Journal of Ornamental Plants
www.jornamental.iaurasht.ac.ir
ISSN (Print): 2251-6433 ISSN (Online): 2251-6441

Research Paper
DOR: https://dorl.net/dor/20.1001.1.22516433.2020.11.1.4.7

Development of Graft Union Formation and Histological Observations in Cactus Influenced by Benzyladenine, Grafting Method and Rootstock

Najme Kamalzade[1], Seied Mehdi Miri[1*] and Noushin Ghazijahani[2]

[1] Department of Horticulture, Karaj Branch, Islamic Azad University, Karaj, Iran
[2] Department of Microbiology, Karaj Branch, Islamic Azad University, Karaj, Iran

Received: 31 August 2020 Accepted: 09 November 2020
*Corresponding author's email: smmiri@kiau.ac.ir

Abstract

Attractive and colorful cultivars of cactus have been developed that are mainly propagated by grafting. The aim of this study was to determine histological events during graft union formation in *Gymnocalycium* cactus influenced by BA (Benzyladenine) application, grafting method and rootstock. The basal end of the scions and the top end of the rootstocks were first dipped into BA solution at concentrations of 0, 100 and 500 mg L^{-1} for 10 s, before being grafted on *Lemaireocereus marginatus* and *Myrtillocactus geometrizans* rootstocks using horizontal or wedge techniques. Longitudinal sections of the graft union were taken weekly to observe the developmental process of graft formation. The rootstocks did not have a significant effect on grafting success and scion growth, while horizontally grafted *Gymnocalycium* showed better grafting success rate. The grafting rate and scion growth were enhanced by application of BA compared with the control. The best results were obtained from treating the graft cut-surfaces of both scions and rootstocks with 100 mg L^{-1} BA. Histological observations showed that the graft union process involved development of a thin necrotic layer, callus formation at the graft interface, cambium differentiation and initiation of new vascular elements, developing the new vascular tissue, and connection of scion and rootstock vascular bundles. BA stimulated rapid proliferation of callus between the scion and rootstock as well as differentiation of vascular elements. We suggest the horizontal graft and application of BA (100 mg L^{-1}) on the cut surfaces of both scion and rootstock as an easy and reliable method for successful graft union in *Gymnocalycium* cactus, which may have potential use in the commercial production of other cactus species as well.

Keywords: Aesthetic, Foliage plants, Low-maintenance, Shade.

INTRODUCTION

Cacti are one of the most diverse and interesting plants in the plant kingdom containing approximately 145 genera and 4300 species (Erwin, 2009; Hunt 2016). They represent a group of plants with potential as ornamental potted, bedding and/or landscape plants. Aside from their ornamental potential, cacti are often drought, heat and/or cold tolerant, and can have minimal nutritional requirements (Erwin, 2009). The genus *Gymnocalycium* has been popular with cactus fanciers for many years and is one of the most well-known and popular species. The chlorophyll-less and variegated forms of this species are very popular. They vary in color from the most commonly seen, bright red to yellow, orange and various combinations of colors where the chlorophyll is partly present (Pilbeam, 1995).

Cacti species may be propagated as seeds, cuttings or by grafting (Pérez-Molphe-Balch *et al*., 2015). Although, the most frequently method of propagating cacti is cuttings, which is easy to perform (Cabahug *et al*., 2018), the main goals in grafting cacti are to produce a larger number of robust offsets in a short time, and to propagate rare or difficult plants much faster than would ordinarily be possible (Bach, 1981). It is not usually necessary to graft *Gymnocalycium*, except for chlorophyll-less, or strongly variegated plants (Pilbeam, 1995).

The histology of developmental process in successful grafting of herbaceous plants roughly includes: (i) The initial adhesion of scion and rootstock, (ii) The formation of the wound callus in both rootstock and scion, and (iii) Differentiation of the vascular tissue to form a functional unit across the scion and rootstock (Wang, 2011). Differentiation is not usually considered to be a problem, but there are often difficulties at initiation and proliferation of callus (Panea *et al*., 1998). Callus formation is important to promote grafting for many plants (Melnyk, 2017). A number of factors such as compatibility of scion and rootstock, plant species, type of graft, environmental conditions, and plant growth regulators can influence graft union success (Hartmann *et al*., 2020).

Grafted cacti are regarded as one of the most admired ornamental indoor plants worldwide, and the demand for them has been increasing sharply as a result of the new cultivars develop (Jeong *et al*., 2004). Therefore, it is necessary to evaluate the factors affecting the improvement of graft success in cacti. The purpose of this study was to study the effects of benzyladenine, grafting method and rootstock on the success of *Gymnocalycium* grafting.

MATERIALS AND METHODS

Gymnocalycium mihanovichii was used as the scion and grafted on the *Lemaireocereus marginatus* (DC.) A. Berger (Syn. *Pachycereus marginatus* (DC.) Britton & Rose) and *Myrtillocactus geometrizans* rootstocks (Fig. 1). The cut surfaces of the scion and rootstock were first dipped into 0 (distilled water), 100 and 500 mg L^{-1} BA for 10 s. The plant materials were grafted using horizontal or wedge techniques, and two rubber bands were then applied to hold rootstock and scion firmly together (Fig. 2). Grafted cacti were placed at 27 ˚C temperature and 80-85 % relative humidity in the greenhouse.

Seventy days after grafting, they were evaluated for the rates of success (%) [grafting survival (%)] as well as the length, diameter, fresh and dry weight of scions. The sample graft combinations were harvested weekly to study the sequence of anatomical events during the graft union formation. Thin transverse sections were cut manually to cross longitudinally through the rootstock and scion. The prepared sections were first soaked in 20 % household bleach for 10 min, washed three times with distilled water and submerged in 10 % acetic acid. The slices were then put in methyl-green for 1 min, washed with distilled water, stained in carmalum solution for 15 min and finally washed with distilled water. The photomicrographs were obtained with the aid of a light microscope with 10 X magnification.

The factorial experiment was conducted in a complete randomized block design with three factors (two rootstocks × three BA concentrations × two grafting techniques) and four replications with 10 grafts per replicate. All data were evaluated by analysis of variance and means were separated by Duncan's Multiple Range Test at P<0.05 and 0.01 using SAS statistical software.

Fig. 1. Plant materials used in the study. 1) *Gymnocalycium mihanovichii* 2) *Lemaireocereus marginatus* 3) *Myrtillocactus geometrizans.*

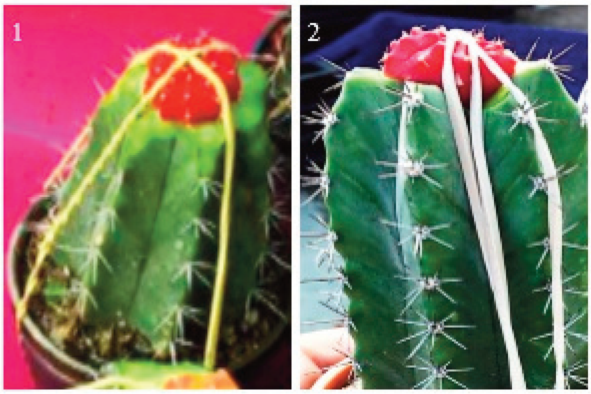

Fig. 2. *G. mihanovichii* grafted on *L. marginatus* using horizontal (1) and wedge (2) techniques.

RESULTS

Based on analysis of variance (Table 1), BA had a significant effect on all traits ($P < 0.01$) except scion diameter, while the type of graft had a significant effect only on the grafting success ($P < 0.05$), and the rootstock did not have a significant effect on any of the traits. The results of interactions also showed that only the triple effect of graft type, BA and rootstock on fresh weight of scion was significant ($P<0.05$). The coefficients of variation (CV) in all traits was less than 15 %, which indicates a high reliability estimate of the experiment (Couto *et al*., 2013).

The success of grafting of *Gymnocalycium* varies according to the type of graft methods. The horizontal graft method was significantly more successful (78.3 % survival rate) than the wedge grafting method (69.4 % survival).

BA promoted both grafting success rate and scion development compared with the control, and 100 mg L^{-1} BA yielded the best results (Table 2).

Table 1. ANOVA of the effects of grafting type, BA and rootstock on grafting success and scion growth of *G. mihanovichii*.

S.o.V	df	MS				
		Grafting success	Scion length	Scion diameter	Scion fresh weight	Scion dry weight
Block	4	10.13^{ns}	0.009^{ns}	0.079^{ns}	0.095^{ns}	0.036^{**}
Grafting type (G)	1	497.36^{*}	0.263^{ns}	0.310^{ns}	0.156^{ns}	0.001^{ns}
BA (B)	2	2072.61^{**}	1.595^{**}	0.781^{ns}	4.466^{**}	0.023^{**}
Rootstock (R)	1	16.27^{ns}	0.185^{ns}	0.012^{ns}	0.093^{ns}	0.001^{ns}
G×B	2	167.78^{ns}	0.029^{ns}	0.132^{ns}	0.006^{ns}	0.0001^{ns}
G×R	1	41.42^{ns}	0.110^{ns}	0.099^{ns}	0.027^{ns}	0.0002^{ns}
B×R	2	88.88^{ns}	0.029^{ns}	0.113^{ns}	0.146^{ns}	0.0001^{ns}
G×B×R	2	74.98^{ns}	0.010^{ns}	0.073^{ns}	0.549^{*}	0.001^{ns}
Error	33	82.89	0.053	0.113	0.110	0.0006
CV (%)		12.52	13.61	13.53	11.81	14.21

^{ns}, *, **: Non-significant and significant at $P < 0.05$ and $P < 0.01$, respectively.

Table 2. Effect of BA on grafting success (%) and scion growth 70 days after grafting.

BA (mg L^{-1})	Grafting success (%)	Scion length (mm)	Scion dry weight (g)
0	60.9 c	3.9 c	0.15 c
100	83.6 a	4.6 a	0.23 a
500	72.8 b	4.3 b	0.20 b

Means in each column followed by different letters are significantly different ($P < 0.01$) as indicated by Duncan's Multiple Range Test.

The highest scion fresh weight was obtained with *Gymnocalycium* treated with 100 mg L^{-1} BA and grafted by horizontal graft on *L. marginatus* or wedge graft on *M. geometrizans* rootstocks (Fig. 3).

The histology of the graft union formation was similar in all treatments, except that the process of callus formation and differentiation of vascular tissue in the BA-treated graft combinations occurred earlier. The observed weekly developmental steps of graft-healing anatomy are as follows:

Beginning of 1st week: There are gaps between rootstock and scion immediately after the grafting (Figs. 4-1 & 2).

1st week: Thin necrotic layers were clearly visible as dark lines of dead cells on the graft junctions caused by the mechanical damage during surface cutting (Figs. 4-3 & 4). The parenchyma cells near the wound dedifferentiated and recovered the ability to divide leading to callus formation. Low callus formation was observed in BA-untreated combinations (Figs. 4-3), but sufficient callus mass originated primarily from cortex areas of rootstocks and spread into gaps between rootstock and scion in BA-treated combinations (Figs. 4-4).

2nd: Callus continued to proliferate and almost fill the grafting area, creating a callus bridge between the rootstock and scion (Figs. 4-5 and 6). Few grafts failed due to low callus formation on the graft union.

3th week: In BA-treated combinations, the procambial cells differentiated at the cut ends of the rootstock cambium (Figs. 4-7 and 8).

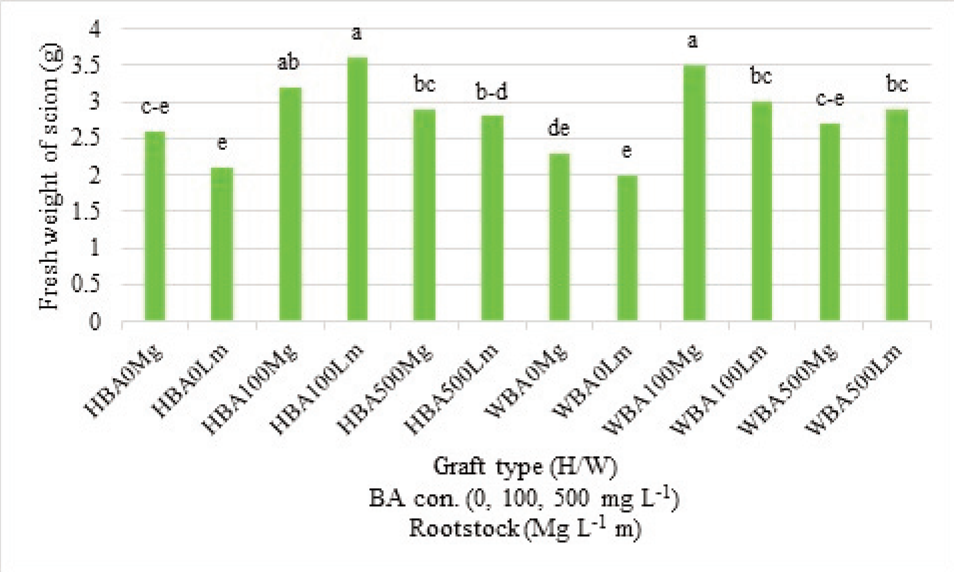

Fig. 3. Effect of grafting type (horizontal/wedge grafts), BA concentration and rootstock (*M. geometrizans*/ *L. marginatus*) on fresh weight of scion 70 days after grafting. Means followed by different letters are significantly different (P< 0.05) as indicated by Duncan's Multiple Range Test.

4th week: Newly formed vascular bundles initiated in the callus tissue between scion and rootstock (Figs. 4-9 and 10), however a higher rate of vascular formation was observed in BA-treated combinations (Figs. 4-9).

5th week: Vascular bundles developed and connected with xylem, of both rootstock and scion. In this stage, the necrotic layers also gradually disappeared (Figs. 4-11 and 12).

6th week: Vascular connection between BA-treated rootstocks and scions was completed (Figs. 4-13 and 14).

7th week: Formation of vascular bridge between scion and rootstock was apparent in all combinations (Figs. 4-15 and 16).

8th-10th week: Successful completion of graft unions was performed in all grafts (Figs. 4-17 to 22). No histological development related to incompatibility was observed.

Fig. 4. Histological observations of the longitudinal sections of the grafting area of *G. mihanovichii*/ *M. geometrizan*. Pictures of BA-untreated grafts are displayed on the left and BA-treated on the right. The weeks after grafting are mentioned on the bottom of the pictures. 1-2) Gaps between rootstock and scion immediately after the grafting. 3-4) Development of thin necrotic layers and callus formation. 5-6) Callus filled the grafting area. 7-8) An early cambium differentiation in BA-treated callus tissue. 9-10) Initiation of vascular bundles. 11-12) Development of vascular bundles and connection to vascular of both rootstock and scion, disappearance of necrotic layers. 13-14) Completion of vascular connection in BA-treated grafts and to some extent in BA-untreated grafts. 15-16) Establishment of vascular bridge between scion and rootstock in all combinations. 17-22) Successful completion of graft unions.

DISCUSSION

Since the colored cacti do not have chlorophyll for photosynthesis, they should be grafted on photosynthesizing rootstocks for normal growth (Lee and Oda, 2003).

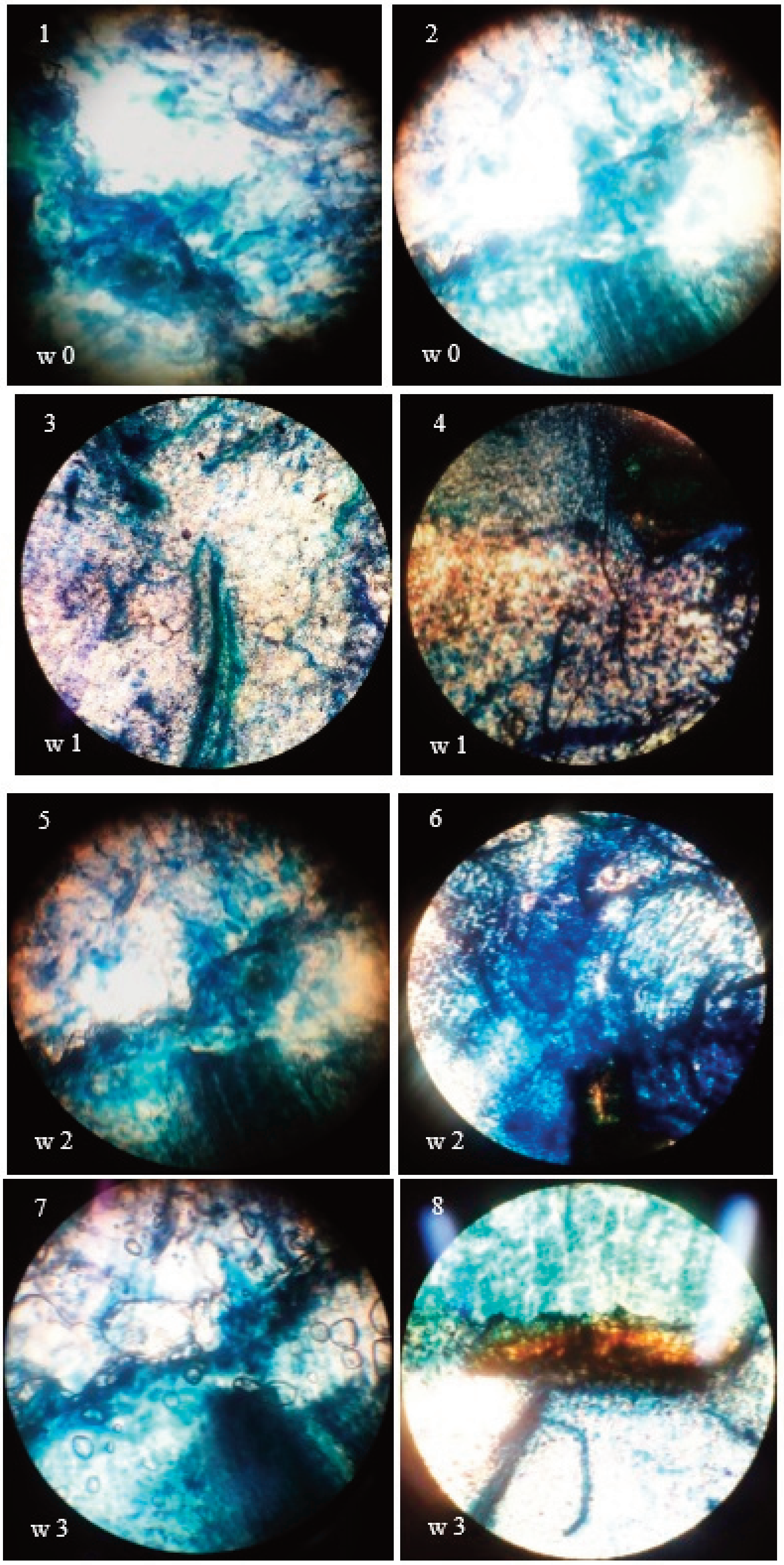

Fig. 4. Histological observations of the longitudinal sections of the grafting area of *G. mihanovichii*/ *M. geometrizan*. Pictures of BA-untreated grafts are displayed on the left and BA-treated on the right. The weeks after grafting are mentioned on the bottom of the pictures. 1-2) Gaps between rootstock and scion immediately after the grafting. 3-4) Development of thin necrotic layers and callus formation. 5-6) Callus filled the grafting area. 7-8) An early cambium differentiation in BA-treated callus tissue.

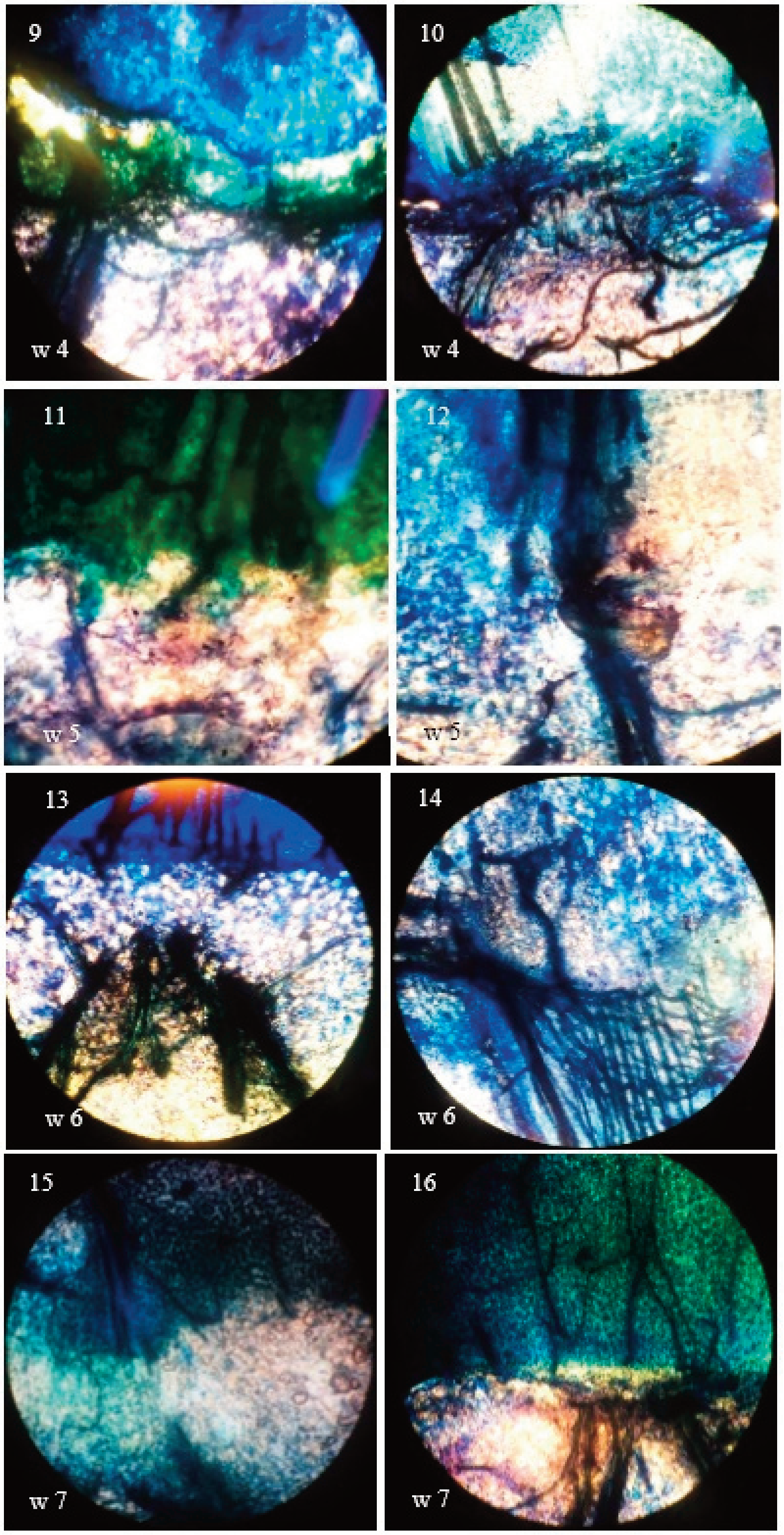

Fig. 4. Continued. 9-10) Initiation of vascular bundles. 11-12) Development of vascular bundles and connection to vascular of both rootstock and scion, disappearance of necrotic layers. 13-14) Completion of vascular connection in BA-treated grafts and to some extent in BA-untreated grafts. 15-16).

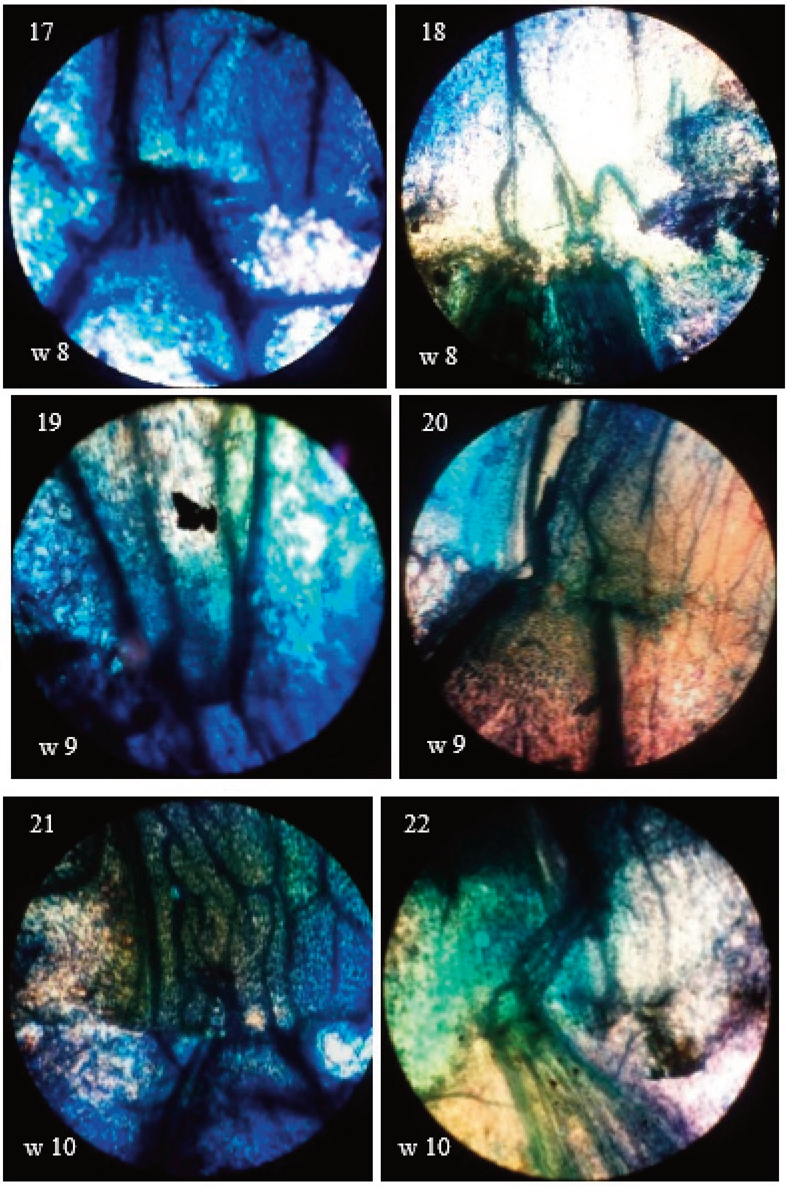

Fig. 4. Continued. 17-22) Successful completion of graft unions.

The success of the graft rate was higher in the horizontal graft, probably because of the contact surfaces in horizontal graft was perfectly smooth, while the lower success in the wedge graft could be due to its difficulty to perform and problems with mismatched tissues between the scion and rootstock (Estarda-Luna *et al*., 2002). Although, Hurst (2016) found that perpendicular wedge graft had a higher success rate than horizontal and parallel wedge grafts in *Opuntia ficus-indica*, Estarda-Luna *et al*. (2002) reported that the horizontal graft method was more successful than the wedge grafting method in *Opuntia* spp. micrografting, which was the case in our experiment as well.

The two rootstocks in this study showed no sign of compatibility problems during grafting. Evidence of this is the high percentage of grafting success (> 70 %), vascular tissue cohesion observed in the histological study, and the excellent growth observed after 70 days of grafting. This agrees with the results obtained by Estarda-Luna *et al*. (2002) and Aruna and Mangaiyarkarasi (2020) who did not observe any grafting incompatibility among cacti genus and species.

It has been proposed that plant growth regulators such as auxins and cytokinins function as morphogenic substances inducing and controlling the regeneration of cambium and vascular tissue (Hartmann *et al*., 2020; Rahimi *et al*., 2012). Cytokinins play an important role in the growth and development of graft union by stimulating the callus proliferation at the site of tissue unions (Sharma and Zheng, 2019). In the present study, exogenous application of BA improved success rate and callus formation at the grafting point as well as scion development. The reason for this is that cytokinins promote cell division, callus formation and differentiation of vascular tissues (Miri and Roughani, 2018; Pérez-Molphe-Balch *et al*., 2015). These effects could be useful in accelerating the formation of a graft union and thus improving graft take percentages (Cary, 2008), as confirmed by our histological observations. Köse and Güleryüz (2006) reported that BA and Kin as cytokinins application increased callus formation at the grafting point of grapevine. Ezan *et al*. (2016) found that the walnut rootstocks treated with DKW (Driver and Kuniyuki, 1984) semi-solid medium containing BA and ascorbic acid had higher bud-take rate. Farsi *et al*. (2018) obtained the highest percentage of graft-take on walnut in hormonal treatment of BA plus IBA.

Dose-response curves of plant growth regulators are bell-shaped, and at optimum concentrations, these stimulant effects are typically maximized, beyond which they become inhibitory (Arteca, 1996). It was also observed in this study that the use of high level of BA (500 mg L^{-1} compared to 100 mg L^{-1}) led to decrease in graft success and scion development.

The development of a compatible graft is typically comprised of five events: Lining up vascular cambiums of the rootstock and scion, wounding response, callus bridge formation, differentiation of vascular cambium across the callus bridge, and production of secondary xylem and phloem from the new vascular cambium in the callus bridge (Hartmann *et al*., 2020). The graft union formation in *Gymnocalycium* showed a similar sequence of events reported previously by Shimomura and Fujihara (1976) and Estarda-Luna *et al*. (2002) in different cacti species. We observed the necrotic layers as dark lines in outer sides of graft interface. Formation of necrotic layers from the cell contents and cell walls of the cut scion and rootstock cells occurred as a wound healing response against pathogens invasion. Undifferentiated callus tissue is generated from uninjured, rapidly dividing parenchyma cells, spread into spaces between the two graft components (Hartmann *et al*., 2020). Callus formation must develop between scion and rootstock for a successful graft union (Melnyk and Meyerowitz, 2015). In our study, callus tissue originated mainly from the rootstocks. The *Gymnocalycium* scion was considerably small and was limited to a short stem piece of 1.5 g, hence, the initiation of callus formation is probably limited by lower phytohormone levels, nutrients and carbohydrates. At the edges of the newly formed callus mass, parenchyma cells touching the cambial cells of the rootstock and scion differentiate into new cambium cells. A vascular cambium layer subsequently forms between the vascular systems of the scion and rootstock (Hartmann *et al*., 2020). The formation of vascular connections between the scion and rootstock is one of the hallmarks of grafting success (Melnyk and Meyerowitz, 2015).

CONCLUSION

Significant increases in callus formation and success rate of the graft union as well as scion development showed that BA treatment may have the potential to improve graft union formation in *Gymnocalycium*. In addition, horizontal graft was found more suitable than wedge graft in terms of graft success rate. The results of the histological analysis revealed the compatibility in the grafting of *G. mihanovichii* on *L. marginatus* and *M. geometrizan* rootstocks, and positive effects of BA application in graft healing process. This method is easy to perform, requires very little equipment, and is most likely to be useful for commercial production of other varieties of colored or unusual forms cacti.

ACKNOWLEDGMENT

The authors would like to thank Mr. S. Moniri for providing plant materials and his collaboration.

Literature Cited

Arteca, R.N. 1996. Plant growth substances. Springer, Dordrecht.

Aruna, P. and Mangaiyarkarasi, R. 2020. Grafting studies in ornamental cacti. The Pharma Innovation Journal, 9 (3): 38-40.

Bach, D. 1981. The double-cut technique for grafting cacti to *Trichocereus pachanoi* rootstock. Desert Plants, 3 (1): 10-12.

Cabahug, R.A.M., Nam, S.Y., Lim, K.B., Jeon, J.K. and Hwang, Y.J. 2018. Propagation techniques for ornamental succulents. Flower Research Journal, 26 (3): 90-101.

Cary, D.J. 2008. The effects of benzyladenine on ornamental crops. M.Sc Thesis of Horticultural Science, North Carolina State University.

Couto, M.F., Peternelli, L.A. and Barbosa, M.H. 2013. Classification of the coefficients of variation for sugarcane crops. Ciência Rural, 43 (6): 957-961.

Driver, J. and Kuniyuki, A. 1984. *In vitro* propagation of Paradox walnut rootstock. HortScience, 19 (4): 507-509.

Erwin, J. 2009. Cacti: Studies on introducing a new group of ornamental plants. Acta Horticulturae, 813: 359-364.

Estarda-Luna, A.A., López-Peralta, C. and Cárdenas-Soriano, E. 2002. *In vitro* micrografting and the histology of graft union formation of selected species of prickly pear cactus (*Opuntia* spp.). Scientia Horticulturae, 92: 317-27.

Ezan, J., Hadavi, E. and Miri, S.M. 2016. Improving the bud take rate in conventional patch budding of Persian walnut by applying a paste of the DKW semi-solid medium enriched with plant growth regulators. Acta Horticulturae, 1109: 133-136.

Farsi, M., Fatahi Moghadam, M.R., Zamanim Z. and Hassani, D. 2018. Effects of scion cultivar, rootstock age and hormonal treatment on minigrafting of Persian walnut. International Journal of Horticultural Science and Technology, 5 (2): 185-197.

Jeong, M.I., Chao, C.H. and Lee, J.M. 2004. Production and breeding of cacti for grafting in Korea. Chronica Horticultural, 44 (3): 7-10.

Hartmann, H.T., Kester, D.E., Davies, F.T., Geneve, R.L. and Wilson, S.B. 2020. Plant propagation: Principles and practices. Pearson, USA.

Hunt, D. 2016. CITES cactaceae checklist. Royal Botanic Gardens, UK.

Hurst, J. 2016. Development of grafting protocols for salt-tolerant cactus pear (*Opuntia ficus-indica*). M.Sc Thesis of Plant Science, California State University.

Köse, C. and Güleryüz, M. 2006. Effects of auxins and cytokinins on graft union of grapevine (*Vitis vinifera*). New Zealand Journal of Crop and Horticultural Science, 34 (2): 145-150.

Lee, J.M. and Oda, M. 2003. Grafting of herbaceous vegetable and ornamental crops. p. 61-124. *In*: J. Janick (ed.), Horticultural Reviews, vol. 28. John Wiley & Sons, New York.

Miri, S.M. and Roughani, A. 2018. Factors affecting tissue culture success in ornamental crops, I. Medium composition. 2nd International and 3rd National Congress on Flower and Ornamental Plants, Mahallat, Iran, 23-25 October.

Melnyk, C.W. 2017. Plant grafting: Insights into tissue regeneration. Regeneration (Oxford, England), 4(1): 3-14.

Melnyk, C.W. and Meyerowitz, E.M. 2015. Plant grafting. Current Biology, 25 (5): R183-R188.

Panea, T., Ungur, I., Panea, I., Varga, N.V. and Mihaiescu, T.C. 1998. The stimulation of callus formation of graft vines cuttings with Romanian bioregulator Calovit. Acta Horticulturae, 463: 185-190.

Pérez-Molphe-Balch, E., Santos-Díaz, M.D.S., Ramírez-Malagón, R. and Ochoa-Alejo, N. 2015. Tissue culture of ornamental cacti. Scientia Agricola, 72 (6): 540-561.

Pilbeam, J. 1995. *Gymnocalycium*: A collector's guide. A.A. Balkema, Rotterdam, Netherlands.

Rahimi, M., Vedadi, C., Naserian, B., Majd, F., Miri, S.M., Rahmani, E. and Neahan, N. 2012. Studies of different sucrose, BAP and 2,4-D concentration on shoot tip grafting responses in tangerine. 3rd Iranian Agricultural Biotechnology Conference, Mashhad, Iran, 3-5 September.

Sharma, A. and Zheng, B. 2019. Molecular responses during plant grafting and its regulation by auxins, cytokinins, and gibberellins. Biomolecules, 9 (9): 397. doi: 10.3390/biom9090397.

Shimomura, T. and Fujihara, N. 1976. Histological observations on graft union formation in cactus. Journal of the Japanese Society for Horticultural Science, 44 (4): 402-408.

Wang, Y.Q. 2011. Plant grafting and its application in biological research. Chinese Science Bulletin, 56: 3511-3517.

How to cite this article:

Kamalzade, N., Miri, S. and Ghazijahani, N. 2021. Development of graft union formation and histological observations in cactus influenced by benzyladenine, grafting method and rootstock. *Journal of Ornamental Plants,* 11(1), 43-53.

URL: http://jornamental.iaurasht.ac.ir/article_680556_50dbebca937abfd14fe2595cf6fa2e39.pdf