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Effect of Cold Stratitication and Gibberellic Acid Treatment on Seed Germination and Seedlings Growth of Quince (*Cydonia oblonga*)

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Abstract: The study aimed to determine the effect of cold stratification, concentrations of gibberellic acid (GA_3) and the interaction between them on the characteristics of germination and growth of quince seedlings. Quince seeds have embryonic dormancy attributed to inhibitory germination in seed covers; thus, seeds exposed to cold stratification (30, 60, and 90 days) and treated with GA_3 at concentrations (0, 250, 500, and 1000 ppm). A randomized complete block design was used, and each treatment contained 40 seeds. The results showed the cold stratification of 90 days gave the highest percentage and speed of germination, the longest seedling, the largest leaf area and a percentage of dry matter with an average of (87%, 1.88, 37.35cm, 31cm^2 and 55%), and the use of a concentration (500 ppm) gave the best percentage and speed for seed germination. The interaction showed a significant effect on the characteristics of germination and growth. The seed treated with cold stratification for 60 days with a concentration of GA_3 at 500 ppm gave the highest percentage of germination (94%), and the stratification for 90 days with a concentration of 1000 ppm recorded the largest leaf area and the highest percentage of dry matter (34. 40 cm^2 and 57.54%) on respectively. While the seeds stratified for 60 days without GA_3 have the highest value for the diameter (2.89 mm). From this, we concluded that the treatment with GA_3 with cold stratification is of economic importance for its effective role in breaking the dormancy of Quince seeds.

Keywords: Quince, Stratification, dormancy. GA_3

تـأثير التنضيد البـارد والمعاملـة بحمـض الجبريليك علـى إنبـات البـذور ونمـو شـتلات السـفرجل *Cydonia oblonga*

المستخلص: أجريت الدراسة بهدف تحديد تأثير طول فترت التنضيد البارد، الغمر في حمض الجبريليك والتداخل بينهما على خصائص الإنبات ونمو شتلات السفرجل، تتميز بذور السفرجل بجود سكون الجنين الراجع الي وجود مثبطـات الانبـات فـي أغلفـة البـذرة. حيـث عرضـت البـذور لـثلاث فتـرات تنضـيد (30، 60، 90 يـوم) وعوملـت بحمـض الجبريليك بتركيـزات (0، 250، 500، 1000 جزء فـي المليـون)، استخدم تصـميم القطاعـات الكاملـة العشوائية في تنفيذ هذه الدراسة واحتوت كل معاملـة علـى 40 بـذرة. أظهـرت النتـائج أن فتـرات التنضـيد أثـرت معنوياً على خواص الإنبات والنمو حيث اعطت فترة التنضيد 90 يوم اعلي نسبة وسرعة للإنبات، اطول شتلة أكبـر مسـاحة ورقـة ونسـبة من المـادة الجافة بمتوسط (87%، 1,88، 37.35 سم، 31سم^2 و55%، كمـا ادى استخدام تركيز (500 جزء في المليون) إلى الحصول على أفضل نسبة وسرعة لانبات البذور، في حين التركيز (1000جزء في المليون) اعطى اطول طول للشتلة وأكبر مساحة للورقة ونسبة من المادة الجافة. أظهر التداخل بين فترات التنضيد وتراكيز حمض الجبريليك تأثيراً معنوياً على خصائص الإنبات والنمو، حيث أعطت البذور المنضدة لفترة 60 يوماً والمعاملة بتركيز (500 جزء في مليون) من حمض الجبريليك أعلى نسبة إنبات (94%) والفتـرة 90 يـوم مـع التركيـز 1000 جـزء بـالمليون سـجلت أكبـر مسـاحة ورقـة وأعلـى نسـبة مـن المـادة الجافـة (34.40سم^2 و57,54%) على التوالي. بينما اعطت البذور غير المعاملة بحمض الجبريليك والمنضدة لمدة 60 يومـاً اعلـى قيمـة لقطـر للشـتلات بمتوسـط (2،89 ملم). ومـن ذلـك نسـتنتج ان المعاملـة بحمـض الجبريليـك مـع التنضيد البارد ذات اهمية اقتصادية لدورها الفعالة في كسر سكون البذور.

الكلمات المفتاحية: السفرجل، التنضيد البارد، السكون، حمض الجبريليك.

INTRODUCTION

Cydonia oblonga Mill is an important fruit for some pome fruit, particularly pear fruit. Quince as rootstock showed high compatibility and induced a significant dwarfing effect on pear fruit trees. Quince rootstock showed also tolerance to calcareous soil and drought conditions (Tatari at el., 2020; Mohammadi et al., 2015). Moreover, Quince rootstock is quite resistant to moist soil and iron chlorosis (Kafkas at el., 2018). Quince rootstocks have important influences on different fruit production such as canopy architecture, nutritional uptake, flowering, yield and fruit quality (Nimbolkar at el., 2016). Cold stratification is routinely employed to break dormancy in water-permeable seeds (Willis at el., 2014). Several researchers demonstrated that the effect of cold stratification on seed dormancy breaking was a result of a decline in abscisic acid (ABA) and an increase in gibbrrens (GA) (Wang at el., 2020; Chen at el., 2015 & Lewak, 2011). Cold stratification broke embryo dormancy in *Malus domestica* seeds by catabolizing lipids, sugars, and proteins with hydrolytic or proteolytic enzymes (Lewak, 2011). Cold stratification broke the embryonic dormancy of *Mallus domestica* and *Juglanas regia* (Lewak 2011) and *Corylus avellana* (Einali & Sadeghipour, 2007; Li & Ross, 1990) by activating of hydrolytic and proeolytic enzymes that catabolize lipids, sugar and proteins. Cold stratification leads to the activation and transcription of genes involved in the biosynthesis of gibberellins and consequently, an increase in level of endogenous GA_3 (Yamauchi et al., 2004).

GA_3 has been widely used for breaking seed dormancy in many plants (Lee et al., 2022; Su, et a.l., 2016 & Rawat, et al., 2008). Cold stratification has been shown to increase the endogenous GA_3 content of stratified seed and consequently seed germination (Hashemirad et al., 2023; Rawat et al., 2008). Subjecting of exogenous GA seeds to exogenous GA_3 treatments increased their germination rate and decreased their germination time (Ge et al., 2023; Hopkins, & Gravatt 2019; Ali & Elozeiri, 2017). The purpose of this study was to determine the effects of various dormancy-breaking treatments, such as stratification and gibberellic acid (GA_3) on the seed germination of Quince.

MATERIALS AND METHODS

This study was conducted on the farm of the Department of Horticulture, Faculty of Agriculture, Omar Al-Mukhtar University. The seeds were collected from farm No. (4) of the Al-Jabal Al-Akhdar Agricultural Project in the Shahat region in 2018 season. The seeds were divided into 3 groups based on perids of stratification as a main factor (30, 60, and 90 days) then each stratification period was divide into four groups based on concentrations of GA_3 (0,250,500 and 1000 ppm), where each individual treatment contained 40 seeds.

The following treatments were used:

Cold stratification 30 days

Cold stratification 60 days

Cold stratification 90 days

GA_3 0 ppm

GA_3 250 ppm

GA_3 500 ppm

GA_3 1000 ppm

For 24 hours, seeds were soaked with different concentrations of GA_3 based on experiments. After that, these seeds were sterilized with the fungicide (Mancozeb). Seeds were mixed with sand in a ratio of 1:3 (seed and sand, respectively). Then the seeds of each treatment were placed in a polyethylene bag to allow gas exchange and sealed. With weekly flipping and wetting the mix if needed. At the time of planting, the bags were prepared, and each bag contained equal amounts of a mixture of soil, sand and peat moss in a ratio of 1: 2: 1, respectively. At a depth of 2 cm seeds were planted, then irrigated as needed.

Germination percentage (GP) was calculated according to (Peters,2000) GP= $\left(\frac{n}{N}\right) \times 100$, where n is the number of germinated seeds, and N is the total number of seeds.

Germination speed (GS): It was estimated according to the equation (Naylor, 1981)

$$GS= \frac{\mathbf{a+(a+b)+(a+b+c)+ \ldots\ldots}}{\mathbf{N(Total\ number\ of\ seeds/treatment)}}$$

where, a: Number of seeds germinated in the first count , b: Number of seeds germinated in the second count,c: Number of seeds germinated in the third count and N: number of times to count. Seedling length (cm): after 150 days of planting. Seedling diameter (mm): 150 days after planting. Leaf area index of seedling: using Planimeter Kp - 90N. The percentage of dry matter was calculated.

$$\text{as follows: } \frac{\mathbf{dry\ weightdy}}{\mathbf{wet\ weight}} \times 100.$$

Statistical Analysis: The obtained data were subjected to a statistical analysis of variance ANOVA of the combined analysis in a completely randomized block design according to (Ott & Longnecker, 2015) and least significant difference (LSD) of 0.05% was used to compare between the means of the treatments using COSTAT Software (Pacific Grove, CA, USA).

RESULTS

Quince seeds were subjected to cold stratifications and GA_3 concentrations and recorded seed quality parameters.

Table 1shows the results of percentage germination and speed of germination. For percentage germination, results showed that there were significant differences between stratification periods, where stratification for 90 days had the highest germination value (87%).For concentrations of GA_3, also there were significant differences between concentration, where GA_3 500 ppm treatments had the highest ratio of germination (67%). The results of the interaction between stratifications and concentrations show that there were significant differences between treatments in percentage of germination, where stratification 60 days with GA_3 500 ppm treatments had the highest mean (94%).

For speed germination trait (table 1), the results show that there were differences between treatments, with 90 days of stratification having the best results compared with other treatments (1.88). While concentration 500 ppm had the highest mean (1.66). The results of the interaction between the two treatments shown that treated seeds with stratification 90 days with GA_3 500 ppm had the highest value (2.26), whereas the seeds treated with stratification 30 days had the lowest value (0.43).

Table (1): Effect of cold stratification (CS) and GA_3 concentrations on percentage and speed germination of Quince seed.

Germination percentage %					
	Concentration of GA_3 ppm				$\bar{x}$
CS	0	250	500	1000	CS
30 days	11^{e}	13^{e}	14^{e}	$16.^{e}$	13.5^{C}
60 days	80^{c}	84^{bc}	94^{a}	90^{ab}	83^{B}
90 days	70^{d}	$83.^{c}$	93^{a}	$86.^{bc}$	87^{A}
$\bar{x}$ GA3	53.66^{C}	60^{B}	67^{A}	64^{A}	
LSD GA_3= 3.97 Interaction = 6.88 Stratification = 3.44					
Germination speed					
	Concentration of GA3 ppm				$\bar{x}$
CS	0	250	500	1000	CS
30 days	0.43^{g}	0.46^{g}	0.62^{f}	0.68^{f}	0.54^{C}
60 days	1.14^{e}	1.53^{d}	2.11^{b}	2.08^{b}	1.72^{B}
90 days	1.45^{d}	1.66^{c}	2.26^{a}	2.15^{b}	1.88^{A}
$\bar{x}$ GA3	1.00^{C}	1.22^{B}	166^{A}	1.64^{A}	
LSD GA_3= 0.051 Interaction= 0.089 Stratification=0.044					

Means not sharing the same letter(s) within each column, significantly different at 0.05 level of probability

Table 2 shows the results of seedling length and seedling diameter for Quince seedling. For seedling length (cm) when comparing between stratification period treatments, we found that stratification for 90 days had the highest value (37.35 cm) while stratification for 30 days had the lowest value (31.57 cm). Additionally, both concentrations of GA_3 at 1000 ppm had the highest value (37.53 cm). Moreover, the interaction between two treatments shows there were significant differences in terms of seedling length (cm). The results show that the treatments stratified 90 days with GA_3 at 500 ppm had the highest value of seedling length (39.9cm), but did not differ significantly between these two treatments. However, the stratification for the 30-day treatment without GA_3 had the lowest value of seedling length (28.6 cm). Also, Table 2 shows the Inverse relationship between seedling diameter (mm) with concentrations of GA_3, where seedlings that were not treated with GA_3 and stratified 60 days had the highest diameter (2.89).

Table (2): Effect of cold stratification (CS) and GA_3 concentrations on seedling length (cm) and seedling diameter (mm) of Quince seedling

Seedling length (cm)					
	Concentration of GA_3 ppm				$\bar{x}$
(CS)	0	250	500	1000	CS
30 days	28.6^{g}	29.2^{g}	32.7^{f}	35.8^{de}	31.57^{C}
60 days	34.6^{e}	35.4^{de}	37.7^{bc}	38.1^{b}	36.45^{B}
90 days	34.4^{e}	36.4^{cd}	39.9^{a}	38.7^{ab}	37.35^{A}
$\bar{x}$ GA_3	32.53^{C}	33.66^{B}	36.76^{A}	37.53^{A}	
LSD GA_3= 0.83 Interaction = 1.44 Stratification = 0.72					
Seedling diameter (mm)					
	Concentration of GA_3 ppm				$\bar{x}$
(CS)	0	250	500	1000	CS
30 days	2.24^{f}	2.13^{g}	2.11^{g}	2.02^{h}	2.13^{C}
60 days	2.89^{a}	2.63^{c}	2.40^{d}	2.37^{de}	2.57^{A}
90 days	2.85^{a}	2.72^{b}	2.31^{ef}	2.03^{h}	2.48^{B}
$\bar{x}$ GA_3	2.66^{A}	2.49^{B}	2.27^{C}	2.14^{D}	
LSD GA_3= 0.039 Interaction = 0.068 Stratification = 0.034					

Means not sharing the same letter(s) within each column, significantly different at 0.05 level of probability

In terms of the influence of treatments on leaf area index (Table 3) there was a significant impact for both main treatments (stratification and GA3 concentrations). Treatment stratification for 90 days with 1000 pm of GA_3 possesses the highest value (34. cm^2). Furthermore, there were differences between treatments in dry matter in seedlings. where stratification 90 days had the highest Values (55.90 %) while GA_3 1000 ppm treatment had the highest percentage of dry matter (56.17%) the treatments stratification 90 days with GA_3 at 1000 ppm had the highest in dry matter (57.54mm).

Table (3): Effect of cold stratification (CS) and GA_3 concentrations on leaf area index(cm2) and percentage of dry matter (%)of Quince seedling.

Leaf area index(cm^2)					
	Concentration of GA_3 ppm				$\bar{x}$
CS	0	250	500	1000	CS
30 days	23.40^{f}	24.70^{ef}	26.20^{de}	27.10^{d}	25.35^{C}
60 days	25.10^{e}	27.50^{d}	31.10^{c}	32.00^{bc}	28.92^{B}
90 days	26.20^{de}	30.60^{c}	32.80^{ab}	34.40^{a}	31.00^{A}
$\bar{x}$ GA_3	24.90^{D}	27.60^{C}	30.03^{B}	31.16^{A}	
LSD GA_3= 0.96 Interaction = 1.66 Stratification = 0.83					
Percentage of dry matter (%)					
	Concentration of GA_3 ppm				$\bar{x}$
CS	0	250	500	1000	CS
30 days	50.10^{i}	53.42^{gh}	54.67^{de}	54.58^{def}	53.19^{C}
60 days	52.84^{h}	53.63^{fgh}	54.72^{cd}	56.39^{bc}	54.40^{B}
90 days	53.91^{efg}	55.48^{cd}	56.65^{ab}	57.54^{a}	55.90^{A}
$\bar{x}$ GA_3	52.29^{D}	54.17^{C}	55.35^{B}	56.17^{A}	
LSD GA_3= 0.57 Interaction = 0.99 Stratification = 0.49					

Means not sharing the same letter(s) within each column, significantly different at 0.05 level of probability

DISCUSSION

The results of the current study demonstrated that stratification for 60 and 90 days or concentrations of 500 and 1000 GA_3 had an obvious effect in terms of improving the speed and germination of seed. These results were explained previously by (Lewak 2011; Han & Yang ,2015; Hopkins & Gravatt 2019; wang et. al 2020 & Ge et. al, 2023). They reported that the effects of cold stratification on seed dormancy breaking occur when the level of abscisic acid (ABA) declines and the level of GAs increases due to cold stratification. Additionally, cold stratification broke embryonic dormancy in seeds by catabolizing lipids, sugars, and proteins with hydrolytic or proteolytic enzymes. Our results are also consistent with the findings of (Sondheimer et. al, 1968; Chen et. al., 2007; Finkelstein et. al, 2008; Leida et.al, 2012 & Ge et. al, 2023). They found that treating with cold stratification and GA_3 markedly increased the speed and germination of seed. The main hormone responsible for maintaining seed dormancy is abscisic acid (ABA). The ABA levels decrease significantly in seeds as the stratification time increases. Because seed dormancy is ultimately controlled by the balance between ABA and gibberellic acid (GA) a decrease in ABA would tip the hormonal balance toward GA and the allowance of germination after sufficient stratification (Guo et al., 2020; El-Yazal 2021 &Ge at el., 2023).

For seedling length (cm), the result shows that the treatments of stratification for 90 days with both GA_3 500 and 1000 ppm have a significant impact on seedling length comparing with other treatments. This confirms the findings by (Yamauchi, at el., 2004), where the cold stratification impacts gene involved in the biosynthesis of gibberellins, thus leading to an increase in the level of bioactive GAs and transcript abundance of GA-inducible genes (Yamauchi, at el., 2004; Chen, at el., 2008). Additionally, our results are consistent with those of (Nedunchezhiyan, at el., 2020) they found that the increase in GA content has an obvious effect in terms of improving the vegetative growth, such as the length of the stem. They reported that the main functions of gibberellins are to increase the intermodal length of the stem, resulting in a reduction in the dwarf stature of the plant.

Stratification 60 and 90 days without GA_3 had the highest value which was markedly and significantly different the rest of the treatments in the seedling diameter (mm). Moreover, the concentrations of GA_3 had an inverse relationship influence on this trait. This may be attributed to the nature of the action and effect of gibberellins, which leads to the division and elongation of cells, giving the highest rate of seedling length, thus having a smaller diameter compared to seedlings produced from untreated seeds. These results agree with what was obtained by (Samaan at el., 2000). Moreover, the treatments with stratification periods and concentrations of gibberellins have a pronounced influence on leaf area and dry matter (%), where stratification of 60 and 90 days with 500 and 1000 ppm GA had a distinct effect. These results agree with (Li at el., 2020; Castro - Camba at el., 2022), they reported that the concentrations of gibberellins play an important role in the enhancement of the efficiency of fruit crops in terms of growth and yield. Therefore, the exogenous applications of GA at different concentrations drastically increase stem elongation and shoot initiation, an addition to modifying several other vital processes in fruit crops.

CONCLUSION

The results of this study revealed that stratification for 60 or 90 days with 500 and 1000 GA_3 ppm had an obvious effect in terms of improving the germination of seed (94% and 93 % respectively). Additionally, it's had a great impact on all other traits, with the exception of the seedling diameter (mm) which revealed that 60 and 90 days of stratification without concentration of GA_3 had the highest value.

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