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Original Research Article

Seed germination criteria and seedling characteristics of *Magnolia grandiflora* L. trees after cold stratification treatments

M.I.Fetouh[1] and F.A.Hassan[1,2*]

[1]Horticulture Department, Faculty of Agriculture, Tanta University, Egypt
[2]Current address: Biology Dep. Fac. of Science, Taif University, Saudi Arabia
*****Corresponding author*

Keywords

Magnolia; Cold stratification; Seed germination; Leaf area.

A B S T R A C T

The present study was carried out to investigate the effect of cold stratification on seed germination and seedling growth of *Magnolia grandiflora* L. Seeds were subjected to 0, 30, 60, 90 and 120 days cold stratification at 5 ˚C. After each stratification period, seeds were putted in polyethylene bags under saran greenhouse till the germination ceased. During germination stage, Imbibitions period (IP), total germination period (TGP), germination percentage (G %), germination index (GI), germination value (GV), mean germination time (MGT) and the speed of emergence (SE) were recoded. At the end of the experiment root main length, lateral root number and root fresh weight, shoot height, seedling total length, vigor index, shoot fresh weight and leaves area were measured. Results obtained clearly indicate that increasing cold stratification period enhanced germination parameters as well as seedling characteristics. The most effective stratification period was 90 days of cold stratification followed, in most cases, by 120 days cold stratification treatment. In order to enhance seed germination and seedling growth of *Magnolia grandiflora*, treating seeds with 90 or 120 days of cold stratification was recommended.

Introduction

Magnolia grandiflora L., belongs to Magnoliaceae family, is one of the most important landscape trees and consider a rare ornamental tree in Egypt as their numbers is in shortage due the low propagation ability under the local conditions. Magnolia seeds exhibit double dormancy, embryos will not develop until seeds are exposed to warm, moist temperatures after cold moist temperatures. During the after ripening period, the oil and proteins are converted to reducing sugars and the water content of a seed increases (Del Tredici, 1981). Stratification enables seeds to germinate more quickly and completely. Even when the total germination percentage does not change, the germination of most trees seeds is more rapid after they have been stratified (Leadem, 1997).

Seeds of *Carpinus caroliniana* exhibited higher germination when treated with 20 - 24 weeks of cold stratification (Lisa *et al.*, 2000). Lohengrin and Arroyo (2000) on *Phacelia secunda* reported that cold stratification is needed to initiate early and to reach maximum germination rates. The best germination of *Illicium parviflorum* and *Illicium floridanum* was occurred after 90 and 60 days cold stratification, respectively (Olsen and Ruter, 2001). The cold moist stratification significantly increased the germination percentage of different seed trees (Harrington and Kraft, 2004; Tilki and Cicek, 2005; Dahkaei, 2009 and Guner and Tilki, 2009). Rawat *et al*. (2010) clearly revealed that 25-30 days stratification of *Punica granatum* seeds resulted in the highest germination percentage, longest radicle, maximum root and shoot length, number of leaves and highest survival of seedlings.

Because of the problems of seed germination of *Magnolia grandiflora*, there is a lack in the previous literature concerning how to solve this problem. In addition, the relationship between cold stratification and seedling characteristics hasn't been well investigated. Therefore, the aim of this work was to study the effect of cold stratification not only onseed germination but also on seedling characteristics of *Magnolia grandiflora* L.

Materials and Methods

The present study was conducted at the Experimental Farm of the Faculty of Agriculture, Tanta University over a period of eight months from mid-November to mid-July during the two successive seasons of 2007/08 and 2008/09.

Seed collection and treatments

Seed pods of *Magnolia grandiflora* L. were collected form some trees in Gharbia Governorate. The red-orange seed coating were removed by soaking them in water then the seeds were well washed to remove the oily coating that prevents them from absorbing moisture.After washing seeds were subjected to some cold stratification treatments.Seeds were divided into 5 groups and stratified for 0, 30, 60, 90 and 120 days at 5 ˚C.

Seed cultivation

Every 30 days seeds were removed from the cooler and sowed in polyethylene bags 10 x 12 cm filled with a light compost of two parts peat, one part loam and one part sand. Each treatment contained seven replicates of 45 bags each were arranged in a shallow tunnel covered with polyethylene until germination ceased. The experiment was conducted under saran greenhouse.

Assessment of seed germination

Germination was periodically recorded every weak from the date of sowing and continued till the germination ceased. Imbibitions period (IP) defined as number of days from sowing to germination beginning and total germination period (TGP) were recorded.

Germination index (GI) was calculated as described in the association of official seed analysis (AOSA, 1983) by the following equation:-

$$\text{Germination index} = \left(\frac{\text{No of germinated seeds}}{\text{days of first count}}\right) + \cdots + \left(\frac{\text{No of germinated seeds}}{\text{days of last count}}\right)$$

Germination value (GV) which combines both germination speed and total germination was calculated using the equation of Djavanshir and Pourbeik (1976)

GV= (DDGs/N) x GP/10 where

DG is daily germination speed obtained by dividing the cumulative germination percentage by the number of days since sowing.

DDGs is total germination obtained by adding DGs value obtained from the daily counts.

N is the total number of daily counts, starting from the date of first germination.
GP is Germination percentage at the end of the test and 10 as a constant.

Mean germination time (MGT), a speed index asquicker germination corresponds to lower values of MGT, was computed according to Schelin *et al*. (2003).

MGT (seed/ day) = $\sum fini / N$ where
fi is day during germination period (between 50 and 120 days).

ni is the number of germinated seeds on day fi.

N is the total number of germinated seeds.

The speed of emergence (SE) was calculated according to Islam *et al.* (2003).

$$\text{The speed of emergence} = \left(\frac{\text{No of seedling emergened 70 days after sowing}}{\text{No of seedling emergened 120 days after sowing}}\right) \times 100$$

Growth Performance

Seedlings were uprooted carefully to estimate the main root length, lateral root number and root fresh weight. Upper ground parts measurements i.e shoot height, seedling total length (shoot height + main root length), shoot fresh weight and leaves area were recorded. Vigor index was calculated as described by Hossain *et al.* (2006)as follows

Vigor index = germination percent x seedling total length.

Leaves area was digitally measured according to the method of Matthew *et al.* (2002).

Statistical design and data analysis

Seven replicates of 45 seed each was used for each treatment. Treatments were arranged in a complete randomized block design. The obtained results were statistically analyzed by using MSTATC program. Analysis of variance was performed and difference between means was separated by LSD test at $P = 0.05$.

Results and Discussion

Effect of cold stratification periods on seed germination

Data presented in Table (1) show that cold stratification treatments had a significant effect on seed germination of magnolia tree. The IP as well as TGP were shortened due to stratification periods compared to the control. The shortest IP were obtained by using 90 days stratification period treatments in both seasons. However, there was no significant difference between 60 and 90 days in this respect in both seasons (Table 1). The treatment of 120 days resulted in the shortest TGP, meanwhile there were no significant difference between 90 and 120 days treatments in both seasons (Table 1).

The highest G %(75.24 and 73.02 %) were recorded after 90 days of cold stratification during both seasons, respectively.Also GI followed the same trend of G% as 90 days stratification treatments resulted in the highest values (0.7824 and 0.7624) in both seasons, respectively. In the same time the highest GV (0.7448 and 0.7100) were recorded after 90 days of cold stratification in both seasons, respectively.

Concerning MGT, different cold stratification treatments significantly shortened this parameter in both seasons compared with the control. The best results in this respect (67.71and 68.21 days) were obtained by applying 120 days of cold stratification treatment in the two seasons, respectively (Table 1). Furthermore, there was no significant difference between 90 and 120 days in this respect in both seasons.

The SE of magnolia seeds was gradually increased with increasing stratification periods and reached its maximum values (73.60 and 71.69) by using 120 days treatment in both seasons respectively (Table 1). On the other hand there was no significant difference in this concern between 90 and 120 days treatments in both seasons. The promotion effect of cold stratification on seed germination of magnolia trees may be attributed to the effect of cold stratification in increasing both proteins and sugars needed for embryos growing. In addition, minimizing the seed oil content may increase the moisture absorption (Del Tredici, 1981). GA biosynthesis is regulated by temperature at the level of transcription in the imbibed seed. The final step in active GA biosynthesis is catalyzed by the enzyme gibberellic acid 3-oxidase (GA3ox), and importantly, the expression of both seed-expressed GA_3oxisoforms is promoted by low temperatures(Powell, 1987 and Penfield *et al.*, 2005).These results are in accordance with the findings of Lohengrin and Arroyo (2000) on *Phacelia secunda*, Tilki and Cicek (2005) on *Fraxinus angustifolia*, Dahkaei (2009) on *Danae racemosa* and Guner and Tilki (2009) on *Cotinus coggygria.*

Effect of cold stratification on root parameters

The main root length of magnolia seedlings was slightly increased by applying different cold stratification treatments. The treatment of 60 and 90 days significantly increased the main root length in comparison with the control or other treatment (30 days) in both seasons (Table 2). The number of lateral roots was promoted by cold stratification. The highest number (29.52 and 29.90) was obtained by 60 days of cold stratification treatments of both seasons, respectively during both seasons, respectively (Table 2). However there were no significant differences among 60, 90 and 120 days treatments in this respect in the first seasons.

Concerning the root fresh weight, data presented in Table (2) clearly showed that cold stratification increased the root fresh weight in both seasons. The best results in this concerned were obtained by 90 days treatments in the first seasons and 60 or 90 days treatment in the seconds one. The promotion effect of stratification on root parameters may be due to the effect of stratification on germination speed (Table2), and consequently took longer growth periods compared to non-stratified seeds. In addition this promotion could be explained through the role of stratification in enhancing gibberellins synthesis which

Table.1 Effect of cold stratification periods on seed germination of *Magnolia grandiflora* L. tree

Stratification periods	IP	TGP	G%	GI	GV	MGT	SE
	2007/ 2008 season						
0	57.05	109.4	64.76	0.5310	0.4462	80.17	46.10
30	56.71	106.0	68.89	0.6133	0.5367	76.64	51.16
60	55.24	95.48	71.75	0.5995	0.5386	78.36	45.13
90	53.43	82.48	75.24	0.7824	0.7448	68.59	70.05
120	55.71	82.10	71.75	0.7614	0.4171	67.71	73.60
LSD at 0.05 %	2.73	3.99	3.60	0.0477	0.0675	2.23	5.61
	2008/2009 season						
0	55.48	111.7	63.81	0.5205	0.4305	80.80	45.37
30	55.14	106.6	66.98	0.5814	0.4867	77.13	50.07
60	53.67	94.29	70.16	0.6262	0.5505	75.58	51.29
90	51.86	83.05	73.02	0.7624	0.7100	68.79	68.77
120	54.14	82.62	70.79	0.7467	0.4071	68.21	71.69
LSD at 0.05 %	2.71	3.95	2.47	0.0390	0.0435	2.08	5.89

IP = Imbibitions period, TGP=Total germination Period, GI=Germination index,GV=Germination value,MGT=Mean germination time,SE=The speed of emergence.

Table.2 Effect of cold stratification periods on root parameters of *Magnolia grandiflora* L. tree

Stratification periods	Main root length (cm)	Lateral root number	Root fresh weight (g)
	2007/ 2008 season		
0	21.23	16.90	0.5387
30	21.94	18.05	0.5203
60	22.72	29.52	0.7599
90	22.72	27.19	0.8529
120	22.31	29.24	0.7872
LSD at 0.05 %	1.14	4.28	0.0702
	2008/ 2009 season		
0	21.78	17.33	0.5333
30	21.89	16.14	0.5608
60	23.16	29.90	0.8796
90	23.16	25.29	0.8789
120	22.64	28.10	0.7977
LSD at 0.05 %	1.25	4.22	0.0616

Table.3 Effect of cold stratification periods on shoot parameters of *Magnolia grandiflora* L. tree

Stratification periods	Shoot height (cm)	Seedling total length (cm)	Vigor index	Shoot fresh weight (g)	Leaf area (cm^2)
	2007/ 2008 season				
0	9.595	30.82	2006	0.6024	21.38
30	10.43	32.38	2244	0.6190	21.97
60	11.73	34.45	2477	0.9062	37.41
90	12.35	35.07	2646	0.9329	38.25
120	11.55	33.86	2436	0.8019	32.53
LSD at 0.05	0.56	1.24	150.7	0.0390	4.08
	2008/ 2009 season				
0	9.700	31.48	2016	0.5587	21.54
30	10.64	32.52	2189	0.6170	22.44
60	12.02	35.18	2470	0.9256	37.68
90	12.29	35.45	2599	0.9575	39.06
120	11.35	34.00	2411	0.8385	33.23
LSD at 0.05	0.50	1.25	124.6	0.0551	3.04

also leads to increase the growth and root branching and overall increased roots fresh weight (Penfield *et al*. 2005). These results are in agreement with Harrington and Kraft (2004) on *Arbutus menziesii Pursh*, Tilki and Cicek (2005) on *Fraxinus angustifolia* and Rawat *et al.* (2010) on *Punica granatum.*

Effect of cold stratification on shoot parameters

Data presented in Table (3) show that cold stratification treatments significantly increased shoot height as well as seedling total length in both seasons. 90 days stratification treatment resulted in the highest shoot (12.35 and 12.29 cm) for both seasons, respectively. Concerning the seedling total length, there was no significant difference among 60, 90 and 120 days treatments in the first season while 90 days treatment recorded the tallest seedling in the second season. Vigor index which combines both seedling growth and germination percentage was highly affected by all stratification periods compared to the control in both seasons. The highestvigor index (2646 and 2599) was recorded by 90 days treatment in both seasons, respectively.

The fresh weights as well as leaves area were increased as a result of using different stratification periods. The highest shoot weight (0.9329 and 0.9575 g) and leaves area (38.25 and 39.06 cm^2) were recorded by 90 days treatment in both seasons, respectively. However, there was no significant difference between 60 and 90 days of stratification in this respect in both seasons. (Table 3). The effect of cold stratification on shoot parameters followed the same trend of its effect on roots. The effect of stratification on enhancing growth could be attributed to the solubility of fats and sugars due to stratification plus the increasing of gibberellins synthesizing enhanced the growth. In addition, the promotion effect of cold

stratification on seed germination may reflect on enhancing the shoot parameters. These results are in agreement with Lohengrin and Arroyo (2000) on *Phacelia secunda*, Harrington and Kraft (2004) on *Arbutus menziesii Pursh*, Tilki and Cicek (2005) on *Fraxinus angustifolia*, Dahkaei (2009) on *Danae racemosa* and Rawat *et al.* (2010) on *Punica granatum*.

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