Research Article

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Impact of seed cold stratification on apricot germination and subsequent seedling growth as well as chemical constituents of seeds during stratification

Abstract

Freshly harvested seeds of apricot varieties "Balady (Amar)" and "Hamawy" were found to be dormant and did not germinate at all. A specific low-temperature stratification treatment was required to overcome seed dormancy. 5°C cold stratification was found to be the best for breaking seed dormancy, germination and seedling growth. Increased seed germination percentage was recorded when the period of stratification prolonged. Seedling developed from stratified seeds had better growth than those developed from non-stratified seeds. Apricot seeds required a cold stratification of about 12-15 days for "Balady variety (Amar)" and15-18 days for "Hamawy" variety "to reach maximum germination and normal seedling growth.

Chemical constituents of apricot seeds (total and reducing sugars, total free amino acids and total indoles were increased while that of free phenols and total soluble phenols were decreased) when stratification period prolonged. Therefore, it can be suggested that breaking of dormancy is coincided with several changes in different chemical constituents of seeds. Some of these materials increased and other materials which decreased at seed germinations.

Keywords: apricot (*Prunus armeniaca L.*), dormancy, stratification, germination, seedling growth, chemical constituents

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Samir Ahmed Seif El-Yazal,[1] Ahmed Abd El-Monem El-Shew,[2] Mohamed Ahmed Seif El-Yazal[2]

[1]Department of Horticulture, Faculty of Agriculture, Fayoum University, Egypt
[2]Department of Botany, Faculty of Agriculture, Fayoum University, Egypt

Correspondence: Mohamed Ahmed Seif El-Yazal, Botany Department, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt, Email mas04@fayoum.edu.eg

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Introduction

Prunus is could be a massive, numerous genus in *Rosaceae* family unremarkably cited as stone fruits. Principle business crops in this genus embrace peaches, nectarines, plums, prunes, apricots, cherries and almonds. Seeds of stone fruits do not germinate immediately after harvest and a period of after ripening is essential for certain chemical and other changes to take place in the seed and for dormant embryo to grow.[1] Dormancy is outlined as a physiological mechanism that completely different metabolic pathways, light-weight perception, hormones, cell cycle and abiotic stress resistance.[2] Seed dormancy may be a physiological development in plants, that is caused by external or internal factors, and forestall of seeds germination, even in best conditions. Seed dormancy is also caused because of laborious testa, immature embryo, rudimentary embryo and inhibitors materials.[3] In this respect, Garcia et al.[4] reported two independent dormancies in seeds of drupe a physical (external) and embryo (internal) dormancy that are essential for higher survival and institution of seedlings within the field. Seeds of temperate fruit species would like an extended time to germinate as a results of their demand of stratification or cold treatment.[1,5] Seeds of temperate fruit species don't germinate as a result of seed dormancy although conditions like water, temperature, and oxygen are appropriate for germination. Seed dormancy is classed as physiological, morphological, morpho-physiological, physical, and combinable dormancy (physical and physiological).[6,7] These mechanisms of dormancy area unit gift within the seeds of all temperature fruit species, as well as peach, cherry, and apricot.[8,9] Generally, these germination issues in temperate fruit species are with success overcome by cold stratification of seeds for many months throughout winter. However, seeds of temperate fruit species need a protracted time to germinate underneath ancient stratification or natural conditions. Therefore, quick and uniform germination techniques are fascinating for fruit-growing and breeding studies.[10] Various ways are tried to beat dormancy of stone fruits. Stratification has been used historically to interrupt seed dormancy in *Prunus sp.*[7] Scarification can be done either mechanically or chemically.[3]

Several researchers working on breaking dormancy such as[12–23] and 2020 on Buds[1,24–29] on seeds. The beneficial effect of stratification on seed germination were studied by[30–32]

Materials and methods

The fruits of apricot varieties" Balady (Amar)" and "Hamawy" were collected from bearing trees grown in private orchard at Ibshawai district, Fayoum governorate, Egypt by hand-stripping in June 1998 and 1999. All the fruits were packed in plastic bags and transported to the laboratory. Seeds obtained by breaking fruits were sampled randomly for all the experiments. Initial viability was obtained using the cutting method. The seeds were washed carefully with tap water and air dried. Hard shell (endocarp) of seeds was removed. Seeds were stored in opened-mouth jams at room temperature before stratification treatments were applied.

Stratification treatments

For stratification treatments, the seeds with removed endocarp were mixed with moistened sand. Afterwards they were subjected to a period of stratification at 5°C. Seeds were stratified in pots of 30×40 cm. Stratified seeds were regularly irrigated once per week. To prevent the water loss during stratification upper surface of pots was covered by a sack. The following stratification were applied for each species: Cold stratification (CS) for 0,3,6,9,12 and 15 days in 1998 and for 0, 3, 6, 9 and 12 days in 1999 years for "Balady (Amar)" variety. And 0, 3, 6, 9, 12, 15 and18 days in 1998 and for 0, 3, 6, 9, 12, and 15 days in1999 years for "Hamawy" variety. Sowing time (ST) in 1st July of 1998 and15th July 1999 years. Control (without pretreatment). Dishes were placed at 25°C in incubators to allow germination. The germination% was calculated at three days interval during a period of 15 days.

Effect of exposed seed to cold stratification on growth characters of seedling after 120days from planting

During 1998 and1999 seasons, four samples divided into three replicates each of 45 seeds from each variety were stratified at 5ºC. The first two samples were stratified for 15 days, the second two samples were stratified for 3 days later (12 days), the third two samples were stratified after another 3 days (9days) while some seeds are left without stratification. The seeds were sown after a given stratification period each in plastic pots (25x12cm) containing sterilized clean sand and kept under shade greenhouse conditions. Seedlings height (cm), seedlings thickness (mm), fresh weight (g) of the above ground portion as well as root fresh weight of seedlings were measured for each treatment 120 days after seed sowing.

Determination of chemical constituents in seeds during cold stratification

In both seasons samples of 20 seeds were taken at 3 days interval and extracted with the methanol being changed every 24 hours.[33] The combined methanolic extracts were filtered and evaporated under vacuum at 40-+2°C and transferred into aqueous phase for the following determinations:

Determination of total and reducing sugars

Total and reducing sugars were determined as mg/g dry weight using phosphomolybdic acid reagent according to A.O.A.C.[34]

Determination of total free amino acids

Total free amino acids were determined as mg/g dry weight according to Jayarman[35] with some modifications.[36]

Determination of total indoles

Total indoles were determined (as ug/g dry weight) according to Larsonet al.[37]

Determination of free and total phenolics

Total and free phenols in seeds were determined as mg/g dry weight using folin-ciocalteu reagent and Sodium carbonate solution according[38] with some modification.

Statistical analysis

All studied treatments were arranged in a complete randomized block design with three replicates for each and were statistically analyzed according to the method of Duncan[39] The values presented in the results obtained in the Table 1 is the mean of the two seasons under the study.

Table 1 Effect of cold stratification period on the items of seedling growth after 120 days from planting

Stratification period(days) at 5°C	Roots weight (g)	Shoot weight (g)	Seedling height (cm)	Seedling thickness (mm)	Roots weight (g)	Shoot weight (g)	Seedling height (cm)	Seedling thickness (mm)
	"Balady Variety Amar"				"Hamawy" variety			
0	(Z) 3.96b	5.7b	42.3b	3.0b	3.4b	4.9c	34.7c	3.0b
9	4.1ab	6.9ab	57.7a	3.5b	3.4b	6.1b	49.2b	3.5b
12	4.7a	8.1a	62.4a	5.0a	4.3a	6.0b	50.6b	4.5a
15	4.6a	7.8a	59.0a	4.5a	4.1a	7.3a	54.3a	5.0a

The values presented in the results obtained in this table is the mean of the two seasons under the study

Mean separation, within columns, by Duncan's multiple range test, 5% level

Results

Seed germination

Data in (Table 2 & 3) indicated that seed germination percentage was significantly increased (after 15 days germination at 25ºC) to94.6% and 96.1% in "Balady (Amar)" and "Hamawy" varieties respectively as compared to the control (non-stratified seeds) 20.8% and 17.3% after 15 and 18 days cold stratification in 1998 season. Moreover, germination percentage was increased to 97.2% and 98% in "Balady (Amar)" and "Hamawy" varieties respectively as compared to the control (non-stratified seeds) 18.2% and13.4% after 12 and 15 days stratification period in 1999 season.

The data also show that cold stratification at 5ºC had a stimulating effect on seed germination. Moreover, the number of days required for seed germination was decreased with the increase of cold stratification period (Table 2 & 3).

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Concerning apricot varieties, it is well noticed that "Balady Variety (Amar)" and "Hamawy"variety varied in their stratification requirements. Thus "Hamawy" seeds required 3 days stratification longer than "Balady Variety Amar)" seeds for their dormancy to be released. Furthermore, stratification period in season 1999 was three days shorter than those in 1998 season (Table 2 & 3).

Table 2 Effect of cold stratification period on germination% of apricot seeds during 1998 season

Stratification period(days) at 5°C	Germination period (days) at 25°C									
	"Balady Variety Amar"					"Hamawy" variety				
	3	6	9	12	15	3	6	9	12	15
0	(Z) 9.4cd	10.0d	15.2d	16.4d	20.8d	07.0de	08.3d	10.2c	12.4c	17.3c
3	4.0d	08.0de	09.1de	10.0e	10.5c	2.4c	03.5de	05.0c	06.4f	07.2f
6	2.0e	04.0e	6.10e	9.00e	09.4c	1.2c	2.60c	03.4c	04.2f	05.8f
9	14.5c	25.5c	32.5c	36.0c	42.0c	12.6d	23.4c	28.3d	30.1d	32.4d
12	28.8b	33.8b	40.0b	53.0b	64.6b	22.8c	26.6c	34.2c	46.3c	58.3c
15*	58.8a	87.5c	94.0a	94.0a	94.6a	46.3b	58.8b	73.2b	78.3b	80.4b
18						62.4a	78.3a	89.2a	96.1a	96.1a

The seeds of "Balady Variety Amar" started to germinate during stratification (Z)

Mean separation, within columns, by Duncan's multiple range test, 5% level

Table 3 Effect of cold stratification period on germination % of apricot seeds during 1999 season

Stratification period(days) at 5°C	Germination period (days) at 25°C									
	"Balady Variety Amar"					"Hamawy" variety				
	3	6	9	12	15	3	6	9	12	15
0	(Z)6.2d	12.0d	14.3d	17.0d	18.2d	5.2d	06.3d	8.2d	9.0e	13.4c
3	2.0d	6.2d	7.3e	15.2d	14. 4d	1.3d	2.0d	3.2d	6.3e	7.8f
6	12.2c	20.6c	28.8c	30.2c	36.4c	10.7c	18.2c	22.4c	28.3d	24.4d
9	35.4b	46.2b	32.5c	60.3b	70.2b	16.4c	19.2c	25.3c	35.0c	46.6c
12*	60.2a	88.2a	95.0a	97.2a	97.2a	40.0b	52.2b	66.0b	72.0b	78.0b
15						58.0a	64.0a	82.0a	95.0a	98.0a

The seeds of "Balady Variety Amar" started to germinate during stratification (Z)

Mean separation, within columns, by Duncan's multiple range test, 5% level

Vegetative growth of apricot seedling

Vegetative growth of apricot seedling after 120 days from planting (Table 1) indicating that seedling developed from stratified seeds for 12 days ("Balady Variety Amar)") and 15 days("Hamawy" variety) had significantly the best vegetative growth compared with the corresponding ones developed from non- stratified seeds.

From the previous results it can be concluded that, apricot seeds ("Balady Variety Amar") and "Hamawy" variety required a cold stratification period of about of 12-15 days for "(Balady Variety Amar)" and about 15-18 days for "Hamawy" variety to reached maximum germination and normal seedling growth.

Endogenous changes occurring in apricot seeds during cold stratification

Total and reducing sugars, total free amino acids and total indoles concentration: Total and reducing sugars, total free amino acids and total indoles (Table 4 & 5) were present at lower levels in the initial extract of non-stratified seeds. A gradual increase was recorded with the advance of cold storage period to reach its maximum levels after 12 and 15 days cold stratification in the first season and 9 and 12 days cold stratification period in the second season for("Balady (Amar") and "Hamawy" varieties, respectively.

Phenolic content: Free and total soluble phenols content in apricot seeds (Table 6) were decreased gradually with prolonging the cold storage period to reach its minimum values after 18 and 15 days stratification in 1998 and 1999 seasons respectively.

Conclusively, from the present results, it is clear that there was a relationship between seed germination and several changes in their chemical contents. Thus, some of these chemical increased such as total and reducing sugars, total free amino acids and total indoles while the other chemical decreased during seed germination as free and total soluble phenols.

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Table 4 Total and reducing sugars concentration (mg/g D.W.) in apricot seeds as affected by period of cold stratification during 1998 season

Days of cold Stratification	Total sugars concentration (mg/g D.W.)				Reducing sugars concentration (mg/g D.W.)			
	"Balady Variety Amar"		"Hamawy" variety		"Balady Variety Amar"		"Hamawy" variety	
	1998	1999	1998	1999	1998	1999	1998	1999
0	64.3	58.15	42.25	54.2	33	30	40	32.1
3	64.9	64.1	58.15	58.18	35.11	40.1	38	38.4
6	71.1	72.1	58.6	64.1	48.1	46.2	36.8	50.1
9	92.15	89.12	60	71	49.1	50	39.1	52
12	95.12	80	64.1	100.11	49.3	35.2	49.2	60
15*	83.1	56.1	98	44.9	47	36.5	60	30.1
18	57.2	--	32	--	30	--	35.4	--

Mean separation, within columns, by Duncan's multiple range test, 5% level

Table 5 Total free amino acids and total indoles concentration (mg/g D.W.) in apricot seeds as affected by period of cold stratification during 1998 season

Days of cold Stratification	Total free amino acids concentration (mg/g D.W.)				Total indoles concentration (ug/g D.W.)			
	"Balady Variety Amar"		"Hamawy" variety		"Balady Variety Amar"		"Hamawy" variety	
	1998	1999	1998	1999	1998	1999	1998	1999
0	25.1	20.6	60.1	50.2	7010	851	615	795
3	55.15	52	45.2	65.1	545	715	600	782
6	70.2	175	80	81.15	610	661	611	732
9	82.15	198.1	78.2	198	655	714	751	784
12	180.1	150	191	200.1	731	748	431	798
15*	145	150	200.1	152	322	321	456	497
18	70	--	179.15	--	293	--	465	--

Mean separation, within columns, by Duncan's multiple range test, 5% level

Table 6 Total and free phenols concentration (mg/g D.W.) in apricot seeds as affected by period of cold stratification during 1998 season

Days of cold Stratification	Total phenols concentration (mg/g D.W.)				Free phenolsconcentration (mg/g D.W.)			
	"Balady Variety Amar"		"Hamawy" variety		"Balady Variety Amar"		"Hamawy" variety	
	1998	1999	1998	1999	1998	1999	1998	1999
0	58	69	77	83	17.5	18	18	19
3	41	54	75	76	17	17.9	16	17.5
6	40	42	50	62	15.5	17.1	15.9	14.1
9	34	39	38	41	13.1	15	8.9	11.3
12	31	30	40	40	10.3	12.5	7.5	8.1
15*	30	28	22	37	9.8	8.1	7	7.6
18	27	--	19	--	9.75	--	5.95	--

Mean separation, within columns, by Duncan's multiple range test, 5% level

Discussion

Apricot seeds are dormant and that dormancy breaking treatments have to be performed to obtain high germination and that the dormancy being caused by the permeability of the seed coat and fruit pericarp rather than by the embryo.[40,41] Stratification, application usually increase the germination percentage of the seeds.[42,43] It is clear from the data that cold stratification at 5ºC had a stimulating effect on seed germination. In this respect, Lewek[44] reported that embryonic dormancy is outline as a group of blocks obligatory upon a process(es) cardinal for growth. In apple seeds, of these blocks square measure removed as a results of cold treatment (stratification). Certain other block are responsible for dormancy of embryo, were removed as a result of a change in hormonal equilibrium.[45] Also, Bogatck & Lewek[46] indicated that the elimination of embryonic dormancy in apple seeds was connected with a change from domination of PPP to domination of glycolysis in sugar catabolism during cold stratification. The presented results also show that "Balady Variety (Amar)" and "Hamawy" variety varied in their stratification requirements. In this respect,[29] reported that apple seed germination relied on the varities and also the manner of stratification. Among the examined cultivars, 'Szampion' seeds germinated at the very best share. Lower seed germination was discovered for 'Ligol', whereas 'Gold Milenium' seeds germinated at the bottom share. The bottom germ in ability of 'Gold Milenium' seeds can be partially caused by a negative influence of germination inhibitors gift in apple fruit extracts, that is, abscisic acids, chlorogenic acids, and so on.[47,48] In turn, the lower proportion of 'Ligol' seeds' germination could in all probability be caused because of their low maturity. Obviously, the variations in their germination may depended not solely on variety however conjointly. Conjointy properties the share of seed germination depended not only on cultivar but also on the year of seed harvest.[49] The present study showed that stratification in water resulted in a rise of share germinated seeds in compared to control (untreated) seeds. The data also indicating that seedling developed from stratified seeds had significantly the best vegetative growth compared with the corresponding ones developed from non- stratified seeds. These results are in agreement with those of Kilany[50] who found that peach seedling height increased by increasing the period of cold stratification of seed up to 60 days. The results also show that cold stratification period increased total and reducing sugars, total free amino acids and total indoles in seeds. In this respect, Jones & Armstrong[51] pointed out that the synthesis of a-amylase elicited by gibberellin usage and this leads to high levels of soluble carbohydrates and maltose as a starch hydrolysis in the endosperm. Moreover, Kilany[50] observed the accumulation of soluble amino acids in peach seed tissues as ripening progressed at cold stratification. In addition, Daskalyuket al.[52] found a decrease in the content of polypeptides in apple seeds with increasing period of stratification. On the opposite hand, Kopecky et al.[53] noted a exact auxin-like activity in part cold-stratified apple seeds. The absence of free IAA in dormant seeds and in seeds submitted to cold treatment was finally confirmed twenty years later.[54]

In contrast the information conjointly shown a decrease within the content of total and free phenols in apricot seeds with increasing amount of stratification. During this concern, Phloridzin (phloretinb-D-glucoside) is the most long monomeric phenol in apple seeds. It amounts up to eight of dry matter and in dormant seeds is found at the side of condensed tannins, within the seed coat chiefly.[55] Its level in the integument decreased to trace amounts during the first 20 days of stratification.[56] The huge concentration of phenolics with in the coat has been postulated to play a task in maintaining the dormancy of the embryo.[57] On the other hand, lower concentrations of phloridzin, its aglyconephloretin and alternative phenolics gift within the embryo (e.g.,chlorogenic acid) might play a secondary role within the control of dormancy, poignant the activity of vital enzymes and therefore at least some of the processes cardinal for the onset, maintenance, or removal of dormancy.[58] Also, Kefeli & Kutacek[59] suggested that plant phenol is also divided into three teams, promotive, inhibitor and inactive. They extra that promotion of plant growth by phenols might proceed through the modulation of either IAA synthesis or its destruction.[60–63]

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Conflicts of interest

The authors declare there are no conflicts of interest.

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References

1. Şan B, Yildirim AN, Yildirim F. An *in vitro* germination technique for some stone fruit species: the embryo isolated from cotyledons successfully germinated without cold pre-treatment of seeds. *American Society for Horticultural Science*. 2014;49(3):294–296.
2. Arora R, Rowland LJ, Tanino K. Induction and release of bud dormancy in woody perennials: a science comes of age. *Hort Science*. 2003;38:911–921.
3. Mousavi SR, Rezaei M, Mousavi A. A general overview on seed dormancy and methods of breaking It. *Advances in Environmental Biology*. 2011;5(10):3333–3337.
4. García-Gusano M, Martínez-Gómez P, Dicenta F. Breaking seed dormancy in almond (*Prunusdulcis* (Mill.) D.A. Webb). *Scientia Hort*. 2004;99:363–370.
5. Shah RA, Arti Sharma VK, Jasrotia WA, et al. Effect of seed priming on peach, plum and apricot germination and subsequent seedling growth. *Indian J Hort*. 2013;70(4):591–594.
6. Baskin JM, Baskin CC. Classification system for seed dormancy. *Seed Sci Res*. 2007:14116.
7. Finch-Savage WE, Leubner-Metzger G. Seed dormancy and the control of germination. *New Phytol*. 2006;171(3):501–523.
8. Han M, Zhang M, Tian Y, et al. Effect of plant hormones on seed dormancy and seedling growth of stone fruits. *Acta Botanica Boreali-occidentalia Sinica*. 2002;22(6):1348–1354.
9. Martinez-Gomez P, Dicenta F. Mechanisms of dormancy in seeds of peach [*Prunuspersica* (L.) Batsch] cv. 2001.
10. Arbeloa A, Daorden ME, Garcia E, et al. *In vitro* culture of 'Myrobalan' (*Prunuscerasifera Ehrh*.) embryos. *Hort Science*. 2009;44(6):1672–1674.
11. Seif El-Yazal MA, Rady MM. Changes in nitrogen and polyamines during breaking bud dormancy in "Anna" apple trees with foliar application some compounds. *Scientia Horticulturae*. 2012;136:75–80.
12. Seif El-Yazal MA, Rady MM. Foliar-applied DormexTM or thiourea-enhanced proline and biogenic amine contents and hastened breaking bud dormancy in "AinShemer" apple trees. *Trees*. 2013;27(1):161–169.
13. Seif El-Yazal MA, Rady MM. Exogenous onion extract hastens bud break, positively alters enzyme activity, hormone, amino acids and phenol contents, and improves fruit quality in 'Anna' apple trees. *Scientia Horticulturae*. 2014;169:154–160.

14. Rady MM, Seif El-Yazal MA. Response of "Anna" apple dormant buds and carbohydrate metabolism during floral bud break to onion extract. *Scientia Horticulturae*. 2013;155:78–84.

15. Rady MM, Seif El-Yazal MA. Garlic extract as a novel strategy to hasten dormancy release in buds of 'Anna' apple trees. *South African Journal of Botany*. 2014;92:105–111.

16. Seif El-Yazal MA, Rady MM, Seif SA. Foliar-applied dormancy-breaking chemicals change the content of nitrogenous compounds in the buds of apple (*Malussylvestris Mill*. cv. Anna) trees. *Journal of Horticultural Science & Biotechnology*. 2012;87(4):299–304.

17. Seif El-Yazal MA, Seif El-Yazal SA, Rady MM. Exogenous dormancy-breaking substances positively change endogenous phytohormones and amino acids during dormancy release in 'Anna' apple trees. *Plant Growth Regul*. 2014;72:211–220.

18. Seif El-Yazal MA, Rady MM, Seif El-Yazal SA. Metabolic changes in polyamines, phenylethylamine, and arginine during bud break in apple flower buds under foliar-applied dormancy-breaking. *International Journal for Empirical Education and Research*. 2018a;1(2):1–18.

19. Seif El-Yazal MA, Rady MM, Seif El-Yazal SA. Foliar-applied mineral oil enhanced hormones and phenols content and hastened breaking bud dormancy in "Astrachan" apple trees. *International Journal for Empirical Education and Research*. 2018b;1(2):57–73.

20. Seif El-Yazal MA, Seif El-Yazal SA, Rady MM. Changes in promoter and inhibitor substances during dormancy release in apple buds under foliar-applied dormancy-breaking agents. *International Journal for Empirical Education and Research*. 2018c;1(4):1–20.

21. Seif El-Yazal MA, Rady MM, Seif El-Yazal SA, et al. Changes in metabolic processes during break dormancy in apple buds under foliar-applied garlic extract. *International Journal for Empirical Education and Research*. 2018d;1(4):36–58.

22. Seif El-Yazal MA, Seif El-Yazal SA, Morsi ME, et al. Onion extract application effects on flowering behavior and yield, and a few chemical constituents of shoots throughout dormancy break in "Anna" apple trees. *Journal of Horticulture and Plant Research*. 2019a7:1–15.

23. Seif El-Yazal MA, Seif El-Yazal SA. Impact of chilling requirements on metabolic changes in nitrogenous compounds in buds during and after dormancy releasing in early and late (*Malussylvestris*, Mill) apple varieties. *Horticult Int J*. 2019;3(5):230–238.

24. Mark R, Jo-Ann B, Paul W, et al. Maturity and temperature stratification affect the germination of Styrax japonicus seeds. *The Journal of Horticultural Science and Biotechnology*. 2015;79:645–651.

25. Jeung Keun S, Ji Hee K, Ae Kyung L. Effect of warm and cold stratification, and ethanol treatment on germination of Corylopsis seeds. *Hort Sci (Prague)*. 2016;43:84–91.

26. Pliszko A, Kostrakiewicz-Gierałt K. Resolving the naturalization strategy of Solidago ×niederederi (*Asteraceae*) by the production of sexual ramets and seedlings. *Plant Ecol*. 2017a;218:1243–1253.

27. Pliszko A, Kostrakiewicz-Gierałt K. Seed germinationin Solidago ×niederederi (*Asteraceae*) and its parental species after two different fruit storage periods. *Biodiv Res Conserv*. 2017b;48.

28. Pliszko A, Kostrakiewicz-Gierałt, K. Effect of cold stratification on seed germination in Solidago × niederederi (*Asteraceae*) and its parental species. *Biologia (Bratisl)*. 2018;73(10):945–950.

29. Górnik K, Grzesik M, Janas R, et al. The Effect of Apple Seed Stratification with Growth Regulators on Breaking the Dormancy of Seeds, the Growth of Seedlings and Chlorophyll Fluorescence. *Journal of Horticultural Research*. 2018;26(1):37–44.

30. Seng M, Cheong EJ. Comparative study of various pretreatment on seed germination of *Dalbergia cochinchinensis*. *Forest Science and Technology*. 2020;16(2):68–74.

31. Yan A, Chen Z. The control of seed dormancy and germination by temperature, light and nitrate. *The Botanical Review*. 2020;86:39–75

32. Guo C, Shen Y, Shi F. Effect of temperature, light, and ltorage lime on the seed germination of *Pinus bungeana Zucc*. exEndl.: The role of seed-covering layers and abscisic acid changes. *Forests*. 2020;11:300.

33. Diaz DH, Martin GC. Peach seeds dormancy in relation to endogenous inhibitors and applied growth substances. *J Amer Soc Hort Sci*. 1972;97:651–654.

34. A.O.A.C. Official Methods of Analysis of the Association of Official Agricultural Chemists. 16th ed. USA: Washington D.C; 1995.

35. Jayarman J. Laboratory Manual in Biochemistry. New York: Wiley Eastern Limited; 1981:61–73.

36. Chen L, Chen Q, Zhang Z, et al. A novel colorimetric determination of free amino acids content in tea infusions with 2,4-dinitrofluorobenzene. *Journal of Food Composition and Analysis*. 2009;22:137–141.

37. Larson P, Herlo A, Klunsour S, et al. On the biogensis of some indoles compounds in *Acetobacter xylimum*. *Physiol Plant*. 1962;15:552–565.

38. Galicia L, Nurit E, Rosales A, et al. Laboratory protocols: maize nutrition quality and plant tissue analysis laboratory. CIMMYT, Mexico, DF. 2009.

39. Duncan DB. Multiple range and multiple F tests. *Biometrics*. 1955;11(1):1–42.

40. Eriş A. Garden Plants Physiology. U.U.Z.F. *Ders Notları*. 1990;11:152.

41. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant*. 1962;15(3):473–497.

42. Serebryakova NV, Kalanova AI. The effect of water soluble vitamins on rose seed germination and rooting of cuttings. *Hort Abst*. 1978;44(4):5819.

43. Tuzcu O, Kaplankıran M, Yesiloglu T, et al. The effects of germination and developing of different preservation methods on pecan (*Carya illinoensis*) seeds. Turkey First Nurseries Symposium. *The Ministry of Agriculture Publication*. 1991:201–211.

44. Lewak S. Metabolic control of embryonic dormancy in apple seed: seven decades of research. *Acta Physiologiae Plantarum*. 2011;33:1–24.

45. Lewak S. Regulatory pathways in removal of apple seed dormancy. *Acta Hortic*. 1981;120:149–159.

46. Bogatek R, Lewak S. Effect of cyanide and cold treatment on sugar catabolism in apple seeds during dormancy removal. *Physiol Plant*. 1988;173(3):406–411.

47. Rudnicki R. Studies on abscisic acid in apple seeds. *Planta*. 1969;86(1):63–68.

48. Kamiński W. Inhibitory effect of apple juice on the germination of apple and cherry seeds and the growth of apple seedlings. *Acta Societatis Botanicorum Poloniae*. 1968;37(1):173–178.

49. Bewley JD, Black M. Seeds. Physiology of development and germination. New York: Plenum Press; 1994:445.

50. Kilany OA. Studies on germination of peach seeds. 1-Effect of seed coat, cold stratification and growth regulators. *Annals of Agric Sc Moshtohor, Egypt*. 1986;24(4):2169–2180.

51. Jones RL, Armstrong JE. Evidence for osmotic regulation of hydrolytic enzyme production in germinating barley seeds. *Plant Physiol*. 1971;48(2):137–142.

52. Daskalyuk AP, Toma OK, Yarotskaya LV, et al. Seed germination and polypeptide composition in early- and late-ripening apple cultivars as related to stratification time. *Russ J Plant Physiol*. 1996;43:504–550.

53. Kopecky F, Sebanek J, Blazkova J. Time course of the changes in the level of endogenous growth regulators during the stratification of the seeds of the "*Panenskeceske"* apple. *Biol Plant*. 1975;17:81–87.

54. Dziewanowska K, Lewak S. Non-decarboxylating transformation of indol-3-acetic acid in apple seeds. *Biol Plant*. 1987;29:110–117.
55. Dziewanowska K, Grochowska MJ, Lewak S. Changes in phloridzin and chlorogenic acid content and in indolylacetic acid oxidase activity during development of apple seeds. *Fruit Sci Rep*. 1974;1:3–9.
56. Bogatek R, Podstolski A, Ostaszewska A, et al. Phloridzin transformation and accumulation during the stratification of apple seeds and the culture of isolated embryos. *Biol Plant*. 1976;18:241–250.
57. Come D. Relationship between oxygen and embryonic dormancy and argumentative inhibition phenomena. *Bull Soc Frane Physiol Veg*. 1968;14:3145.
58. Dziewanowska K, Lewak S. Indolylacetic acid oxidase in dormant apple embryos. *Biol Plant*. 1975;17:207–213.
59. Kefeli V, Kutacek M. Phenolic substances and their possible role in plant growth regulation. *Plant Growth regulator Abs*. 1977;5:472.
60. SeifEl-Yazal MA. Effect of timing of mineral oil spraying on budburst and metabolic changes in “Barkhar” apple trees under conditions of inadequate winter chilling in Egypt. *Horticult Int J*. 2019a;3(2):67–75.
61. Seif El-Yazal MA. Seasonal changes in soluble and non-soluble carbohydrates during and after dormancy release in early and late varieties of apple (*Malus Sylvestris*, Mill) trees. *International Journal For Empirical Education and Research*. 2019b;3(20):1–18.
62. Seif El-Yazal MA. Impact of chilling requirement on budburst, floral development and hormonal level in buds of early and late apple varieties (*Malus sylvestris*, Mill) under natural conditions. *Journal of Horticulture and Plant Research*. 2019c8:1–11.
63. Seif El-Yazal MA. Impact of Chilling Requirements on Metabolic Changes in Phenolic Compounds in Buds during and after Dormancy Releasing in early and late (*Malus sylvestris*, Mill) Apple Varieties. International Letters of Natural Sciences. 2020.