



## Anatomical and Histological Studies of Grafted Tomato with Interspecific Solanaceous Rootstocks

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

A study was carried out at the Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during 2019-2020 to assess the anatomical and histological changes within the graft union of tomato and identify compatible rootstocks for improved propagation of the crop. This experiment involved rootstocks of three wild tomatoes including *Solanum torvum*, *S. sisymbriifolium* and *S. capsicoides* and scions of two tomato hybrids including TNAU tomato hybrid CO3 and Shivam. The grafting was done using the cleft grafting method. The anatomical and histological sections of six graft combinations and two tomato scion samples were viewed microscopically at 7, 14 and 21 days after grafting (DAG). Among the six tomato graft combinations, *S. torvum* rootstock showed complete development of vascular connection at 21 DAG followed by *S. sisymbriifolium* rootstock, whereas *S. capsicoides* rootstock showed only callus growth at 14 DAG. Scion growth dominated the rootstock growth in *S. torvum* rootstock leading to mismatch of scion-rootstock stem thickness and delayed epinasty symptom at the later stages of plant growth. Of the rootstocks of the three species studied, *S. sisymbriifolium* rootstock was compatible for tomato grafting though it exhibited delayed vascular connection between the scion and rootstock.

**Keywords:** Graft union; compatibility; epinasty; vascular connection; *Solanum* species.

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## 1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important protective foods because of its special nutritive value and is a widely grown vegetable crop in the world next to potato and onion in India. Tomatoes are important source of lycopene, Vitamins C and A and are valued for their colour and flavor [1]. In recent years, epidemiological study has shown that tomatoes protect the digestive tract and prostate from cancer. Moreover, tomato interferes with oxidative damage to DNA, lipoproteins and inhibits the oxidation of LDL (Low density lipoprotein) cholesterol [2]. It has a strong naturally present antioxidant that scavenges free radicals which are involved in destruction of healthy body walls causing degenerative diseases [3]. In India, the major biotic constraints of tomato production are the occurrence of many insects and diseases causing considerable economic loss. Among the biotic factors hampering the tomato production, root knot nematode (*Meloidogyne incognita*) and *Fusarium* wilt (*Fusarium oxysporum* f.sp. *lycopersici* (FOL)) are the most devastating diseases that contribute to reduction in fruit yield and quality [4]. Grafted tomato plants showed resistance to biotic stress and consistent yield in many vegetable crops. Grafting of cultivated tomato varieties on to compatible and related wild species as rootstocks, which are resistant to pests and diseases is one of the handy methods. Thus, the aim of this study was to assess the anatomical and histological changes within the graft union of tomato and identify compatible rootstocks for improved propagation of the crop.

## 2. MATERIALS AND METHODS

The present study was carried out at the Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during 2019 - 2020 to examine the anatomical and histological changes occurring during the graft union process. Three wild *Solanum* species (*Solanum torvum*, *S. sisymbriifolium* and *S. capsicoides*) were chosen as rootstocks to graft on to two tomato hybrids such as TNAU tomato hybrid CO3 and Shivam. Cleft grafting method was used in this study and the grafting experiment was conducted in Completely Randomized Design (CRD) with three replications under insect proof nethouse. Observations on the number of days taken for graft union, success per cent of grafted plants,

number of fruits per plant, fruit yield per plant and crop duration were recorded. In order to study the ultra structural changes and compatibility in the grafted tomato plants, histological study was taken up in the graft union region sections of 10  $\mu$ m at 7, 14 and 21 DAG. By using a rotary microtome, sections were stained with safranin/ aniline blue dye and histological observations were carried out under microscope following the method suggested by Johansen [5].

### 2.1 Sample Preparation

The sample preparation involved several steps. The specimens (graft union portion) were first killed and fixed in FAA (FAA: 10:50:5:35 proportion of formalin, alcohol, acetic acid and water) solution for about 24 h. This was followed by washing with 50 per cent ethanol and transferring them to tertiary butyl alcohol series of 60, 70, 80, 90 and 100 per cent for one h followed by further dehydration in 100 per cent tertiary butyl alcohol (TBA) for 12 h. After the dehydration step, the samples were subjected to infiltration by transferring into a solution containing different proportions of TBA + wax (2/3+1/3, 1/2 +1/2, 1/3+2/3), respectively, and absolute wax two times for 30 to 45 min in each series.

The plant materials were then embedded in wax with melting point of 52 – 54°C. The molten wax was poured into a paper boat with the inner side smeared with glycerin. The infiltrated pieces were placed in molten wax in the proper orientation. The blocks were cut such that each block contained one section. The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10 – 12  $\mu$ m.

### 2.2 Dewaxing and Staining of Samples

Dewaxing was done using xylene alcohol mixture. The slides containing sections were kept for 30 min in pure xylene (2 x), ethanol + xylene (1:1), 90, 70, 50 per cent ethanol. The slides were kept in aniline blue dye solution for 12 hours subsequently transferred to 50, 70, 90 per cent ethanol for 10 min in each series. The slides were then dipped in alcohol: ammonia and alcohol: picric acid mixture. This was followed by transferring the slides into 70 per cent alcohol for 2-3 min. Fast green solution was added over the sections and the stain was drained with clove oil and washed with distilled water. The slides were

transferred to alcohol: xylene mixture for 5 min and in pure xylene (2 x) for 10 min.

### 2.3 Mounting and Photomicroscopy

Glycerin mounted temporary preparations were made for macerated / cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell components were studied.

Observations on the cellular changes were made using microscope (Laborlux S, Leitz, Austria) under bright field cold illumination. Sections were micrographed in Kodak photo film using Nikon F 301 camera. Descriptive terms of the anatomical features were done as described [6].

## 3. RESULTS AND DISCUSSION

Grafting is defined as the natural or deliberate fusion of plant parts so that vascular continuity is established and functions as a single plant. However, to achieve the beneficial effects of grafting, the plant has to undergo external and internal processes to establish communication between rootstock and scion. It is an alternative approach to enhance wider adaptability of high yielding genotypes to enhance its yield [7] Grafting with wild rootstocks showed increased tolerance to pests and diseases which is due to the limitation of pathogen movement by rootstocks to the scion from the soil. The performance of the grafted plants on the number of days taken for graft union, success per cent of grafted plants, number of fruits per plant, fruit yield per plant and crop duration is given in Table 1.

From the Table it is evident that the graft combination of TNAU tomato hybrid CO3 with *S. torvum* rootstock showed faster graft union of 9.57 days, followed by Shivam grafted with *S. torvum* (10.03 days) (Table 1). Comparing all the rootstocks *Solanum torvum* showed faster results in graft union with both the scion. However, *S. sisymbriifolium* took periods of 20.58 and 19.56 days for successful graft union formation when grafted with Shivam and TNAU tomato hybrid CO 3, respectively. Graft combinations with three wild species and two tomato scions indicated that success percentage was influenced by both the rootstocks and scion at various intervals of grafting.

Further the results revealed that plants grafted with *S. torvum* rootstock had more successful

grafted union plants of 80.81 and 85.77% with the scions of TNAU tomato hybrid CO3 and Shivam, respectively. Grafted plants of *S. capsicoides* with TNAU tomato hybrid CO3 recorded the least success percentage of 59.89. Significant variation in grafted and non-grafted tomato plants were observed for number of fruits per plant. Shivam and TNAU tomato hybrid CO3 grafted on *S. sisymbriifolium* significantly exhibited higher production of 112.37 and 102.27 fruits per plant compared to the other treatments. The grafts of Shivam with *S. capsicoides* rootstock produced least number of fruits (85.34). Longer crop duration was recorded in TNAU tomato hybrid CO3 grafted on to *S. sisymbriifolium* rootstock (171.34 days) followed by Shivam grafted on to *S. sisymbriifolium* rootstock (170.18 days).

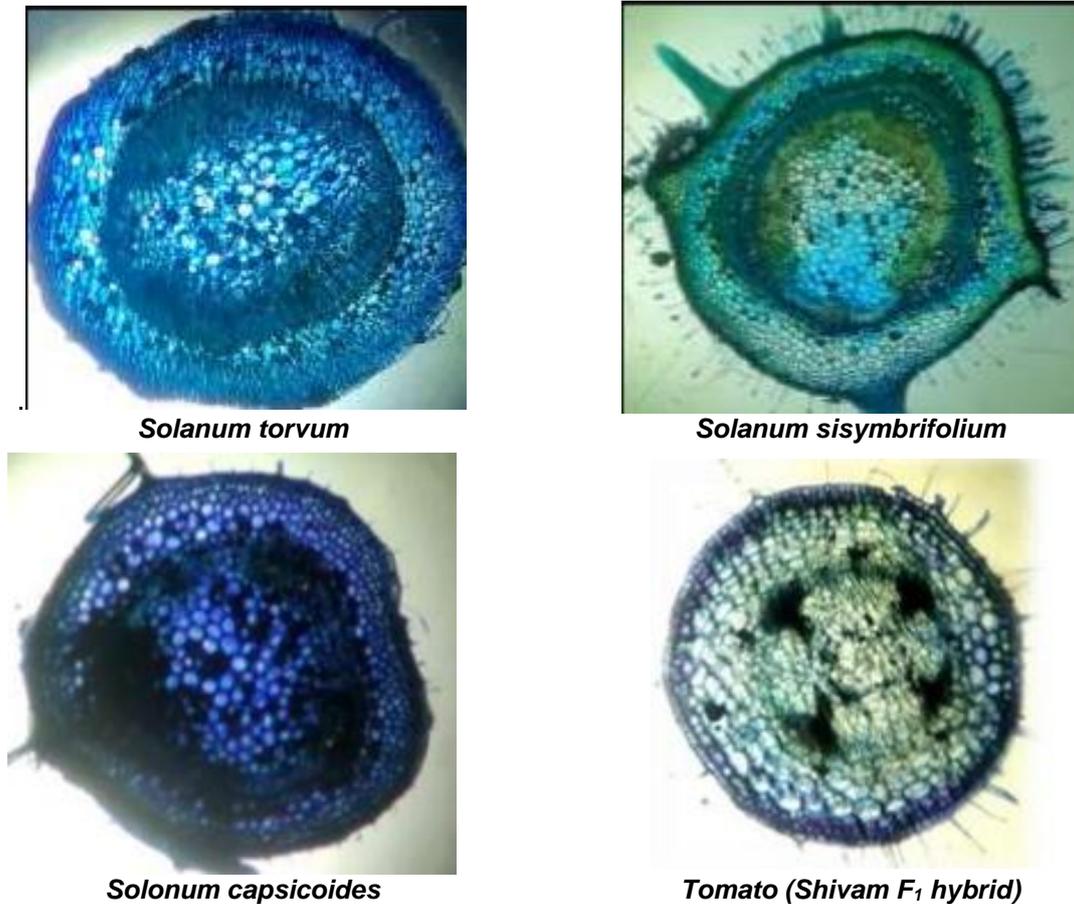
A successful grafting is ensured by the anatomical development between tissue of scion and rootstock after grafting (Fig. 1). The graft union section was viewed under microscope at 7, 14 and 21 days after grafting. The stages of growth were observed through histological studies.

The early stage of graft union (*i.e.* on 7<sup>th</sup> day), is the first step of wound repair process where necrotic/ proliferation layers were seen as dark strands in outer sides and interfaces of TNAU tomato hybrid CO3 grafted on to *S. torvum* rootstock (Fig. 2). and proliferation layer was partially formed in TNAU tomato hybrid CO3 grafted on to *S. sisymbriifolium* rootstock (Fig. 3). The phenolic compounds from the cut surface cells oxidize and produce necrotic layers isolating the surfaces. However, it was not formed in TNAU tomato hybrid CO3 grafted on to *S. capsicoides* rootstock (Fig. 4). Instead of differentiation layers, the gap between the stock and scion is seen in TNAU tomato hybrid CO3 grafted on to *S. capsicoides* rootstock, such that the conduction of water and food material was lost and it automatically led to incompatibility. During 14<sup>th</sup> day of grafting, a limited callus formation was seen in TNAU tomato hybrid CO 3 grafted on to *S. torvum* rootstock followed by TNAU tomato hybrid CO3 grafted on to *S. sisymbriifolium* rootstock. Callus started to spread into gaps between graft partners by fusing into the proliferation layer. Then, the gap between the rootstock and scion are filled and vessel connections between them are re-established. After this stage, the transport of water and nutrients through the grafting area is restored [4,8,9,10, 11].

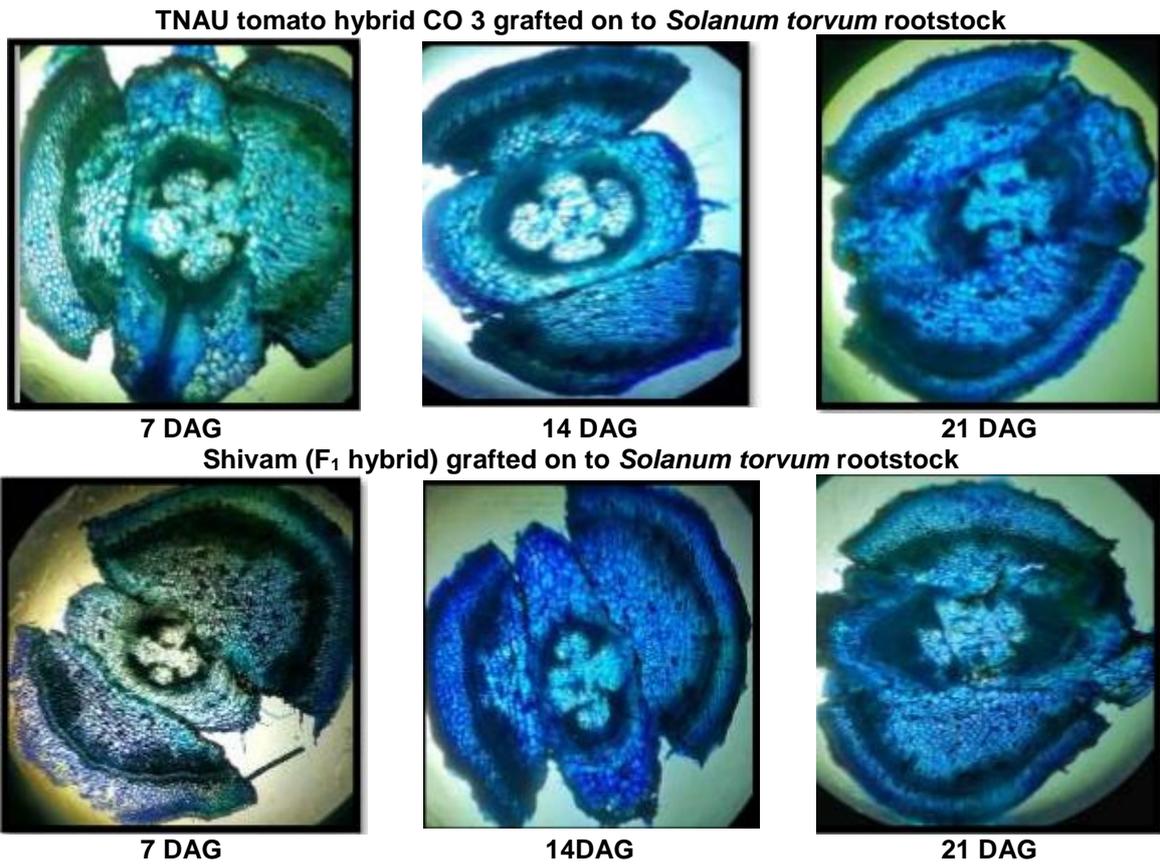
**Table 1. Growth and yield performances of grafted plants of various graft combinations of tomato**

Treatments	Number of days taken for graft union	Success % of grafted plants	Number of fruits per plant	Fruit yield per plant(kg)	Crop duration (days)
TNAU tomato hybrid CO3 grafted on to <i>Solanum torvum</i> rootstock	9.57	80.81	92.13	5.50	167.21
Shivam grafted on to <i>Solanum torvum</i> rootstock	10.03	85.77	98.26	5.54	165.65
TNAU tomato hybrid CO3 grafted on to <i>Solanum sisymbriifolium</i> rootstock	19.56	65.44	102.27	5.73	171.34
Shivam grafted on to <i>Solanum sisymbriifolium</i> rootstock	20.58	63.00	112.37	5.61	170.18
TNAU tomato hybrid CO3 grafted on to <i>Solanum capsicoides</i> rootstock	10.52	59.89	87.18	4.68	156.67
Shivam grafted on to <i>Solanum capsicoides</i> rootstock	11.12	61.73	85.34	4.52	159.99
SEd	0.42	1.95	2.54	0.14	4.41
CD (p= 0.05)	0.95	4.41	5.51	0.31	9.56

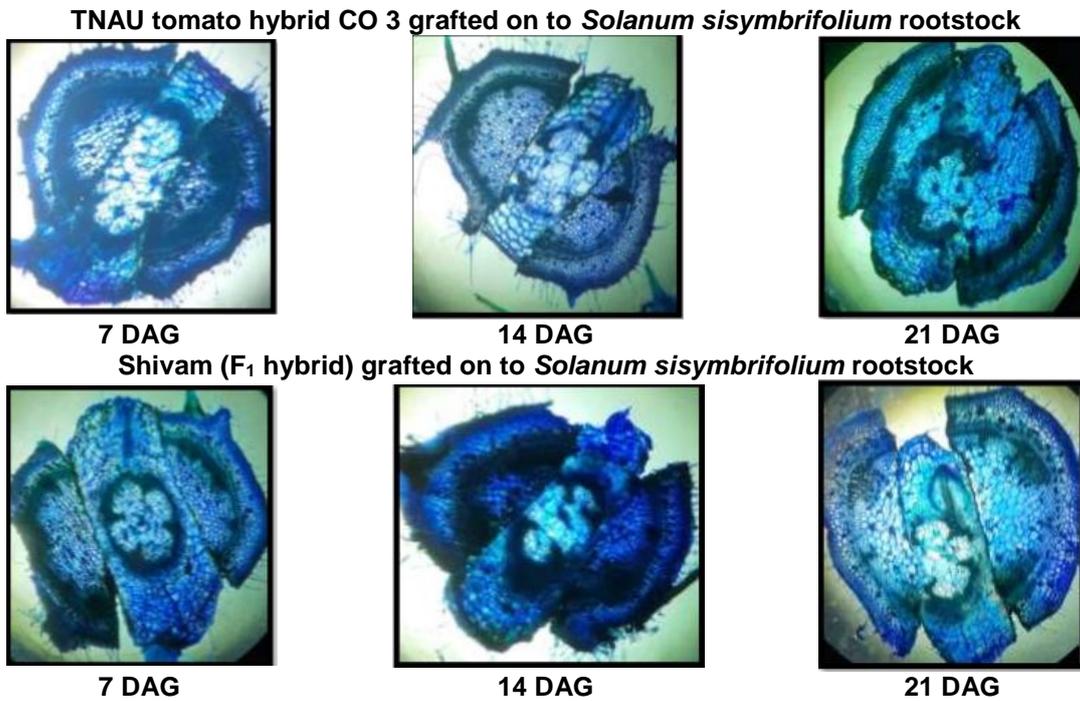
CD – Critical Difference; SEd – Standard Error of Difference



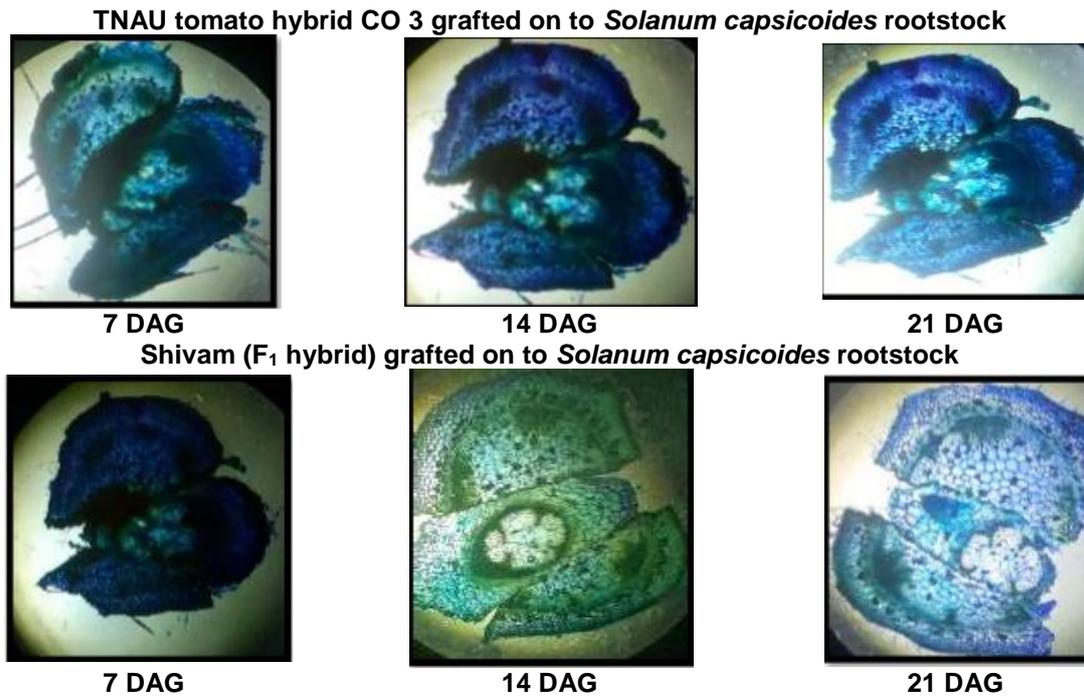
**Fig. 1. Anatomical section of rootstock and scion**



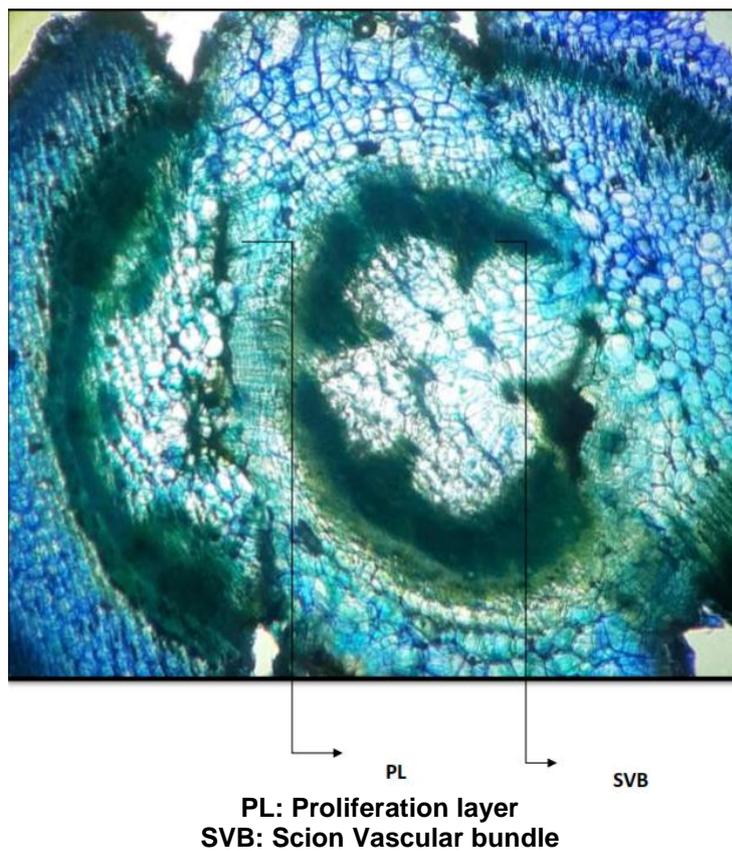
**Fig. 2. Histological view of TNAU tomato hybrid CO 3 and Shivam (F<sub>1</sub> hybrid) grafted on to *Solanum torvum* rootstock**



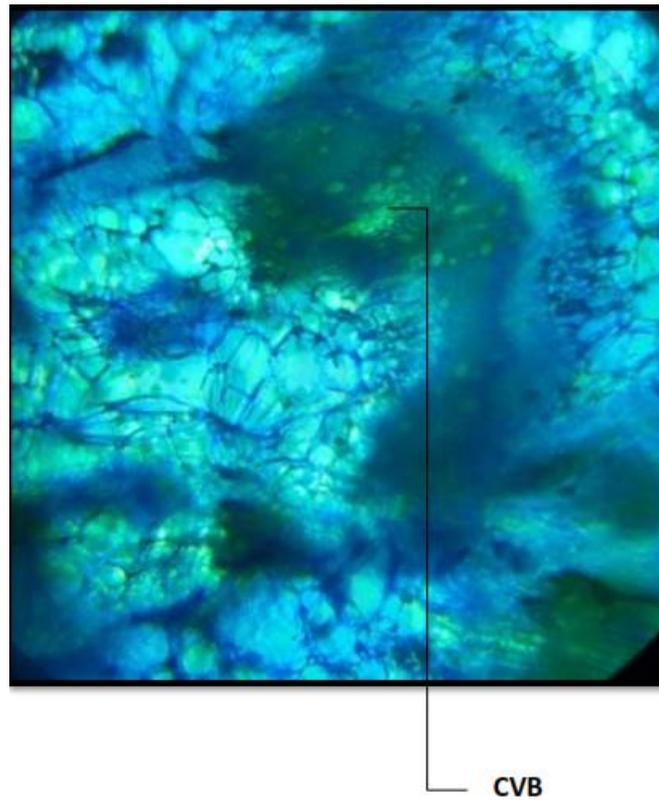
**Fig.3. Histological view of TNAU tomato hybrid CO 3 and Shivam (F<sub>1</sub> hybrid) grafted on to *Solanum sisymbirifolium* rootstock**



**Fig. 4. Histological view of TNAU tomato hybrid CO 3 and Shivam (F<sub>1</sub> hybrid) grafted on to *Solanum capsicaoides* rootstock**



**Fig. 5. Transverse section of TNAU tomato hybrid CO 3 grafted on to *Solanum torvum* rootstock at 21 DAG (40X view)**



**CVB: Complete vascular bundle**

**Fig. 6. Transverse section of Shivam grafted onto *Solanum torvum* rootstock at 21 DAG (40X view)**

At 21<sup>st</sup> day of grafting, complete differentiation of vascular bundles across the callus bridge could be seen in TNAU tomato hybrid CO3 grafted on to *S. torvum* rootstock (Fig. 5). This was followed by the graft union between TNAU tomato hybrid CO3 and *S. sisymbriifolium* rootstock (Fig. 5). At this stage, callus production was satisfactory and vascular bundle differentiation was noticed. Callus proliferation was observed between interspaces of stock and scion. Some of the newly formed cells which were transformed into cambial cells in the callus tissue were also visible. Compared to *S. sisymbriifolium* rootstock, TNAU tomato hybrid CO3 grafted on to *S. torvum* rootstock showed better differentiation of vascular bundles. However, scion growth dominated the rootstock growth in *Solanum torvum* rootstock leading to mismatch of scion-rootstock stem thickness which reveals the delayed incompatibility. Vascular tissue formation is considered the last stage of the successful grafting after the establishment of cambial continuity and a strong connection may occur in a short time in compatible grafts. This finding is in line with [4,11,12,13,14,15]. However, *S. capsicoides* rootstock took more time for graft

union. The hard and slow growing habit of the rootstock delayed the formation of callus and graft union establishment [16] leads to graft incompatibility. Poorly aligned graft components may result in complete failure of cambial union and failure to achieve vascular continuity in the union might result in desiccation of the scion and graft failure. The results are in line with [17,18]. Based on the above observations, it could be concluded that, among the three rootstocks used, *S. sisymbriifolium* rootstock was found to be a compatible one for tomato grafting.

#### 4. CONCLUSION

Successful grafting of two tomato hybrids with three wild *Solanum* rootstocks indicated that TNAU tomato hybrid CO3 and Shivam grafted on *Solanum sisymbriifolium* showed better performance followed by TNAU tomato hybrid CO3 and Shivam grafted on *Solanum torvum*. TNAU tomato hybrid CO3 and Shivam grafted on *Solanum torvum* took the least number of days for graft union (9.5 days). Shivam grafted on *Solanum torvum* rootstock showed the highest success percentage (85.77%) followed by TNAU

tomato hybrid CO3 grafted on *Solanum sisymbriifolium*. The number of fruits per plant was the highest in Shivam grafted on *Solanum sisymbriifolium* (112.37). Shivam and TNAU tomato hybrid CO3 grafted on *Solanum torvum* showed proliferation layers 14 days after grafting. Plants grafted on *Solanum sisymbriifolium* and *Solanum capsicoides* did not show vascular connection on 21st day of grafting. It took more time for graft union. Eventhough *Solanum sisymbriifolium* took more number of days for graft union compared to the other two rootstocks, the number of fruits per plant and fruit yield per plant was higher along with extended crop duration. Hence, it can be concluded that *Solanum sisymbriifolium* is the most compatible rootstock for tomato grafting.

## DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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