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EFFECT OF REMOVAL ENDOCARP AND SOWING DATES ON SEEDS GERMINATION AND SEEDLINGS GROWTH OF BITTER ALMOND ROOTSTOCK

Thanaa Sh. M. Mahmoud[1]*, M.S. Abou Rayya[1], E.K. Nabila[1] and Malaka A. Saleh[2]

[1*]Department of Horticultural Crops Technology, National Research Centre, Dokki, Giza, Egypt.
[2]Pomology Department, National Research Centre, Dokki, Giza, Egypt.

Abstract

Removal endocarp seeds and sowing date are considered basis to get high germination percentage (GP) and produce significant seedling growth Therefore, bitter almond seeds were sown in a private nursery at Kalubia governorate, Egypt during two seasons (2017/2018 and 2018/2019). Selected seeds were divided to two groups, the first with endocarp and the second was removal endocarp. The two groups were sown in four different dates (20th Nov, 5thDec, 20thDec and 5thJan). Results showed that the highest GP (71%) and (69%) were obtained by removing the endocarp when seeds sown on (20thDec) and (5thDec) respectively by no significant difference. GP was (33%) and (67%) from seeds with endocarp at the same dates respectively. Early date of sowing seeds (20thNov) was less effect on improving germination in the two groups of seeds. Successful seedling growth was noticed by removal endocarp. Sowing seeds on (20thNov) was more effective on stem length than other sowing dates, while there were no significant differences between the two sowing early dates (20thNov) and (5thDec) on stem diameter and leaf area. The relatively higher increments of root length, root volume and fresh weight of seedlings observed with sowing seeds on the proper date in early winter (5thDec) when removed endocarp seeds, delaying sowing seeds on (5thJan) has the less values of seedling vegetative growth. The greatest seedling dry weight was obtained by sowing on (5thDec) and (20thNov), while the lowest value was obtained by sowing on (5thJan) that were from seedling of seeds with endocarp. Different sowing dates did not show any differences among chlorophyll (a), while total chlorophyll produces an increment when sowing seeds on (5thDec) compared to delay sowing on (5thJan). It can be concluded that the bitter almond seeds sown at 5th Dec without endocarp are considered as the best performing germination percentage as having healthy seedling as compared to other sowing dates.

Key words: Almond, *Prunus*, germination percentage (GP), sowing date, removal endocarp, seedling growth.

Introduction

Almond (*Prunus amygdalus* L.) is one of the most important subgenera of *Prunus*, and the oldest commercial nut crops of the world (Rahemi *et al.,* 2011). Besides its commercial use as a nut crop, the almond considers an important rootstock species for almonds and well compatible with almond cultivars and other *Prunus* species like plum, peach and nectarine (AK *et al.,* 2001; Rahemi and Yadollahi, 2006; Rahemi *et al.,* 2011). In addition, they are known as drought-resistant, hardy and long-lived plants and they are planted in the poorest and driest soils as the best way of using them (Grasselly, 1990). In this case, bitter almond seeds are preferred because it is believed that they are more resistant to drought and soil pests than grains obtained from sweet almond seeds (Parvaneh *et al.,* 2011).

Seeds of many *Prunus* species including almond have two different types of dormancy; internal (embryo dormancy) and external (endocarp dormancy) (Martinez-Gomez and Dicenta, 2001; Ghayyad *et al.,* 2010). The latter occurs in the seeds of all *Prunus* members and seeds are offer thought to have resistance to germination (Ellis *et al.,* 1985). So, before germination certain chemical and physiological changes must take place in the seeds for contribute to germination (Ikramullah *et al.,* 2017).

Almond endocarp is known as shell, composed of

***Author for correspondence :** E-mail : thanaa_3000@yahoo.com

compact arrangements of lignocellulosic scereid cells morphology, fiber content the outer shell adherence (Ledbetter, 2008). The hardness of a shell (endocarp) is associated with the total amount of lignin formed during the nuts development (Gradziel, 2009). Several mechanical scarification and removal of the endocarp increases germination in some *Prunus* species (Nasir *et al.,* 2001; García-Gusano *et al.,* 2004; Ghayyad *et al.,* 2010; Tewari *et al.,* 2011; Pipinis *et al.,* 2012).

Sowing date is important factor in producing successful seedlings (Gholami *et al.,* 2007). Although suitable moisture, oxygen and soil condition are available many seeds of woody plant species cannot germinate (Ürgenç and Çepel, 2001). So it is necessary to understand the effect sowing date on germination (Yücedað and Gültekin, 2011). Finding the suitable sowing date for each species could help seedling to come established during favorable growing condition (McCreary, 1990). The sowing date and suitable planting media are considered basic requirement to get the maximum yield and good seedling and high profit for their direct and significant impact on seedling quality (Thanaa *et al.,* 2019).

The aim of this study was to investigate the effect of removal endocarp and different sowing dates on seed germination and seedlings growth of bitter almond rootstock.

Materials and Methods

This experiment was carried out in a private nursery at Kalubia governorate, Egypt during two growth seasons (2017/2018 and 2018/2019) and data was represented as average of two seasons.

Plant material

Mature bitter almond fruits (*Prunus amygdalus* L.) were collected from a number of trees (more than 10) grown in Saint Catherine located at South Sinai of Egypt. Fruits were harvested at fully mature stage in August during both seasons. All fruits were packed in plastic bags and transported to the laboratory. The fleshy outer layer (exocarp and mesocarp) was removed by hand, then the clean seeds (with endocarp) stored in burlap bags in dry place and well ventilated at room temperature until sowing dates.

Treatments

Seeds selected were divided to two groups; the first with endocarp and the second was removed endocarp. Almond seeds of two groups were sown in four different dates at interval 15 days (20^{th}November, 5^{th}December, 20^{th}December and 5^{th}January) of 2017/2018 and 2018/2019 seasons. 50 seeds for each date disinfected in a 0.01% Rizolix fungicide solution for 10 minutes. Afterwards, the seeds (with and without endocarp) sown in polyethylene bags (30 cm diameter, 50 cm height) containing 1:3 (v/v) mixtures of loam and sand. One seed was sown in bag at depth equal to three times of the seed size and then placed in a greenhouse and irrigated with water daily.

Germination percentage

Germinated seeds were counted every day for 30 days, from the beginning of the emergence of the cotyledons above the soil (Ghayyad *et al.,* 2010). The germination percentage per treatment was calculated by the equation:

Germination percentage (GP %) =

$$\frac{Nuber\ of\ ger\min ated\ seeds}{total\ number\ of\ seeds} \times 100$$

Seedling growth parameters

At the end of September from both seasons the vegetative properties of seedlings were determined.

Stem length (cm) were measured by measuring tape from surface of soil to the apex of seedling.

Stem diameter (mm) of (5cm) above the soil surface was measured using Vernier caliper.

Leaf area $(cm)^2$ was measured by using Cl-202 portable laser leaf area meter. Root length (cm) and root volume $(cm)^3$ were recorded in seedling.

Seedling fresh weight and dry weight were recorded.

Leaf chlorophyll contents: Three fully extended leaves from each seedling were collected to determine the chlorophyll pigments. The extract was prepared from fresh leaves (1g) by grinding in mortar with 10 ml of 80% aqueous acetone. After filtering, absorbance of extracts was measured at 663 and 645 nm for chlorophyll a and b, respectively using a spectrophotometer according to (Lichtenthaler, 1987).

Statistical analyses

The experiment was arranged in a Complete Randomized Design with five replicates and 10 plants in each replication. Means were compared by Duncan's Multiple Range Test at the 5% probability level (SAS, 2001).

Results and Discussion

Germination percentage (GP)

Fig. 1 concluded that there was significant difference in germination percentage (GP) on seeds with or without

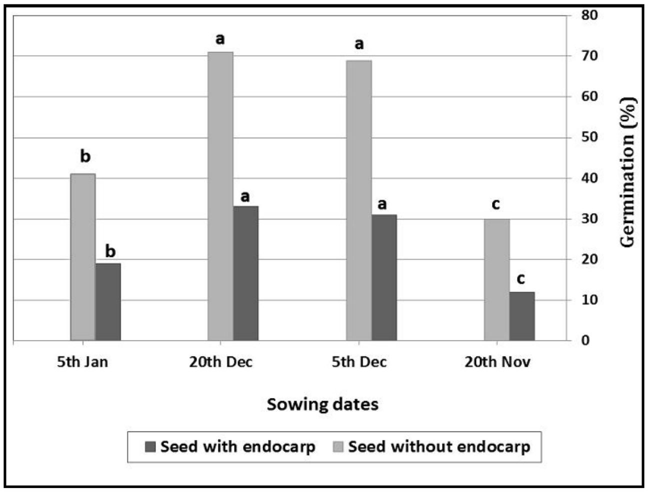

Fig. 1: Effect of removal endocarp and sowing dates on germination percentage of bitter almond rootstock.

endocarp in the different dates of sowing. The highest GP was obtained by the removal endocarp seeds, and that is true in all different sowing dates. There were significant results among sowing times and germination percentage.

According to the effect of almond seeds with endocarp on GP in different sowing dates, it was recorded that the minimum GP (12%) was obtained by sowing seeds on (20^{th}Nov) followed by (5^{th}Jan 19%), (5^{th}Dec 31%) and (20^{th}Dec 33%) respectively. There were no significant differences between GP results when sowing date at (5^{th}Dec) and (20^{th}Dec).

As for removal endocarp seeds, results show that the highest GP (71%) was obtained from seeds sown on (20^{th}Dec) followed by (5^{th}Dec 69%), (5^{th}Jan 41%) and (20^{th}Nov 30%) respectively. In this respect, it is obtained from data illustrated in Fig. 1 there were no significant differences in GP at the date of (5^{th}Dec) and (20^{th}Dec). These results may be due to the earlier breaking seed dormancy at this environmental condition date (5^{th}Dec). Nearly result was reported in other finding McCreary (1990) who found that, peach seeds sown on (10^{th}Nov) get their chilling requirements earlier needed for breaking dormancy thus subsequently germinate earlier.

From the above mentioned results it can be concluded that the cause of the significant differences between the positive values of GP by removing seed endocarp than other seeds with endocarp may due to that the hard shell (endocarp) of seed coat needs a mechanical stimulation to break dormancy. Thus appear the important and major role of removal seeds endocarp hastens and increase germination percentage.

These results are in line to those achieved by Ghayyad *et al.*, (2010) who mentioned that, the hard stony endocarp or the inhibitors around the seed may need another chemical pre-treatment. Endocarp may also offer some resistance to germinate and removed endocarp may hasten or increase GP in stone species (García-Gusano *et al.*, 2004). Rahemi *et al.*, (2011) observed that, seed germination of *Prunus* species are influenced by dormancy. The seed coat and embryo dormancy prevent seed germination. In almond nuts the endocarp has a mechanical effect affecting gas exchange and imbibition and washing out of teguments hormones (DuToil *et al.*, 1979). Pipinis *et al.*, (2012) mentioned that, pre-treatment has been considered to reduce the mechanical resistance of endocarp. Moreover, Nasir *et al.*, (2001), Cetinbas and Koyuncu (2005), Ghayyad *et al.*, (2010) and Tewari *et al.*, (2011) point to remove the endocarp was found to hasten or significant increased germination in some *Prunus* species.

Regarding to the sowing date on germination percentage, it can be concluded that, in this study the proper times for seeds GP were (5^{th}Dec) and (20^{th}Dec) which did not gave significant differences between them. It was (31%, 33%) and (69%, 71%) respectively in the two treatments of seeds with or without endocarp respectively.

These results assure the important role of sowing time in this respect. The obtained results are in agreement with (Yücedað and Gültekin, 2011) who showed that the germination percentage was significantly affected by sowing time and the proper sowing time for almond and wild almond was the period between (1^{st}Oct) and (15^{th}Dec). Gültekin (2007) emphasize that the sowing time of these species was in autumn and early winter.

Seedling growth parameters

Seedling stem length

Seeds with endocarp table 1 presented that the highest mean value of seedling stem length recorded (40.52 cm) with sowing date (5^{th} Dec) while the minimum value was (12.50 cm) obtained when the sowing seeds were on (20^{th}Dec).

Regarding to the seeds of removed endocarp, stem length showed the same previous trend, Maximum value of seedling stem length (46.17cm) recorded at the same date (5^{th}Dec). There were no significant differences between the two late dates of sowing seeds at (5^{th}Jan) and (20^{th}Dec).

The above results, may be due to the early sowing time can help in obtaining high length of seedling, thus it may be concluded that there is a significant correlation between the early date of sowing seeds and the high seedling stem length. These results nearly coincides with

Table 1: Effect of removal endocarp and sowing dates on stem length, stem diameter and leaf area of bitter almond rootstock.

Sowing dates	Stem length(cm)	Stem diameter (mm)	Leaf area (cm)^2
Seed with endocarp			
20th Nov	26.17 b	3.33 a	3.19 a
5th Dec	40.52 a	3.33 a	3.11 a
20th Dec	12.50 c	3.00 b	2.66 b
5th Jan	13.50 c	3.00 b	2.11 c
Seed without endocarp			
20th Nov	37.87 b	3.79 a	3.52 a
5th Dec	46.17 a	3.80 a	3.45 a
20th Dec	23.43 c	3.20 b	2.23 b
5th Jan	24.70 c	3.17 b	2.04 c

Means in column with the same letter are not significantly different at 0.05.

the finding of Ikramullah *et al.,* (2017) who suggested that, the seedling that germinated and established earlier grew better. Better plant growth results from seeds sown on November 10th coincides with the finding of Tocklai (1972) who observed that the best time to sown seed is late October and early November. However, peach stone sown later than or earlier this date usually give rise to inferior stunted plants (Ikramullah *et al.,* 2017).

Seedling stem diameter

It is obvious from table 1 that, the mean value of seedling stem diameter in the two earlier sowing dates (20thNov) and (5thDec) were more effective than the other two later sowing dates (20thDec) and (5thJan) respectively. There were no significant differences between the two earlier dates, also between the two later dates of sowing and that is true for the seedling emergence from seeds with or without endocarp. As for the maximum stem diameter (3.80 mm) it is obtained by the removing of endocarp seeds at (5thDec) sowing date. While the minimum seedling stem diameter (3.17 mm) was recorded at (5thJan). It can be conducted that, there were a positive relationship between the early date of sowing seeds and the high value of seedling stem diameter. Gholami *et al.,* (2007) indicated that early sowing dates were better because it leads to collar diameter, height and survival as compared to delayed sowing.

Leaf area

The large area of the leaf table 1 resulted from the early sowing dates (20thNov) and (5thDec) respectively with no significant differences between them. Where, the smallest seedling leaf area was noted in the delaying sowing seed on (20thDec) and (5thJan). The large area of the leaf was remarked on seedling from seeds removed endocarp. It may be responsible for superiority role of removal endocarp and due to the previous good parameters of seedling growth (stem length and diameter). In agreement with these results, Rahemi *et al.,* (2011) demonstrate, the growth capacity can be determined by stem diameter.

Seedling root length and volume

Results in table 2 show that root length and volume were significantly affected by the planting time and seeds with or without endocarp. The seedling growth in this respect was more effective when planted almond seeds at autumn and early winter. The highest root length and volume was obtained from sowing seeds on (5thDec) but the lowest value was on (5thJan) and that is true by seeds with or without endocarp. It can be noticed that the highest and largest almond seedling root length and volume respectively, were when planted seeds without endocarp.

Regard to the minimum seedling length and volume, it may due to the low temperature which is considered one of the main factor affecting the growth of seedling during the days after plating date on (5thJan). These results coincide with the finding of McCreary (1990), who reported that, date for each species could help seedling become established during favorable growing condition.

Seedling fresh and dry weight

Statistical analysis in table 2 showed that, there was significant effect of sowing dates on (fresh and dry weight) of seedling that is true on seedling which emergence from seeds with or without endocarp. Seeds with endocarp exhibited the maximum seedling fresh weight (5.63g) obtained at (5thDec), while the minimum

Table 2: Effect of removal endocarp and sowing dates on root length, root volume, seedling fresh and dry weight of bitter almond rootstock.

Sowing dates	Root length (cm)	Root volume (cm^3)	Seedling fresh weight (g)	Seedling dry weight (g)
Seeds with endocarp				
20th Nov	22.17 b	2.97 b	4.89 b	4.10 a
5th Dec	29.10 a	3.21 a	5.63 a	5.02 a
20th Dec	16.67 c	2.60 c	3.22 c	2.90 b
5th Jan	11.00 d	1.03 d	3.12 c	1.60 c
Seeds without endocarp				
20th Nov	28.30 b	4.07 b	5.63 b	3.37 a
5th Dec	34.00 a	5.20 a	7.53 a	2.57 c
20th Dec	18.00 c	2.83 c	3.63 c	3.17 b
5th Jan	16.11 d	1.47 d	3.30 d	2.67 c

Means in column with the same letter are not significantly different at 0.05.

seedling fresh weight (3.12g) was at (5th Jan). Because of the beneficial effect of removal endocarp on almond seedling the fresh weight was clearly increased by the different dates of sowing seeds. The highest values in this respect (7.53g) recorded with sowing seeds at (5th Dec) followed by (20th Nov), (20th Dec) and (5th Jan), since they were (5.63g), (3.63g) and (3.30g) respectively. Increasing seedling fresh weight in the early date was considered the cause of increasing shoot fresh weight, root fresh weight and stem diameter at the same early date. The obtained results are in agreement with (Shah *et al.*, 2013) who noticed that the maximum diameter may due to more number of seedling leaves, resulted into more photosynthates which were translocated towards the roots causing over increasing in growth.

According to the dry weight of seedling, it has the same trend of seedling fresh weight, but in this respect the values of seedling dry weight was more effective from endocarp seeds than the other seeds without endocarp. The greatest dry weigh (5.02g) was found with the planting date on (5th Dec), while the lowest dry weigh (1.06g) was noted when seeds of almond were sown on (5th Jan).

Leaf chlorophyll contents

Table 3 presents the effect of different sowing dates on leaf chlorophyll contents. There were no significant differences between the values of chlorophyll (a) in different dates of sowing in seedlings emergence from seeds with endocarp. Meanwhile, removal endocarp affected significantly on chlorophyll (a) the maximum value (0.262) was obtained at (5th Dec) while the minimum value (0.220) was at (5th Jan).

Concerning chlorophyll (b), results show that there were no significant differences between the values of chlorophyll (b) in different dates of sowing in seedlings emergence from seeds with or without endocarp except the planting seeds on (5th Jan) which recorded the lowest values (0.141 and 0.166) respectively.

Table 3: Effect of removal endocarp and sowing dates on chlorophyll a, chlorophyll b and total chlorophyll of bitter Almond rootstock.

Sowing dates	Chlorophyll (a)(mg/g fw)	Chlorophyll (b) (mg/g fw)	Total chloro-phyll (mg/g fw)
Seed with endocarp			
20th Nov	0.184 a	0.172 a	0.356 b
5th Dec	0.185 a	0.173 a	0.358 b
20th Dec	0.186 a	0.176 a	0.362 a
5th Jan	0.183 a	0.141 b	0.324 c
Seed without endocarp			
20th Nov	0.233 b	0.184 a	0.417 b
5th Dec	0.262 a	0.185 a	0.447 a
20th Dec	0.227 c	0.180 a	0.407 c
5th Jan	0.220 d	0.166 b	0.386 d

Means in column with the same letter are not significantly different at 0.05.

Maximum value of total chlorophyll (0.447) was noted when seed sown on (5th Dec) from removed endocarp, that is may due to the increasing of seedling vegetative growth (seedling diameter, leaf area and seedling fresh weight in the same proper date 5th Dec) which led to production of more leaves/ seedling and increased the leaf chlorophyll contents. These results are in harmony with those obtained by (Okunomo *et al.*, 2009, Shah *et al.*, 2013; Meena *et al.*, 2017; Abou Rayya *et al.*, 2019) who reported that increasing growth is accompanied by biosynthesis of new protoplasmic constituents , that would be delays senescence of leaves and increase total chlorophylls in leaves and prolonged photosynthetic activity.

Conclusion

It can be concluded from the study that the removal endocarp of seeds might be a useful method for enhancing growth of bitter almond rootstock to shorten the necessary time needed for reaching the seedlings to transplanting size. 5th Dec is the most suitable sowing date for maximum germination percentage and good growth of seedlings under the same conditions.

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