International Journal of Plant & Soil Science

33(23): 86-94, 2021; Article no.IJPSS.77131
ISSN: 2320-7035

Cytological Studies on Graft Union Development with Perennial Chilli Rootstocks

P. Keerthana [a#*], L. Pugalendhi [a†], R. Swarna Priya [b‡] and H. Usha Nandhini Devi [a¥]

[a] Department of Vegetable Science, HC & RI, Tamil Nadu Agricultural University, India.
[b] Horticultural College and Research Institute, Tamil Nadu Agricultural University, India.

Authors' contributions

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

Article Information

DOI: 10.9734/IJPSS/2021/v33i2330722
Editor(s):
(1) Hon H. Ho, State University of New York, USA.
Reviewers:
(1) Udit Kumar, Dr. Rajendra Prasad Central Agricultural University, India.
(2) Tatyana Boryanova Balabanova, University of Food Technology, Bulgaria.
Complete Peer review History: https://www.sdiarticle4.com/review-history/77131

Original Research Article

Received 29 August 2021
Accepted 09 November 2021
Published 12 November 2021

ABSTRACT

Grafting technology in vegetable crops is becoming increasingly popular as an alternative tool to improve the biotic and abiotic resistance besides improvement in horticultural traits. By utilizing the right combination of resistant rootstock and scion, desired variability can be achieved to improve the yield and quality of vegetables. A study was conducted at the College orchard, Department of Vegetable science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during the year 2020-2021 to evaluate the graft compatibility with two chilli rootstocks. The experimental material consisted of two perennial rootstocks *viz.,* CC-CBE-001 and CF-CBE-007 and three scion materials *viz.,* TNAU Chilli Hybrid CO 1, Ranga hybrid and Bangaram hybrid. Wedge grafting was done using 60 days old rootstock and 45 days old scion seedlings with nine treatments. The adhesion line wall thickness of pith cells were determined at different stages after grafting. The wound healing of the scion-rootstock union was studied using microscopic examination of the grafting region on the 5th, 10th, 15th and 20th day after grafting. It was observed that ten days after grafting, vascular bundle was formed and a large amount of callus was produced to bridge the scion and rootstock. Despite interspecific

[#]M. Sc, Scholar;
[†]Dean (Hort);
[‡] Professor and Head;
[¥]Assitant Professor (Hort);
**Corresponding author: E-mail: keerthipalanivel232@gmail.com;*

grafting, callus formation, subsequent cell differentiation and vascular connection were established, resulting in effective graft compatibility, according to the anatomical and histological analysis.

Keywords: Graft compatibility; callus development; vascular connection; perennial chilli rootstocks.

1. INTRODUCTION

Chilli (*Capsicum sp*) is one of the important vegetable crops widely cultivated around the world, used as spice, vegetable and as medicinal plant. Chilli belongs to the genus *Capsicum* and comes under the family Solanaceae. It is native to Mexico, assumed to have originated from South America and later introduced to Central America. In India, it is cultivated in an area of 7.33 lakh hectares, with a production of 17. 64 lakh tonnes and a productivity of 2400 kg/ ha. Grafting is frequently employed in chilli as it is one of the world's most commercially significant crops. The productivity advantages that grafted plants provide make the practice crucial to many producers.

Growers have been using plant's regeneration through seeds and also by grafting the crops with diverse types or even species since ancient times [1]. Grafting is a technique of combining two genotypes to generate a single plant by physically uniting the aerial component (scion) and the root bearing portion (rootstock) and allowing the graft union to heal. Grafting has recently become a popular alternative strategy for reducing the time taken in conventional breeding process. Using the appropriate resistant rootstock and scion, improvement in the yield and quality can be achieved. A better understanding of the structural and molecular mechanism underlying may aid in improving graft success and quality, thereby increasing the economic outcome of this technique.

The goal of this study was to document the sequence of cytological changes that occurs over time in the graft union of chilli functional homografts, as a model of good scion-rootstock compatibility.

2. MATERIALS AND METHODS

2.1 Raising of Rootstock and Scion

The experiment was conducted at the College orchard, Department of Vegetable science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during the year 2020-2021 to evaluate the graft compatibility of two chilli species. The experimental material consisted of two chilli rootstocks *viz.,* CC-CBE-001 and CF-CBE-007 and three scion materials *viz.,* TNAU Chilli Hybrid CO 1, Ranga hybrid and Bangaram hybrid. A total of nine treatments were used in the study as given in Table 1. The seedlings of rootstock and scion used for grafting were raised in protrays under the shade-net condition. The protrays were filled with well decomposed nutrient- rich cocopeat. Seeds were sown in protrays and irrigated at regular intervals. The rootstock seeds were seeded 15 days earlier than the scion seeds in order to achieve the requisite grafting stem size of rootstock and scion.

2.2 Grafting Method

Wedge grafting was done using 60 days old rootstock and scion seedlings which were 45 days old. Rootstock was decapitated at the top and a 1.5 cm incision cut was given in the centre to implant the scion. The top portion of the scion was removed and 1.5 cm wedge cut was made in the bottom portion, which was then inserted into the rootstock slit and secured it by a grafting clip. The grafted plants were kept in the mist chamber for 15 days, covered in 1000 gauge polythene sleeves, at a relative humidity of above 95 per cent, at a temperature of 25-30^{o} C and kept in darkness. After the sprout appeared on the seedlings, the polythene sleeves were removed and the seedlings were kept under the shade-net for hardening. The clips were removed once the graft union occurred [2-4]. Fig. 1 illustrates the procedure involved in the grafting technique.

2.3 Healing Condition

Before being moved to the shade net, grafted plants were maintained under the mist chamber for 7 to 14 days with the air temperature ranging from 25 to 28^{o} C to aid the healing process. Throughout the process, the grafting clips were left on the plant. The stock growths were periodically removed till the end of the graft union.

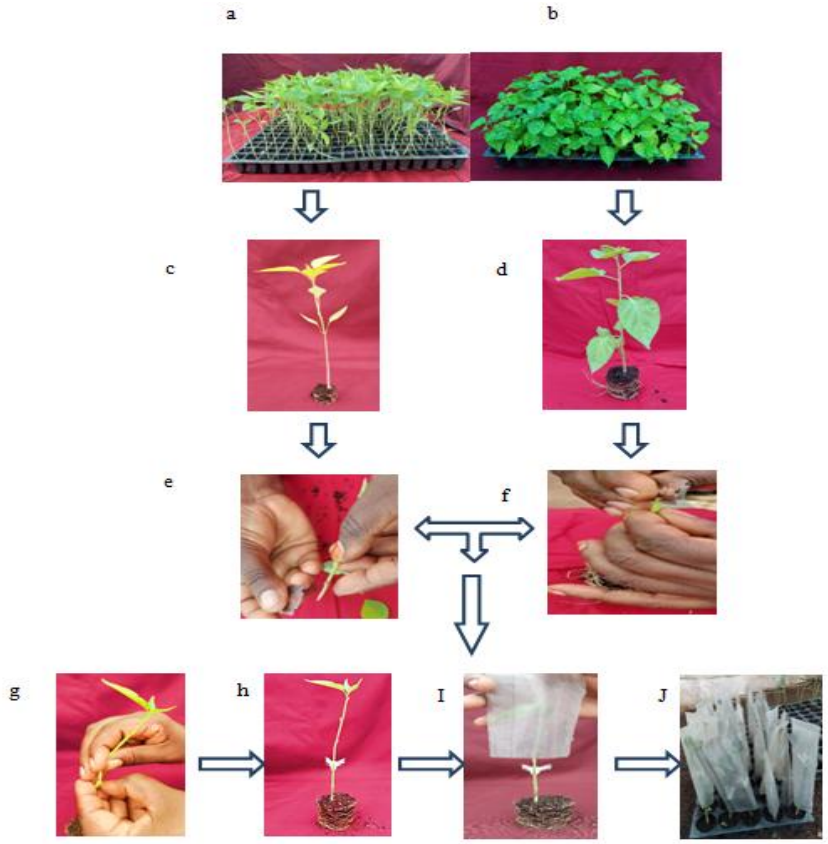

Fig. 1. Steps in grafting

a – Scion in protay

b – Rootstock in protray

c – Scion

d – Rootstock

e – y shape cut in scion

f - Wedge cut in rootstock

g – Insertion of scion into rootstock

h - Graft union with clip

i– Covering with polythene cover

j - Grafted plants under poly tunnel

Table 1. Details of treatments and graft combinations

Treatment	Graft Combination
T_1	TNAU Chilli Hybrid CO 1 grafted on to CC-CBE-001
T_2	Ranga hybrid grafted on to CC-CBE-001
T_3	Bangaram hybrid grafted on to CC-CBE-001
T_4	TNAU Chilli Hybrid CO 1 grafted on to CF-CBE-007
T_5	Ranga hybrid grafted on to CF-CBE-007
T_6	Bangaram hybrid grafted on to CF-CBE-007
T_7	TNAU Chilli Hybrid CO 1
T_8	Ranga hybrid
T_9	Bangaram hybrid

2.4 Cell Wall Thickness

The adhesion line wall thickness of pith cells was determined at various stages after grafting. ImageJ 1.52a software was used to measure the thickness of the cell wall.

2.5 Callus Thickness

At 5th, 10th, 15th and 20th day after grafting, graft union was observed under the stereoscopic microscope and photographed using a Nikon D4 full-frame camera. Using ImageJ 1.52a software the callus thickness was measured.

2.6 Histological Techniques

A histological examination of plant tissue during the development of the graft union between the rootstock and scion was done using paraffin sectioning method. After removing the grafted plant from the protray, a one-centimeter long sample was taken from the fresh rootstock and scion and sections were cut longitudinally and transversely at the grafting junction for all graft combinations using a disinfected razor blade to accurately detect tissue affinity over time. The wound healing of the scion-rootstock union was studied using microscopic examination on the 5th, 10th, 15th and 20th day after grafting.

At all stages, paraffin sectioning technique was employed to observe the structural changes in the grafting process at the graft interface using stereoscopic microscope. Observations on the number of days taken for germination, germination per cent and days taken to attain the graftable size were recorded.

2.7 Statistical Analysis

Student't' test and ANOVA variance analysis were used to examine the differences in cell wall and callus thickness. The analysis was carried out using the statistical software SPSS v.25; statistical significance was defined as p-value less than 0.05.

3. RESULTS AND DISCUSSION

Grafting is a process that joins the rootstock and scion by its biochemical and structural activity. Aloni *et al.* [5] validated the identification of a suitable rootstock that promotes the quick union *via* callus formation and subsequent cell differentiation, followed by vascular connection between the rootstock and scion, as well as the rapid root and shoot growth.

The performance of the different rootstock and scion on the number of days taken for germination, germination per cent and days taken to attain the graftable size is presented in Table.2. The results showed that among the different treatments, Ranga hybrid took the minimum number of days to germinate (8.53) followed by Bangaram hybrid with 10.53 days. However, the germination per cent was higher in TNAU Hybrid CO 1 (75.32) followed by Bangarm hybrid with 71.33 per cent. Similarly, TNAU Hybrid CO 1 took lesser number of days to attain the graftable size (45.98) followed by Bangaram hybrid with 49.65 days.

The days taken for graft union and graft success per cent is given in Table.3. The results revealed that T_1 (TNAU Chilli Hybrid CO 1 grafted on to CC-CBE-001) showed faster union with 7.5 days followed by T_2 (Ranga hybrid grafted on to CC-CBE-001) with 8.3 days. Similarly the graft success per cent was higher in T_1 (TNAU Chilli Hybrid CO 1 grafted on to CC-CBE-001) with (80.45, 75.72 and 70.32 %) at 15, 30 and 45 DAG followed by T_2 (Ranga hybrid grafted on to CC-CBE-001) with 75.61, 69.34 and 65.63 respectively. In a study conducted by Pugalendhi *et al,* [3-4] graft combinations with three wild Solanum species and two tomato scions showed that the graft combination of TNAU Tomato Hybrid CO3 with *S. torvum* rootstock had the faster graft union in 9.57 days followed by Shivam with *S. torvum* (10.03 days) indicating that success per cent was influenced by both rootstock and scion at different intervals of grafting. *S. torvum* rootstock resulted in higher success per cent of 80.81 and 85.77 with the scion of TNAU tomato hybrid CO3 and Shivam respectively in tomato.

The distribution of tissues in the scion and rootstock, particularly the vascular meristematic tissue, is critical for the proper establishment of the graft [6]. Schweingruber *et al.* [7] reported that the features of chilli stems have the characteristic structure of dicotyledons, which starts from the outer cuticle, the epidermis, the central cylinder and the medulla. During the early phase the vascular cylinder is discontinuous which subsequently becomes continuous with the interfasicular region (Fig.3).

A variety of complicated metabolic and structural mechanisms are required for the effective

grafting which results in the formation of a connection between rootstock and the scion. The development of vascular component and their differentiation into xylem and phloem is the first stage in union formation, followed by the adhesion of parenchyma (Fig.2). Because of the wound delivered to the stock and scion during the grafting that interrupts the plants vascular system, the establishment of a vascular link between the stock and scion during wound healing is crucial for the growth of new cambial bridges [8-9]. A similar finding was reported by Ilakiya *et al.* [10] in grafting mechanism in vegetable crops.

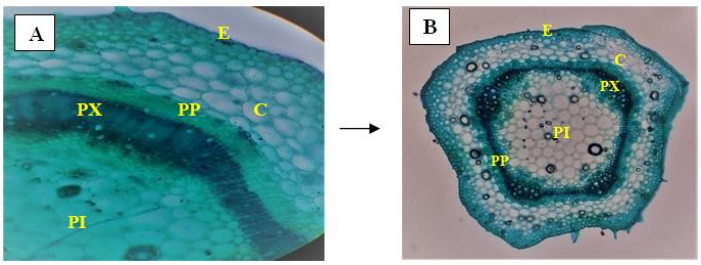

Fig. 2. Cross section of rootstock

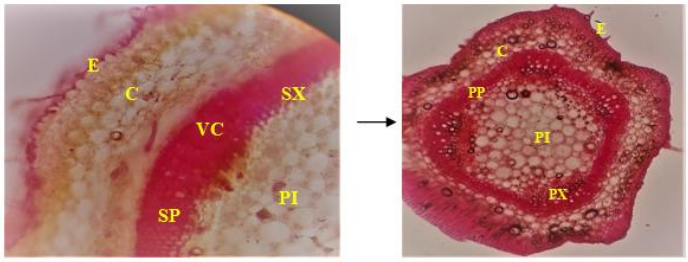

Fig. 3. Secondary growth of chilli rootstock sections under stereomicroscope

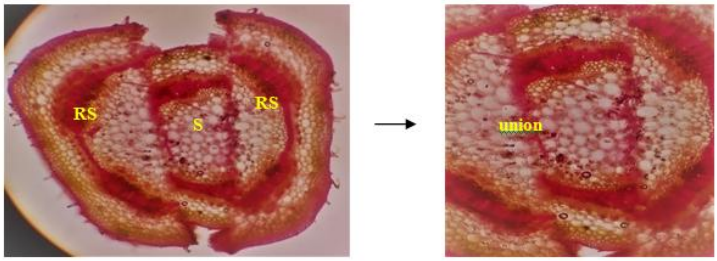

Fig. 4. Graft union formation at 5 DAG

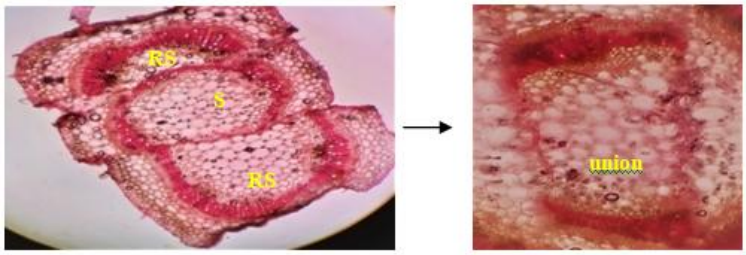

Fig. 5. Graft union formation at 10 DAG

Table 2. Growth parameters of rootstock and scion

Treatments	Days to Germination	Germination percentage		Number of days taken to attain graftable size
		15 DAS	30 DAS	
CC-CBE-001	15.53	48.90	65.45	60.34
CC-CBE-007	20.63	37.78	59.90	65.76
TNAU Hybrid CO 1	10.60	65.45	75.32	45.98
Ranga Hybrid	8.53	58.80	70.51	50.87
Bangaram Hybrid	10.53	63.45	71.33	49.65

Table 3. Number of days taken to attain graft union and graft success percentage

Treatment	Days taken for graft union	Success grafting %		
		15 DAG	30 DAG	45 DAG
T_1	7.5	80.45	75.72	70.32
T_2	8.3	75.61	69.34	65.63
T_3	10.6	66.74	57.65	50.50
T_4	11.9	61.74	50.54	44.67
T_5	8.8	70.83	65.55	58.98
T_6	9.7	65.78	53.64	48.19
S.E (d)	0.26	1.94	1.74	1.60
C.D	0.56	4.28	3.84	3.53

3.1 Callus Proliferation and Interdigitation

Undifferentiated parenchymatous cell adhesion is the first critical stage in the development of graft union. Parenchymal cells split and proliferated in the combinations at the graft interface on the 5^{th}, 10^{th}, 15^{th} and 20^{th} day after grafting and the cells could be detected at the various stages of development. In certain areas of the sliced surface, proliferating callus cells followed a unique pattern of anticlinal and periclinal cell division. Small callus cells were packed firmly with little internal space, whereas large callus cells were loosely linked. However, in other zones, the newly formed walls did not have a preferred direction, resulting in unstructured tissue development. In all combinations, there was substantial cellular activity at the graft interface 10 days after grafting. Smaller cells were densely packed together in places surrounded by large cells. The isolation layer disappeared during the callus development and interdigitation.

The cross section of grafted rootstock and scion at 5 DAG was characterized by a necrotic layer that formed between the grafting region, forming regular condense uniform callus that fills the space between the scion and rootstock and a good connection was cleared without the formation of new vessels (Fig.8). At 20 DAG, callus cells were initiated by xylem and phloem ray parenchyma cells of both the scion and the rootstock and new vessels were initiated within the scion's callus. This indicated a good vascular connection between the rootstock and the scion clearly showed a perfect compatibility (Fig.9). Similar results were reported by Pugalendhi *et al*. [2-4] in tomato with interspecific Solanaceous rootstock. The other histological characteristics of incompatible grafts include the development of collapsed cells, a necrotic layer of dead cells, many free callus sites and separation zones along the grafting edges. These poor structures cause the graft rootstock and scion to separate. No such necrotic layer was observed in all the graft combination suggesting a desired compatibility between stock and scion.

3.2 Vascular Cambium Formation

The plasmodesmata was formed 5 DAG (Fig.4) and a clear graft union was observed 10 DAG (Fig.5), this was followed by the development of structural bridges after 8-10 days (Fig.5). As early as 10 DAG, the development of callus tissues on freshly exposed surface was seen as a first reaction of the stem injury. At 15 DAG, a partial graft union was seen between rootstock and scion (Fig.6). Some filamentous structure between the rootstock and scion was detected under the stereomicroscope during the early phase of the grafting (10 DAG), which produced the first contact between the two tissues, but new xylem and phloem components had not yet developed between the rootstock and scion (Fig.5).

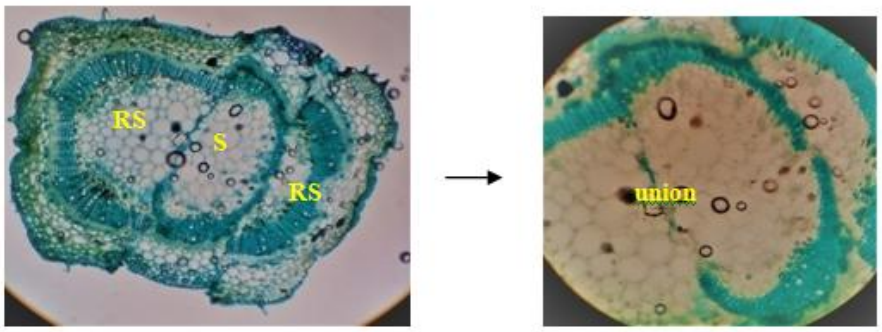

Fig. 6. Graft union formation at 15 DAG

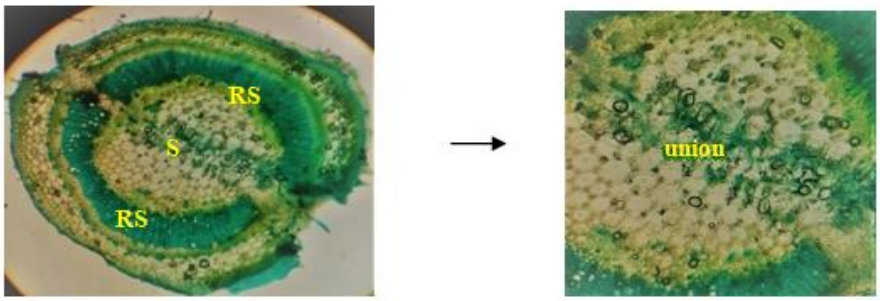

Fig. 7. Graft union formation 20 DAG

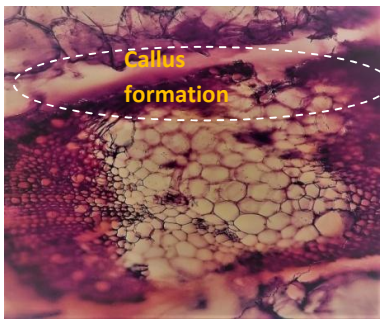

Fig. 8. Callus formation at 5 DAG

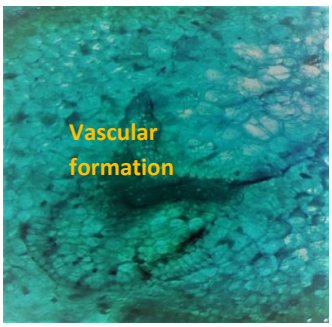

Fig. 9. Callus formation at 20 DAG

Cell division and differentiation resumed at 10th DAG. Cell activity was shown to be unique as the graft developed and a vascular link was formed. At 15 DAG isolated filamentous structures were apparent at the graft interface and were connected between the rootstock and scion (Fig.6). It also showed that the rootstock and scions linked filamentous tissues. The presence of lipid and waxy components on both surfaces and the structural characteristic of filamentous linkages were found 20th DAG (Fig.7). A similar finding was reported in tomato grafted on to *S. sisymbrifolium* rootstock by Pugalendhi *et al.* [2-4].

A complete graft connection appeared at 45 DAG and the vascular connection, on the other hand was better defined and the tissues were more organized indicating the perfect graft compatibility. Tamilselvi and Pugalendhi. [11] reported that vascular regeneration at the graft interface was found in Palee F1 scion grafted on to pumpkin (*C. moschata*) rootstock at four distinct phases *viz.,* 7th, 15th, 21st and 30th day. Incompatibility of graft union was detected in CO 1 bottle gourd scion grafted on to *C. ficifolia* rootstock, which might be attributed to the existence of a necrotic layer at the graft interface.

In the present study, it was observed that there was no distinct necrotic layer between rootstock and scion though they belong to different species suggesting a clear compatibility in the early phase of graft union. Pina *et al.* [12] found that callus proliferation begins one week after grafting in both compatible and incompatible combinations, with the first two weeks important for graft union formation in *Prunus spp.* Before the establishment of plasmodesmata inside callus tissue, an undifferentiated amorphous mass composed of thin-walled parenchyma cells normally arises to ease the symplasmic migration during the grafting process and subsequent callus growth. According to the microscopic image, callus tissues were created at 10 DAG (Fig.5) and more pronounced at 15 DAG (Fig.6).

4. CONCLUSION

The graft union of TNAU Chilli Hybrid CO 1 on to CC-CBE-001 was successful compared to the other combinations used in the study. Though the rootstock and scion belong to different species, callus development and subsequent cell differentiation and vascular connection were established resulting in successful graft union. Because of the vigorous nature of the rootstock, the graft union was faster followed by higher nutrient water translocation triggering faster growth of scion. Chilli graft establishment is indicated by thicker cell walls at the graft connection in the early stages. Some regions of the scion and rootstock's xylem and phloem are linked and start functioning 10 DAG. Even after the graft union is formed, remnants of the connecting process can be seen. The microscopic approach is one of the best ways to investigate the early stages of the grafting process and structural changes at the graft interface because the formation of fine structures between the scion and rootstock aids in understanding the graft union process. It can be concluded that at 10 DAG, the vascular bundle bridge was established, and a considerable amount of callus tissue was created to connect the scion and rootstock, ensuring plant survival.

DISCLAIMER

The products used for this research are commonly and predominantly use 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

A. Primary structure and B. Cross section structure. E-Epidermis; C-Cortex; PP-Primary phloem; PX-Primary xylem; Pi-Pith.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mudge K, Janick J, Scofield S, Goldschmidt EE. A history of grafting. Hortic. Rev. 2009;35:437–493. DOI: 10.1002/9780470593776.ch9.
2. Pugalendhi L, Usha Nandhini Devi H. Grafting technology for biotic and abiotic stress mitigation in vegetables. In: Compendium of lead and research papers of International Horticulture conference Next Generation Horticulture (NextGen-Hort); 2021. ISBN:978-93-849093-8-3.p37-43.
3. Pugalendhi L, Bharathi S, Swarna Priya R, Velmurugan M. Biochemical and quality attributes of grafted tomato (*Solanum lycopesicum* L.) the Pharma innovation. 2021;10(8):333-338.
4. Pugalendhi L, Bharathi S, Tamil Selvi NA, Usha Nandhini Devi H. Anatomical and histological studies of grafted tomato with interspecific solanaceous rootstocks. International Journal of plant and soil science. 2021;33(20):132-140.
5. Aloni B, Karni L, Deventurero G, Levin Z, Cohen R, Katzir N, Lotan-Pompan M, Edelstein M, et al. Physiological and biochemical changes at the rootstock-scion interface in graft combinations between Cucurbita rootstocks and a melon scion[J]. J. Horti.Sci. Bio. 2008;83:777-783.
6. Hartmann HT, Kester DE, Davies FT, Geneve RL. Plant propagation. Principles and practices. 7th ed. Upper Saddle River, NJ: Prentice Hall; 2002. ISBN 0-13-679235-9:849
7. Schweingruber FH, Börner A. The Plant Stem: A Microscopic Aspect; Springer: New York, NY, USA; 2018.
8. Asahina M, Satoh S. Molecular and physiological mechanisms regulating tissue reunion in incised plant tissues. J. Plant Res. 2015;128:381–388.

DOI:10.1007/s10265-015-0705-z
9. Fernandez-Garcia N, Carvajal M, Olmos E. Graft union formation in tomato plants: peroxidase and catalase involvement[J]. Ann. Bot. 2004;93:53-60.
10. Ilakiya T, Parameswari E, Davamani V, Yazhini G, Singh S. Grafting Mechanism in Vegetable Crops. Res J. Chem. Env. Sci. 2021;9(4):01-09.
11. Tamilselvi N, Pugalendhi L. Graft compatibility and anatomical studies of Bitter Gourd (*Momordica charantia L.*) scions with cucurbitaceous rootstocks. International Journal of Current Microbiology and Applied Sciences. 2017;6(2):1801-1810.
12. Pina A, Errea P, Martens HJ. Graft union formation and cell-to-cell communication via plasmodesmata in compatible and incompatible stem unions of Prunus spp[J]. Sci. Horti. 2012;143:144-150.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/77131