J. Plant Production, Mansoura Univ., Vol. 8 (10): 999 - 1009, 2017

Anatomical Study on Watermelon Grafting

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ABSTRACT

The present study was carried out at Al-Amana nursery in Talkha district, Dakahlya Governorate, Egypt, during the summer season of 2014 to study the effects of different graft combinations between rootstocks and scion of watermelon (*Citrullus lanatus* (Thunb.) Matsum et Nakai) var. Lanatus cv. Aswan F_1. The experiment included thirteen treatments resulted from the combinations of four rootstocks [Jumbo F_1 and Nun 6001 F_1 hybrids [*Cucurbita maxima* (Duch.) ex. Lam.× *Cucurbita moschata* (Duch.) ex. Poir.], bottle gourd (*Lagenaria siceraria* Standl.) and pumpkin (*Cucurbita moschata* L.)] and three grafting methods [Hole insertion (HIG), Splice (SG) and Tongue approach (TAG)] as well as non-grafted plants as control. The obtained results illustrated that there was a high compatibility between Watermelon(scion) and Jumbo followed by bottle gourd then Nun 6001 rootstock, where these graft combinations recorded the highest vascular thickness and area of vascular cambium compared to other graft combinations. Although, SG gave the highest area of graft union. In addition, TAG showed the greatest width of graft union which is related to increase of xylem and phloem tissues thickness and area of vascular cambium. Moreover, grafted plants onto Jumbo and bottle gourd using TAG method recorded the highest survival percentage (100 and 98%, respectively) in comparison with other graft combinations. While, grafting onto pumpkin rootstock using HIG method had the lowest values of all studies traits in comparison with other combinations. Generally, the callus development and graft success in Watermeloncv. Aswan F_1can be performed using Jumbo rootstock with TAG followed by SG method.

Keywords: Anatomical, Grafting, Scion, Rootstocks, Watermelon

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum et Nakai) var. Lanatus, an most important crop in Egypt, is grown worldwide on over 6 million acres which produce over 50 million ton of fruits per year. Egypt is one the fifth largest watermelon producer in the world. It produces approximately 1.7 % from globally watermelon production; 1719 thousand metric tons were harvested from around 60554 ha with an average of 31.29 ton/ha (FAOSTAT, 2013). Recently, Watermelon grafting has been strikingly quick to spread across Egypt. The advantages of vegetable grafting commercial cultivars onto desired technique to decline the injuries impact of soil borne pathogens and increased yield and fruit quality (Lee and Oda, 2003; Yetisir and Sari, 2003; Martínez-Ballesta *et al.*, 2010; Bhatt *et al.*, 2013). Graft compatibility/incompatibility is a complex response including a wide range of anatomical and biochemical interactions. The success in grafting technique mainly depends on the appropriate choice for scion and rootstock combinations, using of proper grafting method and grafts maintaining (Lee and Oda, 2003; El-Semellawy, 2005; Aloni *et al.*, 2008). The influence of these factors is mainly depends on the callus and vascular differentiation. Furthermore, the graft compatibility between scion and rootstock depends on the rapid callus formation, differentiation of new vascular tissue (secondary xylem and phloem) (Shehata *et al.*, 2000; Kawaguchi *et al.*, 2008; Martínez-Ballesta *et al.*, 2010). This in turn impact on the survival percentage of the grafted transplants (Hartmann *et al.*, 2002). A complete compatibility was found by grafting Watermelon onto bottle gourd, calabash gourd, pumpkin and figleaf gourd compared to squash rootstock (El-Semellawy, 2005). Grafting watermelon cv. Crimson sweet had the higher union percent with bottle gourd and Shintoza rootstocks than pumpkin (Khankahdani *et al.*, 2012). In this concern, Yetisir and Sari (2003) assessed the effect of watermelon grafting onto 10 different rootstocks and found that the survival rate was (65%) on *Cucurbita* rootstocks and (95%) on *Lagenaria* rootstocks. In addition, Mohsen *et al.*, 2012) found that grafting Nubian watermelon (*Citrullus lanatus* var. *Colocynthoide*) onto *Cucurbita ficifolia* and *Lagenaria siceraria* gave the greatest survival percentage.

On the other hand, various watermelon grafting methods have been developed (Hassell *et al.*, 2008; Kubota *et al.*, 2010). The tongue approach (TAG), hole insertion (HIG), and Splice grafting (SG) are the most succeed methods. The TAG is one of the original grafting methods performed (Lee and Oda, 2003). The TAG continues to be preferred by inexperienced growers due to its technical simplicity, high success rate, and the little care needed since it does not require healing chambers (Oda, 1995; Lee and Oda, 2003; Hassell *et al.*, 2008). However this methods requires severing of the rootstock top and the scion bottom after the graft succeed has healed (Hassell *et al.*, 2008). The HIG is favored by experienced watermelon growers because of growing space and performance (Lee and Oda, 2003). On the other hand, it requires slightly more skill and more time to graft than most various grafting techniques, and careful control of environmental conditions i.e. humidity, temperature, light after grafting (Cushman, 2006; Hassell *et al.*, 2008). Despite SIG is a relatively simple technique, it is unpopular due to the failure of vascular bundles to align sufficiently if the healing environment is not optimal (Cushman, 2006; Memmott, 2010). Using Splice method to graft watermelon cv. Aswan F_1 onto bottle gourd achieved rapidly and strongly connect, and increased graft union area compared to Tongue-approach and Hole insertion methods (Abd El-Wanis *et al.*, 2013). Similar results were obtained by Khankahdani *et al.* (2012) who found that the greatest union percent was in Splice grafting and the lowest in Hole insertion. The grafting watermelon onto bottle gourd (*L. siceraria*) by Cut method recorded the greatest callus area and vascular tissues (xylem and phloem) area, while using squash rootstock with Cut or Tongue approach method recorded the lowest values (El-Semellawy, 2005). In the same line, Khankahdani *et al.* (2012) grafted watermelon cv. Crimson sweet onto three rootstocks including bottle gourd, Shintoza and pumpkin using Hole insertion, Splice approach and Tongue approach methods. They found that using bottle gourd and Shintoza rootstock with Splice approach method had the highest graft union percent compared to other combinations. However, there is a lack of information about the anatomical changes that occur during graft union formation in watermelon.

The our investigation aimed to clarifies the histological features of various grafting area of watermelon c.v Aswan F_1 and four rootstocks [Jumbo F_1 and Nun 6001 F_1 hybrids (*Cucurbita maxima* Duch. × *Cucurbita moschata* Duch.), bottle gourd (*Lagenaria siceraria* Standl.) and pumpkin (*Cucurbita moschata* L.)] using three grafting methods (Hole insertion, Splice and Tongue approach).

MATERIALS AND METHODS

Plant materials and experimental design

Watermelon (*Citrullus lanatus* (Thunb.) Matsum et Nakai) var. Lanatus cv. Aswan $F_1$was used as scion. The used rootstocks were [Jumbo F_1 and Nun 6001 F_1 hybrids (*Cucurbita maxima* Duch. × *Cucurbita moschata* Duch.), local cultivar of bottle gourd (*Lagenaria siceraria* Standl.) and local cultivar of pumpkin (*Cucurbita moschata* L.)].

A completely Randomized Blocks (CRB) with three replicates were performed. Four rootstocks were assigned to main plots. The sub-plots were devoted to three grafting methods [Hole insertion (HIG), Splice (SG) and Tongue approach (TAG)].

Culture conditions:

The pot experiment was performed in plastic house at Al-Amana nursery located in Talkha district, Dakahlya Governorate, Egypt. The averages of minimum and maximum air temperature and humidity in the plastic house during the transplants production were 21.5º C and 60 %, respectively.

Watermelon (scion) seeds were sown in seedling foam trays filled with a mixture of peatmoss: vermiculite (1:1 v/v) under plastic house on 1st February 2014. Rootstock and control seeds were sown after 15 days from scion seeds sowing (on 16th February) in seedling trays filled with the same batch. The grafting was performed after 20 days from rootstock seeds sowing (on 8th March) because rootstocks seedlings growth is faster than that of watermelon. The grafted plants were transplanted into plastic cups of 7 cm diameter, containing BVB (Al-Kalthoum Company) peatmoss consisting (90 % black peat, 10 % white peat and 1.2-1.7 kg /m³ fertilizer PG mix 12-14-24+TE) with pH (5.5–6.0). Each combination contained 50 cups with one plant.

Grafting methods

Three grafting methods were carried out according to vegetable grafting technique that described by Kubota *et al.* (2010) i.e.:

- **Hole insertion grafting method (HIG).**

Rootstock seedlings were harvested 30 min before grafting to reduce rootstock tissue turgid, and avoid cracking. The first true leaf and the apical and lateral (axillary) meristem of rootstock were removed with a razor blade (Fig. 1.1). The rootstock top was perforated in an angle where the edge of the tool comes out through hypocotyls below the opposite cotyledon with leaving the perforating tool (Fig. 1.2). The scion cutting was prepared with two cuts to have a sharp edge with about 7-10 mm hypocotyl. Second sharp cut of the scion was done after rotating the scion by 90 degrees in which the end cut is not V-shaped (Fig. 1.3). The scion was inserted into the rootstock hole (Fig. 1.4). The grafted transplants were inserted to the cups and sprayed with water to avoid excessive dehydration. Then, the cups was placed into boxes and moved to the healing chamber for up to 9 days healing and rooting.

Fig. 1. Hole insertion grafting method.

- **Splice (One cotyledon/ Slant cut) grafting method (SG).**

When both cotyledons and true leaf started to develop, the root stock plant was ready to graft about 20 days after sowing. One cotyledonary leaf with apical and axillary buds were cut from rootstock at 45 degree angle (Fig. 2.1). Scion was prepared with matching hypocotyl width and cut at the same angle at about 10-15 mm below the cotyledons (Fig. 2.2). Both scion and rootstock were matched together (Fig. 2.3) and hold with a grafting clip (Fig. 2.4). The grafted transplants were inserted into cups and sprayed with water immediately. Thereafter, the cups was placed into boxes and moved to the healing chamber for up to 9 days healing and rooting. The grafting clip was removed after the union is taken.

Fig. 2. Splice (One cotyledon/ Slant cut) grafting method.

- **Tongue approach grafting method (TAG).**

After rootstock and scion have developed the first true leaf, plants were pulled out from the tray and laid on the table (before daring the grafting). A downward cut was made at a sharp angle (60 degree relative to the horizontal plane), and a half way across the rootstock hypocotyl was made with a razor blade

(Fig. 3.1). An upward cut was made at the same angle, and a half way across the scion hypocotyl was made (Fig. 3.2). The upward 'tongue' of rootstock hypocotyl was hooked with the downward 'tongue' of scion hypocotyl by a grafting clip (Fig.3. 3 and 4). The joined plants were inserted into cups and placed into boxes. The grafted plants were misted with water and leaved in the greenhouse conditions, and watered as needed. The top of the rootstock was cut off 9 days after grafting then the bottom portion of the scion was cut off 3 days later.

Fig. 3. Tongue approach-grafting method.

Healing process

The grafted plants via were placed in misty chamber in the day of grafting. The healing stage was conducted according to Miles *et al.* (2013) with some modifications as follows:

Day 1: The plastic of healing chamber was closed; the chamber was covered with two layers of shaded cloths and two other layers were placed above the greenhouse.

Day 2: The chamber was kept closed and covered with shaded cloths.

Day 3: The chamber was opened and misted from the inside (sides and top), the chamber was closed, and one layer from shaded cloths was removed.

Day 4: The chamber was leaved closed.

Day 5: The chamber was opened for 30 minutes, then misted from the inside, the plants were misted, and the chamber was reclosed.

Day 6: The chamber was opened for 1 hour, then misted from the inside, the plants were misted and the chamber was reclosed.

Day 7: The chamber was opened for 3 hours, and then misted from the inside, the plants were misted and the chamber was reclosed.

Day 8: The chamber was opened for 6 hours, then misted from the inside, the plants were misted and the chamber was reclosed.

Day 9: The plants were removed then the hardening off was started.

Anatomical study:

The histological study was carried out on the primary structure of scion and rootstocks at grafting as well as the graft union region between them after 15 days from grafting (50 and 35 days from sowing for scion and rootstock respectively). Five samples were taken from scion and rootstocks in the hypocotyl below the cotyledons as well as the graft union region in grafted plants. The obtained materials were killed and fixed in FAA solution (90 ml ethyl alcohol 70%, 5 ml formalin and 5 ml glacial acetic acid) for 48 hours. Then, the samples were washed and dehydrated in series of ethanol, cleared in ethanol: xylene (3:1- 1:1- 1:3 and 100% xylene) and embedded in paraffin wax (52-54^{o}C m.p.). Transverse and longitudinal sections were done at 15-20 µm thick using rotary microtome and double stained with saffranin-light green, cleared in clove oil finaly mounted in canada balsam (Gerlach, 1977). The sections were examined by light microscope (Olympus CX41) supplemented with photographic unit (Tuscan, USB 2.0 H Series). The following measurements were recorded:

- **The hypocotyl structure characters of scion and rootstocks:** hypocotyl diameter (µ), cortex thickness (µ), length and width of large vascular bundle (µ), pith thickness (µ), xylem tissue thickness (µ), phloem tissue thickness (external and internal) (µ), vascular cambium dimensions (length and width) (µ), area of vascular cambium (μ^2) and metaxylem diameter (µ). Then, the percentage of rootstock change from scion was calculated (± % to scion).
- **Graft union dimensions:** length of graft union (µ), width of graft union (µ) and area of graft union (mm^2).

Survival percentage:

The survival percentage of grafted watermelon plants was calculated after 15 days from grafting by accounting the successful transplants number and dividing it on the total number of transplants.

Statistical Analysis:

Data were statistically analyzed according to the technique of analysis of variance (ANOVA) described by Gomez and Gomez (1984) using CoStat computer software program. The treatment means were compared using Duncan's multiple range test at probability of 0.05 according to Duncan (1955).

RESULTS AND DISCUSSION

Structure of scion:

The anatomical study of hypocotyls of watermelon (scion) plants (Fig 5A) consists of an epidermis, ground tissue and vascular system. The epidermal cells consist of single orbicular or rectangular, which cell layer is covered with thin layer of cuticle. Ground tissue is differentiated into cortex and pith. The cortex is composed of 5-6 layers of chlorenchyma whereas; those at ridges are collenchyma. The vascular bundles are open bicollateral bundles type (Fig 5A). The primary inter phloem had form 10 cells layers, while the primary xylem had form 6 layers. The bicollateral bundles are common feature in cucurbitaceous family (Esau, I960). Moreover, the bicollateral bundles are found to be in two forms *i.e.* large (6) and small bundles (5).

Structure of rootstocks:

Comparing the primary structure of four rootstocks and scion (Fig. 5B-5E and 4) was found that most of anatomical characters are different. All studied anatomical characters in Jumbo rootstock were found to be more developed as compared to the scion (Fig. 5D and 4). Hypocotyl diameter, large vascular bundle dimensions (length), internal and external phloem tissue thickness and metaxylem diameter were decreased in pumpkin by 8.8, 7.2, 36.3, 29.1 and 19 % less than the scion, respectively (Fig. 5E and 4). Bottle gourd rootstock contained approximately the same thickness and phloem tissues as in the scion (Fig. 5C and 4).

Differentiation of graft union between rootstock and scion:

• Necrotic layer and Callus formation

Contact layer was observed at the graft union region on the surface of both rootstock and scion. It combined of dead cells and deposition of suberin in the dead cells and mashed cells. Callus was differentiated from various living cells in the contact region of both sides of the graft partners. Callus tissue was produced at both side of the graft partners and they combined to make connection between scion and rootstock. Furthermore, callus continued to proliferate and filled the region between scion and rootstock forming a callus bridge (Fig 7-10).

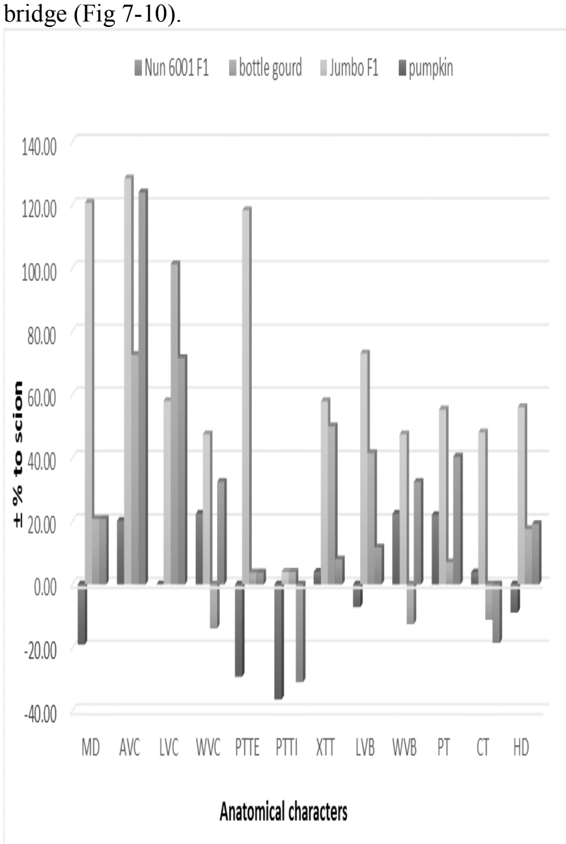

Fig. 4. The anatomical characters of rootstocks as percentage of change from watermelon cv. Aswan F1 (scion).

MD: Metaxylem diameter (μ), AVC: Area of vascular cambium (μ^2), LVC: Length of Vascular cambium (μ), WVC: Width of Vascular cambium (μ), PTTE: Phloem tissue thickness (external) (μ), PTTI: Phloem tissue thickness (internal) (μ), XTT: Xylem tissue thickness (μ), LVB: Length of Large vascular bundle (μ), WVB: Width of Large vascular bundle (μ), PT: Pith thickness (μ), CT: Cortex thickness (μ) and HD: Hypocotyl diameter (μ).

The present anatomical studies on grafts introduced detailed information on structure of revascularization throughout graft union development. The amount of callus varies within plant species, the formation of vascular connections was considered as the basic requirement for a successful graft. Formation of new vascular tissues allows the connection between scion and rootstock, vascular connection is an important feature for grafting success. The grafting success may be due to that callus tissue formation between scion and rootstock connected together and began to differentiate to vascular connection elements (xylem and phloem) as shown in Figures (7-10).

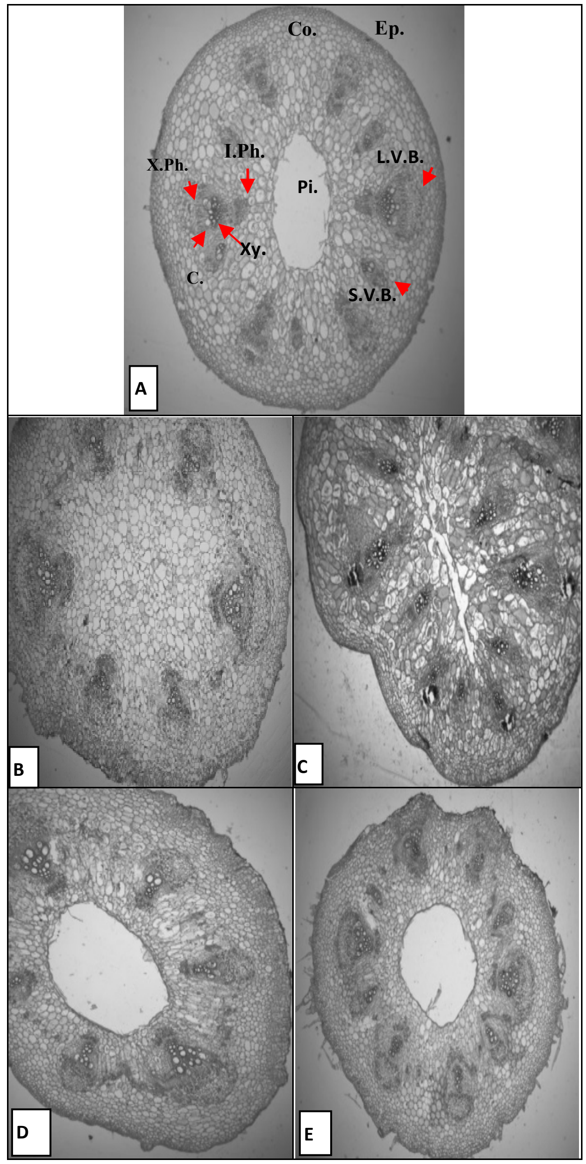

Fig. 5. Transversal section below the cotyledons, showing the primary structure of hypocotyl of scion (Watermelon cv. Aswan F1) "A" after 50 days from grafting, and rootstocks Nun 6001 F1 hybrid "B", Bottle gourd "C", Jumbo F1 hybrid "D" and pumpkin gourd "E" after 35 days from sowing (Oc. 10x * Obj. 4x).

Ep.: Epidermis, C.: Cambium, Co.: Cortex, L.V.B.: Large Vascular Bundle, I.Ph.: Internal Phloem, S.V.B.: Small Vascular Bundle, Pi.: Pith, X.Ph.: External Phloem and Xy.: Xylem.

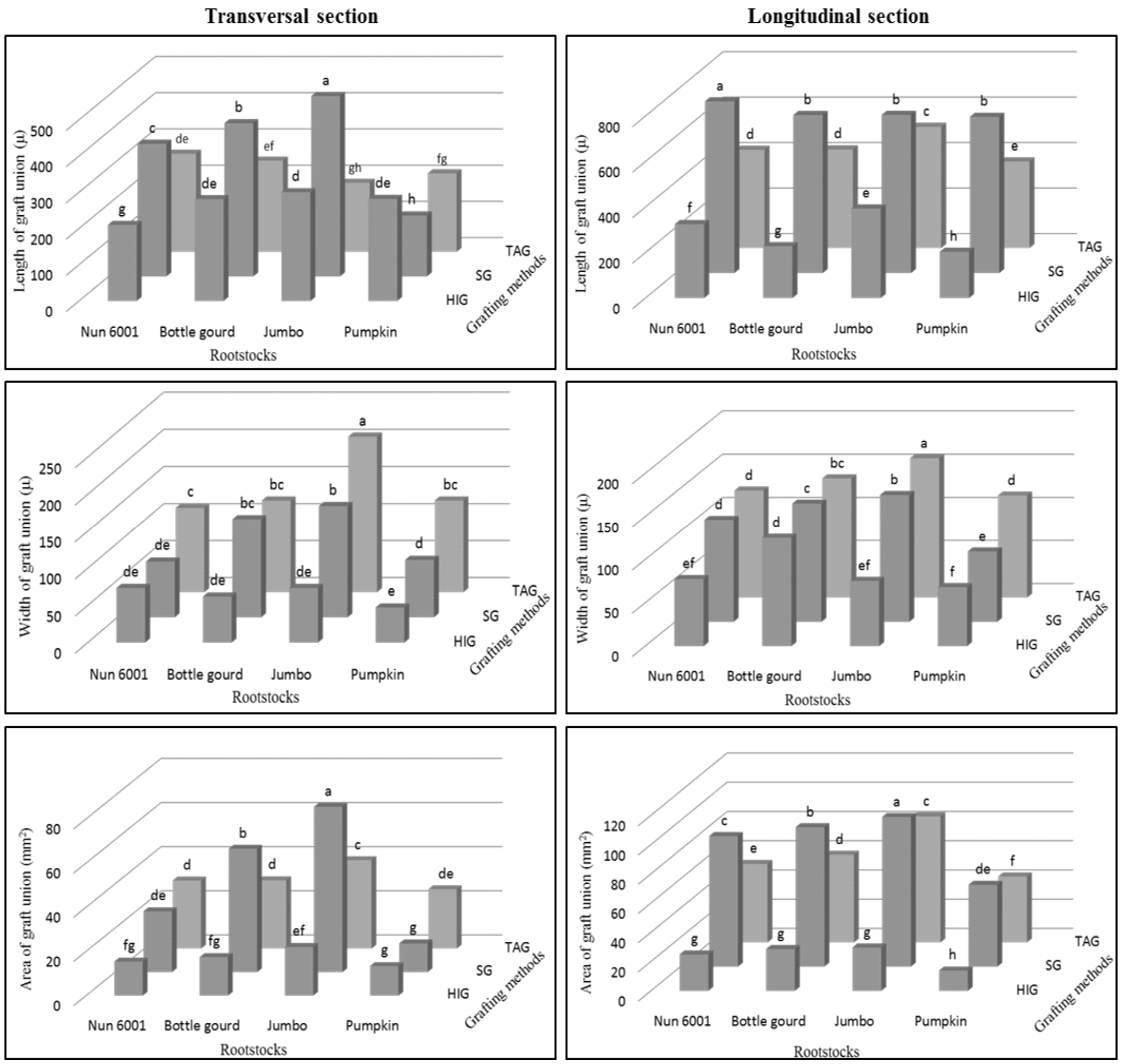

Fig. 6. Length, width and area of graft union at transversal and longitudinal sections after 15 days from grafting watermelon cv. Aswan F1 onto four rootstocks using three grafting methods (HIG: Hole insertion grafting, SG: Splice grafting, TAG: Tongue approach grafting). Columns with the same letter are not significantly different at the 0.05 % level of probability according to Duncan's multiple range test.

In our observation of cross and longitudinal sections (Figures, 4-10) it could be mentioned that the hypocotyls structure of all rootstocks except pumpkin was increased in area and thickness of vascular tissues (phloem and xylem) as compared to scion. Also, the observation reveals that thickness of phloem tissue, diameter of metaxylem and area of vascular cambium in Jumbo rootstock were larger than in other rootstocks. These characters reflected on the strength of graft union, differentiation of vascular connecting tissues as well as area of graft union. In this respect, vascular differentiation begins after establishment of cambial continuity and the strong connection occur in a short time in the compatible grafts.

Successful grafting depends mainly on the suitable choice for scion-rootstock combinations and using of appropriate grafting method. Several experiments have confirmed different models of graft union development with the entire process typically divided into stages. In this respect, callus formation with grafting site, vascular cambium differentiation, secondary xylem and phloem formation, and finally vascular connection between scion and rootstock (Hartmann *et al.*, 2002; Dolgun *et al.,* 2008). Moreover, Aloni *et al.* (2008) revealed that this process depends on the identification of a compatible rootstock that promotes the rapid formation of vascular connections between the rootstock and the scion and rapid resumption of root and shoot growth. In this concern, Trinchera *et al.* (2013) reported that callus formation at the graft interface followed by differentiation of wound vascular elements of scion and stock; establishment of a continuous wound repair xylem and phloem interconnecting cut vascular bundles of scion and rootstock and, finally, the formation of a wound cambium giving rise to secondary vascular elements in the graft union. The present study also revealed similar activities of graft union formation between the scion and rootstock that probably resulted in the graft success or failure observed.

There was a high compatibility between watermelon (scion) and Jumbo rootstock using Tongue approach grafting method, where this graft combination recorded the highest xylem and phloem tissues thickness and area of vascular cambium as compared to other graft combinations. Good established vascular connections provide a good water and nutrient flow from rootstock to scion. In this respect, Tiedemann (1989) reported that phloem development in the graft union resulted in different numbers of connecting sieves tubes in each individual graft. Continuous vascular tissues from rootstocks to scions were observed at the graft unions of using Jumbo rootstock in grafting, especially using Splice and Tongue approach grafting methods, which increased area of graft union and outnumbered vascular tissues as compared to Hole insertion method. The highest obtained area of graft union using SG method is resulted from the highest length of cutting surface, which is related to the grafter performance. Furthermore, for a successful grafting, the vascular cambium of the rootstock and scion must be well aligned and in contact with one another. The scion and rootstock plants must therefore have similar stem diameters at the time of grafting. In this respect, Salama and Abd El-Wanis (2016) revealed that good compatibility in grafting can be observed by more number of vessels with less necrotic layer at graft union. This in turn impact on the survival percentage of the grafted transplants (Hartmann *et al.*, 2002). Similar results were obtained by Mounir (1965) who found that watermelon grafting rootstock caused a special growth in vascular bundles of rootstock lying near the scion, such bundles are characterized by the presence of internal cambium, which provide the bundles with the internal secondary tissues of xylem and phloem. Moreover, Kabeel (1999) revealed that the differentiation of callus increased the vascular connection between cucumber and rootstock, where this connection led to success of grafting.

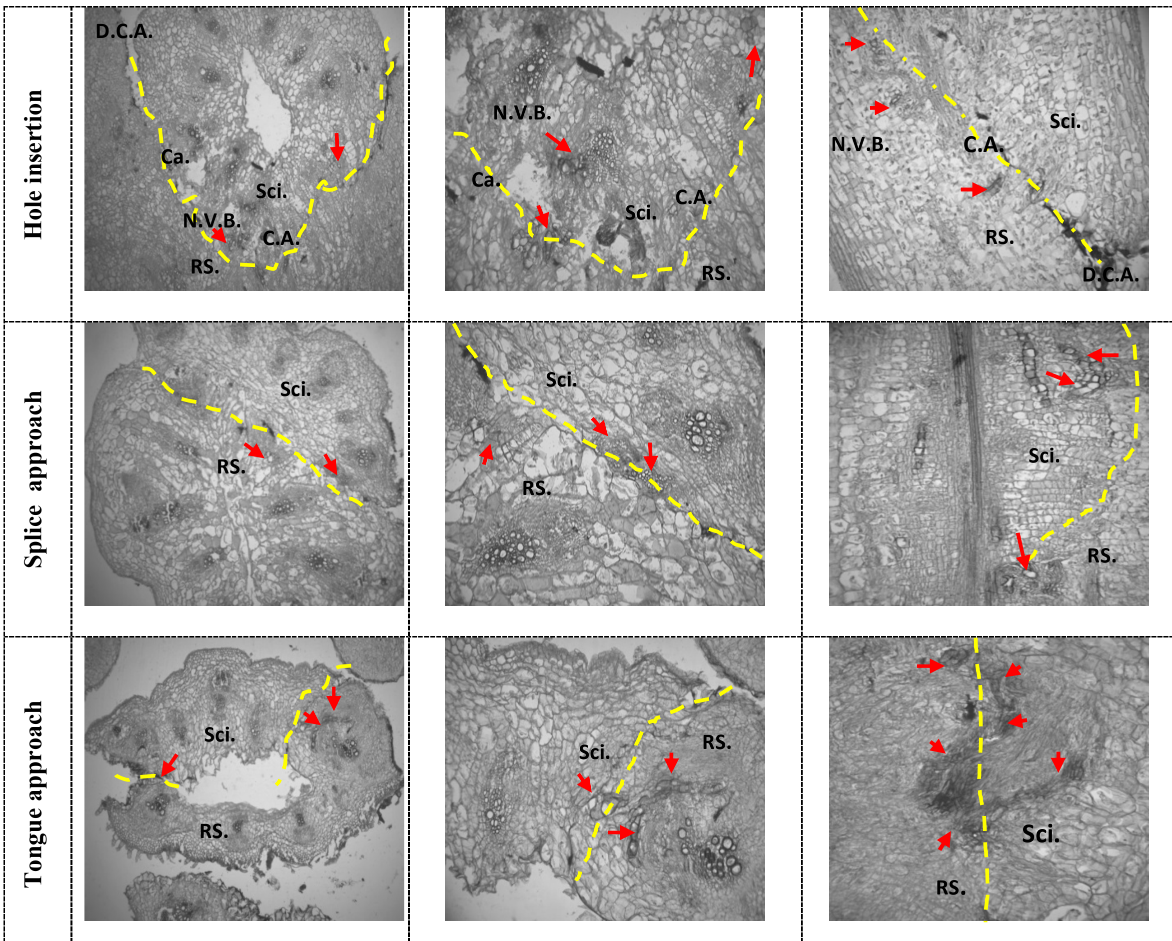

Fig. 7. Cross and longitudinal sections of the grafting union after 15 days from grafting watermelon Aswan F1 (scion) onto Nun 6001 F1 hybrid rootstock, (40X and 100X), showing connected vascular tissues between scions and rootstock. The red arrows show the new vascular bundles.
Abbreviations: Ca., Callus; C.A., Connected Area; D.C.A., Disconnected Area.; N.V.B., New Vascular bundle; RS., Rootstock; Sci., Scion.

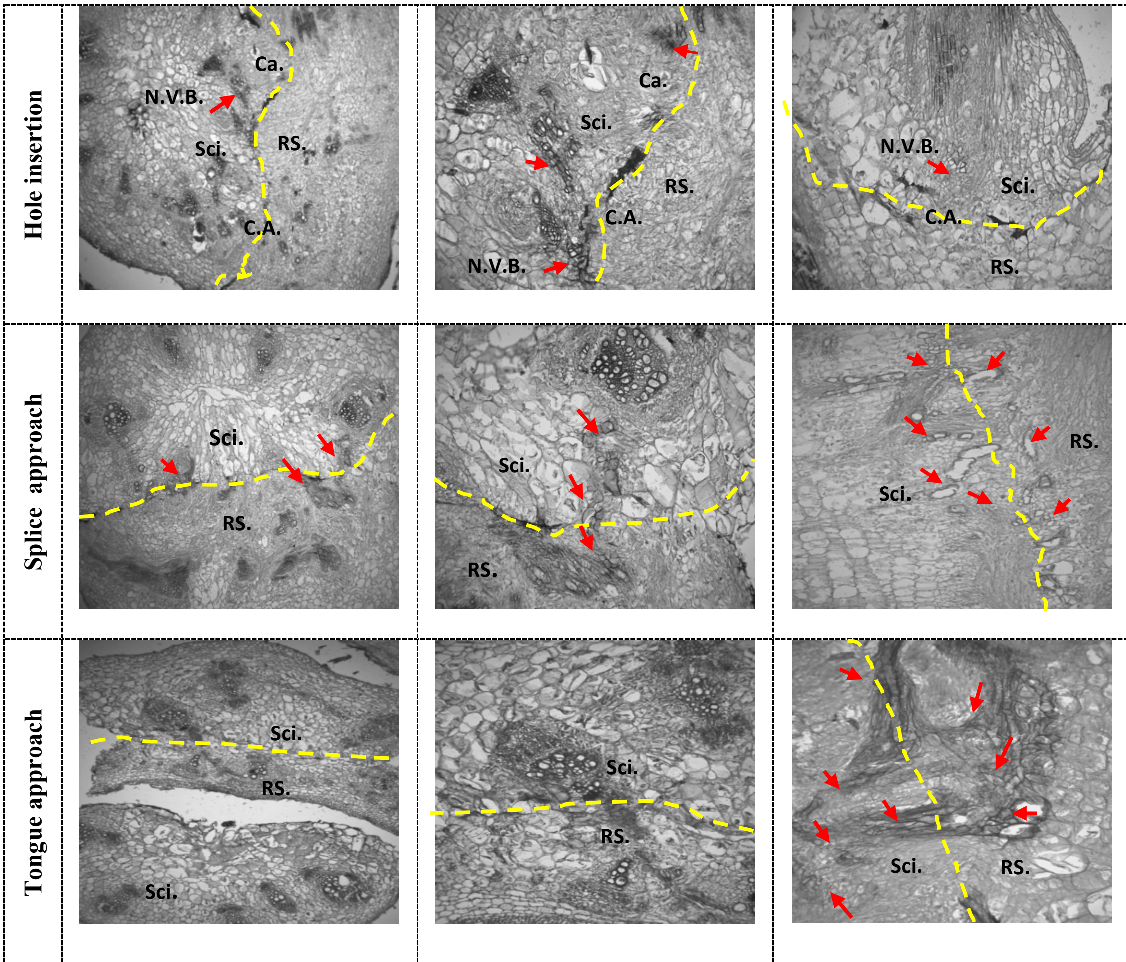

Fig. 8. Cross and longitudinal sections of the grafting union after 15 days from grafting watermelon Aswan F1 (scion) onto Bottle gourd rootstock, (40X and 100X), showing connected vascular tissues between scions and rootstock. The red arrows show the new vascular bundles. Abbreviations: Ca., Callus; C.A., Connected Area; N.V.B., New Vascular bundle; RS., Rootstock; Sci., Scion.

- **Differentiation of connecting vascular tissues (xylem and phloem)**

Grafting plants onto Jumbo followed by bottle gourd rootstock with Splice grafting method had the highest significant area of graft union (Fig. 6). It's had the highest significant width of graft union with Tongue approach grafting method and had the highest vascular thickness and area of vascular cambium (Fig. 6, 8 and 9). Fig. 9 shows that some parenchyma cells differentiated into vascular cambium, which produces new xylem and phloem tissues. The smallest area and width of graft union (Fig. 6 and 10) and poor grafting compatibility or weakness of cohesion between scion and pumpkin rootstock were detected with using Hole insertion method (Fig. 11).

Poor grafting compatibility or weakness of cohesion between scion and pumpkin rootstock under Hole insertion method (Fig. 11) mainly due to the no connecting between scion and rootstock in many places which lead to lower produced of callus tissues (Fig. 11 A, B) and failure to from a new vascular bundles (Fig 11. C, D) or may be due to the callus tissues formation on both sides of scion and rootstock that covered with surprised cells, which prevented the normal connection and/or failure of vascular connection and formation of impermeable perception between both (Sallam, 2010). On the other hand, the increase of phenolic compounds and peroxidases (Usenik *et al.*, 2006; Pina and Errea, 2008; Zarrouk *et al.*, 2010; Pina *et al.*, 2012) or low concentrations of starch (Yano *et al.*, 2002) in the graft union were combined with the reduced compatibility, that reduced auxin biosynthesis and influenced the vascular tissues differentiation (Errea, 1998). Furthermore, badly matching cambial layers between rootstock and scion may be delay the successful grafting or may be prevent the graft union (Hartmann *et al.*, 2002). Trinchera *et al.* (2013) they reported that graft incompatibility may be cause the delayed constitution of cell-to cell communication through plasmodesmal connectivity at cellular level. However, failure of grafts will occur due to water stress when scions are unable to receive water from the rootstocks before the graft union is formed.

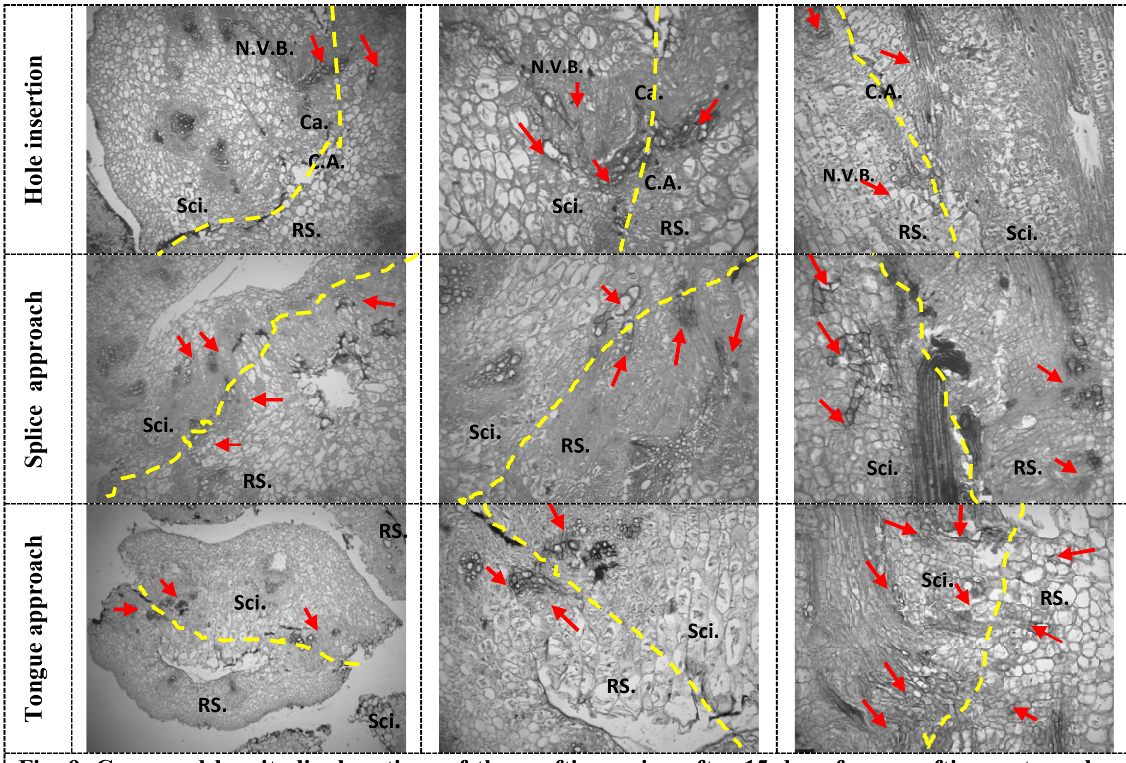

Fig. 9. Cross and longitudinal sections of the grafting union after 15 days from grafting watermelon Aswan F1 (scion) onto Jumbo F1 hybrid rootstock, (40X and 100X), showing connected vascular tissues between scions and rootstock. The red arrows show the new vascular bundles.

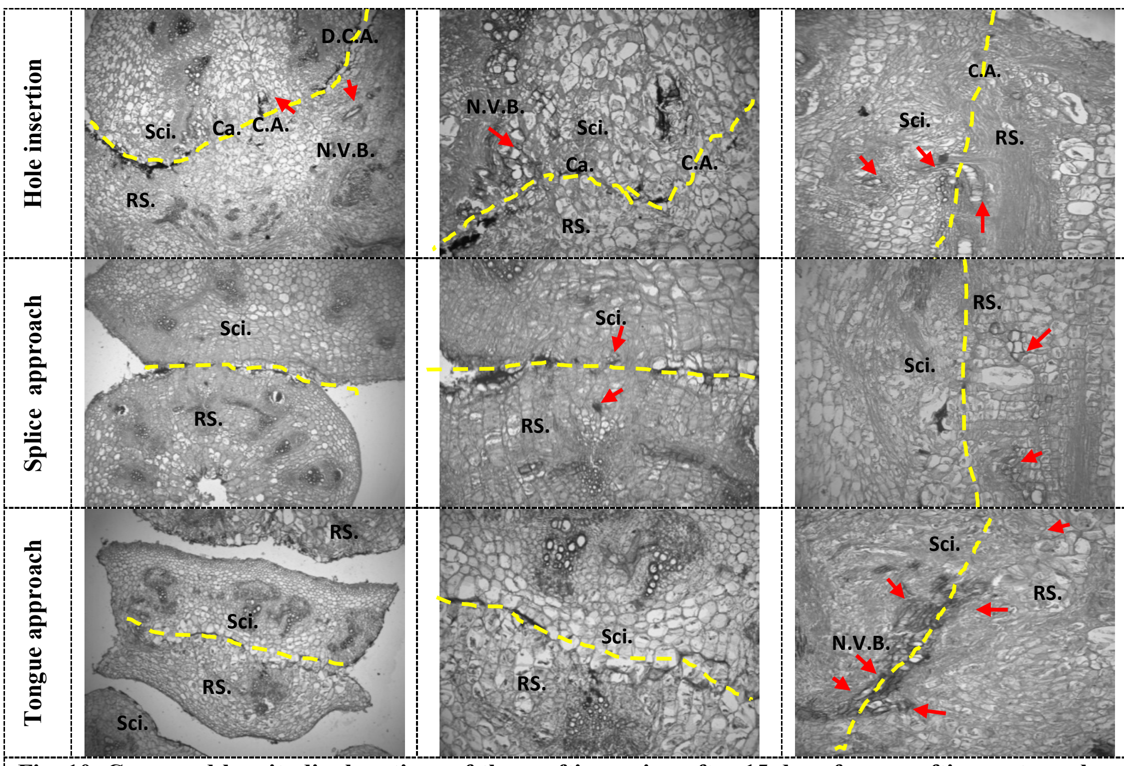

Fig. 10. Cross and longitudinal sections of the grafting union after 15 days from grafting watermelon Aswan F1 (scion) onto Pumpkin rootstock, (40X and 100X), showing low connected vascular tissues formation between scions and rootstock. The red arrows show the new vascular bundles. Abbreviations: Ca., Callus; C.A., Connected Area; D.C.A., Disconnected Area; N.V.B., New Vascular bundle; RS., Rootstock; Sci., Scion.

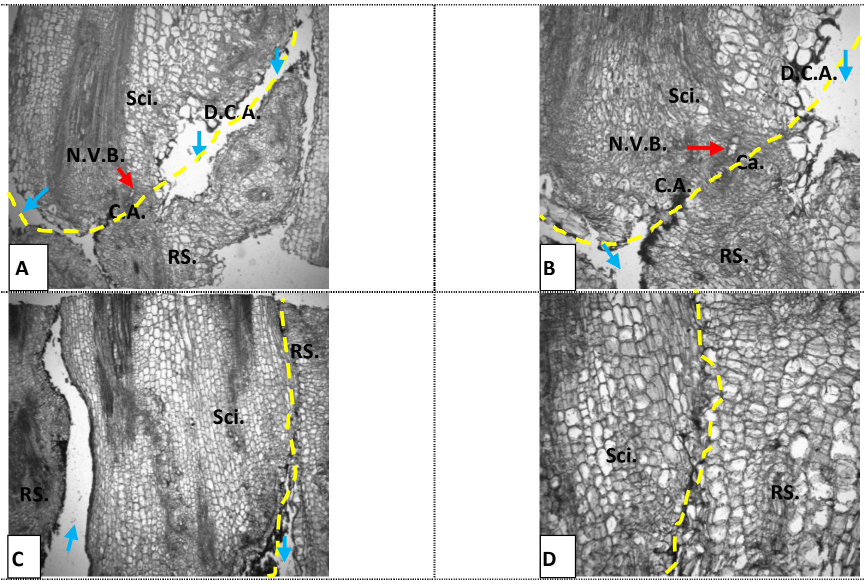

Fig. 11. Longitudinal sections of the grafting union after 15 days from grafting watermelon Aswan F1 (scion) onto Pumpkin rootstock with using hole insertion method, (40X and 100X), showing no connecting between scion and rootstock in many places (Figs. A and B) which led to lower produced callus and connected vascular tissues between scions and rootstock (Figs. C and D). The red and blue arrows show the new vascular bundles and unconnected area respectively.
Abbreviations: Ca., Callus; C.A., Connected Area; D.C.A., Disconnected Area; N.V.B., New Vascular bundle; RS., Rootstock; Sci., Scion.

- **Survival percentage of grafted watermelon plants**

The highest successful grafting percentages were obtained from grafting plants onto Jumbo followed by those grafted onto bottle gourd rootstock using Tongue approach method. While the lowest values were obtained from grafting plants onto pumpkin rootstocks using Hole insertion grafting method in comparison with other graft combinations (Fig. 12). Using the Tongue approach method resulted in a better survival percentage over all rootstocks.

The grafting survival percentage is a criterion of how scion-rootstock compatibility. Increases in survival percentages are important due to grafting is labor-intensive and losses resulting from graft failure can represent significant economic loss. The results of this study show that the survival percentage was significantly influced by the grafting methods and rootstock type. According to, El-Semellawy (2005), found that both grafting methods and rootstocks had significant differences in callus area. The highest survival percentage may be attributed to the callus that was rapidly formed in both tissues of the stock and scion and filled the space between them (El-Semellawy, 2005). These findings are in conformity with those of by Yetisir and Sari, (2004), Heidari *et al.* (2011), Mohsen *et al.* (2012), Islam *et al.* (2013) and Farhadi *et al.* (2016) who observed that plant survivability and graft union integrity differ according to the rootstocks used. Moreover, Rojas and Riveros (1999) stated that grafting methods caused significant differences on plant survival rate. Furthermore, Abd El-Wanis *et al.* (2013) and Khankahdani *et al.* (2012) indicated that rapid and strong connect, and graft union area were affected significantly by different grafting methods.

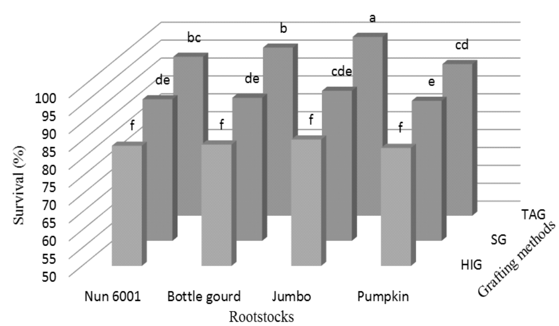

Fig. 12. Effect of different graft combinations on the survival percentage of grafted transplants of watermelon cv. Aswan F1 after 15 days from grafting. Columns with the same letter are not significantly different at the 0.05 % level of probability according to Duncan's multiple range test.

These results suggest that rootstock and grafting methods influence the structural development of the graft union formation. The good compatibility between scion and rootstock had quicker production of new vessel elements and the earlier connection of the callus and conducting rootstock and scion tissues. In conclusion, this study demonstrated that the highest grafting compatibility between watermelon cv. Aswan F1 and rootstock can be obtained using Jumbo rootstock with Tongue approach followed by Splice grafting method.

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دراسة تشريحية علي التطعيم في البطيخ

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أُجريت هذه الدراسة في مشتل الأمانة، مركز طلخا، محافظة الدقهلية، مصر خلال موسم الزراعة الصيفى ٢٠١٤ لدراسة تأثير التركيبات التطعيمية المختلفة على مدى التوافق بين الأصول والطعم لنبات البطيخ هجين أسوان. وشملت التجربة ثلاث عشرة معاملة نتجت من توليفات أربعة أصول (هجن جامبو و نـــن ٦٠٠١ واليقطين والقرع العسلى) وثلاث طرق تطعيم [الإيلاج بالحفرة (الثقب)، اللصق (الورقة الفلقية الواحدة) واللسانى] بالإضافة إلى النباتات غير المطعومة (الكنترول). اوضحت النتائج المتحصل عليها أن أعلى درجة توافق كانت بين البطيخ (الطعم) و أصول جامبو واليقطين ثم نـــن ٦٠٠١. حيث سجلت هذه التركيبات أعلى سمك لأنسجة الخشب واللحاء وأكبر مساحة للكامبيوم الوعائى مقارنة بالتركيبات التطعيمية الأخرى. وكذلك فقد اوضحت النتائج على الرغم من أن التطعيم باللصق (الورقة الفلقية الواحدة) أعطى أكبر مساحة إلتحام، إلا أن طريقة التطعيم اللسانى أظهرت أكبر عرض لمنطقة التطعيم والمرتبط بزيادة مساحة الأنسجة الوعائية. إلى جانب ذلك، أوضحت النتائج أن طرق التطعيم المختلفة لها تأثير مباشر على تكشف الأنسجة الوعائية الجديدة (الخشب واللحاء). حيث سجل التطعيم اللسانى أكبر مساحة و عدد لأوعية الخشب عند التطعيم على جامبو ثم يتبعه اليقطين ثم نـــن ٦٠٠١ ، بينما جاء القرع العسلى فى المرتبة الأخيرة. أوضحت النتائج المتحصل عليها أن النباتات المطعومة على أصل جامبو ثم اليقطين بطريقة التطعيم اللسانى حققت أعلى نسبة نجاح (١٠٠ و ٩٨% على التوالي) مقارنة بباقى التركيبات التطعيمية. بينما سجلت النباتات المطعومة على أصل القرع العسلى بطريقة الإيلاج بالحفرة (الثقب) أقل نسبة نجاح للشتلات (٨٢%). بصفة عامة، فإن تطور الكالس ونجاح التطعيم في نبات البطيخ صنف أسوان ١ يمكن أن يتم بإستعمال أصل جامبو مع استخدام طريقة التطعيم اللساني يليه طريقة اللصق (الورقة الفلقية الواحدة).