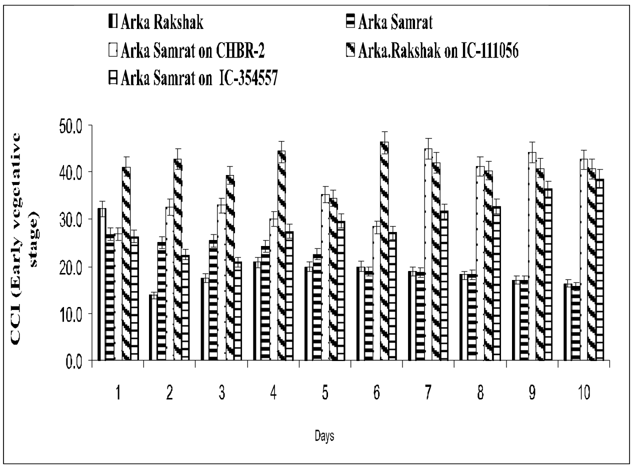



**Fig. 1c**

i- Arka Rakshak on IC-111056
ii- Arka Samrat on IC-354557
iii- Arka Rakshak
iv- Arka Samrat

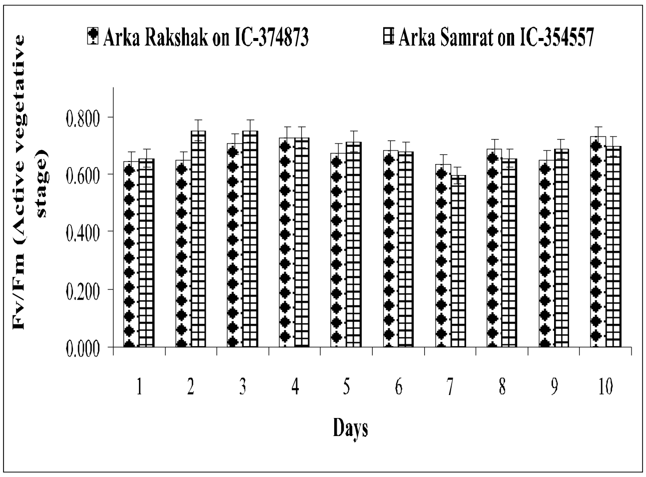
**Fig. 1** showing wilting during early vegetative, active vegetative and reproductive stages. 0 = dead plant, 1 = 100-75% of wilt from tip to the base, 2 = 74-50% wilting of leaves from tip to the middle, 3 = leaves between base and middle undulating, 4 = recurved leaves margins and 5 = green plant with no sign of stress (Yeboah *et al*., 2008). (Error bar shows $P<0.05$ level of significance.)

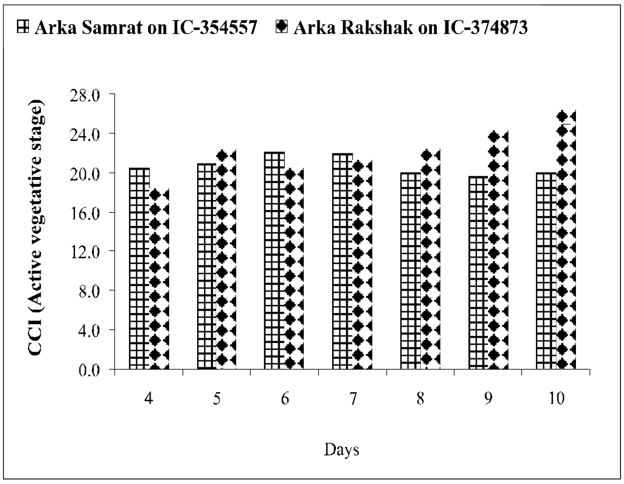



**(2a)** **(2b)**

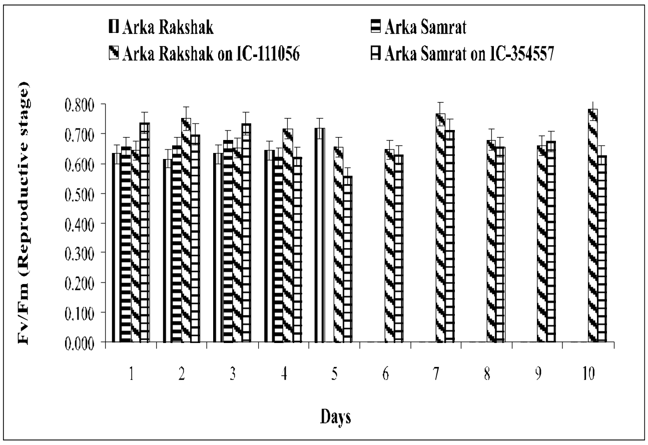
**Fig. 2** Chlorophyll fluorescence **(2a)** and chlorophyll content index **(2b)** at early vegetative stage of 72 hr waterlogging. (Error bar shows $P<0.05$ level of significance).

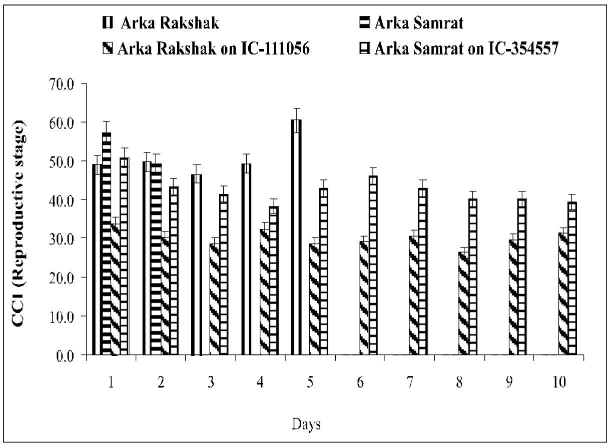



**(3a)** **(3b)**

**Fig. 3** Chlorophyll fluorescence **(3a)** and chlorophyll content index **(3b)** at active vegetative stage of 96h waterlogging. (Error bar shows $P<0.05$ level of significance)




**(4a)** **(4b)**

**Fig. 4** Chlorophyll fluorescence **(4a)** and chlorophyll content index **(4b)** at reproductive stage of 96 hr waterlogging. (Error bar shows $P<0.05$ level of significance) non-stomatal factors may also have contributed in reduced photosynthetic rate as indicated by significant reduction in Fv/Fm. Decrease in Fv/Fm indicated damage to thylakoid membranes and/or photosynthetic electron transport system (Havaux and Lannoye, 1983). Flooding induces progressive impairment of photosynthetic machinery, starting with reduction in efficiency of the light-harvesting complexes that leads to an electron overflow in the photosynthetic system, and to severe impairment in the electron transport chain. The relatively higher Fv/Fm is associated with healthy, non-stress, evergreen plants (Demming and Bjorkman, 1987; Maki and Columbo, 2001; Percival, 2004).

As far as chlorophyll content index (CCI) was concerned, during vegetative stage CCI did not reduce significantly upto 7 days after flooding treatment in grafted and non-grafted plants, however at reproductive stage, after $5^{th}$ day of flooding the non-grafted plant could not survive (CCI value zero). In contrast, the tomato plant grafted over eggplant noticed the higher CCI value (46.0) at reproductive stage. First to ten days after flooding, the CCI range from 40.3 to 44.3 for Arka Rakshak on IC-111056; 26.9 to 44.9 for Arka Samrat on CHBR-2; 20.9 to 38.6 for Arka Samrat on IC-354557; 13.9 to 32.2 for non-grafted Arka Rakshak and 15.7 to 26.7 for non-grafted Arka Samrat at early vegetative stage (fig. 2b). At active vegetative stage, the CCI values were 18.4 to 26.3 for Arka Rakshak on IC-374873, 19.7 to 22.1 for Arka Samrat on IC-354557 (fig. 3b), whereas the CCI was registered zero for non-grafted Arka Rakshak and Arka Samrat. Similarly, at reproductive stage, the CCI values ranges between 26.4 to 33.8 for Arka Rakshak on IC-111056; 39.5 to 50.7 for Arka Samrat on IC-354557; 0.0 to 60.4 for non-grafted Arka Rakshak and 0.0 to 57.3 for non-grafted Arka Samrat (fig. 4b). In non-grafted plants, considerable reduction (41-100%) in CCI was observed as compared to about 40-43% reduction in grafted plants, 8 days after flooding exposure. Earlier, Kato *et al.* (2001) also reported reduction in chlorophyll content of cucumber leaves induced by waterlogging which was enhanced by grafting onto squash rootstocks. When grafting watermelon *cv*. 'Crimson Tide' onto bottle gourd (Landrace) the decrease in chlorophyll content was less pronounced compared to non-grafted watermelons (Yetisir *et al*., 2006; Liao and Lin, 1996). Recently, Bhatt *et al.* (2015) also noticed that chlorophyll reduction at 6 days of flooding to the tune of 24-28% in non-grafted or self-grafted tomato while 4-19% reduction was observed over eggplant grafted tomato.

All the flooded tomato plants grafted over eggplant rootstocks were recovered after relieving flooding, indicating better efficiency of rootstock for conferring flood tolerance to tomato scion. Eggplant rootstocks may be of great use in improving physiological tolerance to flooding through a resilient root system. Grafting of tomato on eggplant rootstocks improved waterlogging tolerance on early vegetative, active vegetative and reproductive stages in comparison to the non-grafted plants reflected by wilting/survival of plants, Chl fluorescence and CCI. Grafting of tomato onto eggplant rootstocks can improve flooding tolerance of flood sensitive tomato crop. It may conclude from the present study that grafting tomato onto eggplant rootstocks, particularly on IC-354557 and IC-111056 may improve waterlogging tolerance for 72-96h in tomato hybrids during all three stages of the plant. Using these eggplant rootstocks for tomato would be a potential tool for growing tomatoes in rainy season, where waterlogging situations frequently occur.

## Acknowledgements

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## सारांश

टमाटर में जल–भराव के प्रति सहनशीलता विकसित करने हेतु बैंगन के मूलवृन्त पर वर्ष 2013–14 में ग्राफ्टिंग पर प्रयोग किया गया। टमाटर सायन हेतु दो प्रजातियों : अर्का रक्षक एवं अर्का सम्राट तथा बैंगन मूलवृन्त के लिए 4 लाइनें यथाः आई.सी.354557, आई.सी. 111056, आई.सी. 374873 तथा सी.एच.बी.आर. 2 प्रयोग में लायी गई। ग्राफ्टिंग तथा बिना ग्राफ्टिंग पौधों में विभिन्न अवस्थाओं में 72 घंटे तथा 96 घंटे का जल भराव का पौधे की विभिन्न आकारिकी एवं दैहिकी गुणों का प्रभाव आँका गया। निष्कर्ष में यह पाया गया कि जिन टमाटर पौधों को बैंगन के मूलवृन्त, विशेषकर आई.सी.354557 तथा आई.सी. 111056 पर लगाया गया था उनकी पत्तियों में पीलापन या मुरझापन के लक्षण 96 घंटों तक जल भराव होने के बावजूद नहीं देखा गया जबकि बिना ग्राफ्टेड या स्वयं के मूलवृन्त वाले टमाटर में जल भराव से मुक्ति होने के 4 से 7 दिन में क्लोरोफिल सांद्रता, क्लोरोफिल फ्लोरोसेन्स में 40–100 प्रतिशत गिरावट दर्ज की गई, तथा पौधे बाद में पूरी तरह सूख गये इसके विपरीत ग्राफ्टेड पौधे जल भराव के बाद पूरी तरह 5–7 दिनों में स्वस्थ हो गये। इस प्रयोग से यह निष्कर्ष निकाला जा सकता है कि टमाटर का बैंगन पर ग्राफ्टिंग जल भराव की स्थिति से निपटने के लिए एक बेहतर माध्यम है।

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