

### Micro-grafting of Florida prince peach cultivar

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**Abstract:** This work was an attempt to develop an *In vitro* technique for micro-grafting of Florida prince peach variety on Nemaguard or Okinawa peach rootstocks. Factors affecting micro-grafting technique success have been studied. Results indicated that MS media free of hormones and amino acids resulted in the maximum seeds germination (%). Inverted T cleft method was superior to surface ring vascular one regarding the micro-grafting success. Shoot tips  $\geq 0.5$  cm cultured on MS liquid media supplemented with 6% sucrose possessed the highest micro-grafting success values in comparison to other treatments.

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**Key words:** Micro-grafting, Peach (*Punus persica* L.), Florida prince.

#### 1. Introduction

Peach tree is one of the most important deciduous fruit grown in Egypt, while the harvested area reached about 33017 ha and produced 332487 tons (FAO, 2011). The extension of the cultivated area nowadays is due to its highly economic value, exporting potential and introducing new low chilling peach cultivars such as Florida Prince which is an early ripening variety and exhibited a high adaptation to the local environmental conditions. Therefore, it is considered as promising cultivar in Egypt (Shaltout, 1988). Micro-grafting is an *in vitro* technique that can be used to the placement of a meristem tip or shoot tip explants onto a decapitated rootstock that has been grown aseptically from seed or micro-propagated plants (Hartmann *et al.*, 2001). In Egypt, the demand of Egyptian's farmers for stone fruit rootstocks is usually met by importing them from abroad which costs the government high amounts of money. On the other hand, propagation of such rootstocks by cuttings is rather difficult and characterized by very low rooting success. Thus, production of good deciduous rootstocks is one of the most important objectives in horticulture industry. Grafting and budding of seedling rootstocks is the usual methods used for vegetative propagation (Yildirm *et al.*, 2010). Production of new xylem and phloem thus permits the vascular connection between the scion and rootstock (Aloni *et al.*, 2010). Micro-shoots that are difficult to root, or do not form roots at all *in vitro* can be micro-grafted on rootstocks to obtain rooted plants (Thimmappaiah *et al.*, 2002). *In vitro* micro-grafting has been reported in many plants such as almond (Channuntapipat *et al.*, 2003), *Prunus avium* L. (cherry var.) (Mohammad, 2006) and Citrus (Ali *et al.*, 2007). This technology has the potential for large scale of *Pyrus spp* production in a short period and presents several advantages and could offer serious opportunities for

rapid mass propagation for healthy plant materials and might be an efficient way for overcoming conventional peach propagation problems such as resolving the seasonal dependence for grafting.

The aim of this study was conducted to establish a micro-grafting protocol suitable for clonal propagation of true-to type mature Florida prince peach variety on some peach rootstocks.

#### 2. Material and Methods

**Preparation of rootstocks:** The experiments were carried out at the laboratory of plant tissue culture, Department of Horticulture, Faculty of Agriculture Al-Azhar University during the period from 2012 to 2013. The seeds of the two rootstocks namely; Nemaguard and Okinawa were chosen carefully to be free of infection by any seed born-pathogen and free of fungus. The endocarp of the seeds was removed, and then the seeds were put in sand mix beat moss in exchangeable layers at the refrigerator under 4°C for one month then soaked in GA<sub>3</sub> solution at 1000 ppm for six hours to breakdown its dormancy (Silva *et al.*, 2003). Thereafter seeds were sterilized with dipping in 70 % Ethanol for (1min) then washed in double sterilized distilled water (dsd) water (1x) and then immersed in 0.1% (HgCl<sub>2</sub>) solution for (3min) then immersed in 20% of Clorox Sodium hypochlorite solution (NaOCl) for 20 min then washed in dsd water (3x). Sterilized explants were cultured on different media as follows:

- 1-MS medium free of hormones and amino acids. (Control).
- 2-MS medium supplemented with BA at 0.5 mg / L
- 3-MS medium supplemented with BA at 1.0 mg / L
- 4-MS medium supplemented with BA at 1.0 mg / L and NAA 0.1mg / L
- 5-MS medium supplemented with adenine sulphate at 100 mg / L

6-MS medium supplemented with adenine sulphate at 150 mg / L

7-MS medium supplemented with Tyrosine at 100 mg / L. The pH was adjusted at 5.7. All media were sterilized by autoclaving at 121°C and 1.1 kg/cm for 20 min. Cultures were incubated under 25± 2°C in the growth room under darkness conditions. After two weeks, germinated seeds were selected as rootstock materials for *in vitro* grafting operation. The measurements were as follows: 1- Percentage of contamination 2- Percentage of germination and 3- Percentage of dead seeds.

#### Preparation of Scion:

Shoots of the scion 1-5 cm were collected from peach trees grown in greenhouse of Department of Horticulture, Faculty of Agriculture, Al -Azhar University. Explants were dipped in tap water with soap for 30 min., to eliminate dust then they transferred to the laminar flow where they firstly immersed in 70% ethanol solution for 30 sec., then dipped in 0.1% of mercuric chloride (HgCl<sub>2</sub>) solution for 3 min., then washed in distilled water (1x), thereafter sterilized by 10% sodium hypochlorite and 1% Tween 20 for 15 min, and then washed three to five times with sterile distilled water. Then axillary buds (1-5mm) were excised and cultured on MS medium supplemented with 1.0 mg / L of BA and 0.1 mg / L of IAA and incubated for four weeks at 27±2 °C with a 16 h photoperiod. Shoot tips of Florida prince peach cultivar which obtained through *in vitro* technique were used as micro-scions for *in vitro* grafting.

#### Micro-grafting procedure.

This experiment was carried out to study the effect of some factors such as size of shoot tips, state of the media, position of the shoot tips on the rootstock and sucrose on peach shoot-tip grafting *in vitro* (STG) success. Before grafting, all leaves of the scion of shoots except 2-3 leaf primordial were removed from the lateral shoots which were about 3-4 cm. The shoot tips were excised to be 1-1.5 cm in length then grafted on rootstocks. Different experiments were conducted to achieve the micro-grafting technique as follow.

Experiment 1): Effect of scion size on grafting success %:

Shoot tips with sizes from 1-15 mm were used to be grafted on rootstocks to test the suitable size.

Experiment 2): Effect of sucrose:

Sucrose at different concentrations (3%, 4.5% and 6%) was used to test the effect of sucrose on grafting success.

Experiment 3): Effect of kind of media.

Different kinds of media were used such as Liquid or semi-solid to test the effect of media on grafting success.

Experiment 4): Effect of scion position:

Ring vascular or inverted cleft T methods were tested to determine their effects on micro-grafting success. Cultures were incubated at 27±2 °C under darkness for 2 and 4 weeks then subjected to 16 hours photoperiod daily 1200 lux (cool-white florescent) for 2 weeks. The measurements as follows Leaves number. Shoot length (cm), Percentage of success and Percentage of death.

#### Statistical Analysis:

A complete randomized block design was followed and analysis of variance (ANOVA) was performed using two ways ANOVA. Co-stat software according to Stern (1991) was followed.

### 3. Results and Discussion

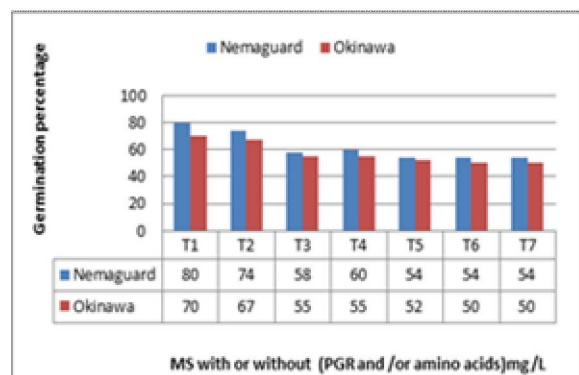
#### Micro-propagation of peach rootstock through seed culture

#### Effect of growth regulators and amino acids on seed germination percentage of Nemaguard and Okinawa peach rootstocks.

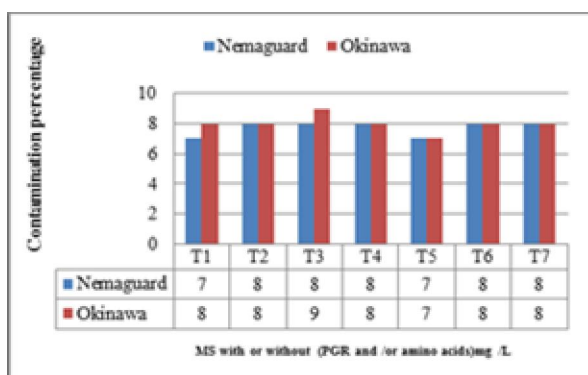
Data presented in Figs. 1 and 2 showed the effect of MS media supplemented with some growth regulators and some amino acids on *in vitro* seed germination of Nemaguard and Okinawa peach rootstocks. It was clear that the germination, contamination and death percentages as a parameters indicating the success of seed germination were enhanced by culturing on MS media. Data cleared that MS media free of PGR and amino acids significantly possessed the maximum values of seed germination percentage of both Nemaguard and Okinawa peach rootstocks followed by those cultured on MS media supplemented with BA at 0.5 mg / L, while the least seed germination percentage were gained when cultured on MS media supplemented with either adenine sulphate at 150 mg / L or MS plus Tyrosine at 100 mg / L. Results showed that Nemaguard rootstock was superior in this regard to Okinawa peach rootstock. Maximum contamination percentages were recorded when seeds were cultured on T4 where Nemaguard and Okinawa peach rootstock gained 8 or 9% respectively. Also results cleared that the minimum seed death percentage of the two rootstocks were recorded when cultured on MS media free of growth regulators and amino acids where Nemaguard peach rootstock gained 13% in comparison with Okinawa peach rootstock which possessed 24%. On the other hand, maximum dead seed percentages of the two peach rootstocks were recorded when cultured on MS media supplemented with adenine sulphate at 100 mg / L where Nemaguard recorded 39%, compared with Okinawa which possessed 41%. These results are in line with findings of (Can *et al.*, 2006) who found that the best seeds germination of Pistachio (*Prunus terebinthus*) obtained when cultured on MS media free

of hormones, while the maximum seeds germination rate of *Prunus mutica*, gained when cultured on MS media supplement with 0.5 mg / L BA. The increase in seed germination as cultured on MS media free of

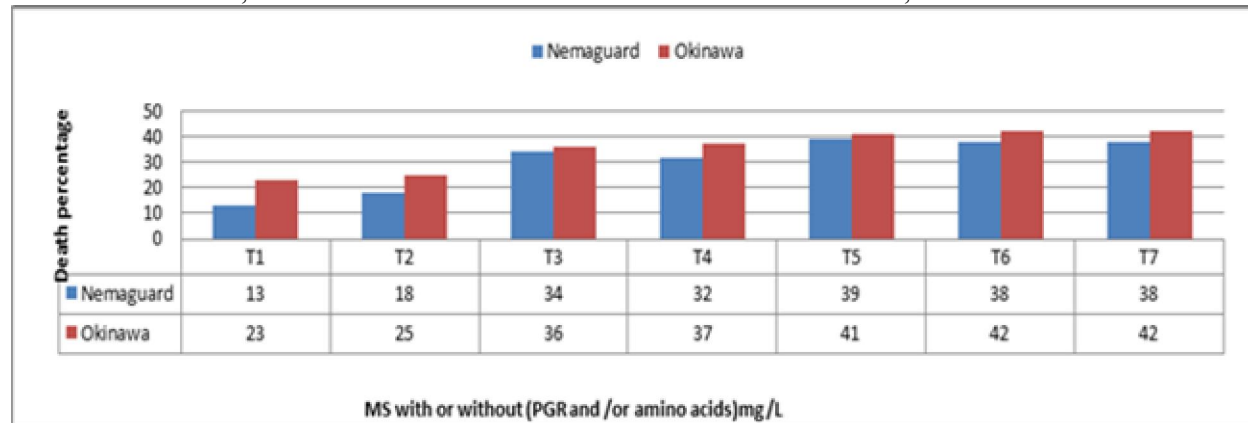
hormones might be due to that embryos do not need active substances such as hormones at this stage because they are at autotrophic stage (Raghavan., 2003).



LSD at 5% T = 4.29, R = 2.29 and T x R = 6.06



LSD at 5% T = 1.44, R = 0.77 and T x R = 2.04



LSD at 5% = T = 2.24, R = 1.19 and T x R = 3.17

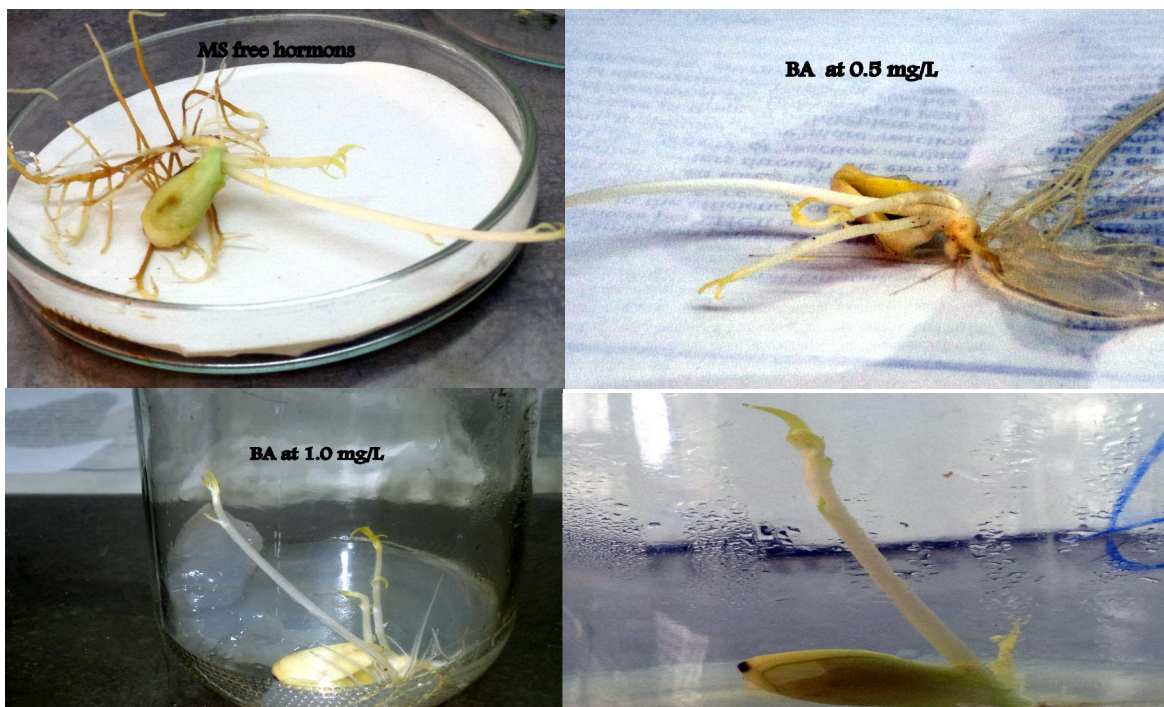
Fig. 1. The effect of growth regulators and amino acids on seed germination of some peach rootstock.

### Micro-grafting of Florida prince on some peach rootstocks

#### Effect of size of scion:

Data in in Fig. 3 cleared that all micro-grafting parameters such as micro-grafting percentage, shoot length, leaves number were enhanced by increasing the scion size of Florida prince peach variety to be bigger than 5mm where the data revealed that scion size at 6-15 mm possessed the maximum values in comparison with those of 1-5 mm ones. In most cases, Okinawa peach variety was superior to Nemaguard one. The results also cleared scion at 6-15 mm size caused the least values of death percentage in comparison to those of size 0.1- 5 mm. In this regard, Nemaguard peach rootstock possessed the least death percentage in comparison to those of

Okinawa which possessed the highest values. Data also revealed that the highest grafting death percentage values were recorded when using size at 0.1- 5 mm. These results are in agreement with that obtained by Hassanen (2013) who reported that the maximum percentage of micro-grafting success (83%) were obtained when scions of pear were higher than 0.5 cm length, while the scion with 0.5 cm long gave (37%) of successful pear micro-grafts. Increasing of micro-grafting success by increased size of scion may be due to the small size of shoot tip organ making problematic the excision, handling, grafting and subsequent maintains of grafted assembly and leading to the drying of shoot tips and low graft integration where larger size of shoot tip is making handling of grafting easier (Jonard, 1986).



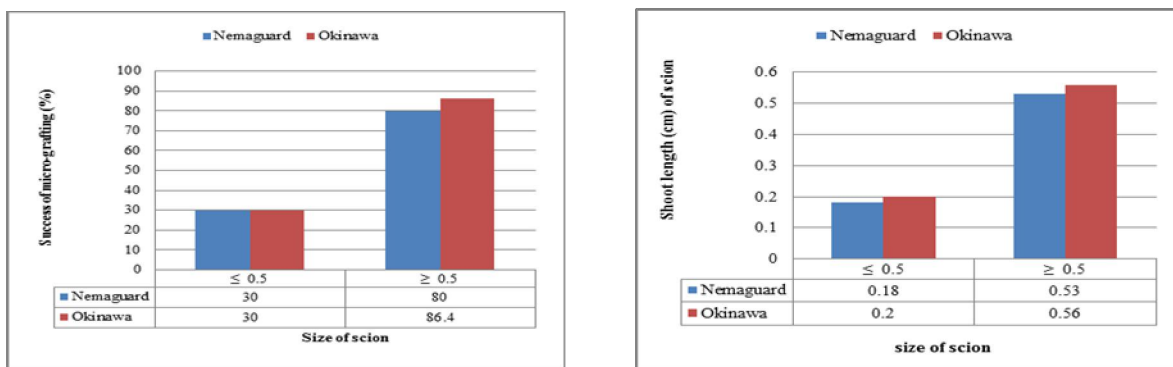
**Fig.2.** The effect of some growth regulators and amino acids on seed germination of the two peach rootstocks.

#### Effect of state of media:

Data in Fig. 4 indicated that MS media free of Agar (Liquid) were superior to those supplemented with Agar whereas micro-grafting success related of Florida prince possessed the highest values when cultured on Liquid MS media in comparison to the solid one. In this regard, Florida prince budded on Okinawa rootstock possessed higher values of success (80%) then those of budded on Nemaguard which possessed the lowest values (70.6%). Data also revealed that minimum death percentage in micro-grafting was obtained when Florida prince peach variety budded on both rootstocks cultured on Liquid MS media. Florida prince micro-grafted on Okinawa rootstock possessed the least values of death percentage (20% in comparison to those budded on Nemaguard which gained (29.4%). Culturing micro-grafts on MS media supplemented with Agar increased in general micro-grafting death percentage since it reached (73.3% and 70 %) for Florida prince budded on Nemaguard and Okinawa rootstocks respectively. These results are in agreement with that obtained by **Toma and Mosleh (2010)** who found that using liquid medium raised successful grafts percentage to 60 and 70% from only 10% in case of solidified media with agar for both apple and pear micro-grafts, respectively. Liquid medium was easy to culture micro -grafted seedling without disturbance and damage to micro-grafts and better availability and absorption of nutrients (**Thimmappaiah et al., 2002**).

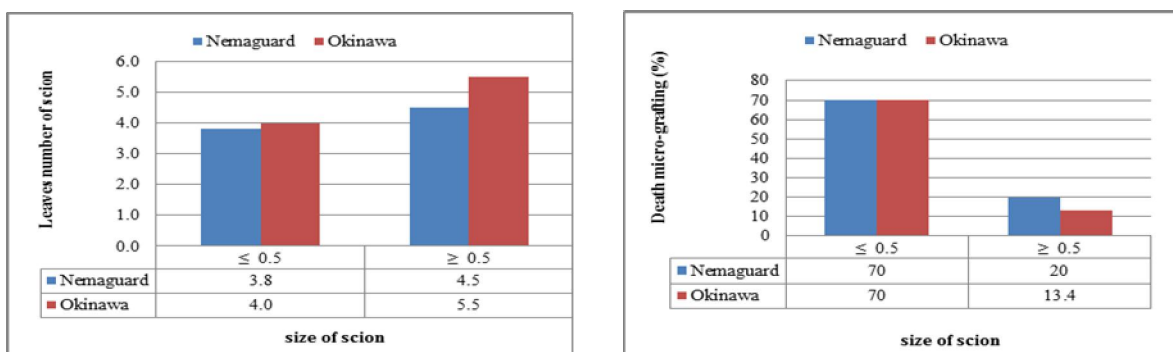
#### Effect of scion position

The data presented in Fig. 5 showed that, the two tested scion position (inverted cleft T and ring vascular surface methods), enhanced the micro-grafting parameters of Florida prince peach cultivar budded on Nemaguard and Okinawa peach rootstocks. The results reported that using Inverted cleft T Micro-grafting method of scion caused the highest values of micro-grafting success percentage in comparison to those of ring vascular surface one. In this regard, Florida prince budded on Okinawa peach rootstock was superior to those budded on Nemaguard Rootstock. Least grafting success percentage values were recorded when using surface ring vascular method in comparison to those grafted using inverted cleft T method. Data also, reported that using Inverted cleft T method caused the least values of death micro-grafting percentage in comparison to those grafted using ring vascular surface method. Florida prince budded on Nemaguard peach rootstock was superior to those budded on Okinawa rootstock. These results are in agreement with those of (**Toma and Mosleh, 2010**) who reported that there were 80 and 90% of successful micro-grafts when Anna budded on MM106 apples and Aly-Sur budded on *P. calleryana* pears, respectively when T budding method was used while lower success of 70% in both apple and pear was observed when scion was grafted by surface placement using cleft grafting.



LSD at 5% T = 2.86, R = 2.86 and T x R = 4.05

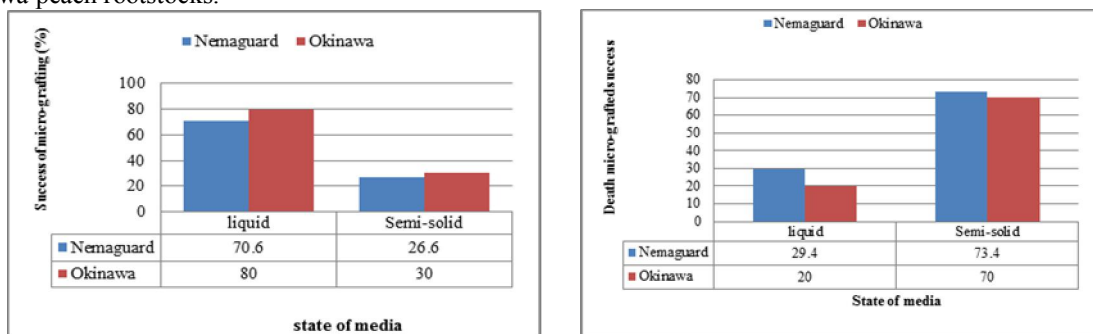
LSD at 5% T = 0.12, R = 0.12 and T x R = 0.17



LSD at 5% T = 0.78 R = 0.78 and T x R = 1.10

LSD at 5% T = 2.86, R = 2.86 and T x R = 4.05

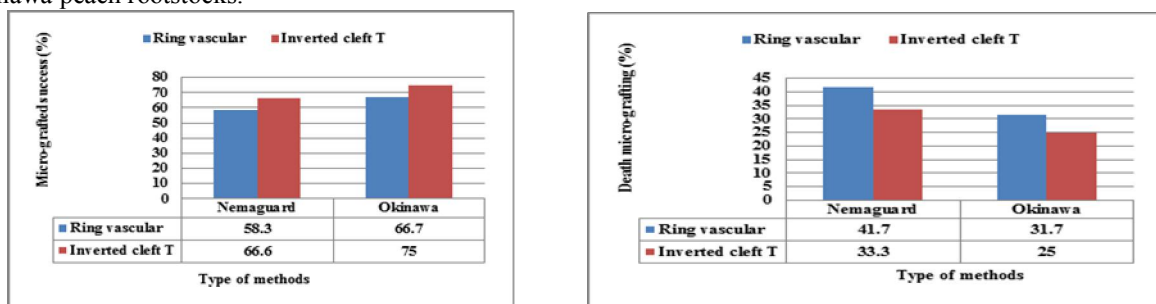
**Fig. 3.** The effect of scion size on some micro-grafting parameters of Florida prince budded Nemaguard and Okinawa peach rootstocks.



LSD at 5% T = 3.19, R = 3.19 and T x R = 4.51

LSD at 5% T = 3.19, R = 3.19 and T x R = 4.51

**Fig. 4.** Effect of the state of media on some micro-grafting parameters of Florida prince budded on Nemaguard and Okinawa peach rootstocks.



LSD at 5% T = 3.13, R = 3.13 and T x R = 4.43

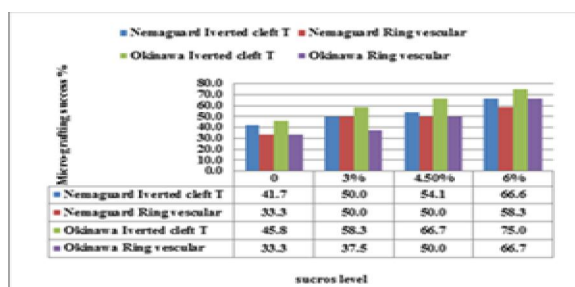
LSD at 5% T = 3.13, R = 3.13 and T x R = 4.43

**Fig. 5.** The effect of scion position on some micro-grafting parameters of Florida prince budded on Nemaguard and Okinawa peach rootstocks

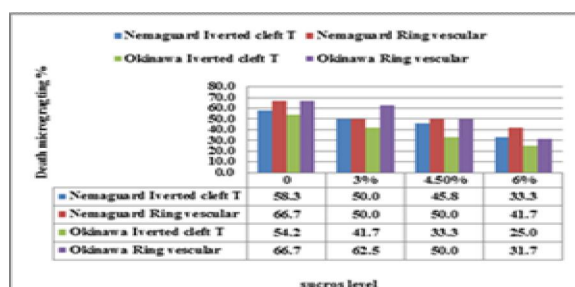
**Effect of sucrose**

The data presented in Fig. 6 showed the effect of different sucrose concentrations on some micro-grafting parameters such as micro-grafting percentage, shoot length, leaves number as well as death percentage. In general, all tested sucrose concentrations enhanced micro-grafting parameters of Florida prince peach cultivar budded on Nemaguard and Okinawa peach rootstocks in comparison to those of control treatment except death percentage. The results also, cleared that MS media supplemented with 60 g / L of sugar caused the highest values of micro-grafting success percentage in comparison to those cultured on MS media supplemented with other sugar concentrations or control. In this regard, Florida prince budded on Okinawa peach rootstock was superior to those budded on Nemaguard rootstock. Data also revealed that the least grafting success percentage values were recorded when micro-grafted plants were cultured on MS media free of sucrose. MS media supplemented with 60 g/L of sugar possessed the highest values of both shoot length (cm) and leaves number of Florida prince budded on either Nemaguard or Okinawa rootstocks in comparison to those cultured on MS media supplemented with other sugar concentrations or control. No- significant difference between the two tested rootstocks was noticed when the media were supplemented with sugar at any concentration

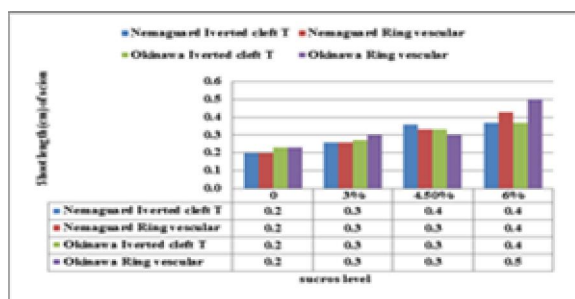
used. Data also revealed that the least shoot length values were recorded when micro-grafted plants were cultured on MS media free of sucrose. The results also, ensured that MS media free of sugar caused the highest values of micro-grafting death percentage of Florida prince budded on either Nemaguard or Okinawa rootstocks in comparison to those cultured on MS media supplemented with other sugar concentrations. Florida prince budded on Nemaguard peach rootstock possessed higher death percentage than those budded on Okinawa rootstock. Data also revealed that the least micro-grafting death percentage was recorded when micro-grafted plants were cultured on MS media supplemented with sugar at 60 g / L. Our results are in conformity with those of (Anderson and Byrne, 2001) who cleared that increasing concentration of sucrose increased survival percentage of micro-grafts. Many other workers have also used sucrose at 7.5 % for growing micro-grafted seedlings (Vijaykumari and Singh, 2000; Kobayashi et al., 2003). However (Thimmappaiah et al., 2002) claimed that no beneficial effect of increasing sucrose concentration. Sugar added in culture medium does not only act as a carbon source but also plays a role in osmotic regulation of water stress, however, increasing the concentration of sucrose (0.2-0.3 M) and sorbitol (0.3 M) could enhance phenolic compound formation. (Aslan and Sompong 2005).



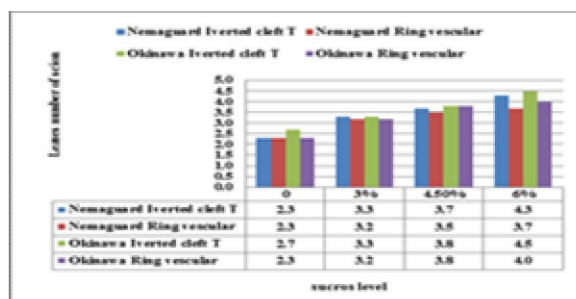
LSD at 5% T = 9.35, R = 6.61 and T x R = 13.22. (Cleft T)  
LSD at 5% T = 1.74, R = 1.23 and T x R = 2.47. (Ring)



LSD at 5% T = 9.35, R = 6.61 and T x R = 13.22  
LSD at 5% T = 1.74, R = 1.23 and T x R = 2.47

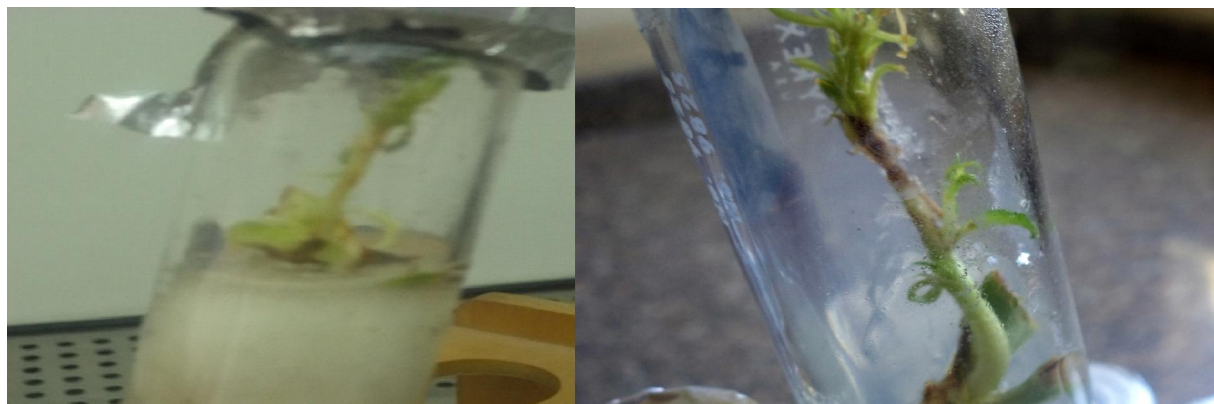


LSD at 5% T = 0.06, R = 0.04 and T x R = 0.09. (Cleft T)  
LSD at 5% T = 0.06, R = 0.04 and T x R = 0.09. (Ring)



LSD at 5% T = 0.62, R = 0.44 and T x R = 0.88  
LSD at 5% T = 0.56, R = 0.39 and T x R = 0.80

Fig.6. Effect of sucrose on some micro-grafting parameters of Florida prince budded on Nemaguard and Okinawa peach rootstocks using different grafting methods.



**Fig. 7:** complete micro-grafted plants of Florida prince budded on peach rootstocks

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