

Effect of Pre-Grafting Incubation and Grafted Cuttings Position on Grape Grafting Success

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THIS EXPERIMENTS was carried out during two successive seasons 2015 and 2016 at the nursery of pomology department, Faculty of Agriculture, Cairo university, Egypt. The study was aimed to investigate the effect of two factors namely *i.e.*, pre-grafting incubation and grafting materials position during callusing on grafting success of flame seedless grapes onto Salt Creek rootstock. Callusing was carried out in three positions *i.e.*, horizontal, upright and upright down either with or without pre-grafting incubation. The results showed that either pre-grafting incubation or callusing at upright position increased significantly callus percent and degree, sucker number, rooting rate, bud break percent during callusing period and grafting success percent. The interaction, between treatments revealed that callusing at upright position accompanied with pre-grafting incubation gave the best results in this respect. It can be concluded that callusing at upright position either with or without pre-grafting incubation increased grafting success significantly compared to callusing at horizontal or upright down position. Chemically, higher grafting success is accompanied with lower phenols, reducing sugars and total indoles. It may be concluded that there is no antagonistic effect between root and callus formation in grafted cuttings. Also, upright position for incubation the grafted cuttings are better than pre-grafted incubation in increasing grape grafted cuttings.

Keywords: Callus, Propagation, *Vitis*.

Grapevine (*Vitis vinifera*) is one of the most popular fruit in Egypt with total harvested area of 64,253 Ha and a total production 21.62 ton/ha (FAO, 2016). For safety agriculture Flame seedless, one of the most exported grape variety in Egypt due to early and high production (Hegazi and Omar, 2000 and Diab *et al.*, 2009), is. Grafted on rootstocks like Salt Creek with both nematodes resistance (Mckenry and Anwar, 2006) and salt tolerance (Walker *et al.*, 1997).

It has recently demonstrated that bench grafting technique by using directly recently dormant wood of both scion and rootstocks reduce the time for transplants production to only 4 months (Halbrooks, 1985). This technique with increasing grafting success percent led to lowering the costs for transplants production. So, the grafted cutting needs to formation rooting and grafting union together, which needs for more care. So, Preparing grafts material prior to grafting either by storing it in cold environment in moist sawdust (Porebski

et al., 2002), or storing it at low temperature before grafting (Ferhato1997, Achim and Botu, 2001), or forcing it at 26 to 28°C for 2-3 days (Lantos, 1990 and Ozkanand Gumús, 2001) or finally, soaking scion wood in running water for several hours (Erdogan, 2006 and Halbrooks, 1985), has a vital role for callus formatio position. While in grafted walnut seedling should be placed uprightly (Zachej, 1976). in Egypt, many nursery prefer to callusing their grafts cuttings in a horizontal position to avoid more rooting and to be easier for planting in pots.

There are many factors involved in the grafting success such as the rooting ability of the rootstock, type of graft, fungal infection of cut surfaces and particularly callus formation (Halbrooks, 1985). Callus formation is a key factor in the development of the graft union and subsequent grafting success (Phillips *et al.*, 2015). Carbohydrates, plant hormones (Vrsic *et al.*, 2015 and Hartmann *et al.*, 2002), low amount of phenols and high amount of indoles are required for grafted material at grafting unioneearly for successful callus formation to ensure grape grafting success (Stino *et al.*, 2011). Moreover, degree of grapes incompatibility are not well known and are often ascribed to inability for callusing, root formation, budding, and growth during callusing (Hunter *et al.*, 2004).

In order to know how pre-grafting incubation and grafting material position during callusingaffecting callus formation and grafting success the following experiment was done

Materials and Methods

The exprement was carried out during two successive seasons 2015 and 2016 at the nursery of the Pomology Department, Faculty of Agriculture, Cairo university, Egypton 'Flame seedles's (*Vitis vinifera*) grapevines grated into Salt creek (*Vitis champanii*) rootstock. Position Dormant cuttings of the scion and rootstock were taken at January in both years and stored at 5°C. t mid of FebruaryScions were cut to leave one bud, while rootstocks were cut to leave three ones.The treatments includedcallusing after grafting in three positions: horizontal, upright and upright down with or without pre-grafting horizontal incubationat 27°C for one week. Bench graft technique consisted of cleft grafting using parafin wax after wrapping, then placed into callusing boxes containing peat: sawdust mixture (1:4 ratio) at 27°C and relative humidity up to 90% for 4 weeks.After incubation grafts were planted out in greenhouse.with 75 grafting material plantsin three replicates were used for each of the six treatments.

At the end of callusing period, the percent of callused grafts (number of callusedgrafts/ total number of grafted cuttings), callusing degreearing scale0, 1, 2, 3 and 4 for callusing percent at grafting point ,i.e. no callusing, 1-25%, 26-50%, 51-75% and 76-100%, respectively (Kose and Guleryuz, 2006 and Paunovic *et al.*,2011). Sucker number were counted and then removed, bud break percent (total number of breaked scion bud /total number of grafts), root

weight (g) and rooting rate (number of rooted grafts/ total number of grafted plants) were determined according to Kamiloglu and Tangolar (1997). After two months of growth under green house suckers number were counted then removed weekly, percent of grafting success (number of succeeded grafts / total number of grafts cuttings) and plant weight (gm) were determined.

Chemical analysis includes total indoles content according to Larsen *et al.* (1962), total phenols according to Singleton *et al.* (1999) and reducing sugars according to Dubois *et al.* (1956) in one gram fresh weight of scion and rootstock buds immediately after finishing the callusing period.

Data were analyzed of variance (ANOVA) according to Snedecor and Cochran (1989) using MSTAT-C statistical package (Freed *et al.*, 1990) software, and means of the treatments were compared by Least Significant Difference (L.S.D.) according to Duncan (1955) at significance level of 0.05.

Results

Callus percent

Concerning the effect of pre-grafting incubation on callus percent the data in table 1 indicated that pre-grafting incubation increased it in both seasons with a significant value in the first one.

With regard to the effect of position of grafting material in incubation room on plant callus formation percent, the data indicated that incubation of grafted plant materials in upright direction increased it in the two seasons beside horizontal position in the second season with a significant effect than upright down position.

With respect to the interaction, it is cleared that pre-grafted incubation accompanied with upright position recorded the highest significant callusing percent, followed by pre-grafting incubation in horizontal position.

TABLE 1. Effect of pre-grafting incubation and position of grafted materials during callusing on callus percent of grafted 'Flame seedless' grapevines.

pre-grafting	position during callusing 2015			Mean
	Horizontal	Upright	Upright down	
no incubation	83.80 c	90.40 b	53.47 d	75.889
incubation	91.67 b	95.40 a	89.33 b	*92.133
Mean	87.73 B	92.90 A	71.40 C	
2016				
no incubation	92.13 b	87.07 c	71.80 d	83.667
incubation	94.47 b	99.00 a	91.67 b	95.044
Mean	93.30 A	93.03 A	81.73 B	

Callus degree

Concerning the effect of pre-grafting incubation on callus degree the data in Table 2 indicated that, pre-grafting incubation increased it significantly in both seasons. With regard to the effect of grafting material position, the data indicated that incubation of grafted plant material in upright position increased it significantly in the two seasons compared to the other treatments.

TABLE 2. Effect of pre-grafting incubation and grafted material position during callusing on callus degree of grafted 'Flame seedless' grapevines.

pre-grafting	position during callusing 2015			Mean
	Horizontal	Upright	Upright down	
no incubation	1.400 e	2.520 b	1.617 d	1.846
incubation	1.317 f	2.817 a	2.403 c	**2.179
Mean	1.358 C	2.668 A	2.010 B	
2016				
no incubation	2.000 c	1.600 d	1.000 e	1.533
incubation	2.333 b	3.533 a	2.400 b	*2.756
Mean	2.167 B	2.567 A	1.700 C	

With respect to the interaction, it is cleared that pre-grafted incubation accompanied with upright incubation callus degree recorded the highest significant treatments.

Sucker number after callusing

The effect of pre-grafting incubation at 27C on total sucker number after callusing is presented in Table 3. It indicates that this treatment increased it significantly in both seasons.

With regard to the effect of grafting material position during callusing, the data indicated that incubation of grafted plant material in upright position increased sucker numbers with a significant effect in the two seasons compared to the other two positions.

With respect to the interaction, it is cleared that pre-grafted incubation combined with upright position recorded the highest significant sucker numbers followed by upright incubation position with no pre-grafting incubation.

Rooting rate percent

The data in Table 4 show the effect of pre-grafting incubation on rooting rate. Pre-grafting incubation increased rooting rate with a high significant values in both seasons.

With regard to the effect of grafting material position, the data indicated that incubation of grafted plant material in upright position increased rooting rate in the two seasons with a significant effect than the other positions.

TABLE 3. Effect of pre-grafting incubation and grafted material position on suker number of grafted flame seedless grapevines after callusing.

pre-grafting	position during callusing 2015			Mean
	Horizontal	Upright	Upright down	
no incubation	7.000 d	12.06 b	5.443 e	8.167
incubation	8.443 c	15.56 a	8.557 c	*10.852
Mean	7.722 B	13.81 A	7.000 C	
2016				
no incubation	1.490 d	5.443 b	0.4333 e	2.456
incubation	3.057 c	9.943 a	2.443 c	**5.148
Mean	2.273 B	7.693 A	1.438 C	

Values of the interaction cleared that pre-grafted incubation accompanied with upright and upright down in the two seasons besides up right position with or without incubation in the second season recorded the highest significant values compared to the other treatments.

TABLE 4. Effect of pre-grafting incubation and grafted material position on rooting rate percent of grafted 'Flame seedless' grapevines after callusing.

pre-grafting	position during callusing 2015			Mean
	Horizontal	Upright	Upright down	
no incubation	74.80 c	85.27 b	47.07 d	69.044
incubation	84.33 b	99.33 a	95.73 a	**93.133
Mean	79.57 B	92.30 A	71.40 C	
2016				
no incubation	81.47 c	93.07 a	57.00 d	77.178
incubation	79.13 c	93.67 a	87.20 b	*86.667
Mean	80.30 B	93.37 A	72.10 C	

Root weight

The effect of pre-grafting incubation on root weight after callusing is presented in Table 5. It indicated that pre-grafting incubation increased root weight with a significant value in the first season.

With regard to the effect of grafting material position, the data indicated that incubation of grafted plant material in upright position and in horizontal position significantly increased root weight in the first and second season, respectively.

Values of the interaction cleared that pre-grafted incubation accompanied with upright position and horizontal position in the first and second season, respectively recorded the highest significant values compared to the other treatments.

TABLE 5. Effect of pre-grafting incubation and grafted material position on root weight 4of grafted 'Flame seedless' grapevines after callusing.

pre-grafting	position during callusing 2015			Mean
	Horizontal	Upright	Upright down	
no incubation	1.700 de	1.967 bc	1.567 e	1.744
incubation	2.133 b	2.400 a	1.800 cd	*2.111
Mean	1.917 B	2.183 A	1.683 C	
2016				
no incubation	2.607 b	2.120 c	0.4533 f	1.727
incubation	2.913 a	1.253 d	0.8600 e	1.676
Mean	2.760 A	1.687 B	0.6567 C	

Bud break percent

Data in Table 6 indicated that pre-grafting incubation increased the scion bud break percent significantly in both seasons.

TABLE 6. Effect of pre-grafting incubation and grafted material position on scion bud break percent of grafted 'Flame seedless' grapevines after callusing.

pre-grafting	position during callusing 2015			Mean
	Horizontal	Upright	Upright down	
no incubation	22.27 d	32.40 c	18.00 e	24.222
incubation	36.67 b	74.20 a	9.867 f	**40.244
Mean	29.47 B	53.30 A	13.93 C	
2016				
no incubation	32.73 b	31.53 b	7.167 d	23.809
incubation	33.40 b	64.57 a	11.20 c	**36.389
Mean	33.07 B	48.05 A	9.183 C	

With regard to the effect of grafting material position, the data indicated that incubation of grafted plant material in upright position increased significantly the bud break percent followed by horizontal then upright down position in both seasons.

With respect to the interaction, it is cleared that pre-grafted incubation accompanied with upright incubation position recorded the highest significant percentage of bud break followed by horizontal position with pre-grafted incubation.

Sucker number after planting

The effect of pre-grafting incubation on sucker number after planting is shown in Table 7. The data indicated that pre-grafting incubation increased sucker number with a significant value in the second season.

With regard to the effect of grafting material position, the data indicated that incubation of grafted plant material in upright down position increased sucker number in the two seasons with a significant effect than the other two positions.

As for the interaction, it is cleared that pre-grafted incubation accompanied with upright down position recorded the highest significant values in the first season.

TABLE 7. Effect of pre-grafting incubation and grafted material position on sucker number of grafted 'Flame seedless' grapevines after planting.

pre-grafting	position during callusing 2015			Mean
	Horizontal	Upright	Upright down	
no incubation	8.277 e	16.94 b	10.98 d	12.066
incubation	7.557 e	12.04 c	20.44 a	13.348
Mean	7.917 C	14.49 B	15.71 A	
2016				
no incubation	21.89 d	19.89 e	19.44 f	20.408
incubation	26.89 b	24.89 c	34.55 a	**28.778
Mean	24.39 B	22.39 C	27.00 A	

Plant weight

Concerning the effect of pre-grafting incubation on plant weight, the data in Table 8 indicated that pre-grafting incubation increased it with a significant value in the first seasons.

As for the effect of grafting material position, the data indicated that incubation of grafted plant material in upright position increased it significantly in both seasons compared to the other positions.

With respect to the interaction, it is cleared that pre-grafted incubation accompanied with upright incubation position recorded the highest significant values followed by upright position with no pre-grafting incubation.

TABLE 8. Effect of pre-grafting incubation and grafted material position on plant weight of grafted 'Flame seedless' grapevines.

pre-grafting	position during callusing 2015			Mean
	Horizontal	Upright	Upright down	
no incubation	23.44 cd	27.27 b	21.42 d	*24.046
incubation	24.13 c	31.33 a	22.30 cd	25.919
Mean	23.78 B	29.30A	21.86 C	
2016				
no incubation	23.38 c	34.56 a	28.35 b	28.763
incubation	25.07 c	34.67 a	29.86 b	29.863
Mean	24.22 C	34.61A	29.10 - B	

Grafting success percent

Concerning the effect of pre-grafting incubation on grafting success, the data in Table 9 indicated that pre-grafting incubation increased it significantly in both seasons.

TABLE 9. Effect of pre-grafting incubation and grafted material position on grafting success of grafted 'Flame seedless' grapevines.

pre-grafting	position during callusing 2015			Mean
	Horizontal	Upright	Upright down	
no incubation	45.12 d	70.50 a	37.60 e	51.073
incubation	67.93 b	70.58 a	48.28 c	**62.266
Mean	56.53 B	70.54 A	42.94 C	
2016				
no incubation	59.00 b	70.53 a	50.27 c	59.933
incubation	69.27 a	70.93 a	57.07 b	**65.756
Mean	64.13 B	70.73 A	53.67 C	

With regard to the effect of grafting material position, the data indicated that incubation of grafted plant material in upright position increased the success of grafts highly significant in both seasons compared to the other positions.

The interaction cleared that, upright incubation position recorded the highest significant values either with or without pre-grafting incubation in both seasons as well as pre-grafted in horizontal position with pre-grafting incubation in the second season.

Total indoles content

From the data shown in table 10 it can be found that, generally, scion contain more total indoles than rootstock. Also, incubation pre-grafting decreased significantly indoles content in rootstocks.

With regard to the effect of grafts position it can be found that, upright position during callusing recorded the highest total indoles in scion and rootstock beside the scion in horizontal positions.

With respect to the interaction the data showed that upright position recorded the highest indoles content, while horizontal accompanied with pre-grafting incubation recorded the lowest content.

Total phenols content

The effect of pre-grafting incubation and scion position during callusing on total phenols is shown in Table 11. Pre-grafting incubation increased significantly phenols content especially in rootstock. It can be observed that scion contain more phenols than rootstock.

TABLE 10. Effect of pre-grafting incubation and position of grafted materials after callusing on total indole content of grafted 'Flame seedless' grapevines.

pre-grafting	Flame seedless scion mg/L FW			Mean
	Horizontal	Upright	Upright down	
no incubation	5.617 ab	4.127 bc	7.630 a	5.791
incubation	4.853 bc	2.393 c	4.300 bc	n.s.3.849
Mean	5.235 A	3.260 B	5.965 A	
Salt Creek rootstock				
no incubation	2.117 b	2.080 b	8.080 a	*4.092
incubation	3.053 b	3.157 b	1.283 b	2.498
Mean	2.585 B	2.618 B	4.682 A	

With regard to the effect of grafts position, it can be found that, upright position during callusing recorded the highest total phenols either in scion or rootstock beside the rootstock in horizontal positions, while the upright positions recorded the lowest content.

With respect to the interaction the data showed that upright position recorded the highest scion and rootstock indoles beside horizontal rootstock a combined with pre-grafting incubation.

TABLE 11. Effect of pre-grafting incubation and position of grafted materials after callusing on total phenols content of grafted 'Flame seedless' grapevines.

pre-grafting	Flame seedless scion mg/L FW			Mean
	Horizontal	Upright	Upright down	
no incubation	43.35 cd	39.32 d	48.69 abc	43.788
incubation	46.79 bc	51.05 ab	53.81 a	*50.55
Mean	45.07 B	45.18 B	51.25 A	
Salt creek rootstock				
no incubation	25.16 b	27.14 b	34.91 b	29.071
incubation	34.52 b	50.47 a	56.83 a	**47.274
Mean	29.84 B	38.81 AB	45.87 A	

Reducing sugars content

The effect of pre-grafting incubation and scion position during callusing on reducing sugars content shown in Table 12. Pre-grafting incubation increased reducing sugars content highly significant in rootstock. It can be observed that scion contain more reducing sugars content than rootstocks.

With regard to the effect of grafts position, it can be found that, upright and horizontal position during callusing recorded the highest sugars content in scion and rootstock.

With respect to the interaction the data showed that pre-grafting incubation accompanied with horizontal position recorded the highest scion and rootstock value.

Generally scion contains more sugars, phenols and indoles than rootstocks. This may be due to the more scion activity than rootstocks and it may help more in scion break and building the grafting union.

TABLE 12. Effect of pre-grafting incubation and position of grafted materials after callusing on reducing sugars content of grafted 'Flame seedless' grapevines.

pre-grafting	Flame seedless scion mg/L FW			Mean
	Horizontal	Upright	Upright down	
no incubation	354.0 c	287.9 c	435.7 ab	359.189
incubation	314.9 c	456.8 a	361.7 bc	n.s.377.822
Mean	334.5 B	372.4 AB	398.7 A	
Salt creek rootstock				
no incubation	160.8 d	182.0 cd	265.2 bc	202.667
incubation	222.2 cd	355.5 a	320.4 ab	**299.4
Mean	191.5 B	268.8 A	292.8 A	

Discussion

The results showed that pre-grafting incubation together with upright incubation position during callusing processing increased callus degree and percent which results in increasing scion bud break and rootstock rooting then grafting success. Since according to Rongting & Pinghai (1993 scion moisture may be positively correlated with callus formation and growth these increases and the consequently grafting success can be explained by their effect on grafts moisture. Its effect may be similar to those obtained by hot water treatments as soaking scion wood in water for 12 hr prior to grafting ensures hydration of the plant tissues necessary for vigorous shoot growth and also, increased total sugars soluble sugars while decreased starch, total carbohydrates in some grapevine rootstocks (Phillips *et al.*, 2015).

The positive effect of the upright position during callusing is in line with the observations of Zachej (1976) indicating that walnut trees should be positioned uprightly. This was mostly for practical reasons throughout the callusing period easier. Also, Porebski *et al.* (2002) developed a hot callus system in Poland that was fixed on a glasshouse table in an upright position. Moreover, Exadaktylou *et al.* (2009) found that Cuttings placed vertically gave the best rooting percentage of Cherries cuttings compared to horizontal, Angle and inverted position. Similar results reported by Tsipouridis *et al.* (2005). It may be for a correlation between polarity and both of root differentiation, and auxin movement (Hartmann *et al.*, 2002). Moreover, Polarity reversal resulted in

decreasing rooting percentage of hardwood cuttings of 'Kober 5BB' grapevine rootstock up to 60% (Cristoferi *et al.*, 1988).

The reasons for their effects may be due to this is the right trend for sap water inside plant, stimulate and fasting growth of grafting material which may be shown on the higher number of sucker specially than the upright down position. This reflects in early induction of scion bud break with a good amount of rooting due to a good and early connection between rootstocks and scion.

Increasing callus degree and percent (Tables 1 and 2) in grafting union helps more in increasing bud breaking percent as in Table 6 which may be considering an indicator for grafting success. Since, inflated buds of scion synthesize auxin and gibberellin hormones which accumulate at the cut injured surfaces of the graft union which activate cambium tissue for callus induction and formation beside accelerating differentiation and building transport tissues between scion and rootstock (Rongting and Pinghai, 1993). Callus tissue mostly comes from scion when the graft partners are of equal (Copes, 1969, Stoddard & McCully, 1980 and Tiedemann, 1989). This may be explained that the more scion bud break associated with the formation and building of union grafting.

Movement of auxins is predominantly in basipetal direction (Bleasdale 1984, Rost *et al.*, 1984 and Salisbury & Ross 1992). As for the relationship between callus formation and rooting of grafted cuttings, the present results showed a positive correlation found between rooting and callusing in grafting cuttings. since stimulating rooting and suckering of rootstock stimulated callus formation and scion bud break subsequently grafting success (Arteca 1984 and Salisbury and Ross 1992).

The positive effect of the rootstock in the grafting success may be explained because of the rootstock has the ability to produce roots, suckers induced, grafting union and inducing scion bud break with a positive correlation between them during callusing period without competition which may be responsible for run out of rootstock growth promoters. So the highest rootstock stimulation the highest grafting success during callusing period.

In addition roots of rootstock may be acts as source of cytokines, which make a balance between it and exogenous auxins, applied for rootstock, for calluses induction (Nogueira, 2007).

The lower indoles content and the increase of phenol and sugars contents accompanied with higher grafting success in upright position with similar trend to pre-grafting can be explained by a depletion of reducing sugars, indoles concomitant with the increase of reducing sugars and phenols compounds in the growth processes of root formation, callus formation and start of bud break and growth either in scion or rootstock. Also, the grafting success may depend on the balance between indoles, phenols and sugars.

Furthermore, the effect of phenolic compound on rooting is complicated and varying according to the concentration, phenols type and rooting stage. Low phenolic concentrations may stimulate adventitious root formation and grafting success (Stino *et al.*, 2011), while higher concentrations may restrict them (Wilson and Van Staden 1990). On the other hand, Somkuwar *et al.* (2011) reported that high levels of phenols in grape rootstock cuttings at planting may be correlated with the formation of root primordia and stimulate the elongation of the roots. With respect to the phenols type, some phenolic compounds had a positive effect such as phloroglucinol and chlorogenic acid on rooting (Wilson and Van Staden, 1990, Wang 1991 and Shiozaki *et al.*, 2013). Also, gallic acid found in 'Campbell Early' cuttings which may be prevent IAA oxidation. Moreover, Catechin and gallic acid had a positive effect due to prevent peroxidase-catalyzed auxin oxidation (Osterc *et al.*, 2008)

Conclusion

From this study, it can be concluded that there is no antagonistic effect between formation of rooting and grafting union in grape grafted cuttings, since upright position recorded the highest callus percent and degree together with the highest rooting percent. Also, the upright position surpassed pre-grafting incubation in increasing grape grafted cuttings success.

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تأثير التحضين قبل التطعيم وإتجاه العقل المطعومة على نسب نجاح التطعيم في العنب

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أجريت هذه التجربة خلال موسمى ٢٠١٥ و ٢٠١٦ فى المشتل التابع لقسم الفاكهة بكلية الزراعة جامعة القاهرة، مصر. وكانت تهدف هذه التجربة لدراسة عاملى التحضين قبل التطعيم، وإتجاه العقل المطعومة أثناء التحضين، وذلك على نسب نجاح تطعيم صنف العنب الفليم سيدليس (Falme Seedless) على أصل السولت كريك (Salt Creek). حيث تم تخزين العقل المطعومة فى ثلاثة إتجاهات وهى الأفقى، والرأسى، والمقلوب، وذلك مع عامل التحضين أو بدون التحضين قبل التطعيم. وقد أشارت النتائج إلى أن التحضين قبل التطعيم أو وضع العقل المطعومة فى الإتجاه الرأسى أثناء التحضين زاد معنوياً كلا من نسبة التكليل، ودرجته، وعدد السرطانات، ومعدل التجذير، ونسبة تفتح البراعم أثناء التحضين، ونسبة التطعيم. والتأثير المتداخل بين العاملين أوضح أن التحضين قبل التطعيم، مع التحضين فى الوضع الرأسى أعطى أفضل النتائج فى هذا الصدد. كما يمكن استنتاج أن التحضين فى الوضع الرأسى سواء مع أو بدون التحضين قبل التطعيم زاد من نسبة التطعيم معنوياً مقارنة بالتحضين فى الوضع الأفقى، والمقلوب. كذلك لوحظ كيميائياً انخفاض كمية الفينولات، والسكريات، والإندولات مع ارتفاع نسبة التطعيم. كما يمكن استنتاج عدم وجود تضاد بين تكوين الكلس والجذور فى العقل المطعومة. كما أن التحضين فى الوضع الرأسى أفضل من التحضين قبل التطعيم.