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Callusing soil of grafted grape cuttings as a positive feature for climate change

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Abstract: Nowadays, some relative warming temperatures related to climate change may be provided at the grafting time. Therefore, this study was conducted during two seasons (2018-2019) to study the effect of three callusing method (callusing room, callusing soil, callusing tunnel) and four grafting date (15 Jan., 1Feb., 15 Feb., 1 Mar.) for early (Flame seedless), medium (Thompson seedless) and late (Crimson seedless) grape varieties on grafted grape cuttings as short methods for transplant production. The results indicated that, the early grapes variety achieved higher grafting success on 1st Feb. grafting date as well as the late grape variety in callusing room and callusing soil methods. Also, Callusing soil achieved grafted success by 72.9%, 68.55% and 77.94% compared to callusing tunnel 37.3%, 45.9% and 55% for Flame seedless, Thompson seedless and Crimson seedless, respectively as mean of both seasons. High grafting success resulted from the high content of indole and sugars, along with low phenol content before callusing stage, as well as high indole and low sugars of grafts partner after callusing stage. while, higher phenols was accumulated in rootstock after callusing stage. There is no antagonistic effect between grafts partners. Callusing soil may be considered as an eco-friendly, sustainable and cheaper alternative tool for callusing of grafts cuttings.

Index terms: indole, phenol, rootstock, sugar, *Vitis vinifera*.

Calosidade do solo de mudas de uva enxertadas como uma característica positiva para as mudanças climáticas

Resumo: Atualmente, algumas temperaturas relativas de aquecimento relacionadas às mudanças climáticas, podem ser fornecidas no momento da enxertia. Portanto, este estudo foi conduzido durante duas temporadas (2018-2019) para estudar o efeito de três métodos de calosidade (sala de calosidade, solo caloso, túnel de calosidade) e quatro datas de enxertia (15 de janeiro, 1º de fevereiro, 15 de fevereiro e 1º de março .) para castas precoces (sem sementes Flame), médias (sem sementes Thompson) e tardias (sem sementes Crimson), em estacas de uva enxertadas como métodos curtos para a produção de transplante. Os resultados indicaram que a variedade de uvas precoces obteve maior sucesso de enxertia na data de enxertia de 1º de fevereiro, bem como a variedade de uva tardia, em sala de calos e solo com calos. Além disso,

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o solo caloso obteve sucesso de enxertia em 72,9%, 68,55% e 77,94% em comparação com o túnel caloso 37,3%, 45,9% e 55% para sem sementes Flame, sem sementes Thompson e sem sementes Crimson, respectivamente como média de ambas as estações. O alto sucesso da enxertia resultou do alto teor de indol e de açúcares, juntamente com baixo teor de fenóis antes do estágio de calosidade; bem como alto teor de indol e baixo teor de açúcares dos enxertos parceiros após o estágio de calosidade; enquanto, maiores fenóis foram acumulados no porta-enxerto após a fase de calosidade. Não há efeito antagônico entre parceiros de enxertos. O solo caloso pode ser considerado uma ferramenta alternativa ecologicamente correta, sustentável e mais barata para calosidade de estacas de enxertos.

Termos de indexação: indol, fenol, porta-enxerto, açúcar, *Vitis vinífera*.

Introduction

Fruit crops because they grow in a wide range of climate zones

Grapevine (*Vitis vinifera*) is one of the most economically important fruit crops due to the fact that it grows in a wide range of climatic region, it is consumed either fresh, juice, compote, wine or raisin production and is highly nutritional values. In Egypt, grapes consider the first deciduous fruit in terms of production (1,586,342 tons) from a harvested area of 71,889 ha (FAO, 2020). However, both abiotic and biotic stresses have limited grape production. Therefore, grafting technique has provided the selection of resistant rootstock to drought, salinity and disease which have great importance in many viticulture countries (REYNOLDS; WARDLE, 2001; COOKSON, et al., 2014; FAYEK et al., 2022).

Grafting grapes using specific rootstocks has become a common practice widely spread throughout the world since the introduction of Phylloxera in some European countries in the nineteenth century (WHITING, 2012). Rootstock plays an important role in improving scion development, fruit quality and adaptation to different environmental conditions (ABDEL-MOHSEN; RASHEDY, 2015; ZOMBARDO et al., 2020). In Egyptian viticulture, Freedom rootstock is one of the most important grape rootstocks due to its resistance to nematodes, high nutrition use efficiency and vigor as well as being highly compatible with most of the cultivated grape varieties (ABDEL-MOHSEN and RASHEDY 2015; FAYEK et al., 2016). Also, Flame seedless, Thompson seedless and Crimson seedless were the most common and exported grapes, whose bud burst dates are February, mid-February and March, respectively. However, graft zone formation is affected by several factors such as plant genetics, propagation techniques, growth regulators application, the scion –rootstock combinations, pest and disease control (HARTMANN et al., 2002; YOUQUN, 2011; FAYEK et al., 2016; FAYEK et al., 2022). Also, environmental conditions during grafting, rootstock activity and the biochemical and physiological status of grafted materials affected grafting success (FAYEK et al., 2016; RASHEDY, 2016).

Callusing date, which depends on the biochemical and physiological status of grafts partners (rootstock and scion) as well as the callusing method which controls in the environment (temperature and humidity) are the most critical factors in grape grafted cuttings success that needs to both root formation and grafting union formation at the same time (RASHEDY, 2016). In addition, the incubation of grafted partners two weeks before grafting increased the grafting success of grape cuttings (RASHEDY, 2016). Grafting date is one of the factors that greatly affected the success of grape grafted cuttings, as Singh and Kaur, (2018) indicated that grafting grape cuttings during the third week of February showed superiority in the success rate and other parameters of vegetative growth compared to the grafts made during early February, August and September. Also, Abourayya et al., (2019) indicated that Flame cv. grafted onto Freedom rootstock in mid-February had a significantly higher success percentage, shoot length and leaf area than those grafted in mid-January. Meanwhile, when Abu-Qaoud, (1999) grafted four grape cultivars (Beiruiti, Halawani, Beituni and Zaini) on four grape rootstocks (Richter, B41, Rugerri and Paulsen) it was found that, the grafting method and the scion had no effect on rooting of rootstocks. Also, the growth of the grafts did not vary by grafting method or rootstocks.

The Middle East and North Africa are at risk from the consequences of climate change due to extreme changes in exposure to the strongest temperature, the availability of fresh water and population growth (WAHA et al., 2017). Climatic change has allowed a warmer climate at the end of winter, which may be useful in using alternative cheaper, sustainable and more safety callusing methods for callusing rooms, which is the most costly method. Therefore, this study aimed to investigate the efficiency of callusing soil and callusing tunnel as new tools versus callusing room under climatic change conditions for three grape varieties different in bud burst date during four grafting dates.

Material and Methods

The experiment was carried out in the nursery of the Pomology Department, Faculty of Agriculture, Cairo University, Egypt (30°01’04” N and 31°12’30” E) during the seasons of 2018 and 2019. The aim of the study was to study the effect of three callusing methods and four grafting dates on the grafting process of three grape cultivars (*Vitis vinifera*) grafted onto Freedom rootstock (*Vitis champinii* x 1613C). Permission was obtained to use the grape variety in this study which complies with relevant institutional, national and international guidelines and legislation.

Preparation of the rootstocks and scion materials:

Three grape cultivars, Flame seedless, Thompson seedless, and Crimson seedless, were used to represent early, middle and late bud bursts. Freedom was used as a rootstock for grafting onto it. Every year, the cuttings of cultivars and Freedom rootstock were prepared in the beginning of January and stored in the refrigerator at a temperature of 5 °C after being wrapped in polyethylene bags until grafting date (SABIR; SABIR, 2018).

The rootstock cuttings were prepared with a length of 30-35 cm and a diameter of 1-1.5 cm. As for the cuttings of the grafts, they were prepared from canes of the same thickness and a length of 50 cm. The cuttings of rootstock or cultivars were largely homogeneous and apparently free of disease and insect infections.

Grafting technique:

The grafting was carried out on four different dates, starting on January 15, February 1, February 15, and ending on March1. The grafting process was done by the cleft grafting method and the cuttings of Freedom rootstock were disbudded just before grafting. Grafting was carried out using scion cuttings from a single-bud. The place of grafting was tied with polyethylene and then the scion and the grafted area were waxed with paraffin wax. Finally, the grafted bases of the cuttings (rootstock) were dipped in indole butyric acid at a concentration of 1000 ppm (ABDEL-MOHSEN; RASHEDY 2015).

Callusing treatments:

The grafted cuttings of each cultivar were divided into three groups each one callused for one month and all grafting partners, boxes and sawdust were treated with the fungicide Rizolex (Tolclofos-Methyl 500g/kg). The first group was called a callusing room which was callused at controlled callus room at 26±4 °C and 85-90% RH, where the grafts were placed horizontally in perforated plastic boxes in layers of pre-soaked sawdust layers and finally was covered with a polyethylene sheet.

The second group was called a callusing soil which callused in furrow under the soil surface, with a depth of 30 cm. The base of the furrow was covered with a perforated polyethylene sheet and the grafts were placed horizontally between pre-soaked sawdust layers under the greenhouse finally it covered with a polyethylene sheet.

The third group was called a callusing tunnel which directly grafts were planted vertically in prepared polyethylene bags under a tightly knit plastic tunnel under the Saran shade house, as will be described in planting conditions. Temperature and humidity in the callusing soil and callusing tunnel were recorded twice a week.

One month later (after finishing the callusing stage) the grafts were transplanted in the Saran shade house in black 5 liters polyethylene bags, packed with a cultivation medium consisting of silt: sand: compost in proportions of 1:1:1 v/v, under polyethylene tunnels (height and width of 70 cm * 70 cm) as described by Hussain et al. (2020), and Rashedy et al. (2021). All grafts after finishing the callusing stage were maintained under the polyethylene tunnel (callusing tunnel) for one month to complete callus formation and acclimation.

Measurements:

Biochemical analysis

A sample (0.5 g FW) was taken from the cuttings of the scion cultivars and the Freedom rootstock to estimate the cuttings chemical content of total sugars, total phenols and total indoles before each grafting date and again one month later after finishing of the callusing stage, as follows:

Total sugars (mg g^{-1} FW)

The phenol-sulfuric acid method was used to determine total soluble sugars as mg glucose per g fresh weight (DUBOIS et al., 1956). Bud samples were extracted in 10 mL of ethanol (70 %). One mL of the previous extract was mixed with 1 mL of phenol (5%) and then 4mL of concentrated sulfuric acid, followed by carefully shaking the tube. After stopping the reaction and cooling the mixture (1hr) total sugars were measured at 490 nm using a spectrophotometer.

Total phenols (mg g^{-1} FW)

The Folin Ciocalteu method was used to determine total phenol content (SHARMA et al., 2019) which was expressed as mg gallic acid per g of fresh weight. Bud samples were extracted by 20 mL methanol (80%). Then 1 ml of the previous extract was mixed with 1 mL of Folin reagent in the test tube, then 5 mL of Na_2CO_3 (20%) was added and finally the volume was adjusted to 10 mL using distilled water. After the tube was shaken well, the mixture was kept for one hour in the dark, and then the total phenols content were measured at 765 nm by a spectrophotometer.

Total indoles (mg g^{-1} FW)

Total indole content was expressed as mg IAA per g of fresh weight was determined according to LARSEN et al. (1962). Bud samples were extracted in the dark with 20 mL (80%) methanol. Then 4 mL of P-dimethyl amino benzaldehyde (1 g of P-dimethyl amino benzaldehyde dissolved in 100 of ethanol (95%) and hydrochloric acid at ratio 1:1) was mixed with 1 mL of the previous extract. After the tube was shaken well, the mixture was kept for 90 min at 30°C. Total indole was finally measured by a spectrophotometer at 530 nm.

Morphological parameters

After 30 days of the callusing stage, the following parameters were recorded:

The callus degree in the grafting zone was assessed based on visible observations: 4 = 76-100% callus, 3 = 51- 75% callus, 2 = 26-50 callus, 1 = 1-25% callus and 0 = no callus

(KOSE; GULERYUZ, 2006; PAUNOVIC et al., 2011). The grafts callusing% was calculated from the following equation (total number of callused grafted at grafting zone / total number of grafts) *100) according to Rashedy, (2016). The grafted rooted percent was calculated from the number of rooted grafts (rootstock) divided by the total number of grafts (KAMILOGLU; TANGOLAR 1997). Root rate or degree at the base of rooted grafts was assessed based on visible observations: 4 = grafts rooted from 4 sides, 3 = grafts rooted from 3 sides, 2 = grafts rooted from 2 sides, 1 = grafts rooted from 1 side and 0 = grafts rootless (ÇELIK, 2000). Also, bud burst percent was recorded after finishing the callus stage from the number of bud burst scions divided by the total number of grafts.

Also, after four months from each grafting date the following parameters were recorded: The grafting success percentage was calculated from the following equation (total number of successful grafts/total number of total grafts) x 100). Plant length of the scion (cm) as well as shoot and root fresh weight (g) were recorded.

Experimental design and statistical analysis

The Completely Randomized Block Design was arranged for this experiment. The experiment included three callusing methods and four grafting dates as 12 treatments for each cultivar. Each treatment consisted of three replicates with 15 grafted cuttings for each one. Data from the analytical determinations were subjected to the analysis of variance by the two-way ANOVA test. The least significant difference test was used to analyze the differences between the treatments at the significance level of $p < 0.05$ (SNEDECOR; COCHRAN 1989). The statistical analysis was carried out by MSTAT-C software (FREED et al., 1990). The provided data as means ± standard error for independent replicates (n = 3).

Results

Meteorological data of callusing methods at different grafting date

The relative humidity during February was relatively higher than March. For planting conditions or callusing tunnel treatments Figure 1 (A, B), maximum temperature under tunnels during January and February is lower than the other months. The maximum and minimum humidity during May are lower than the maximum humidity in the other months. Also, under callusing soil (Figure 1 C, D), the maximum and minimum temperature during January and February are greater than in March.

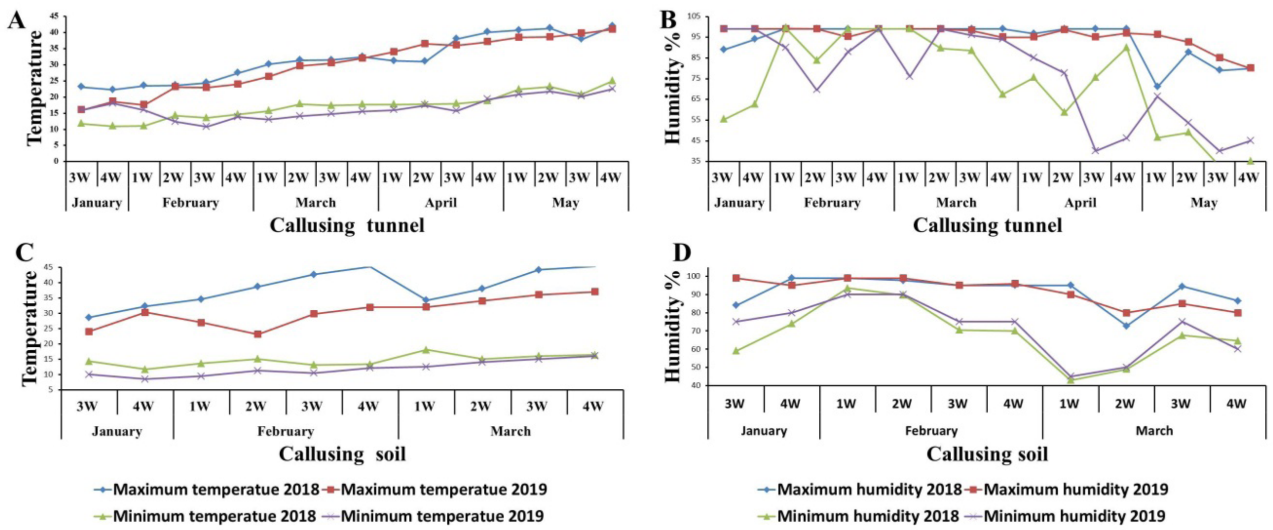

Figure 1. Maximum and minimum temperature and relative humidity in callusing tunnel or planting conditions (A, B) and in callusing soil (C, D) during two season (2018 and 2019).

Generally, the variation in temperature under callusing soil is lower than under callusing tunnel. Also, early and late grafting dates under both callusing soil and callusing tunnel were subjected to low and high temperature, respectively.

Total endogenous sugars, phenol and indole at different grafting date

The results indicated that, generally there is no significant difference between cultivars in total sugars content and total indoles content (Table 1). Also, the grafting date on 1st Feb. recorded the highest significant total sugars content and total indole content while, 15th Jan. grafting date recorded the lowest sugars content and total indole content. The highest sugars content and total indole content were recorded in Freedom rootstock, and Thompson seedless cultivars on 1st Mar. and 1st Feb. grafting date, respectively.

Table 1. Endogenous total sugars, total phenol content and total indole content of three grape cultivars and freedom rootstocks at grafting date

Grape materials		Grafting dates 15 Jan.	1 Feb.	15 Feb.	1 Mar.	Mean
Total sugars (mg g^{-1})	Freedom	11.94±1^{d}	27.81 ±1.57^{a-c}	26.76±3.16^{a-c}	28.37± 13.53^{a-c}	23.72^{A}
	Flame	16.83±1.45^{cd}	26.32±0.66^{a-c}	30.84±5.51^{ab}	26.17±1.09^{a-c}	25.04^{A}
	Thompson	20.60±1.71^{b-d}	35.33±4.25^{a}	24.69 ±4.06^{a-d}	32.84±0.82^{ab}	28.36^{A}
	Crimson	21.13±1.63^{b-d}	23.14±5.25^{a-d}	20.395.39^{b-d}	21.99±1.33^{b-d}	21.66^{A}
	Mean	**17.62^{B}**	**28.15^{A}**	**25.67^{A}**	**27.34^{A}**	
Total phenols content (mg g^{-1})	Freedom	4.14±0.66^{a-c}	4.33±0.17^{ab}	3.39±0.06^{c-g}	2.63±0.62^{g}	3.62^{AB}
	Flame	3.43±0.03^{c-g}	4.37±0.41 ^{a}	4.43±0.14^{a}	3.89±0.18^{a-e}	4.03^{A}
	Thompson	3.98±0.08^{a-d}	2.82±0.46^{fg}	2.59±0.22^{g}	3.61±0.36^{a-f}	3.25^{B}
	Crimson	3.48±0.57^{b-g}	3.15±0.04^{d-g}	3.23±0.11^{d-g}	3.06 ±0.17^{e-g}	3.23^{B}
	Mean	**3.76^{A}**	**3.67^{AB}**	**3.41^{AB}**	**3.30^{B}**	
Total indoles (mg g^{-1})	Freedom	0.106±0.003^{c}	0.115±0.000^{c}	0.115±0.008^{c}	0.134±0.001^{bc}	0.117^{A}
	Flame	0.110±0.001^{c}	0.182±0.005^{abc}	0.127±0.001^{bc}	0.126±0.002^{bc}	0.136^{A}
	Thompson	0.113±0.001^{c}	0.255±0.004^{a}	0.127±0.003^{bc}	0.110±0.087^{c}	0.151^{A}
	Crimson	0.118 c± 0.000	0.126±0.053^{bc}	0.211±0.001^{ab}	0.114±0.003^{c}	0.142^{A}
	Mean	**0.111^{B}**	**0.169^{A}**	**0.145^{AB}**	**0.121^{B}**	

The means in the same parameter followed by different letters were significantly different according to Snedecor and Cochran, [26] ($p < 0.05$).

While Crimson seedless recorded high sugars content on 15th Jan. and 1st Mar. grafting date and total indole content on 15th Feb. grafting date. Moreover, higher total sugars and total phenols were recorded in Flame seedless on 15th Feb. and 1st Feb. grafting date, respectively. Flame seedless recorded the highest phenols content while Crimson seedless and Thompson seedless recorded the lowest values at the same dates. Also, 15th Jan. grafting date recorded the highest significant total phenols content compared to 1st Mar. grafting date. The highest total phenol for Freedom, Flame, Thomson and Crimson were recorded on 1st Feb., on 1st 1st Feb. 1&15th Feb., 15th Jan. and 15th Jan., respectively.

Effect of grafting date and callusing method on some grape grafts

Freedom rootstock

The results indicated that, generally under the callusing room conditions, the Freedom rootstock had higher significant content of sugars, phenols and indoles compared to callusing soil. Also, grafting date on 15th Feb. recorded higher significant sugars, phenols and indoles content compared to 15th Jan. and 1st Mar. grafting dates (Table 2).

Table 2. Effect of grafting date and callusing method on total endogenous sugars, phenol and indole after callusing stage of Freedom rootstock.

Callusing method		Grafting date				
		15 Jan.	1 Feb.	15 Feb..	1 Mar.	Mean
Total sugars (mg g^{-1})	Callusing room	13.27±0.37^{ef}	18.59±1.21^{c}	18.09±1.05^{c}	22.34±1.09^{a}	18.08^{A}
	Callusing soil	12.40±0.97^{f}	15.60±0.84^{de}	14.56±0.27^{ef}	21.72±0.75^{a}	16.07^{B}
	Callusing tunnel	17.30±0.84^{cd}	19.20±1.04^{bc}	18.06±0.78^{cd}	21.19±1.13^{ab}	18.94^{A}
	Mean	14.32^{C}	17.79^{B}	16.90^{B}	21.75^{A}	
Total phenols (mg g^{-1})	Callusing room	4.197±0.105^{ef}	9.513±0.271^{a}	5.630±0.189^{c}	5.263±0.419^{cd}	6.151^{A}
	Callusing soil	4.007±0.097^{ef}	4.433±0.207^{e}	5.893±0.328^{c}	4.483±0.096^{e}	4.704^{B}
	Callusing tunnel	4.257±0.187^{ef}	4.710±0.275^{de}	7.097±0.461^{b}	3.543±0.279^{f}	4.902^{B}
	Mean	4.153^{B}	6.218^{A}	6.206^{A}	4.429^{B}	
Total indoles (mg g^{-1})	Callusing room	0.1223±0.003^{g}	0.1367±0.003^{b}	0.1413±0.006^{a}	0.1287±0.002^{d}	0.132^{A}
	Callusing soil	0.1197±0.001^{h}	0.1267±0.002^{e}	0.1347±0.002^{c}	0.1177±0.002^{i}	0.1247^{B}
	Callusing tunnel	0.1197±0.001^{h}	0.1243±0.002^{f}	0.1250±0.001^{ef}	0.1187±0.002^{hi}	0.1219^{C}
	Mean	0.1205^{D}	0.1292^{B}	0.1336^{A}	0.1217^{C}	

The means in the same parameter followed by different letters were significantly different according to Snedecor and Cochran, [26] ($p < 0.05$).

Flame seedless grafts

The results indicated that, after the callusing stage the higher sugars content recorded in Flame grafts with the callusing tunnel method with high significant value compared to the other two callusing methods (Table 3). Also, the grafting date on 1st Mar. recorded the highest significant sugars value compared to the other grafting dates, while the lowest sugars value was recorded on 15th Jan. grafting date. Generally, the highest phenols content recorded by callusing room and at 1 Mar. grafting date while the lowest values recorded by callusing tunnel and on 15th Jan. grafting date. Furthermore, the highest indole content was observed by callusing room method and on 1st Feb. grafting date, while the lowest values were recorded by the callusing soil method and grafting date on 1st Mar.

Table 3. Effect of grafting date and callusing method on total endogenous sugars, phenol and indole after callusing stage of Flame seedless grafts cuttings.

Callusing methods		Grafting date				
		15 Jan.	1 Feb.	15 Feb..	1 Mar.	Mean
Total sugars (mg g^{-1})	Callusing room	13.36±0.69^{g}	15.55±0.35^{f}	22.18±0.65^{d}	24.35±0.69^{c}	18.86^{B}
	Callusing soil	14.15±0.81^{fg}	20.50±0.66^{de}	19.08±0.53^{e}	25.22±0.71^{c}	19.74^{B}
	Callusing tunnel	18.55±0.82^{e}	24.73±0.13^{c}	29.87±0.54^{b}	34.12±0.56^{a}	26.82^{A}
	Mean	15.35^{D}	20.26^{C}	23.71^{B}	27.90^{A}	
Total phenols (mg g^{-1})	Callusing room	3.560±0.69^{cd}	4.887±0.35^{b}	4.833±0.65^{b}	5.713±0.69^{a}	4.748^{A}
	Callusing soil	3.303±0.81^{d}	3.187±0.66^{d}	4.250±0.53^{bc}	5.710±0.71^{a}	4.113^{B}
	Callusing tunnel	3.143±0.82^{d}	3.583±0.54^{cd}	3.733±0.56^{cd}	3.893±0.13^{cd}	3.588^{C}
	Mean	3.336 ^{C}	3.886 ^{B}	4.272 ^{B}	5.106 ^{A}	
Total indole (mg g^{-1})	Callusing room	0.1467±0.003^{cd}	0.1657±0.001^{a}	0.1327±0.003^{f}	0.1263±0.001^{h}	0.1428^{A}
	Callusing soil	0.1480±0.002c	0.1507±0.002^{b}	0.1240±0.002^{i}	0.1223±0.001^{i}	0.1363^{B}
	Callusing tunnel	0.1423±0.004^{e}	0.1457±0.003^{d}	0.1283±0.002^{g}	0.1197±0.001 j	0.1340^{C}
	Mean	0.1457^{B}	0.1540^{A}	0.1283^{C}	0.1228^{D}	

The means in the same parameter followed by different letters were significantly different according to Snedecor and Cochran, [26] ($p < 0.05$).

The effect of the callusing methods and grafting date on the root rate, the callus degree, grafts callusing % and grafts rooting% and bud burst% of Flame seedless/Freedom grafts were presented in Figure 2. One month after grafting, the callusing room on 1st and 15th Feb. grafting dates recorded the highest significant of rooted grafts, root rate, callus degree, grafts callusing% and bud burst% as well as callusing soil on 1st Feb. grafting date while, callusing tunnel especially at the early grafting date recorded the lowest significant values of grafts rooting%, root rate, callus degree, and grafts callusing%.

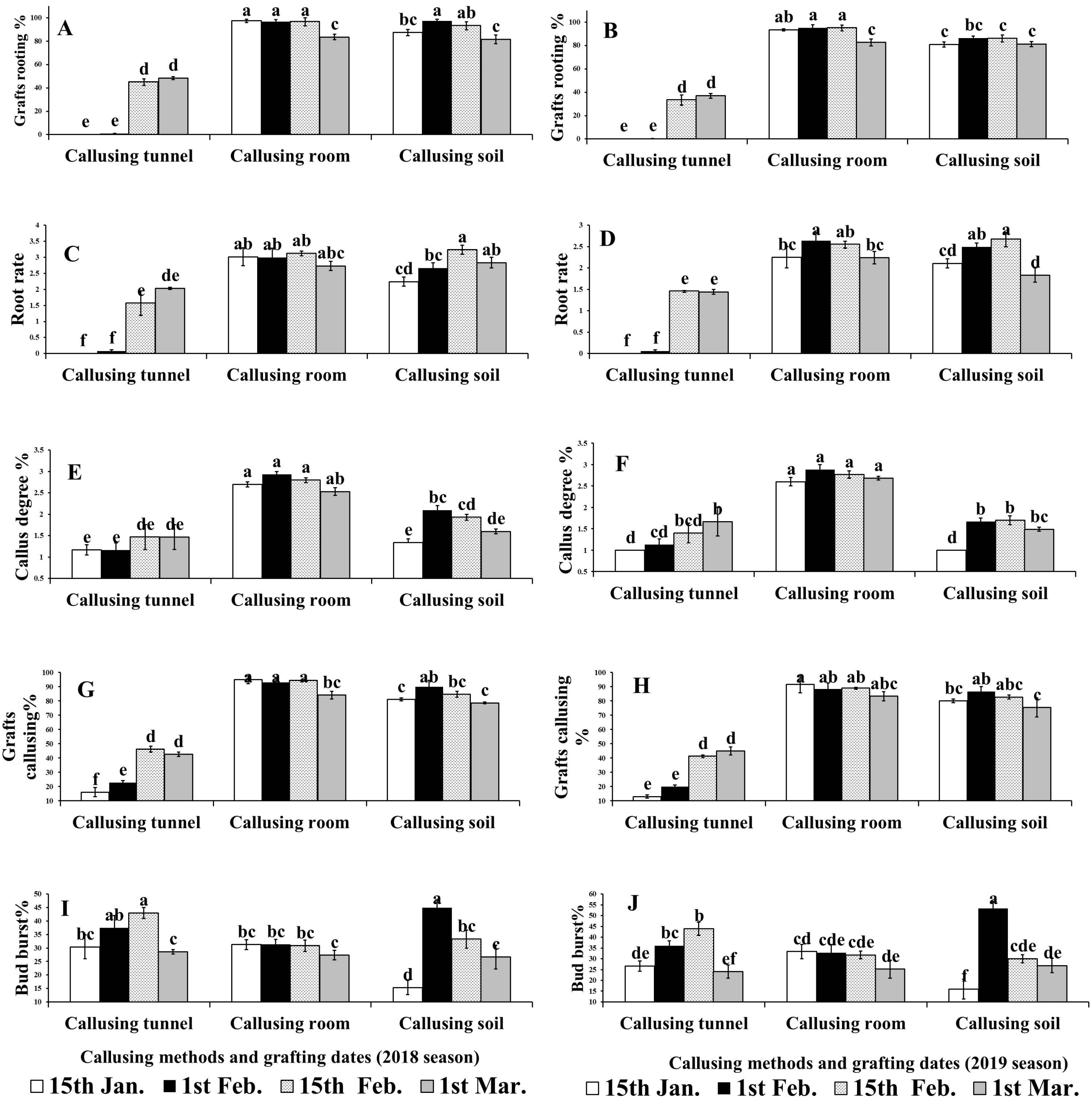

Figure 2. Effect of grafting date (15 Jan., 1 Feb., 15 Feb., 1 Mar.) and callusing methods (callusing tunnel, callusing room, callusing soil) on rootstock rooting% (A, B), root rate (C, D), callus degree (E, F), grafts callusing% (G, H), bud burst (I, J) of grafted cuttings Flame seedless grafted onto Freedom rootstock after finishing callusing stage during two seasons (2018-2019). Data are mean ± standard error (n=3). letters represent significant differences between treatments at $p< 0.05$ level according to the LSD test.

Generally, the early or late grafting date was accompanied by lower rooting of grafts%, root rate, callus degree and grafts callusing % (callus%) especially with the callusing tunnel or the soil callusing treatments.

The data cleared that, one month after planting grafted cuttings, callusing soil treatments recorded the highest bud burst % in both seasons at 1st Feb. grafting date followed by callusing tunnel at 15th Feb and 1st Feb. grafting dates. On the other hand, the callusing soil at 15th Jan and callusing tunnel at 1st Mar. recorded the lowest significant bud burst%.

The effect of the methods and grafting date on the grafting success%, plant length, shoot fresh weight and root fresh weight of grafts cuttings (Flame seedless/Freedom) were presented in Figure 3.

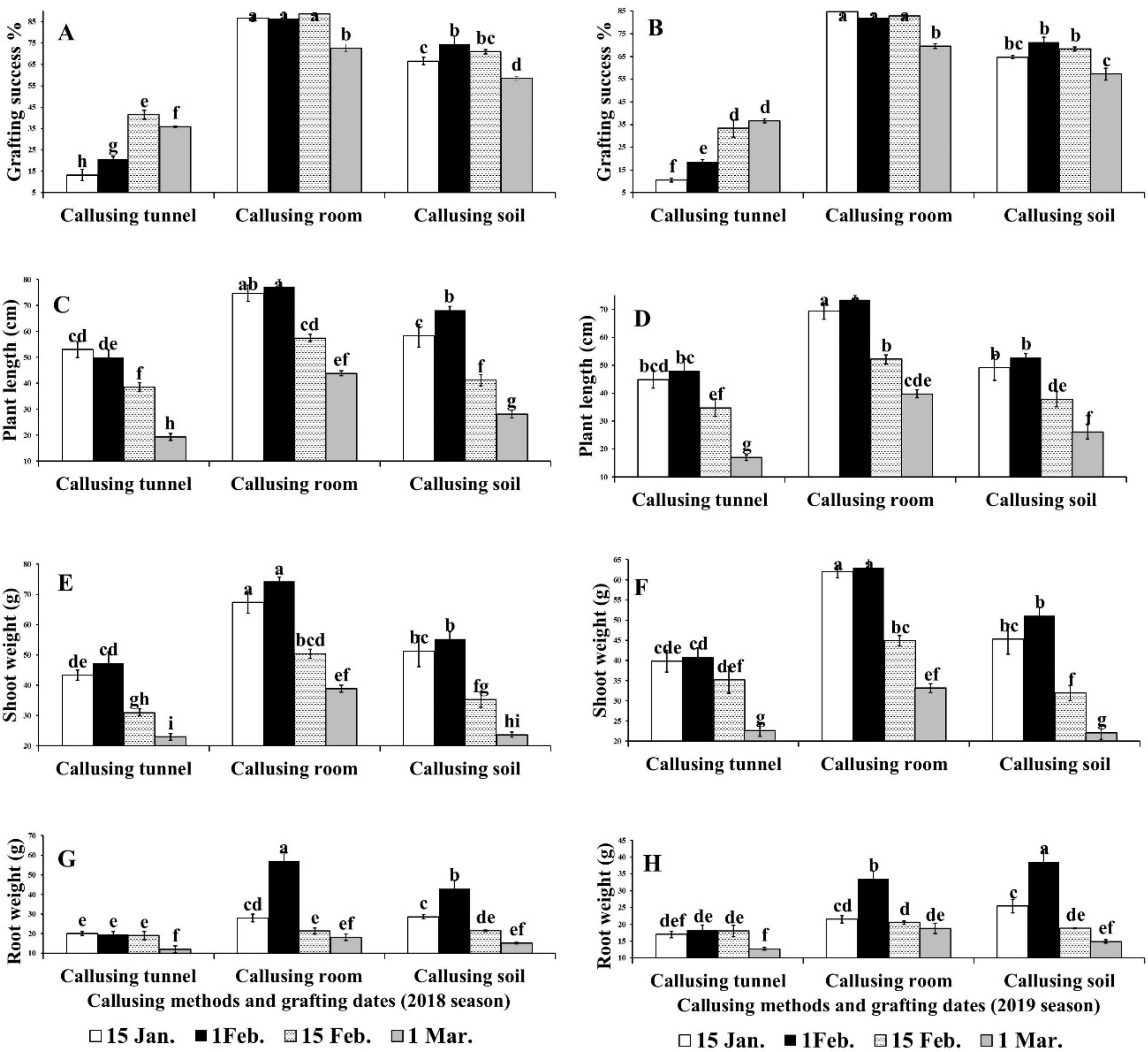

Figure 3. Effect of grafting date (15 Jan., 1 Feb., 15 Feb., 1 Mar.) and callusing methods (Callusing tunnel, callusing room, callusing soil) on grafting success% (A, B), plant length (C, D), shoot fresh weight (E, F), root fresh weight (G, H) of grafted cuttings Flame seedless grafted onto Freedom rootstock four month after grafting (2018-2019). Data are mean ± standard error (n=3). letters represent significant differences between treatments at p< 0.05 level according to the LSD test.

The data cleared that, one month after planting grafted cuttings, the callusing soil treatments recorded the highest bud burst % in both seasons on 1st Feb. grafting date followed by callusing tunnel on 15th Feb and 1st Feb. grafting dates. Also, callusing room recorded the highest significant grafting success% at the early three grafting date followed by callusing soil on 1st Feb. grafting date and callusing soil at 1st Mar. grafting date.

Concerning plant length and shoot weight, the results showed that, callusing on 1st Feb. either with callusing room or callusing soil recorded the highest plant length and shoot weight in both seasons. On the other hand, the callusing soil on 15th Jan and callusing tunnel on 1st Mar. grafting dates recorded the lowest significant bud burst%. Also, callusing tunnel treatment and a late grafting date on 1st Mar significantly decreased grafting success%, plant length and shoot weight.

Thompson seedless grafts

The results indicated that, after the stage, higher significant values of total sugars content were recorded in Thompson seedless grafts with the callusing tunnel method with high significant value compared to the other two callusing methods (Table 4). Also, 1st Mar. grafting date recorded the highest significant sugars value compared to the other grafting dates, while the lowest sugars was recorded on 15th Jan. and 1st Feb. grafting dates.

Moreover, the highest phenols content recorded in callusing room, callusing soil and on 1st Mar. grafting date, while the lowest values were recorded by callusing tunnel and at 15th Feb. grafting date. Furthermore, the highest indole content was observed by callusing room method and on 15th Feb. grafting date, while the lowest values recorded by callusing tunnel method and 1st Mar. grafting date.

Table 4. Effect of grafting date and callusing method on total endogenous sugars, phenol and indole after callusing stage of Thompson seedless grafts cuttings.

Callusing method		Grafting date				
		15 Jan.	1 Feb.	15 Feb..	1 Mar.	Mean
Total sugars (mg g^{-1})	Callusing room	12.30± 0.101^{gh}	16.34±0.723^{e}	11.22±0.652^{h}	20.44±0.482^{d}	15.07^{B}
	Callusing soil	13.54$^{±\ 0.675fg}$	14.74±0.418^{ef}	11.49±0.770^{gh}	23.60±0.865^{c}	15.84^{B}
	Callusing tunnel	16.43± 0.622^{e}	20.29±0.785^{d}	29.11±0.803^{b}	34.43±0.672^{a}	25.07^{A}
	Mean	14.09^{C}	17.12^{B}	17.27^{B}	26.16^{A}	
Total phenols (mg g^{-1})	Callusing room	3.183±0.098^{cde}	3.907±0.149^{c}	2.677±0.241^{ef}	5.120±0.562^{b}	3.722^{A}
	Callusing soil	3.280±0.207^{cde}	2.867±0.287^{de}	1.960±0.191^{fg}	7.127±0.495^{a}	3.808^{A}
	Callusing tunnel	2.730±0.025^{def}	1.69±0.1063^{g}	2.650±0.274ef	3.547±0.180^{cd}	2.655^{B}
	Mean	3.064^{B}	2.822^{B}	2.429^{C}	5.264^{A}	
Total indoles (mg g^{-1})	Callusing room	0.1213± 0.001^{e}	0.1260± 0.003^{c}	0.1450± 0.004^{a}	0.1207± 0.003^{e}	0.1283^{A}
	Callusing soil	0.1173± 0.001^{f}	0.1233± 0.003^{d}	0.1343± 0.004^{b}	0.1133± 0.003^{g}	0.1221^{B}
	Callusing tunnel	0.1027± 0.003^{h}	0.1130± 0.002^{g}	0.1243± 0.000^{cd}	0.1177± 0.003^{f}	0.1144^{C}
	Mean	0.1138^{C}	0.1208^{B}	0.1345^{A}	0.1172^{C}	

The means in the same parameter followed by different letters were significantly different according to Snedecor and Cochran, [26] ($p < 0.05$).

The effect of the callusing methods and grafting date on root rate, callus degree, grafts callusing %, Grafts rooting% and bud burst% of the Thompson seedless/freedom grafts were presented in Figure 4. The results revealed that, both the callusing room and callusing soil on 1st Feb. recorded the highest grafts rooting%, root rate, callus degree and grafts callusing%.

On the contrary, callusing tunnel treatments besides late grafting date with the different callusing methods were recorded the lowest significant grafts rooting%, callus degree and grafts callusing%. Also, the callusing tunnel treatments especially at early grafting date recorded the lowest root rate. Generally, 15th Feb. grafting date recorded the highest bud burst % in all the callusing methods.

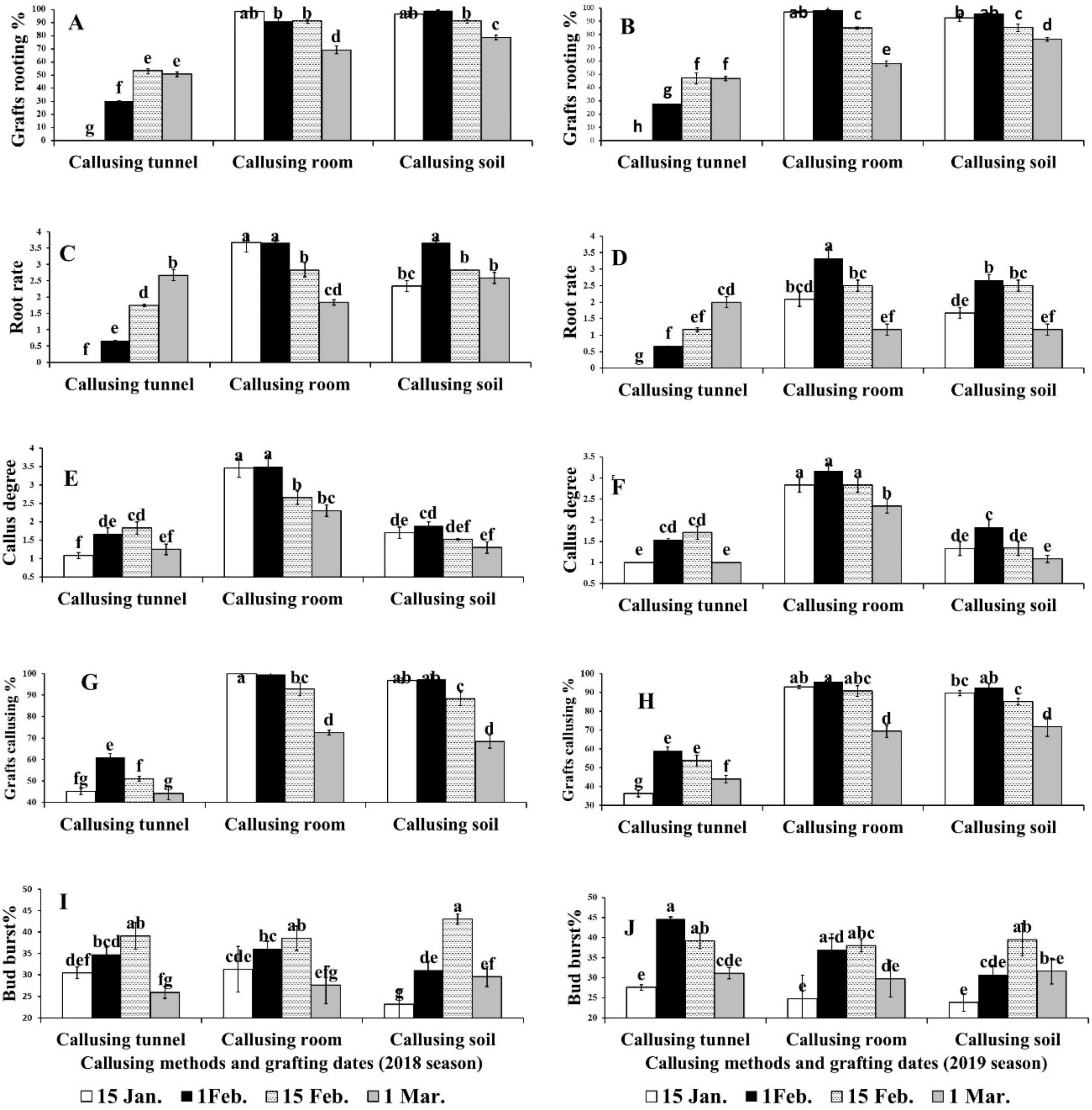

Figure 4. Effect of grafting date (15 Jan., 1 Feb., 15 Feb., 1 Mar.) and callusing methods (Callusing tunnel, callusing room, callusing soil) on grafts rooting% (A, B), root rate (C, D), callus degree (E, F), grafts callusing% (G, H), bud burst (I, J) of grafted cuttings Thompson seedless grafted onto Freedom rootstock after finishing callusing stage during two seasons (2018-2019). Data are mean ± standard error (n=3). letters represent significant differences between treatments at p< 0.05 level according to the LSD test.

The effect of callusing methods and grafting date on bud burst%, grafting success%, plant length, root weight and shoot weight of grafts cuttings (Thompson seedless/ Freedom) were presented in Figure 5.

The results indicated that, callusing room at early three grafting dates followed by soil callusing on 1st Feb. grafting date recorded the highest significant grafting success%, plant length, root weight and shoot weight. Meanwhile, the early grafting date (15 Jan.) as well as late grafting (1 Mar.) recorded the lowest bud burst %. In all callusing methods, the early grafting time recorded the lowest bud burst % and grafting success%. While, the late grafting date significantly decreased bud burst %, grafting success%, plant length and shoot length.

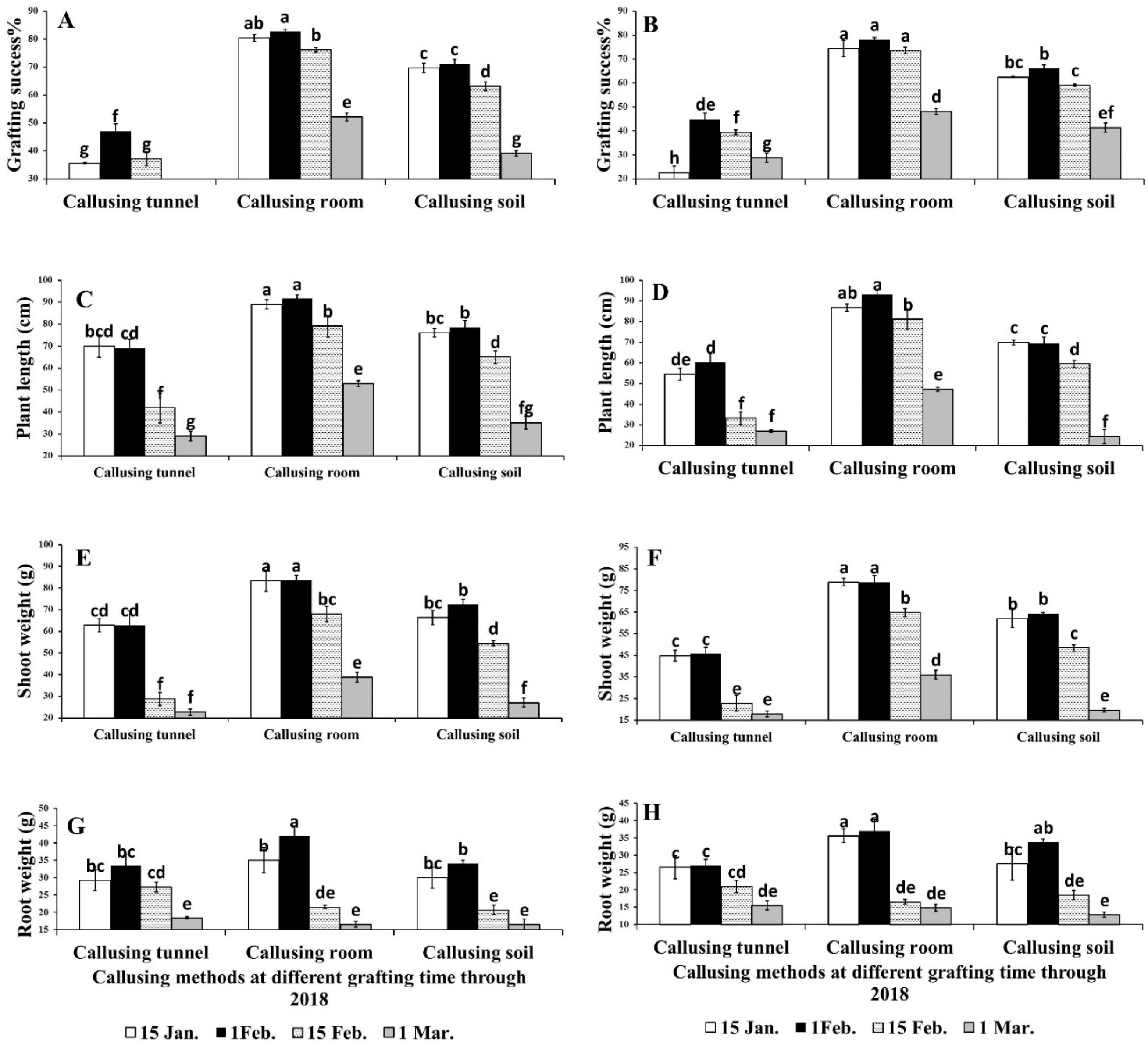

Figure 5. Effect of grafting date (15 Jan., 1 Feb., 15 Feb., 1 Mar.) and callusing methods (Callusing tunnel, Callusing room, Callusing soil) on grafting success% (A, B), plant length (C, D), shoot fresh weight% (E, F) and root fresh weight (G, H) of grafted cuttings Thompson seedless grafted onto Freedom rootstock four month after grafting (2018-2019). Data are mean ± standard error (n=3). letters represent significant differences between treatments at $p < 0.05$ level according to the LSD test.

Crimson seedless grafts

The results indicated that, after the callusing stage, higher significant values of total sugar content were recorded in Crimson seedless grafts with the callusing tunnel method with high significant value compared to the other two callusing methods (Table 5).

Also, 1st Mar. grafting date recorded the highest significant sugars value compared to the other grafting dates. while the lowest sugars content was recorded on 15th Jan. and 15th Feb. grafting dates. Moreover, total phenols content was not affected by callusing methods, while, the callusing soil and 1 Mar. grafting date recorded the highest significant values compared to the other grafting dates. The lowest phenols content was recorded on 15th Feb. grafting date.

Furthermore, the highest indole content was observed by the callusing room method and on 15th Feb. grafting date, while the lowest values recorded by the callusing tunnel and 15th Jan. grafting date.

Table 5. Effect of grafting date and callusing method on total endogenous sugars, phenol and indole after callusing stage of Crimson seedless grafts cuttings.

Callusing methods		Grafting date				
		15 Jan.	1 Feb.	15 Feb..	1 Mar.	Mean
Total sugars (mg g^{-1})	Callusing room	11.81± 0.91^{g}	14.30± 0.54^{f}	15.50± 0.77^{f}	23.39± 0.37^{c}	16.25^{B}
	Callusing soil	11.71± 0.17^{g}	15.34± 0.84^{ef}	15.38± 0.32^{ef}	19.49± 0.65^{d}	15.48^{B}
	Callusing tunnel	21.63± 0.89^{c}	28.39± 0.70^{b}	16.63± 0.73^{e}	31.52± 0.43^{a}	24.54^{A}
	Mean	15.05^{C}	19.41^{B}	15.83^{C}	24.71^{A}	
Total phenols (mg g^{-1})	Callusing room	3.180± 0.12^{def}	3.090± 0.11^{def}	2.133± 0.43^{g}	4.673± 0.47^{a}	3.269^{A}
	Callusing soil	3.470± 0.50^{cde}	2.667± 0.09^{efg}	2.383± 0.06^{fg}	4.550± 0.16^{ab}	3.267^{A}
	Callusing tunnel	3.797± 0.35^{bcd}	3.157± 0.11^{def}	2.187± 0.17^{g}	4.050± 0.21^{abc}	3.297^{A}
	Mean	3.482^{B}	2.971^{C}	2.234^{D}	4.424^{A}	
Total indole (mg g^{-1})	Callusing room	0.1243±0.002^{d}	0.1247± 0.002^{cd}	0.1353±0.004 ^{a}	0.1223± 0.003^{e}	0.1268^{A}
	Callusing soil	0.0986±0.006^{h}	0.1330±0.003^{b}	0.1330±0.003^{b}	0.1167± 0.001^{f}	0.1213^{C}
	Callusing tunnel	0.1123± 0.001^{g}	0.1263±0.003^{c}	0.1313±0.001^{b}	0.1213± 0.002^{e}	0.1230^{B}
	Mean	0.1117^{D}	0.1280^{B}	0.1332^{A}	0.120^{C}	

The means in the same parameter followed by different letters were significantly different according to Snedecor and Cochran, [26] ($p < 0.05$).

The effect of the callusing methods and grafting date on root rate, callus degree, grafts callusing % and grafts rooting% of Crimson/ Freedom grafts were presented in Figure 6.

The results indicated that, the callusing room at the early three grafting dates and callusing soil for 1st Feb grafting date significantly increased rooting of grafts cuttings, root rate, callus degree, bud burst% and grafts callusing% in both seasons.

While the callusing tunnel especially at the two early grafting date gave the lowest rooting of grafts cuttings, root rate and grafts callusing%. Also, at early (15Jan.) and late grafting dates (1Mar.) significantly decreased callus degree and grafts callusing %. While, the callusing tunnel treatments specially at early and late grafting time recorded the lowest significant bud burst %.

The effect of the callusing methods and grafting date on bud burst%, grafting success%, plant length, root weight and shoot weight of grafts cuttings of Thompson seedless grafted onto Freedom rootstock were presented in Figure 7.

It can be observed that, grafting date on 1st Feb. and 15th Feb. with both room and soil callusing methods significantly increased grafting success%. Moreover, grafting date on 1st Feb with any callusing methods significantly increased plant length and shoot weight. While, the callusing tunnel treatments especially at the early and late grafting time recorded the lowest significant bud burst %, grafting success%.In addition to the late grafting date (1Mar.) with different callusing methods recorded the lowest plant length and shoot weight.

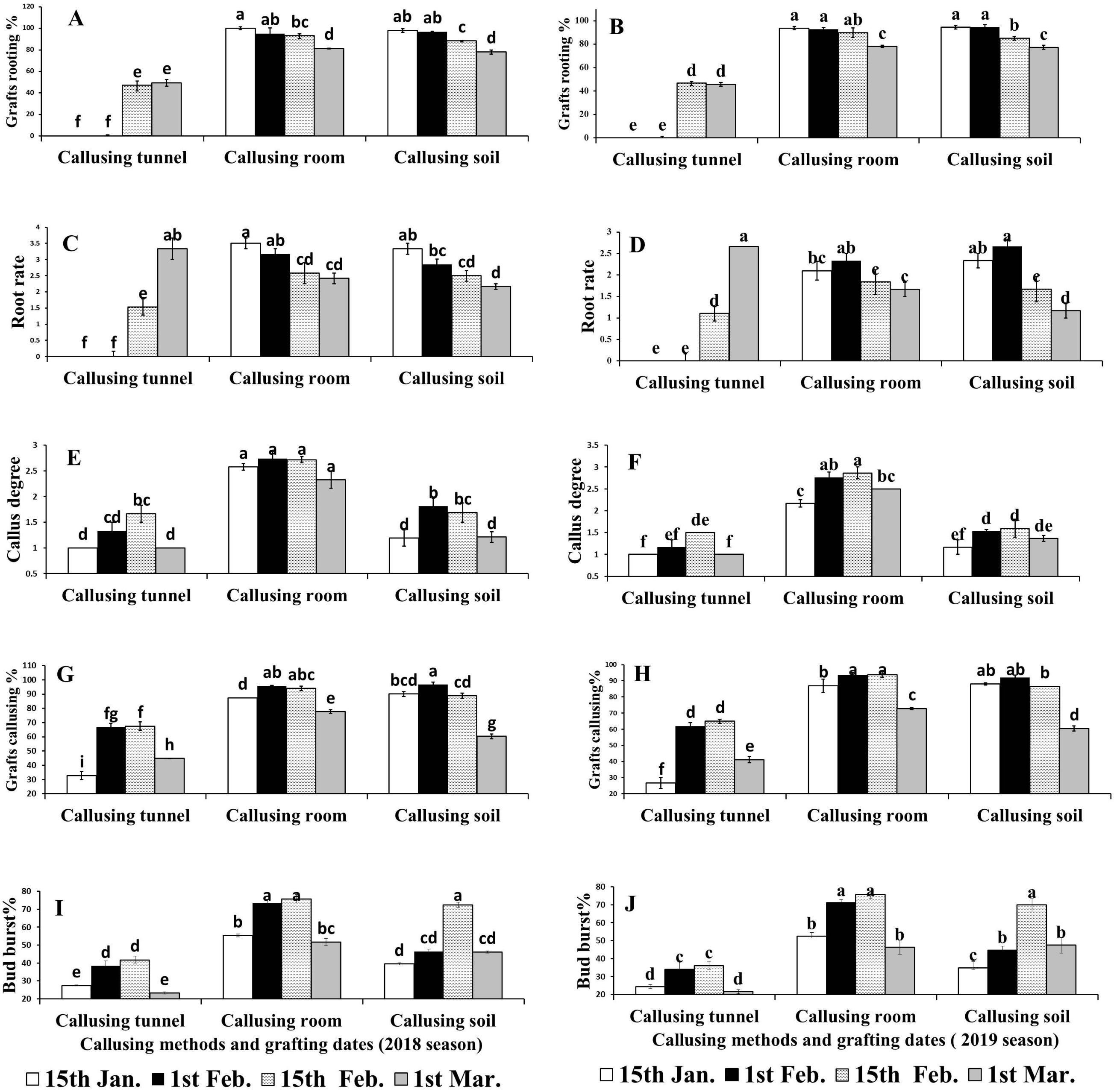

Figure 6. Effect of grafting date (15 Jan., 1 Feb., 15 Feb., 1 Mar.) and callusing methods (Callusing tunnel, callusing room, callusing soil) on grafts rooting% (A, B), root rate (C, D), callus degree (E, F), grafts callusing% (G, H), bud burst (I, J) of grafted cuttings Crimson seedless grafted onto Freedom rootstock after finishing callusing stage during two seasons (2018-2019). Data are mean ± standard error (n=3). letters represent significant differences between treatments at $p< 0.05$ level according to the LSD test.

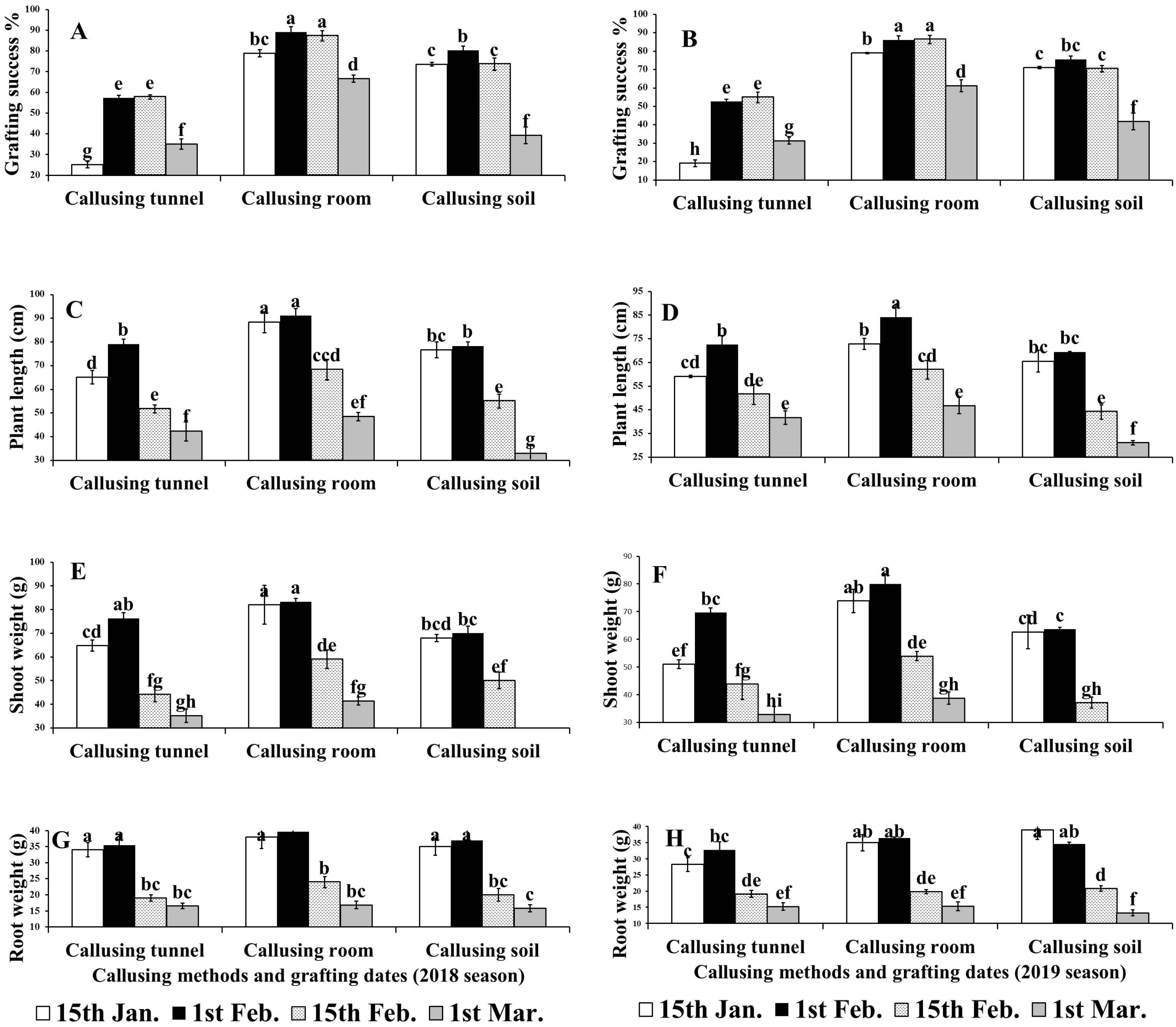

Figure 7. Effect of grafting date (15 Jan., 1 Feb., 15 Feb., 1 Mar.) and callusing methods (Callusing room, Callusing room, Callusing soil) on grafting success% (A, B), plant length(C, D), shoot fresh weight (E, F), root fresh weight (G, H) of grafted cuttings Crimson seedless grafted onto Freedom rootstock four months after grafting during two seasons (2018-2019). Data are mean ± standard error (n=3). letters represent significant differences between treatments at p< 0.05 level according to the LSD test.

Discussion

From the previous results it can be noticed that, the callusing tunnel technique as new callusing tools or planting grafted cutting directly under a polyethylene tunnel recorded the lowest grafting success%. The highest grafting success% recorded by the callusing tunnel (41.39% &33.26%) for Flame seedless on 15th Feb. grafting date, (47.01% &44.92%) for Thompson seedless on 1st Feb. grafting date and (57.39%& 52.62%) for Crimson seedless on 15th Feb. grafting date, respectively. Also, the callusing tunnel was more affected by the variation in temperature between the two seasons since flame seedless recorded 41.39% and 33.26% in the first and second season, respectively. These results may be due to that callusing tunnel conditions provide a huge range of variation in temperature and humidity especially at early and late grafting dates compared to the other callusing methods.

On the other hand, the callusing room methods recorded the highest grafting success% by 86.56%&86.55%, 88.455 &84.80%, 82.13%&82.69% for Flame seedless grafted on 15th Jan., 1st Feb. and 15th Feb. grafting dates, 80.43%&82.65%, 74.38%&78.08% for Thompson seedless grafted on 15th Jan. & 1st Feb. grafting dates and 89.05%&87.36%, 86.08%&86.34% for Crimson seedless grafted at 1st Feb. and 15th Feb. grafting dates. Callusing room which controlled in temperature and humidity also provide a narrow range of grafting success% between seasons.

For callusing soil as a new strategy takes place between callusing tunnel and callusing room treatment. It recorded the highest grafting success% by 74.55%&71.26%, 71.04%&66.06% and 80.35%&75.53% for Flame seedless, Thompson seedless and Crimson seedless, respectively on 1st Feb. in both seasons. Callusing soil provides more constant and lower variation in humidity and temperature (Figure 2) which supported it to become more suitable and sufficient than callusing tunnel methods.

The effectiveness of the callusing methods comes from maintaining optimum humidity and temperature during callusing period especially at suitable grafting time. While soil callusing show a promising effects during 1st Feb. followed by 15th Feb. due to warm temperature provided from the incubation under the soil. By contrast callusing tunnel didn’t provide a suitable temperature and humidity during callusing period but it achieved the best results during 15th Feb.

Grafting date the most influential in grafting success% which did not related to cultivar bud burst. Since, Flame seedless the most earlier cultivars achieve the highest grafting success% at early grafting dates (1 Feb.) as well as both Thompson seedless the medium cultivar in bud burst and Crimson seedless the late cultivar.

The superiority of callusing soil than callusing tunnel comes from callusing soil being more protected from weather variation therefor it preserve more temperature, humidity and low water loss of grafts partners (Figure 2). Furthermore, in all callusing methods the late grafting date on 1st Mar. recorded the lowest grafts callusing, callus degree and grafting success% due to high temperature in both callusing soil and callusing tunnel as well as lower endogenous total indole and total sugars content in all grafting partners before callusing (Table 1) beside higher phenols content in freedom rootstock before (Table 2) and after callusing stage (Table 3) which may be inhibited the activity of grafting partners in formation grafting union. Moreover, after callusing period all grafting partners (different Scions and rootstock) on 15th Jan. contain lower endogenous indoles (Table 2, 3, 4, 5).

In Egypt, Abourayya, (2019) found that, Flame seedless grafted onto Freedom rootstock on mid-February gave the highest shoot length. Also, grafting on mid-February was better than in mid-January. for Freedom, Salt creek and Harmony rootstocks. In India, Somkuwar et al., (2009) tested 8 different grafting dates from July till the mid of October every two weeks intervals in Tas-A-Ganesh grafted onto Dog Ridge rootstock. They found that, grafting on15 September and 15 August grow faster than grafting on 1 July. Also, the thickest shoot was found with grafting at the end of July and mid-August with no significant differences for shoot length. Also, in India, Singh and Kaur, (2018), found that, grafts of Flame Seedless grafted on Dog Ridge rootstock during third week of February gave the highest grafting success% compared to the other grafting dates (early February, August, September).

Higher grafting success% might be due to active growing meristematic stage exhibited by both the scion and rootstock, which supports the callus formation and grafting success (STINO et al., 2011). Therefore, early grafting success recorded early grafting zone formation, early root formation and early plant growth subsequently higher shoot weight and plant length. Planting date was followed grafting date by one month. Under low temperature during callusing or planting the rate of growth and callus formation was decreased. While, in late grafting and planting date extremely high temperature may be increase water loss from recently uncompleted vascular connections in the grafted cuttings, as a result, the temperature and humidity during February are more suitable. Meanwhile, the warmer temperature during April and May faced hot temperature during callusing or acclimation. Scion moisture positively associated with callus formation subsequently grafting success (RONGTING; PINGHAI 1993).

The effects of endogenous growth substances before grafting indicated that, 15th Jan. grafting date (Table 1) recorded the lower total sugars, total indoles and higher total phenols content which decreased grafting success% in all studied cultivars except for Flame seedless. While 1 Feb. grafting date recorded high total total indoles in all studied cultivars (Table 1). These results were in line with Phillips et al., (2015) who found that, at the time of grafting total sugars in scion-wood were accompanied by grafting success%. However, total carbohydrates in February at the time of scion-wood did not correlate to grafting success%. Also, Regina, (2002), reported that, stored carbohydrates in the cuttings was transferred to root formation then shoot formation. Moreover, graft union formation and root formation were affected by stored carbohydrates and water content in the grafts partner (VRSIC et al., 2015) Furthermore, upright position for grafts cuttings recorded higher grafting success% associated with a depletion of sugars compared to the upright down position (RASHEDY, 2016). By contrast, after the callusing stage, decreasing total soluble sugars were recorded in Flame seedless on 15th Jan. and 1 Feb. grafting date which recorded highest callus degree and grafting success% (Table 3). Similar results were found in Thompson seedless on 15th Feb. grafting date (Table 4) and Crimson seedless at 1 Feb. (Table 5) refer to that successful grafts cuttings during callusing period (1month) consumed total sugars as energy for callusing formation in grafting zone as well as root formation. By contrast, higher total sugars content were observed in Crimson (Table 5), Freedom rootstock (Table 2), Flame seedless (Table 3), Thompson (Table 4) on 1st Mar. grafting date which accompanied with lower grafts callusing, callus degree and grafting success% due to no demand for depletion it in callus or root formation.

Increasing of the grafts callusing% and grafting success% were coincided with decreasing total phenols in all scions and rootstock before the grafting (Table 1) as well as in Flame Seedless (Table 3), Thompson seedless (Table 4) and Crimson seedless (Table 5) after the callusing stage. These results were in agreement with Fayek et al., (2016) who reported that, phenolic compounds are biochemical markers for incompatibility in grapevines (FAYEK et al., 2016). Since, the high concentrations of phenolic compounds were obtained in less compatible graft combinations (ERREA et al., 2001; MNGOMBA et al., 2008; GAINZA et al., 2015). In plum higher incompatible degrees were coincided with high phenolic compounds (RODRIGUES et al., 2001). Many phenolic compounds such as, caffeic acid, gallic acid, sinapic acid, ferulic acid, epicatechin and catechin may be suitable as graft incompatibility markers for *vitis vinifera* (CANAS et al., 2015). Also, Assunco et al., (2016) found high content of gallic acid was exhibited in less compatibility grape grafted combinations (Syrah383/SO4 and Syrah383/110R). Moreover, Phenol can disrupt cell functions and inhibit callus formation in grapevines (STINO et al., 2011).

On the contrary, high phenols content was observed in Freedom rootstock after the callusing stage. Although, some phenolic compounds such as m-diphenols and monophenols inhibited root formation while coumarins, p-diphenols, o-diphenols, and polyphenols stimulate root formation (BANDURSKI et al., 1995; TROBEC et al., 2005; DENAXA et al., 2021). Also, Bastos et al., (2006) stated that phenolic compounds related positively with some enzymes stimulate biosynthesis of indole-3-acetic acid (IAA). In olive cuttings, Martins et al., (2022), detected accumulation of phenolic compounds at the bases of the cuttings which may be related with root formation. More recently higher accumulation of phenolic compounds were correlated with the lowest grafting success in olive plants (RASHEDY; HAMED, 2023). The role of phenolic compounds may be resulting from protecting auxin from decarboxylation and oxidation (WILSON; VAN STADEN 1995; TROBEC et al., 2005; OSTERC et al. 2007), improving auxin transport (OSTERC et al., 2007) and conjugating with auxin to form a more active rooting compound (HAISSIG et al., 1974).

Grafting success% was accompanied by higher total indoles after and before the callusing stage in all grafts partner which may be endogenous supporter for callusing and root formation during callusing stage. Generally during January total sugars and indoles as growth stimulator substances were at low level as well as total indoles at the late grafting date (1Mar.). The lowest sugars content on 15th Jan. grafting date come from that the carbohydrates in form of starch and no available at late time of grafting. While for Mar. grafting date lower sugars may be due to low grafts activity from long storage period (Table 1). Callus formation was positively correlated with sugar and IAA at the graft union (ZHOU et al., 2020). These results were in harmony with Vrsic et al., (2015), as they mentioned that, carbohydrates, low amount of phenols as well as high indoles amount are required for successful callus formation and grafting success. Also, grafting success was positively coincided with low phenols content and the high total indols content. Therefore, water soaking of scion and rootstock significantly decreased total phenols and increased total indols which improved grafting success (FAYEK et al., 2022). Furthermore, increasing auxins levels at grafting union stimulated cell division and vascular differentiation (YIN et al., 2012). By contrast, increasing some phenols compounds such as catechins and proanthocyanidins led to a poor callus formation due to reduce cell division and differentiation (MNG'OMBA et al., 2008; GAINZA et al., 2015). This is the first report on the use of soil callusing strategy as environmentally friendly, sustainable, and cheaper alternative tools to the use of grafted cuttings.

The results confirmed that, there is no antagonistic effect between root formation of grafted cuttings and grafting zone formation. Since the higher bud burst, callus formation after callusing stage was accompanied by higher grafts rooting% which led to higher grafting success%. The activity of grafting bud and rootstock together stimulate grafting zone formation. Also, rootstock total indoles stimulate root formation. These results were in line with (RASHEDY, 2016) as he proved that, upright position of grafted grape cuttings during callusing gave the highest grafting success% compared to upside down position which decrease root growth and bud burst of the grafted cuttings. Moreover, the roots of grafts cuttings may be acts as cytokines source, which necessary for calluses induction (NOGUEIRA 2007).

Conclusion

Grafting date on 1st Feb. recorded the highest grafting success for both grape cultivars under both callusing room and callusing soil methods, as well as callusing tunnel for Thompson seedless and Crimson seedless. Also, high grafting success% was accompanied by high total sugars, total indole and low total phenols before the callusing stage as well as high indoles beside low total sugars and total phenols after callusing stage. Regarding to the new alternatives callusing tools, callusing soil achieved grafted success% by 72.9%, 68.55%, 77.94% while callusing tunnel 37.3%, 45.9, 55% for Flame seedless, Thompson seedless and Crimson seedless, respectively as mean of both seasons. This is the first report in using callusing soil as an eco-friendly, sustainable and cheaper alternative tool for callusing of grafts cuttings.

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References

ABDEL-MOHSEN, M.A.; AND RASHEDY, A.A. Nitrogen and potassium uptake and utilization of four grapevine rootstocks. **Journal of Plant Production**, Athens,v.6, n.12, p.1941-56, 2015.

ABOURAYYA, M.S.; NABILA, K.E.; ABD-ALLAH, A.S.E.; AMAL, R.A.M. Effect of grafting date and rootstock type on vegetative growth parameters of Flame seedless grape grafted on three nematode resistant rootstocks. **Middle East Journal of Agriculture Research**, Giza, v.8, n.4, p. 967-72, 2019.

ABU-QAOUD, H. Performance of different grape cultivars for rooting and grafting. **An-Najah University Journal for Research - A Natural Sciences**, West Bank, v.13, p.1–8, 1999.

ASSUNCO, M.; CANAS, S.; CRUZ, S.; BRAZÃO, J.; ZANOL, G.C.; EIRAS-DIAS, J.E. Graft compatibility of Vitis spp.: the role of phenolic acids and flavanols. **Scientia Horticulturae**, New York, v.207, p.140-5, 2016.

BANDURSKI, R.S., COHEN, J. D., SLOVIN, J.P., REINECKE, D. M. Auxin biosynthesis and metabolism. *In*: DAVIES, P.J. (ed.). **Plant hormones**. Dordrecht: Kluwer Academic Publishers, 1995. p.39-65.

BASTOS, D. C.; PIO, R.; SCARPARE FILHO, J.L.; LIBARDI, M.N.; ALMEIDA, L.F.P.; GALUCHI, T.P.D.; BALCKER, S.T. Propagation of red pitaya (Hylocereus undatus) by cuttings. **Ciência e Agrotecnologia**, Lavras, v.30, n.6, p.1106-9, 2006.

CANAS, S.; ASSUNCÃO, M.; BRAZÃO, J.; ZANOL, G.; EIRAS-DIAS Phenolic compounds involved in grafting incompatibility of vitis spp: development and validation of an analytical method for their quantification. **Phytochemical Analysis**, Chichester, v.26, n.1, p.1–7, 2015.

ÇELIK, H. The Effects of different grafting methods applied by manual grafting units on grafting success in grapevines. **Turkish Journal of Agriculture and Forestry**, Ankara, v.24, n.4, p. 499–504, 2000.

COOKSON, S.J.; MORENO, M.J.C.; HEVIN, C.; MENDOME, L.Z.N.; DELROT, S.; TROSSAT-MAGNIN, C.; OLLAT, N. Heterografting with nonself rootstocks induces genes involved in stress responses at the graft interface when compared with autografted controls. **Journal of Experimental Botany**, Oxford, v.64, n.10, p.2997–3008, 2014.

DENAXA, N.K.; ROUSSOS, P.; KOSTELENOS, G.; VEMMOS, S. chlorogenic acid: a possible cofactor in the rooting of ‘kalamata’ olive cultivar. **Journal of Plant Growth Regulation**, New York, v.40, p.1–11, 2021.

DUBOIS, M.; GILLES, K.A.; HAMILTON, J.K.; REBERS, P.A.; SMITH, F. Colorimetric method for determination of sugars and related substances. **Journal of Analytical Chemistry**, Dordrecht, v.28, p.350-6, 1956.

ERREA, P.; GARAY, L.; MARÍN, J.A. Early detection of graft incompatibility in apricot (Prunus armeniaca) using in vitro techniques. **Physiologia Plantarum**, Oxford, v.112, n.1, p.135-41, 2001.

FAO - Food and Agriculture Organization. Crops and livestock products. Rome, 2020. Disponível em: *https://www.fao.org/faostat/en/#data/QCL*.

FAYEK, M.A.; RASHEDY, A.A.; MAHMOUD, R.A.; ALI, A.E.M. Biochemical indicators related to grafting compatibility in grapevine. **Research Journal of Pharmaceutical, Biological and Chemical Sciences**, v.8, n.3, p.574-81, 2016.

FAYEK, M.A.; ALI, A.E.M.; RASHEDY, A.A. Water soaking and benzyladenine as strategy for improving grapevine grafting success. **Revista Brasileira de Fruticultura**, Jaboticabal, v.44, n.3, p.e-946, 2022.

FREED, R.; EISENSMITH, S.P.; GOETZ, S.; REICOSKY, D.; SMAIL, V.M.; WOLLBERG, P. **MSTAT-C a microcomputer program for the design, management and analysis of agronomic research experiments**. East Lansing: Michigan State University, 1990. Disponível em: *https://www.msu.edu/~freed/disks.htm*.

GAINZA, F.; OPAZO, I.; MUÑOZ, C. Graft incompatibility in plants: Metabolic changes during formation and establishment of the rootstock/scion union with emphasis on Prunus species. **Chilean Journal of Agricultural Research**, Santiago, v.75, p.28–34, 2015.

HAISSIG, B.E. Influence of auxin and auxin synergists on adventitious root primordium initiation and development. **New Zealand Journal of Forestry Science**, v.4, p.311-23, 1974.

HARTMANN, H.T.; KESTER, D.E.; DAVIES, F.T.; GENEVE, R.L. **Plant propagation**: principles and practices. 7th ed. Upper Saddle River: Prentice Hall, 2002.

HUSSAIN, K.; QADRI, R.; AKRAM, M.T.; NISAR, N.; IQBAL, A.; YANG, Y.; KHAN, M.M.; IMRAN-UL-HAQ; KHAN, R.I.; IQBAL, M.A. Clonal propagation of olive (olea europeae) through semi- hardwood cuttings using iba under shaded polyethylene tunnels (spts). **Fresenius Environmental Bulletin**, Freising, v.29, n.9A, p.8131-7, 2020.

KOSE, C.; GULERYUZ, M. Effects of auxins and cytokinins on graft union of grapevine (Vitis vinifera). **New Zealand Journal of Crop and Horticultural Science**, Abingdon, v.34, n.2, p.145-50, 2006.

LARSEN, P.; HARBO, A.; KLUNGRON, S.; ASHEIN, T.A. On the biosynthesis of some indole compounds in Acetobacter Xylinum. **Physiologia Plantarum**, Oxford, v.5, p.552-65, 1962.

MARTINS, M.; GOMES, A.F.G.; DA SILVA, E.M.; DA SILVA, D.F.; PECHE, P.M.; MAGALHAES, T.A.; PIO, R. Effects of anatomical structures and phenolic compound deposition on the rooting of olive cuttings. **Rhizosphere**, Amsterdam, v.23, n.100557, 2022.

MNG'OMBA, S.A.; DU TOIT, E.S.; AKINNIFESI, F.K. The relationship between graft incompatibility and phenols in Uapaca kirkiana Müell Arg. **Scientia Horticulturae**, New York, v.117, n.3, p.212-8, 2008.

NOGUEIRA, R.C. Indução de calosemexplantesfoliares de murici-pequeno (Byrsonimaintermedia A. Juss.). **Ciência e Agrotecnologia**, Lavras, v.31, n.2, p.366-70, 2007.

OSTERC, G.; STEFANCIC, M.; SOLAR, A.; STAMPAR, F. The effect of severance date on rooting adility of chestnut cuttings and associated changes in phenolic content during adventitious root formation. **Phyton**, Horn, v.46, n.2, p.285-94, 2007.

PAUNOVIC, S.A.M.; MILETIC, R.; MITROVIC, M.; ANKOVIC, D.J. Effect of callusing conditions on grafting Success in walnut (Juglans regia l.). **Journal of Fruit and Ornamental Plant Research**, Berlin, v.19, n.2, p.5–14, 2011.

PHILLIPS, N.; REYNOLDS, A.; PROFI, F.D. Nonstructural carbohydrate concentrations in dormant grapevine scion wood and rootstock impact propagation success and vine growth. **HortTechnology**, Alexandria, v.25, n.4, p.536-50, 2015.

RASHEDY, A.A. Effect of pre-grafting incubation and grafted cuttings positionon grape grafting success. **Egyptian Journal of Horticulture**, Cairo, v.43, n.2, p.225-40, 2016.

RASHEDY, A.A.; HAMED, H.H. Morphological, physio-biochemical and nutritional status as potential markers for grafting compatibility in Kalamata olive cultivar. **BMC Plant Biology**, London, v.23, p.334, 2023. https://doi.org/10.1186/s12870-023-04346-0

RASHEDY, A.A.; ELDEEB, W.A.M.; HAMED, H.H. Antioxidant Procedure Improve Olive Cuttings Rooting During The Cool Season. **Egyptian Journal of Horticulture**, Cairo, v.48, n.2, p. 267-75, 2021.

REGINA, M.A. Produção e certificação de mudas de videira na França 2. Técnica de produção de mudas pela enxertia de mesa. **Revista Brasileira de Fruticultura**, Jaboticabal, v.24, n.2, p.590-6, 2002.

REYNOLDS, A.G.; WARDLE, D.A. Rootstocks impact vine performance and fruit composition of grapes in British Columbia. **HorTechnology**, Alexandria, v.11, n.3, p.419-27, 2001.

RODRIGUES, A.C.; MACHADO, L.B.; DINIZ, A.C.; FACHINELLO, J.C.; de LUCES FORTES, G.R.; Evaluation of the graft compatibility in prunus sp. **Revista Brasileira de Fruticultura,** Jaboticabal, v.23 n.2, p.359-64, 2001.

RONGTING, X.; PINGHAI, D.A study on the uniting process of walnut grafting and the factors affecting. **Acta Horticulturae**, The Hague, v.31, p.160-70, 1993.

SABIR, F.K.; SABIR, A. Effects of different storage conditions on rooting and shooting performance of grapevine (Vitis vinifera l.) Cuttings in hydroponic culture system. **International Journal of Sustainable Agricultural Research**, Punjab, v.5, n.3, p.46–53, 2018.

SHARMA, A.; SHAHZAD, B.; REHMAN, A.; BHARDWAJ, R.; LANDI M.; ZHENG, B. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. **Molecules**, Basel, v.24, n.13, p.1–22, 2019.

SINGH, N.; KAUR, G. Study on time and method of grafting on the graft success in grape. **Journal of Krishi Vigyan**, Kolkata, v.6, n.2, p.264-71, 2018.

SNEDECOR, W.; COCHRAN, W.G. **Statistical methods**. 8th ed. Ames: Iowa State University Press, 1989. 503p.

SOMKUWAR, R.G.; SATISHA, J.; RAMTEKE, S.D. Propagation success in relation to time of grafting in Tas-A-Ganeshgrapes. **Journal of Maharashtra Agricultural Universities**, Pune, v.34, n.1, p.113-4, 2009.

STINO, R.G.; GHONEIM, I.E.; MORWAD, I.A.; FADL, T.R. Performance of summer grafted superior seedless grape grafts on different rootstocks. **Journal of Horticultural Science & Ornamental Plants**, v.3, n.1, p.86–90, 2011.

TANGOLAR, S.; ERGENOGLU, F.; GÖK, S.; KAMILOGLU, O. Research on determination of callus formation capacity in different grape rootstocks and cultivars. **Acta Horticulturae**, The Hague, v.441, p.399–402, 1997.

TROBEC, M.; STAMPAR, F.; VEBERIC, R.; OSTERC, G. Fluctuations of different endogenous phenolic compounds and cinnamic acid in the first days of the rootingprocess of cherry rootstock 'GiSelA 50 leafy cuttings. **Journal of Plant Physiology**, Jena, v.162, n.5, p.589-97, 2005.

VRSIC, S.; PULKOA, B.; KOCSIS, L. Factors influencing grafting success and compatibility of grape rootstocks. **Scientia Horticulturae**, New York, v.181, p.168–73, 2015.

WAHA, K.; KRUMMENAUER, L.; ADAMS, S.; AICH, V.; BAARSCH, F.; COUMOU, D.; FADER, M.; HOFF, H.; JOBBINS, G.; MARCUS, R.; MENGEL, M., OTTO, I.M.; MAHÉ P.; ROCHA, M.; ROBINSON, A.; SCHLEUSSNER, C. Climate change impacts in the Middle East and Northern Africa (MENA) region and their implications for vulnerable population groups. **Regional Environmental Change**, Berlin, v.17, p.1623-38, 2017.

WHITING, J. **Rootstock breeding and associated R&D in the viticulture and wine industry.** Adelaide: Grape and Wine Research and Development Corporation, 2012. p.52-4.

WILSON, P.J.; VAN STADEN, J. Rhizocaline, rooting co-factors, and the concept of promoters and inhibitors of adventitious rooting. A review. **Annals of Botany**, Oxford, v.66, n.4, p.479-90, 1990.

YIN, H.; YAN, B.; SUN, J.; JIA, P.; ZHANG, Z.; YAN, X.; CHAI, J.; REN, Z.; ZHENG, G.; LIU, H. Graft-union development: a delicate process that involves cell–cell communication between scion and stock for local auxin accumulation. **Journal of Experimental Botany**, Oxford, v.63, n.11, p.4219-32, 2012.

YOUQUN, W. Plant grafting and its application in biological re- search. **Chinese Science Bulletin**, Beijing, v.56, p.3511-7, 2011.

ZHOU, Q.; GAO, B.; LI, W.; MAO, J.; YANG, S.; LI, W.; MA, Z.; ZHAO, X.; CHEN, B. Effects of exogenous growth regulators and bud picking on grafting of grapevine hard branches. **Scientia Horticulturae**, New York, v.264, n.109186, 2020.

ZOMBARDO, A.; CROSATTI, C.; BAGNARESI, P.; BASSOLINO, L.; RESHEF, N.; PUCCIONI, S.; FACCIOLI, P.; TAFURI, A.; DELLEDONNE, M.; FAIT, A.; STORCHI, P.; CATTIVELLI, L.; MICA, E. Transcriptomic and biochemical investigations support the role of rootstock-scion interaction in grapevine berry quality. **BMC Genomics**, London, v.21, p.468, 2020.