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ORIGINAL ARTICLE

Improvement of Seed Germination of Date-plum *(Diospyros lotus* L.*)* by Physical and Chemical Treatments

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KEYWORDS

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ABSTRACT: Persimmon (*Diospyros kaki*) is an important subtropical, monoecious, dioeocious and polygamous tree which belongs to Ebenaceae family. Because of low total seed and low viability seed in persimmon, grafting on seedling rootstock is generally used for its propagation. The common rootstock is *Diospyros lotus*, but its seeds have long dormancy. So, the study was carried out to investigate the effects of stratification (0, 25, 50 and 70 days) at 4-7˚C, scarification with (97%) sulphuric acid (0, 2.5, 5 and7.5 minute) and GA_3 (0, 250, 500 and 750 mgL^{-1}) on seed germination of *D. lotus*. Results showed that the most germination rate was observed in GA_3 at 250 mgL^{-1}. Stratification for 70 days had the most germination percent. The most germination uniformity was observed in GA_3 at 500 mgL^{-1}. In scarification plus stratification, the germination percent was lower than control and stratification.

INTRUDACTION

Persimmon (*Diospyros kaki*) is a subtropical fruit native to China. It is monoecious, dioecious and polygamous tree, which belongs to Ebenaceae family. This genus has almost 200 species that many of them are subtropical and tropical [1]. Only four species have been used commercially for the production of fruit. They are *Diospyros kaki*. L., *D. lotus* L., *D. virginiana* L.,and *D.oleifera* Cheng [1]. Date-plum (*D. lotus*) is a tree with a rounded crown that may attain 30 meter in height. It is valued in Asia for its small, yellowish-brown to bluish-black fruits, which has a taste similar to date and also often are dried for winter consumption. Its fruits attain a diameter of about 2 cm with brown, oblong and flattened seeds. Japanese persimmon (*D. kaki*) has about 3400 seeds/kg. Date-plum has about 8910 seeds/kg. Natural germination of common persimmon usually occurs in April or May, but 2 to 3-year delays have been observed [2]. The main cause of the delay is the seed covering, which caps the radicle, restricts the embryo, and causes a decrease in water absorption. After removal of this cap, germination was secured with mature seeds collected in the autumn. Seed dormancy also can be broken by stratification in sand or peat for 60 to 90 days at 3 to 10°C [2]. Stratification is an old and simple method of pre-germination seed treatment to break dormancy. Cold stratification led to seed dormancy removal [3]. Japanese persimmon does not have strong dormancy [4]. Although stratification was not essential, it improved germination [4]. It was stated that Cold stratification induced to these events; (1) broke embryo dormancy of seeds via catabolism of lipids, sugars and proteins by hydrolytic or proteolytic enzymes in *Malus domestica* (2) raised seed germination by activating hydrolases that catabolized proteins and thus increased the amino acid content in *Juglans regia* and (3) broke dormancy in seeds increased starch content, probably due to gluconeogenesis.from products of reserve lipid hydrolysis in in fruit trees such as *(Corylus avellana)* [5]. Germination of stratified

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common persimmon seeds were examined in sand or peat flats at diurnally alternating temperatures of 20 to 30 °C. Germinative energies ranging from 54 to 94% were obtained in 20 to 34 days; and germinative capacities at 60 days varied from 62 to100% [2]. Fresh Japanese persimmon seeds taken from ripe fruits and sown immediately germinate best. Germination ranged from 20 to 77% in a study of 18 cultivars with fresh seed sown immediately [6]. Date-plum seeds germinated best without light at alternating18 to 30°C with 10 weeks stratification at 5°C. Germination of seeds stratified for 2 weeks was increased by treating them with 500 mgL^{1-} gibberellin (GA3) [4]. Fresh Texas persimmon seed sown immediately after extraction germinated 33%. Germination was reduced with all other treatments [6]. Date-plum seeds due to the long stratification period needed to overcome dormancy. During germination, GA3 promotes embryo growth and reduces the physical restraint imposed by the endosperm and testa that allowes radicle protrusion [7]. Clipping the radicle end of a seed with toenail clippers and soaking the seed for several days in water or 500 mgL^{1-} GA3 will soften the seed. Seeds scarification treatments weakened the hard structure seeds and let more water absorption and gases exchange by seeds and may improve stratification efficiency [8]. The objective of the present study was to evaluate effects of scarification, stratification and GA_3 on seed germination of date-plum *(D. lotus* L.*)*

MATERIALS AND METHODS

The present study was conducted at the Horticultural Science Department of Shiraz University. Fruits were collected in mature stage (brown color) from Eram Botanic Orchard in Shiraz in mid-November in 2009. After they were brought to laboratory, the fleshy segment was eliminated. Seeds were washed with water for three times and then infest with 10% chlorax (tradename for any commercial bleach containing 5-6% sodium hypochlorite) and then dried and kept in room temperature (25°C). Research was done in 4×4 factorial experiment arranged in a completely randomized design with 3 replicates. Treatments include stratification (0, 25, 50 and 70 days) at 4-7°c, scarification with (97%) sulphuric acid (0, 2.5, 5 and 7.5 minute) and GA_3 (0, 250, 500 and 750 mgL^{1-}) on seed germination of *D. lotus*. Germination parameters in terms of seed germination percent [9], seed germination rate [9] and seed germination uniformity were measured [10]. Peatmoss was used for seed planting bed in order to preserve moisture. 15 seeds were placed in petridish that had 2 filter papers wetted with distilled water and also 15 seeds in 200 g of peatmoss in plastic bag with 6 or more pores in order to drain extra water and then put it in the refrigerator with temperature between 4-7°C for stratification. Number of germinated seeds and seeds germinated related days were recorded during stratification periods. After spending each stratification period, seeds were taken out from the bags and washed with water and then with deionized water for three times. Then, they were placed in petridishes and put them in the growth chamber at 25°C. Petridishes included control and treatments with chemical scarification or two different concentrations of GA_3 alone, were placed in the growth chamber from beginning of experiment.

RESULTS

According to Table1, the main effects of stratification were significant on germination percent and rate.

The highest germination percent was observed in stratification for 70 days alone (Table 2). Germination percent in GA_3 concentration at 250 mgL^{1-} with stratification for 50 days, and GA_3concentration at 500 and 700 mgL^{1-} alone were lower than control. The most germination rate was observed in GA_3 at 250 mgL^{1-}. Increased GA_3 concentration plus lengthening stratification periods also reduced the germination rate (Table 3). The most germination uniformity was observed at 500 mgL^{1-} GA_3 concentration (Table 4).

Scarification by 95%sulphuric acid was significant on germination percent (Table 5). In scarification with sulphuric acid alone, no data was attained (Table 6). According to germination percent, in stratification for 25 days, scarification at 7.5 minute is better than 2.5 minute and 2.5 minute is better than 5 minute. In stratification for 50 and 70 days scarification at 2.5 minute is the best, 5 minute is the worst. There were no significant effect on seed germination rate among treatments for scarification with sulphuric acid (Table 5).

Table 1. ANOVA analysis of seed germination properties date palm by Stratification and GA_3 Treatments.

source of variation	Df	Means of Squares		
		Germination Percent	Germination Rate	Germination Uniformity
GA_3	3	276.77 ns	0.09 ns	82.01 ns
Stratification	3	824.3 *	0.297 **	5843.7 **
GA3*Stratification	9	425.746 ns	0.039 ns	70.96 ns
Error	32	7651	0.0431	70.63

** indicate significance at 5 and 1% level of probability respectively and ns means not significant.

Table 2. Effects of stratification and GA_3 on seed germination percent of *D.lotus*

Stratification(days)	GA_3 (mg/lit)			
	0	250	500	750
0	53.33 l	75.55 d	48.88 n	48.88 n
25	75.55 d	71.11 f	60 j	57.77 k
50	73.33 e	51.11 m	63.33 i	76.66 c
70	90 a	68.88 g	84.44 b	64.44 h

Column with the same letter(s) were not significantly different according to Duncan's Multiple Range test at 5% level of propability

Table 3. Effects of stratification and GA_3 on seed germination rate of *D.lotus*

Stratification(days)	GA_3 (mg/lit)			
	0	250	500	750
0	0.84 b	0.95 a	0.65 c	0.58 c
25	0.56 c	0.71 d	0.51 cd	0.47 cd
50	0.38 cd	0.45 cd	0.32 d	0.35 d
70	0.85 b	0.47 cd	0.61 c	0.52 cd

Column with the same letter(s) were not significantly different according to Duncan's Multiple Range test at 5% level of propability

Table 4. Effects of stratification and GA_3 on seed germination uniformity of *D.lotus*

Stratification(days)	GA_3 (mg/lit)			
	0	250	500	750
0	-20 b	-20.33 b	-17.33 a	-20.33 b
25	-29.33 d	-24.66 c	-31.33 e	-39.66 f
50	-48.66 h	-42.66 g	-53.5 i	-57.5 j
70	-70.5 l	-70 l	-74 m	-64.33 k

Column with the same letter(s) were not significantly different according to Duncan's Multiple Range test at 5% level of propability

Table 5. ANOVA analysis of seed germination properties date palm by Stratification and Scarification by 97% sulphuric acid Treatments.

Source of variation	Df	Means of Squares	
		Germination Percent	Germination Rate
Scarification	3	8727.62 **	30.711 ns
Stratification	3	824.3 *	0.297 **
Scarification*Stratification	9	52.065ns	23.85 ns
Error	32	225.91	20.32

** indicate significance at 5 and 1% level of probability respectively and ns means not significant.

Table 6. Effects of stratification and scarification on seed germination percent of *D.lotus*

Stratification(days)	Scarification by 97% sulphuric acid (minute)			
	0	**2.5**	**5**	**7.5**
0	53.33 g	0	0	0
25	75.55 j	8.88 c	6.66 b	15.55 e
50	73.33 i	13.33 d	2.22 a	8.88 c
70	69.33 h	20 f	8.88 c	13.33 d

Column with the same letter(s) were not significantly different according to Duncan's Multiple Range test at 5% level of propability

DISCUSSION

In our study, higher germination percent was achieved by stratification for 70 days alone or along with GA_3 at 500 mgL^{1-} . Chen et al. (2008) explained that GA_3 treatment on hazelnut seeds had higher germination than the control, germination increased as GA_3 concentration rose, but higher concentration (200 and 400 ppm) had a reverse effect on germination [11]. The result was in accordance with Chen et al., 2008, Aygun et al., 2009 and Yin et al., 2009 [11-14]. Both application of GA_3 concentrations at 500 and 750 mgL^{1-} and stratification could demonstrate higher germination percent than control in our experiments. On the other hand, increased cold stratification period led to increase in seed germination in *Diospyrus lotus* (Table 2). This result was in agreement with Hwan et al, 1988, Yin et al 2009, Debska et al., 2013 and Parvin et al., 2015 and Darrudi et al., 2015 [3, 13, 15 and 16]. Chen et al. (2015) stated that seeds of the Persian walnut (*Juglans regia* L.), *Magnolia grandiflora* and *Cercis siliquastrum* treated with two months of chilling also had higher germination percentage compared to one month chilling treatment and significantly improved seedling characteristics [5].

Treatment of two months of chilling stratification affects metabolic processes including changes in hormones, disappearance of ABA, activation of GA_3 and beginning of germination [16]. Stratification affects metabolic and physiological changes in seeds that involve both the embryo and its covering layers. Seed stratification induces a rapid decline in the abscisic acid (ABA) content and ABA sensitivity and increases GA_3 sensitivity of imbibed dormant seeds [16]. It was explained that the prolonged stratification treatment resulted in stimulation of embryo germination. The impact of Reactive oxygen Species (ROS) accumulation in dormancy alleviation by cold stratification may also involve the regulation of hormonal balance [3]. It is possible that H_2O_2 controls seed dormancy by activation of genes involved in ABA catabolism (CYP707A) [3]. ROS also regulate GA biosynthesis by stimulating expression of GA_3ox; GA_{20}ox genes in the boost in H_2O_2 concentration observed in embryos after 90 days of cold stratification is required for nitrogen oxide (NO) synthesis. NO participates in activation of GA_3ox genes [3].

In our experiment, germination rate (day to 50% seedling emergence) for germinating seeds decrease with rising stratification periods plus GA_3 concentration. These results are similar to results in papaya (*Carica papaya)* seeds [17]; coffee (*Coffea arabica* L.) seeds [18]. It was reported that rate of germination of *D. lotus* increased as the stratification period increased to 10 weeks (80 days) [4]. Effects of stratification on olive seeds do not represent any significant difference in two temperatures 10 and 15°C [19].

GA_3 improved the uniformity of seedling emergence in papaya [17]. GA acts by promoting the growth potential of the embryo and by mediating the weakening of tissues that enclose the embryo. Cold treatments do not stimulate GA synthesis, but increases sensitivity to GA. Synthesis of GA is up-regulated during moist chilling [20].

GAs act in two different stages of germination [9] In first stage, they play a role in enzyme synthesis. They are very effective in later stage, because they activate the enzymes involving in translocation nutrients in third stage (seedling growth). After seed dehydration GAs appear in embryo and translocate to aleurone (the layer was composed of 3-4 cell which surround the endosperm) In conclusion, there was produced new product which is so

called alpha amylase. This enzyme moves to endosperm and converts starch to sugar and translocate to active growing point of embryo in order to provide necessary energy for its growth [9].

In seeds of M. *domestica*, proteolytic activity increased markedly during cold stratification, indicating proteolytic solubilization of insoluble reserve proteins, and an increase in quantity of free amino acids paralleled the increase in seed germinability [15]. GA increases the growth potential of embryo and promotes germination and is necessary to overcome the mechanical restraint conferred by the seed covering layers by weakening of the tissues surrounding the radicle. Chilling stratification along with GA_3 pretreatment has been reported to improve germination in *Prunus* species [15].

Breaking of dormancy and germination stimulating effect of low temperatures could possibly be controlled by increased synthesis of and or sensitivity of the seeds to plant growth regulators such as GAs, CKs and ethylene [15]. Stratification might decrease enzymatic reaction rate, enzyme concentrations or productions in seeds of *Morus nigra* stratified at 4° C for 100 days. The effect of GA_3 as a germination promoter is hypothesized to increase with chilling treatment. Stratification also plays a key role in providing the stimulus required to overcome dormancy [15].

In chemical scarification alone, no data was attained. In scarification with sulphuric acid plus stratification, stratification deleted the detrimental effect of chemical scarification such as infection and embryo death. Sulfuric acid scarification for 2 hours proved to be less effective in breaking dormancy than stratification [2]. Two reasons may be for non germinated seeds by scarification with sulphuric acid, one of them relating to the short time for the treatment due to thick outer seed layer and the other one was damage of seed embryo because of absorbing sulphuric acid [21].

CONCLUSIONS

Stratification for 70days alone or along with GA_3 at 500mg/lit were the best treatments in order to raise seed germination percent and rate, respectively.

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