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Research and Full Length Article:

Cryopreservation of *Ammodendron persicum* (Bunge ex Boiss.) Seeds and Evaluation of the Cryogenic Seeds under Various Conditions

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Received on: 14/02/2017
Accepted on: 18/06/2017

Abstract. *Ammodendron persicum* (Bunge & Boiss.) is a desert shrub specie which grows on some sand dunes and sandy areas of South Khorasan and Sistan-va-Baluchestan provinces in east of Iran. Low distribution and narrow ecological range have put this species under threat. In order to evaluate the possibility of long-term preservation of *A. persicum* seeds in cryogenic conditions (-196°C), the seeds of species were collected from its natural habitats and three pre-cryopreservation treatments including PVS2, Desiccation, and 30% Glycerol as well as non-treated (Control) were applied before transferring the seeds into Liquid Nitrogen (LN) or at -196°C. The treated seeds were incubated in LN for a period of 1 week. Subsequently, the cryopreserved seeds were removed from the LN and subjected to post-cryopreservation treatment. Seed germination and establishment were evaluated under laboratory, greenhouse and natural conditions. The laboratory results showed that seeds of the *A. persicum* species can tolerate cryogenic conditions. The effects of the pre-cryopreservation treatments including Desiccation, 30% Glycerol, and PVS2, and non-treated (Control) one on germination of cryopreserved seeds were significantly different. The non-treated (Control) and Desiccation, respectively showed the best effects on the survival rate (51%) and other attributes of the cryopreserved seeds. The results revealed that the cryopreserved seeds were also able to germinate and establish under greenhouse and desert conditions. In this study, the appropriate seeding depth, the seed sowing time and factors affecting the seed germination as well as establishments under natural conditions were evaluated. The results revealed that cryopreservation approach is the most promising method for long-term preservation of the *A. persicum* seeds. Long-term seed preservation via cryopreservation is an important approach to prevent this species from loss of genetic diversity and risk of extinction

Key words: *Ammodendron persicum*, Cryopreservation, Seed, PVS2, 30% Glycerol, Desiccation

Introduction

A. persicum (Bunge & Boiss.) is a native desert shrub of the Fabaceae family adapted to dunes and sandy areas of semi-arid to arid desert climates occurring in Southern Khorasan and Sistan-va-Baluchestan provinces in east of Iran (Rechinger, 1984). *A. persicum* is a cross-pollinated species of desert environment; thus, it could be an interesting and useful candidate for combating desertification through re-vegetation of dunes and sandy areas of desert environments. Based on the published result (Safarnejad and Abbasi, 2010), *Ammodendron persicum* has 2n=18 chromosomes with a karyotypic formula of 3M + 2 SM + 4T.

Although the natural habitats of the species are managed, conserved or protected to some extent, soil degradation, land conversion, damage to ecosystems, reduced plant regeneration, human impacts, over grazing, and some other factors may lead to its loss of genetic diversity and genetic erosion in future. Protection of the species' habitats as well as maintaining genetic diversity ensures sustainability of the species. Seed collection and *ex situ* conservation in gene banks are also an important approach to preserve the species and maintain the genetic diversity. Collecting and conserving the seeds of the endangered or under threat species are an important issue for protecting such species. However, depending on the plant species, only short to medium term seed storage is possible in gene banks.

Long-term preservation of seed samples collected from diverse and wide range of natural habitats is possible under cryogenic (-196°C) conditions. In cryogenic conditions, due to a great decrease in metabolic activities of the cells, longevity of the seeds or plant organs extremely increases (Walters *et al.*, 2004, Caswell and Kartha, 2009). Long-term conservation of seeds, especially seeds of the endangered and threatened species under cryogenic conditions is the most important approach to restore the extinct species through re-establishment of the cryopreserved seeds in the near and distant future. If plant species are threatened in near or far future, it will be possible to use the cryopreserved seeds to replant the species and rehabilitate its natural habitats (Naderi Shahab *et al.*, 2017).

Most of the recalcitrant seeds are hydrated, sensitive to desiccation, and metabolically active and suffer from ice crystal damage at subzero temperatures. Consequently, these seeds cannot be stored under conventional seed-banking conditions and at subzero temperatures (Roberts, 1973). Recalcitrant phenomenon of seeds occurs in only a small proportion of the worldwide flora and is much more common in the mesic tropics and subtropics (Roberts, 1973; Yabor *et al.*, 2015; Pammenter and Berjak, 2014; Sacande´ *et al.*, 2004). In Recalcitrant seeds, higher water content of the cells causes lethal crystal ice formation in cryogenic conditions. In contrast, orthodox or desiccation tolerant seeds mostly tolerate drying to at least 5% moisture content (Berjak and Pammenter, 2002).

Seeds of wide range of rare and endangered forest and range species, as well as other plant species have been successfully preserved under cryogenic conditions via applying various pre-cryopreservation methods (Popova *et al.*, 2012; Wen *et al.*, 2010; Jitsopakul *et al.*, 2008; Wood *et al.*, 2003). The seeds of two Apiaceae species with orthodox nature including *Ferula gummosa* and endangered *Kelussia odoratissima* were treated with PVS2, Desiccation and 30% Glycerol and incubated in LN or -196°C up to 26 months. The cryopreserved seeds germinated, grew normally, and did not show any abnormalities as compared to those of control plants (Naderi Shahab *et al.*, 2013).

Before transferring seeds or samples into cryogenic conditions, reducing cell water content to an appropriate level in most cases has positive impacts on the survival of seeds or plant organs under cryogenic conditions. Decreasing seed water content before transferring them into cryogenic conditions may enhance the viability of the cryopreserved seeds due to low ice crystal formation in the cell environment. In this regard, lowering seed moisture content or seed desiccation before transferring the seeds into cryogenic conditions showed positive effects on seed survival under cryogenic conditions. It is important to note that reduction of seed moisture content is highly related to the plant species. Although in general, a 5-10% seed moisture reduction based on total seed moisture content before transferring the seeds into LN or at -196°C is recommended in some plant species (Stanwood, 1985), 3-5% (Wood *et al*., 2003) and 6.2-8.9% (González-Benito and Pérez-Garcia, 2001) reduction in sample moisture content has shown appropriate results in other plant species. Interestingly, in some forest species such as *Biota orientalis* reduction of 31.2% and in range species of *Ferula gummosa* and *Kelussia odoratissima*, reduction of 17.77 and 9.20% seed moisture content (respectively) based on total seed moisture content showed the highest seed survival rates after removal from cryogenic conditions. In cryogenic storage, cryoprotectant substances and solutions such as plant vitrification solution 2 (PVS2) showed significantly positive effects on seed survival, cell and vegetative organ recovery and viability after removal from LN environment (Chaireok, *et al*., 2016; Mišianiková. *et al*., 2016; Höfer, 2016; Mata-Rosas and Lastre-Puertos, 2015; Park and Kimm, 2015; Rafique *et al*., 2015; Funnekotter *et al*., 2015; Zhang *et al*., 2015; Jitsopakul *et al*., 2008; Kushnarenko *et al*., 2009; Li *et al*., 2009; Sakai and Engelmann, 2007). However, negative or detrimental effects of the solution have also been reported in some published cryopreservation studies (Ferrari *et al*., 2016; Pital *et al*., 1998). Glycerol is also widely used in treating seeds, cells, and plant organs before being transferred into cryogenic conditions. Although positive effects of the glycerol on survival of the cryopreserved samples have been reported (Kim *et al*., 2005), negative effects of this component on seed survival and vigority have also been observed in some plant species (Naderi Shahab *et al*., 2009; 2013).

The objectives of this study were to evaluate the possibility of long-term preservation of *A. persicum* seeds under cryogenic (-196°C) conditions and to determine the effects of pre-cryopreservation treatments on the viability of seeds after removal from cryogenic conditions in order to develop an effective cryopreservation protocol for long-term preservation of *A. persicum* seeds with the highest post-cryopreservation seed recovery, survival and establishment. This study also aimed to evaluate the possibility of establishment of *A. persicum* seeds under desert conditions.

Materials and Methods

Origin of seed samples:

The research was carried out from 2010 t0 2012. Seed pods of *A. persicum* were collected in late June from native mature shrubs growing in the protected area in south of Hajiabad (5Km towards Ahangarn), 85km east of Ghaen in South Khorasan province, Iran (Fig. 1). The pods were trashed and the undamaged clean seeds were used in subsequent experiments. It should be noted that the 1000 seed weight of the species was 36.25g.

Fig. 1. *A. persicum* in its natural habitat with ripened pods attached to the shrub (left), young plant establishment in natural habitat (right).

Seed germination method

A series of preliminary experiments was conducted to determine the optimum seed germination method. Based on the preliminary experiments, the following germination method was applied:

1- Seed scarification with emery paper.
2- Approximately 10g of scarified seeds were transferred into 30ml tube, filled with tap water, capped and washed 3 times with shake.
3- Tubes were drained off, filled with 15% bleach and incubated at room temperature (+22°C) for 15 minutes.
4- Bleach was discarded and the seeds were washed 3 times with sH_2O under aseptic conditions in laminar airflow.
5- Seeds were transferred between sterile moist papers in petri-dishes.
6- The petri-dishes were transferred to a +22ºC germinator under 16/8h (L/D) photoperiod. The intensity of light during light period was $10W/m^2$.
7- In greenhouse and natural environment experiments, the seeds were also sterilized and used.

Laboratory experiments

Pre-cryopreservation treatments

1. PVS2: 30g of *A. persicum* seeds was placed in 50ml screw-capped tube and filled with PVS2 solution and subjected to PVS2 treatment (Naderi Shahab *et al.,* 2013).

2. Desiccation: Fresh weigh (FW) of the seeds was determined through weighing 10g of seeds. The seeds were oven dried at +75°C for 72h, weighed and recorded as seed dry weight (DW). The total seed moisture content percent was obtained using the (FW-DW)/FWx100 formula and the total seed moisture content of the collected seeds was recorded as 4.23%. Approximately 30g of fresh seeds was weighed and placed in air tight desiccators containing 300g silica gel for 7 days at +4°C. The moisture content of the seeds dropped from 4.23% to 2.93% and the reduction of the seeds moisture content was approximately 30.73% based on the total moisture content. The seeds were removed from desiccator and immediately transferred into 50ml screw-cap tubes and submerged in a LN container.

3. 30% Glycerol: 30g of *A. persicum* seeds was placed in 50ml screw-capped tube and filled with 30% glycerol and transferred into LN. This method has also been previously described in detail (Naderi Shahab *et al.,* 2017).

4. Non-treated (Control) seeds: seeds that were not treated with any chemical or non-chemical substances were transferred into cryovials and submerged in LN.

Post-cryopreservation treatments:

The seeds were removed from LN after 1 week and transferred to +42°C sterile

sH_2O for 2 minutes under aseptic conditions in order to receive the post-cryopreservation treatment. The seeds were surface sterilized and subjected to germination tests under laboratory conditions (as described before). In laboratory conditions, the following attributes were recorded: seed vigor index or VI (Abdul-Baki and Anderson, 1973), seed germination percent, germination speed, shoot length, root length and root/shoot length ratio (R/S).

Experimental layout:
The experimental design was a factorial design consisting of 2 factors: 1) four pre-cryopreservation treatments including PVS2, 30% Glycerol, and Desiccation as well as non-treated (Control) seeds which formed 4 levels of factor A, and 2) two LN storage periods of seeds including 0 week (not incubated in LN) and 1 week incubation in LN which made up two levels of factor B using a Completely Randomized Design with three replications. The experimental units were single petri-dishes. The above mentioned six attributes were measured and data analysis was carried out using SAS software. The differences between the treatment means were tested using Duncan's Multiple Range Test.

Greenhouse experiments:
Round plastic bowels with 60 cm diameter and 15 cm depths were filled with sandy soil obtained from Rigboland sand dunes. The analysis of Rigboland sand dunes soil has been reported before (Naderi Shahab *et al.*, 2017). Approximately, 40 seeds from each of the treatments (PVS2, Desiccation, 30% Glycerol and Control) were sown in each washing bowel and maintained at field capacity with tap water**.** Each washing bowel was determined as a replication. The greenhouse temperature was maintained at 22±4°C and the seedling establishment was recorded.

The experimental design was a Completely Randomized Design with 3 replications. The data were subjected to an analysis of variance using SAS software. The differences between the treatment means were tested using Duncan's Multiple Range Test. Seed sowing depth was carried out observationally in depths of 2.5, 5.0, 7.5 and 10.0 cm under greenhouse conditions.

Seedling establishment in desert environment:
An observational study was conducted in Rigboland sand dunes near Aran and Bidgol town, close to Kashan city in center of Iran. Seeds either pre-treated with PVS2, Desiccation, 30% Glycerol or non-treated (Control) were incubated in LN for 1 week. The 0 week seeds (non-cryopreserved seeds) were pre-treated (as mentioned above) but were not incubated in LN. The cryopreserved and non-cryopreserved seeds were sown in Rigboland sand dunes at the end of December. Pit-seeding method was applied and approximately, 10 seeds were located in each pit and covered with 5 cm of the sandy soil.

Results

Laboratory experiments:
Results of ANOVA showed significant differences among different levels of incubation periods and pre-cryopreservation treatments for only seed germination percent and vigor index. The Incubation Period by pre-cryopreservation treatment interaction effect was not significant for all the traits (Table 1).

Table 1. Analysis of variance and mean of squares of A. persicum seed attributes under laboratory conditions

Source of variation	DF	MS					
		Germination	Root length	Shoot length	Seedling length	Vigor Index	R/S
Incubation Period	1	84.37**	0.198^{ns}	0.531^{ns}	1.363^{ns}	9.04**	0.010^{ns}
Treatment	3	48.26**	0.599^{ns}	1.134^{ns}	2.709^{ns}	7.06**	0.032^{ns}
Period x Treatment	3	14.93^{ns}	0.227^{ns}	0.102^{ns}	0.339^{ns}	0.995^{ns}	0.005^{ns}
Error	16	9.375	0.829	0.423	1.575	0.883	0.020
CV%		6.10	5.16	7.35	4.73	7.05	7.05

ns and**: non-significant and significant at P=0.01 level.

For clear understanding of pre-cryopreservation treatments' effects in LN incubation (1 week) and non-LN incubation (0week) conditions, the means comparison of the interaction effects for all traits is indicated in Table 2. Seed germination percent (Fig. 2) of pre-cryopreservation treatments including PVS2 over incubation periods of 0 week (not preserved in LN) and 1 week incubation in LN showed significantly lower germination percent as compared to those of non-treated and Desiccation pre-treatments. The results revealed that regardless of cryopreservation or non-cryopreservation conditions, PVS2 has detrimental effects on seed germination. As shown in Table 2 and Fig. 3, in 0 week period (no incubation in LN), seed germination percent of the PVS2 treated seeds (48.33%) is significantly lower than non-treated (Control) seeds (53.33%). The data revealed the exclusive negative effects of the PVS2 solution on *A. persicum* seed germination. 30% Glycerol also showed negative effects on seed germination only on cryopreserved seeds (45.00%).

Cryopreserved seeds pre-treated with 30% Glycerol and PVS2 and 1 week incubation in LN showed significantly lower germination percent as compared to the non-cryopreserved (0 week) seeds. In this regard, germination percent of the seeds pre-treated with 30% Glycerol in 1 week incubation in LN was 45.00% and it was 53.33% in non-cryopreserved (0 week) condition. In PVS2, seed germination percent in 1 week incubation in LN was 45.00% and it was 48.33% in non-cryopreserved (0 week) conditions (Table 2). The results indicated that PVS2 and 30% Glycerol have detrimental effects on seed survival in cryogenic conditions. In general, in non-cryopreserved (0 week) condition, non-treated (Control), Desiccation and 30% Glycerol showed the highest germination percent (53.33%) as compared to the PVS2 (48.33%). However, in cryogenic conditions (1 week), germination percent of the seeds pre-treated with Desiccation and non-treated seeds (51.67%) was significantly higher than that of 30% Glycerol and PVS2 (45.00%) pre-treatments (Table 2, Fig. 3). Although seed vigor index attribute showed almost a similar pattern to that of seed germination percent, the other attributes did not show significant differences under LN incubation and non-LN incubation conditions. For these attributes, pre-cryopreservation treatments also did not show significant differences under LN incubation and non-LN incubation conditions (Table 2).

Table 2. Means comparison of A. persicum seed attributes affected by different LN incubation periods at different pre-cryopreservation treatments (including Control) under laboratory conditions

Incubation period	Treatment	Germination %	Root length (mm)	Shoot length (mm)	Seedling length (mm)	Vigor Index (VI)	R/S
0 week	non-treated	53.33 a	17.59 a	9.63 a	27.21 a	14.53 a	1.85 a
	30% Glycerol	53.33 a	17.67 a	8.46 a	26.13 a	13.94 ab	2.09 a
	Desiccation	53.33 a	18.34 a	9.28 a	27.62 a	14.72 a	1.98 a
	PVS2	48.33 ab	17.41 a	8.65 a	26.07 a	12.57 bc	2.01 a
1 week	non-treated	51.67 a	17.93 a	9.18 a	27.11 a	14.04 ab	1.96 a
	30% Glycerol	45.00 b	17.40 a	8.37 a	25.77 a	11.55 c	2.08 a
	Desiccation	51.67 a	17.75 a	8.69 a	26.45 a	13.65 ab	2.05 a
	PVS2	45.00 b	17.20 a	8.59 a	25.79 a	11.60 c	2.01 a

Means with the same letter are not significantly different ($\rho < 0.01$).

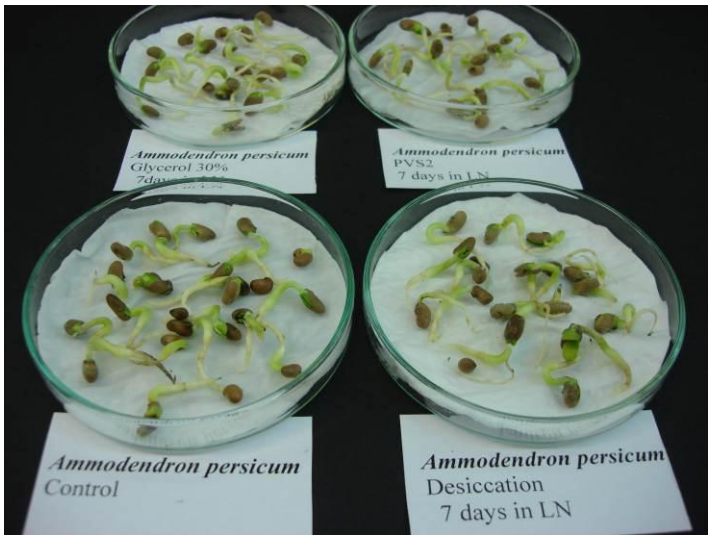
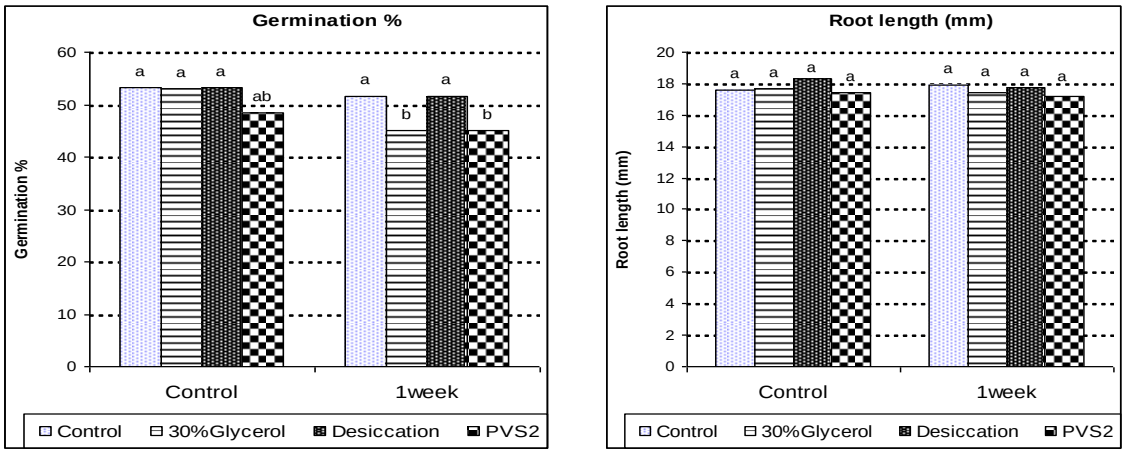


Fig. 2. Germination of cryopreserved and non-cryopreserved *A. persicum* seeds treated with different pre-cryopreservation treatments



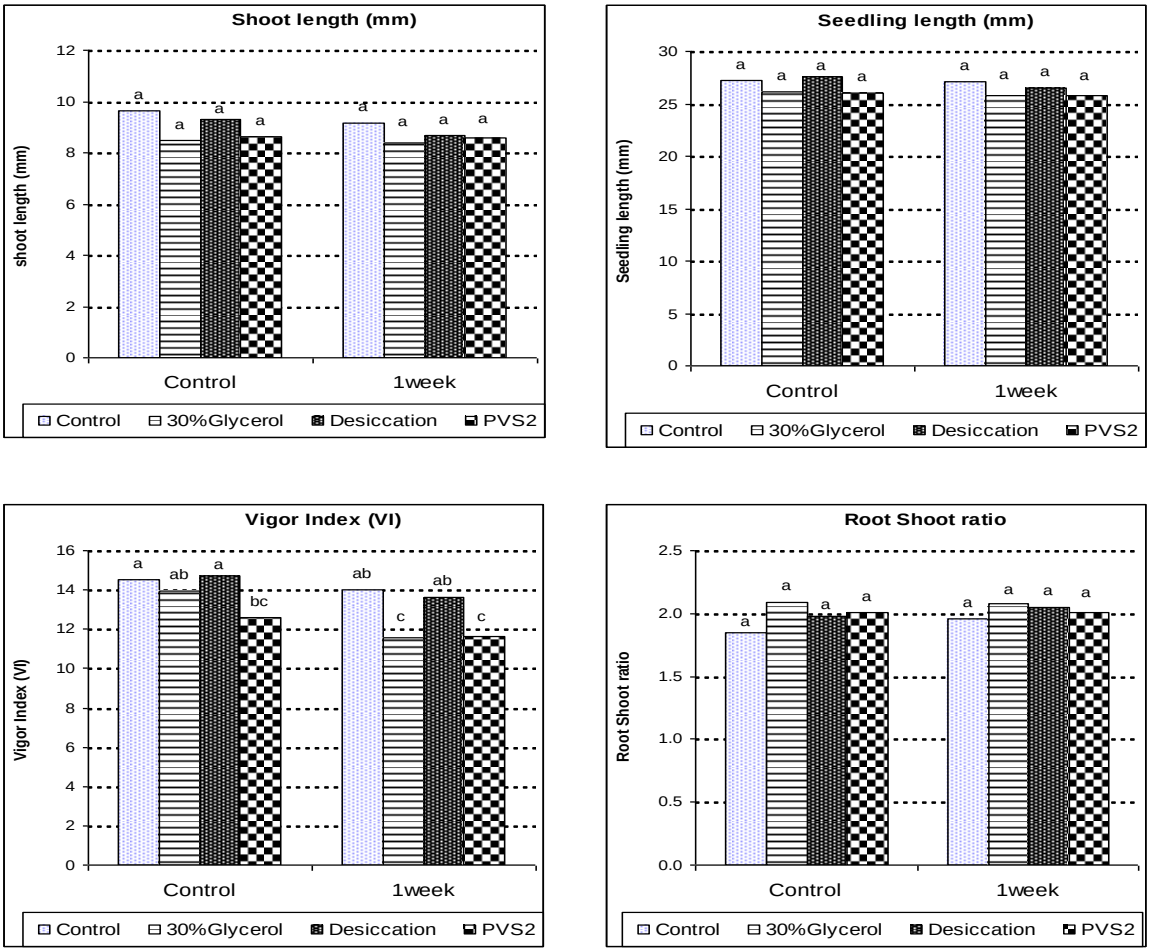


Fig. 3. Seed germination percent, root length, shoot length, seedling length, vigor index and root/shoot ratio of *A. persicum* seeds treated with different pre-cryopreservation treatments and incubated in LN for 1 week and no incubation in Liquid Nitrogen (0 week) under laboratory conditions (means with the same letter are not significantly different ($\rho < 0.01$))

Greenhouse experiment

In the greenhouse experiment, non-cryopreserved or Control seeds were established and developed to seedling significantly higher than those of cryopreserved seeds treated with different pre-cryopreservation treatments. Statistically, pre-cryopreservation treatments did not show significant differences regarding seedling establishment. The seedling establishment percent in cryopreserved seeds ranged from 35 to 40% (Table 3) which was significantly lower than the seedlings developed from Control (non-cryopreserved) seeds (54.67%). This indicated that cryogenic conditions have adverse effects on seedling establishment percent of *A. persicum* species under greenhouse conditions.

Table 3. Seedling establishment of *A. persicum* under greenhouse conditions. Seeds treated with different pre-cryopreservation treatments (including Control) and incubated in LN for 1 week

Pre-cryopreservation Treatment	Seedling Establishment %
Control (non-cryopreserved)	54.67 a
30% Glycerol	35.00 b
Desiccation	40.00 b
PVS2	38.00 b

Means with the same letter are not significantly different ($\rho < 0.01$).

Plant establishment in desert sand dunes

The seedlings emerged in early March 2010 and started to grow up until mid-May. Most of the seeds germinated, emerged and grew to bunch of strong seedlings (Fig. 4). In early spring, the growth of the seedlings was rapid, and vertical root growth was quite faster than the shoot growth. Vertical roots rapidly penetrated into the wet sandy soil and root length of the seedlings by mid-May was approximately 35 cm (Fig. 4, bottom right). By June, the growth of the seedlings was promising. However, the growth stopped in early June and the seedlings started senescing from mid-June. At the time, soil moisture content dropped significantly, especially in the upper layer of soil.

Fig. 4. *A. persicum* seedlings established in Rigboland sand dunes (16 May)

Discussion

Laboratory experiments

Under laboratory conditions, the maximum seed germination percent and maximum seed vigor index of *A. persicum* in non-cryopreservation condition were 53.33% and 14.43, respectively (Table 2, columns 3 and 7). However, seed germination percent and seed vigor index significantly decreased in seeds treated with PVS2 pre-treatment (48.33% and 12.57 respectively). Under cryogenic storage conditions, PVS2 and 30% Glycerol had deleterious effects on seed germination and seed vigor index which could be due to the penetration of chemical components of PVS2 solution such as ethylene glycol, DMSO or glycerol into the seeds. Similar results were observed on some of the range species including *Ferula gummosa, Kelussia odoratissima* and *Smirnovia iranica* (Naderi Shahab *et al*., 2013;

2017). Although plant vitrification solutions (PVS2 and similar PVS2 based solutions) showed positive effects on seed and organ cryopreservation of some of the plant species (Schoenweiss *et al*., 2005; Volk *et al*., 2006; Liu *et al*., 2003), there are several reports indicating negative effects of PVS2 on recovery and survival of seeds and organs of some plant species (Ferrari *et al*., 2016; Pital *et al*., 1998). Furthermore, seed germination percent of the cryopreserved seeds (1 week) treated with PVS2 and 30% Glycerol were significantly lower than those of Desiccation and non-treated (Control) seeds. The results revealed that the seeds treated with chemical components (including PVS2 and 30% Glycerol) have negative impacts on seed germination and vigor index of the *A. persicum* seeds preserved under cryogenic conditions.

In cryopreserved seeds except for vigor index attribute, other attributes including root length, shoot length, seedling length and root/shoot ratio were not affected by pre-cryopreservation treatments either under cryogenic storage or non-cryogenic conditions (Table 2). The seed vigor index of the cryopreserved seeds significantly reduced as compared to the non-cryopreserved seeds (Table 2). Although seed germination percent and seed vigor index decreased in cryogenic conditions, seed germination and vigor remain almost constant over long period of cryogenic storage (Walters, *et al.* 2004). As reported by Walters *et al.* (2004) in cryogenic conditions, seed viability can be extended over 3400 years. In the present study, decrease of seed moisture content (Desiccation) before being transferred into cryogenic condition did not show positive effects on seed germination and seed vigor index as compared to the non-treated (Control) seeds. Although positive effects of the Desiccation treatment on seed germination, vigor index and seedling establishment of cryopreserved seeds of some forest and range species have been reported (Naderi Shahab *et al*., 2009; 20013; 2017), they did not show positive effects on seed germination and vigor of cryogenically stored *A. persicum* seeds.

Results of the laboratory experiments showed that *A. persicum* seeds are able to tolerate cryogenic conditions. Cryogenically, stored seeds are able to germinate with a high percentage after removal from cryogenic conditions. In cryogenic storage of the *A. persicum* seeds, non-treated or Control approach was the most effective method in long-term preservation of *A. persicum* seed under cryogenic conditions.

Greenhouse experiment

Under greenhouse conditions, Control and cryopreserved seeds were developed to seedlings and the highest establishment percent was 54.67% for Control seeds (Table 3). Although seedling establishment percent of the cryopreserved seeds was lower than the Control seeds to some extent, the cryopreserved seeds were developed to normal and vigor seedlings. In contrast to the laboratory experiments, the pre-cryopreservation treatments (30% Glycerol, Desiccation and PVS2) under greenhouse conditions showed similar effects on seedling establishment.

Plant establishment in desert sand dunes

In late winter, most of the seeds germinated emerged and subsequently grow to bunch of strong seedlings (Fig. 4). In early spring, the growth of the seedlings was rapid, and vertical root growth was quite faster than shoot growth. In middle of May, vertical roots quickly penetrated into the wet sandy soil, and the root length of the seedlings was approximately 35 cm (Fig. 4) and by May, growth of the seedlings was promising. By the increase in temperature and drought during early summer, seedlings gradually senesced

and suffered fom drought stress. Several environmental factors could be involved in this regard:

-Altitude of the protected natural habitat of the species in south of Hajiabad (around 6 Km towards Ahangarn) is around 1060m above sea level with annual mean precipitation of 130 mm. While the altitude of seed sowing area in Rigboland is around 1030m, similar to that of Hajiabad, the mean annual precipitation of Rigboland is 90 mm (Naderi Shahab *et al.*, 2017) which is significantly lower than the Hajiabad annual precipitation (130 mm). Annual precipitation and mean temperature of Rigboland are higher than that of plant natural habitat (Hajiabad). Low rainfall and higher temperature could account for water and heat stresses during summer.

-In pit seeding method, most of the seeds grow into bunches of seedlings. Higher number of seedlings per pit resulted in low water availability per single seedling. Moreover, the seedling bunches (pits) were close to each other; once again, low distances between pits resulted in more water shortage for each seedling. Furthermore, differences in latitude, longitude, soil property, temperature and other climatic and environmental factors between Hajiabad (plant natural habitat) and Rigboland (experimental location) could adversely impact the *A. persicum* establishment in a new location.

Conclusion

A. persicum is one of the most important native or indigenous shrub species of sand dunes, grown in arid to semi-arid environments in east of Iran. In natural habitats, the species regenerated vegetatively by root suckers and stump sprouts (on cut stems) generatively by seeds while vegetative propagation is more prevalent than sexual reproduction. Although the habitats of the species is protected, grazing excluded or managed, narrow geographical distribution of the species in addition to desertification, soil erosion, natural or human-induced factors may encounter *A. persicum* with a decline of genetic diversity or risk of existence in far future. To preserve genetic variability and diversity of the species from genetic erosion, population or species extinction, long-term preservation of the species' seed is one of the strategic approaches. Therefore, cryopreservation of *A. persicum* seed is an important and reliable method for long-term conservation and protection of genetic resources of the species.

Laboratory and greenhouse experiments revealed that *A. persicum* seeds are able to tolerate cryogenic (-196°C) conditions. In this regard, seeds of the species can be collected from a wide range of habitats and preserved under cryogenic conditions for thousands of years (Walters *et al*., 2004). If the species encountered the threat of losing its genetic diversity or risk of extinction, the cryopreserved seeds can be removed from the cryogenic conditions and used as plant material to re-establish the species. The cryopreservation experiments also showed that the *A. persicum* seeds before being transferred into cryogenic (-196°C) conditions do not need any pre-cryopreservation treatment.

In this study, under greenhouse conditions, the cryopreserved seeds also germinated and grew into normal seedlings. The final and crucial stage of the experiments was the establishment of the cryogenic and Control seeds under natural environment in sand dunes of Rigboland. The observational results revealed that the seeds were able to germinate and develop into normal seedlings up to early summer. Hot and dry summers caused seedlings suffering from drought stress and wilting. It is recommended that in order to overcome the drought stress and facilitate more space and more water for single seedling in summer, seeding method should be changed from pit seeding method to

broadcast seeding method with more distance between the seeds. As a recommendation to the plant genetic conservation institutes or organizations with the aim of conserving *A. persicum* genetic resources and diversity, seed cryopreservation is the most reliable and applicable approach in this regard.

Acknowledgement

The authors would like to thank Dr. H. Tavakoli, Agriculture and Natural Resources Research Center of Khorasan Razavi province, for his helpful assistance and addressing the plant species locations.

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امکان نگهداری بذر دیودال (*Ammodendron persicum*) در شرایط فراسرد (Cryopreservation) و بررسی بذرهای فراسردی در شرایط مختلف

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تاریخ دریافت: ۱۳۹۵/۱۱/۲۶

تاریخ پذیرش: ۱۳۹۶/۰۳/۲۸

چکیده. دیودال (*Ammodendron persicum* Bunge ex Boiss.) گونه‌ای است درختچه‌ای که بر روی تپه‌های شنی و شن‌زارهای بیابانی شرق ایران در استان‌های خراسان جنوبی و سیستان و بلوچستان مستقر می‌شود. این گونه به‌دلیل دامنهٔ اکولوژیک محدود و گسترهٔ رویشی کم، در معرض خطر می‌باشد. به‌منظور بررسی امکان نگهداری بلندمدت بذر دیودال (*A. persicum*) در شرایط فراسرد (۱۹۶°C-)، بذر این گونه از رویشگاه طبیعی آن جمع‌آوری و با سه پیش تیمار Desiccation, PVS2 و 30% Glycerol تیمار و همراه با بذرهای تیمار نشده یا شاهد به درون نیتروژن مایع (۱۹۶°C-) منتقل شدند. سپس بذرها از درون نیتروژن مایع خارج و در معرض تیمار پس از خروج از نیتروژن مایع قرار گرفتند. جوانه‌زنی بذرها تحت شرایط آزمایشگاه، گلخانه و محیط طبیعی بررسی گردید. نتایج بررسی‌های آزمایشگاهی نشان داد که بذر گونه *A. persicum* توان تحمل شرایط فراسرد را دارد. اثرات پیش تیمارهای فراسردی شامل Desiccation, PVS2, 30% Glycerol و شاهد بر روی جوانه‌زنی بذرها به‌طور معنی‌داری متفاوت بود. بذرهای تیمار نشده یا شاهد و Desiccation به ترتیب بیشترین تاثیر مثبت را در بر زنده مانی (۵۱٪) بذرهای فراسردی و سایر صفات داشتند. نتایج نشان داد که بذرهای فراسردی توان جوانه زنی و استقرار در شرایط گلخانه و بیابان را دارند. در این پژوهش عمق مناسب کشت بذر و اسقرار بذر در شرایط طبیعی نیز بررسی گردید. نتایج به‌دست آمده از این بررسی نشان داد که روش ذخیره سازی بذر در شرایط فراسرد یک روش مطمئن برای نگداری بذر *A. persicum* می‌باشد. نگهداری بسیار طولانی مدت بذر در فراسرد روشی مهم برای جلوگیری از خطر انقراض و از بین رفتن تنوع ژنتیکی این گونه می‌باشد.

کلمات کلیدی: فراسرد، دیودال، بذر، *Ammodendron persicum*، Cryopreservation، PVS2، 30% Glycerol، Desiccation