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Original Article

Effect of Priming on Germination and Enzyme Activity of *Achillea vermicularis* Seeds after Naturally and Accelerated Aging

Liela Rasoolzadeh[1], Parvin Salehi Shanjani[2*] and Hamid Madani[3]

[1]*Department of Agronomy, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran*
[2]*Natural Resource of Gene Bank Group, Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization, Tehran, Iran*
[3]*Department of Agronomy, Arak Branch, Islamic Azad University, Arak, Iran*
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Abstract

Ageing induces seed deterioration expressed as the loss of seed vigor and/or viability. Priming treatment, which consists in soaking of seeds in a solution of low water potential, has been shown to reinvigorate aged seeds. An experiment was conducted to evaluate the effects of naturally and accelerated aging on seed germination traits and enzyme activities (proxidase, catalase and superoxid dismutase) in three *Achillea vermicularis* accessions. Naturally aged seeds were provided from base (stored 10 or15 years at -18 °C) and active (stored 10 or 15 years at 4 °C) cold rooms of Natural Resources Gene Bank of Iran. A two years harvested seeds of the accessions were aged under moisture of 100% and temperature of 40 °C for 48 and 72 hours. The seeds were primed by incubation for 24 hours at 15 °C in solution of polyethylene glycol 6000 (PEG6000) −0.3 Mpa (as osmo-priming), gibberellic acid 250 and 500 ppm (as hormone-priming). Non-primed seeds were used as control. ANOVA suggested significant different among three accessions of *A. vermicularis* for all germination traits and enzymes activities. Results showed that responses of different accessions to aging and priming treatments were significantly different. The data demonstrated that catalase is a key enzyme for seed repair against ageing ROS-induced damage during priming treatment.

Keywords: *Achillea*, Deterioration, Priming, Germination, Catalase

Introduction

Medicinal plants play a critical role in the development of human cultures around the whole world [1]. Recently, these plants gained a considerable importance in agricultural production, pharmacy and exportation because of their use as a raw material for the pharmaceutical industry [2]. Iranian Natural Resource Gene Bank (Research Institute Forest and Rangeland) conserves 22000 accessions from wild medicinal plant species. Seed banking is now widely used for the *ex situ* conservation of wild plant species in gene banks. Seed banks generally store seeds according to the gene bank standards [3] whoever, there are no specific standards for the conservation of wild plant species. Although some statistical equations developed to predict deterioration periods of crop species [4], there are less reports for determine the best times of regeneration of wild species in seeds bank. FAO [3] recommended monitoring the seeds viability every 10 or 15 years for seeds in medium- or long-term storage, respectively. One of the major problems in wild species germplasm is lack of knowledge dormancy breaking at the arrival time and for regenerating deteriorated seeds after years. In most cases the seed dormancy is likely to be lost during storage, and the conditions required for germination (in particular temperature) become less specific [5]. However there are some instances that deteriorated accessions failed to germination using the same treatments and/or conditions that were found to be optimum at the start of storage [5]. Storage of orthodox seeds for prolonged period

[*]Corresponding author: Natural Resource of Gene Bank Group, Research Institute of Forests and Rangelands P.O. Box 13185-116, Tehran, Iran
Email Address: psalehi@rifr-ac.ir, psalehi1@gmail.com

induces their deterioration leading ultimately to loss of their viability. The rate of seed deterioration varies among plant species and seed lots, but higher moisture content and temperature accelerate this process [6,7]. One of the simple techniques which can improve seedling vigor and establishment and consequently field performance of plants is seed priming [8]. This method is useful particularly if the seeds have already aged during storage [9]. Interestingly, priming repairs damage of aged seeds [9-10] or seeds exposed to abiotic stresses such as salinity [11], improving germination performance. Priming treatment consists of soaking seeds in an osmotic of low water potential to control the amount of water supply to the seed. At the cellular level, few processes have been described to act during priming some of these being: activation of cell cycle [12,13], endosperm weakening [14,15] and mobilization of storage proteins [16,17]. The priming-induced increase in the rate of seed germination has been associated with the initiation of germination-related processes [18–19], repair processes [20,21] and increase in various free radical scavenging enzymes, such as superoxide dismutase, catalase and peroxidase have also been demonstrated [17,22,23]. It is plausible that the beneficial effect of priming is due to the competing effect of a number of these physiological processes but the importance of each is to be determined. One of main problem in maintenance of seed in gene bank is regeneration of aged seeds that lose their viability over times. For increasing of seed germination traits, it is necessary to apply some seed dormancy breaking and seed priming treatments.

The genus *Achillea* consists of more than 100 species which are mostly distributed in northern hemisphere [24]. This genus has been noted medically from antiquity and the healing properties have been listed by Dioscorides [1]. The present study focuses on *Achillea vermicularis* Trin. Which has been mentioned in Iranian Traditional Medicine for its ability to dissolve tumors and a former study has tried to somehow relate its claimed use in Iranian Traditional Medicine with modern cancer researches [7,24]. Iranian Natural Resource Gene Bank (Research Institute Forest and Rangeland) conserves different accessions of this species from different parts of Iran. This research was conducted to study effects of different aging and priming treatments on seeds of three accessions of *A. vermicularis* in greenhouse condition.

Material and Methods

Seed of three native accessions of *Achillea vermicularis* consisted Baneh-1 (origin: latitude 35° 57', longitude 46° 03'; storage duration: 15 years), and Baneh-2 (origin: latitude 36° 03, longitude 45° 57'; storage duration: 10 years) and Zanjan (origin: latitude 36° 43', longitude 48° 42'; storage duration: 10' years) were provided from Natural Resource Gene Bank (NRGB), Iran. A factorial experiment was conducted to evaluate the effects of naturally and accelerated aging on seed germination traits and enzyme activities from three *A. vermicularis* accessions. Naturally aged seeds were provided from base (stored 10 or15 years at -18 °C) and active (stored 10 or15 years at 4 °C) cold rooms of NRGB. A two years harvested seeds of the accessions were aged under moisture of 100% and temperature of 40 °C for 48 and 72 hours. The seeds were primed by incubation for 24 hours at 15 °C in solution of polyethylene glycol 6000 (PEG6000) −0.3 Mpa (as osmo-priming), gibberellic acid 250 and 500 ppm (as hormone-priming). Non-primed and hydro primed (water soaked) seeds were used as control. The treated seeds were sown in 15 cm diameter plastic pots filled with sandy soil, and irrigated with tap water in greenhouse at 22±3 °C. The pots were maintained at field capacity. Data collected for germination percent, seed vigor index, shoot length (mm), root length (mm), seedling length (mm) and seedling fresh weight (mg) [25]. Meanwhile, peroxidase, catalase and superoxid dismutase (SOD) enzymatic activities were measured [26]. Data analysis was carried out using SAS software and the differences between treatment means were tested using Duncan's Multiple Range Test.

Results and Discussion

Analysis of variance showed highly significant differences among accessions, aging and priming treatments for all traits (Table 1). The interaction between accessions, aging and priming methods was also significant (Table 1). In *A. vermicularis* seeds, germination decreased significantly with ageing duration at 40 °C (Table 2). Effect of priming treatments (gibberellic acid 250 and 500 ppm as hormone-priming, and PEG −0.3 Mpa as osmo-priming) on seed traits and enzyme activities (peroxidase, catalase and superoxid dismutase) was

significantly different in the three accessions (Table 3).

Naturally Aging

Mean comparison at two naturally aging methods (base and active store) showed that the highest seed germination traits, root length, shoot length, seedling length and seedling fresh weight were obtained in naturally aged at -18 °C, (base store) indicating the effect of low temperature in keeping seed viability (Table 2). The seeds preserved in base store with low humidity and temperature had low metabolic activity and causes it late deterioration. In contest, in active store there was more traffic of staff and open/close the door and also repeated power fluctuations and humidity cause early seed deterioration. Similar to this research, Rincker [27] showed that during the 20 years of storing 37 accessions of alfalfa seeds at (-15 °C) with a relative humidity of 60%, germination decreased from 91 to 81%, whereas, this value reduced to 50% in open storage conditions during 10 years. Comparison of mean germination characteristics of three accessions showed that stored seeds of accession Baneh-1 at base and active store had lower germination percentage, seedling vigor index, root length, shoot length and seedling fresh weight (Table 2). Undesirable effects of seeds priming with gibberellic acid (500 ppm) on the germination traits were obvious for all three accessions. Farooq *et al*. [28] reported higher effect of priming on root length in rice. Similar to our study, gibberellic acid was effective on recovery of deteriorated seeds of rapeseed [29]. Sajjadi Jaghoroghi *et al*. [30] studied effect of osmo-priming, hydro-priming and pre-chilling on seed emergence enhancement and seedling vigor of four medicinal species of *Anthemis* under greenhouse conditions. Their result showed that primed seeds with gibberellic acid and potassium nitrate (osmo-priming) improved germination potential and seedlings growth [30].

Accelerated Aging

According to results of variance analysis, effect of accelerated aging treatments on germination traits, were significant among different accessions ($P<0.01$) (Tab. 1). In agreement with the results, earlier reports Bailly [31], Goel [32], McDonald [33] and Siadat [34] have shown negative effect of aging on germination characteristics. Results showed that in all three accessions the highest germination percentage and vigor index were obtained under control (Table 3). Increase of aging duration reduced the germination percentage and vigor index, therefore the minimum amount of these traits was attained under 72 hours of aging (Table 3). This result is in accordance with reports of Alizadeh [35] in 17 medicinal species, Jan-Mohammadi *et al*. [36] and Ghassemi-Golezani *et al*. [37] in rapeseed, Bhattacharjee *et al*. [38] in common bean and sunflower and Saha and Sultana [39] in soybean. Also, earlier reports Bailly [31], Goel [32], McDonald [33], Siadat [34] Moradi and younesi [40] have shown negative effect of aging in relation to seed performance, germination percentage and seedling indices [41] suggested that decreasing in germination percentage was related to chromosomal aberrations that occur under long storage conditions. Decreasing of germination percentage in aged seeds can be due to reduction of α-amylase activity and carbohydrate contents or denaturation of proteins [31-42]. According to Abdalla and Roberts [43] in barley and pea seeds treated with different combinations of accelerated aging treatment showed that the amount of genetic damage was solely a function of loss of viability. Comparison of peroxidase, catalase and superoxide dismutase activities showed that ageing induced a considerable increase in catalase activity. (Table 2) This result is in accordance with Kibinza *et al*. [44] reported that the catalase is a key enzyme in seed recovery from aging during priming. This result suggest, in accordance with Butler *et al*. [9] that priming repair is possible if accumulated damage is not irreversible. Irreversible damage at the cellular level was designated as the point of non-return which is the point when the cell becomes irreversibly committed to die [45]. Seeds that had been irreversibly damaged, whatever the ageing period, were not repaired by priming. The increase of half-cell reduction potential was shown in response to ageing in *Pisum sativum* seeds [46]. This increase was proposed to be related to a programmed cell death (PCD)/DNA fragmentation suggesting an active and genetically regulated cell death in response to ageing in seeds. Interestingly, catalase was also associated to the reduction of hypersensitive response induction of PCD [47].

If PCD is the common way for seeds to lose their viability during ageing, catalase involvement seems to be highly significant.

Table 1 Analysis of variance and mean of squares of *A. vermicularis* seed traits and enzyme activities (proxidase, catalase and SOD) under greenhouse conditions

Source of variation	d.f.	Germination (%)	Vigor index	Root length (mm)	Shoot length (mm)	Seedling length (mm)	Fresh weight (mg)	Peroxidase activity	Catalyze activity	SOD activity
Accession	2	9524.6**	23864.4**	6583.1**	2392.4**	12549.3**	0.00005^{ns}	236.37**	7148.2**	53.37**
Aging	4	46472.2**	42525.4**	8318**	6109**	3386.3**	0.003**	313.35**	37540**	84.94**
Priming	4	1839**	19122.1**	25053.7**	2321.5**	40132.3**	0.015**	738.37**	16796**	10.44**
Acc* aging	8	2444.3**	6145.2**	7741.3**	442.2**	11657.5**	0.0013**	37.11^{ns}	2743.8**	21.80**
Acc* priming	8	536.4**	2695.5**	2509.3**	464.4**	4611.8**	0.0038**	90.74*	613.7**	6.52**
Aging *priming	16	1240**	4871.6**	9441.9**	1793.4**	18721.2**	0.012**	71.07^{ns}	1792**	6.23**
Acc*aging*priming	32	366.4**	1882.7**	1593.9**	260.9**	2743.1**	0.002**	122.5**	1833.8*	10.81**
Error	150	19.76	78.46	337.6	58.7	610.01	0.0005	33.92	10.76	0.48
CV%		8.14	14.03	25.8	21.7	23.2	36.07	38.26	3.4	17.05

^{ns}: no significant; *, **: significant at 0.05 and 0.01 levels, respectively.

Table 2 Mean comparison of three accessions of *A. vermicularis* seed traits and enzyme activities (peroxidase, catalase and SOD) at naturally and accelerated aging treatments.

Accession	Aging	Treat	Germination (%)	Vigor index	Root Length (mm)	Shoot length (mm)	Seedling length (mm)	Fresh weight (mg)	Peroxidase activity	Catalyze activity	(SOD) activity
Baneh-1	N. aging	-18 °C	65.87 de	69.10 hi	58.28 kl	41.11 d	99.39 kl	56.93 ij	-	-	-
	-	4 °C	43.20 fg	41.38 kl	52.23 l	35.8 ef	88.10 n	44.13 k	-	-	-
	A. aging	Control	64.89 de	77.63 f-h	77.39 i	41.06 d	118.45 ij	83.33 d-f	14 b	42 h	7.03 b
	-	48 h	28.00 ij	43.51 kl	123.60 d	28.9 fg	151.22 de	103.42 bc	12.08 c	85 cd	5 bc
	-	72 h	22.67 j	36.81 l	121.3 d	27.61gh	154.75 d	105.83 b	7.71 d	111.5 a	3 c
Baneh-2	N. aging	-18°C	90.67 a	110.12 bc	68.47 i-k	52.93a	121.40 hi	63.33 hi	-	-	-
	-	4 °C	89.00 b	90.31 f	58.37 kl	47.83bc	106.20 jk	54.73 ij	-	-	-
	A. aging	Control	83.73 c	108.56 d	79.42 hi	43.7 cd	123.21 e-i	66.33 hi	15 b	44 gh	7 b
	-	48 h	34.67 gh	63.48 i	141.82 a	35.10 ef	175.82 a	92.08 cd	5.16 e	73 e	2.57 d
	-	72 h	29.00 i	50.00 i-k	129.7 bc	38.28de	167.99 b	75.83 fg	8.48 de	98 b	3.8 c
Zanjan	N. aging	-18°C	93.40 a	110.86 bc	68.23 i-k	53.97 a	122.20 hi	65.27 g-n	-	-	-
	-	4 °C	90.00 a	100.51 e	61.53 k	50.67ab	112.20 j	58.73 i-q	-	-	-
	A. aging	Control	88.53 ab	135.94 a	96.4 e-g	51.2 ab	147.63 e	76.58 e-i	17.5 a	46.4 gh	9 a
	-	48 h	7.80 j-l	19.13 l-n	116.7 d	40.99de	164.17 bc	114.17 a	10 cd	68 ef	2.67 d
	-	72 h	28.67 ij	44.82 k	108.6 e	43.4 cd	166.65 bc	99.50 a-d	7.08 d	85 cd	2.85 d

Means with the same letter are not significantly different (P=0.01); N: Naturally, A: Accelerated

Table 3 Mean comparison of three accessions of *Achillea vermicularis* seed traits and enzyme activities (peroxidase, catalase and superoxid dismutase) under priming treatments (at gibberellic acid 250 and 500ppm as hormone-priming, and PEG −0.3 Mpa as osmo-priming).

Accession	Priming	Germination (%)	Vigor index	Root Length (mm)	Shoot length (mm)	Seedling length (mm)	Fresh weight (mg)	Peroxidase activity	Catalyze activity	Superoxide dismutase activity
Bane-1	Control	79.11 cd	86.20 cd	58.67 f-h	49.72 c	108.39 hi	55.22 hi	11.26 ef	79.50 e	5.01 b
	GA250ppm	41.67 g	56.31 ef	95.95 bc	40.34 de	136.28 de	84.92 c	56.03 a	120.37 bc	6.05 a
	GA500ppm	22.00 i	21.54 h	84.35 de	27.49 gh	116.33 e-g	91.00 b	19.12 de	139.33 a	5.71 b
	PEG-0.3MP	55.20 ef	73.13 de	97.59 bc	37.26 ef	134.84 de	93.93 a	8.67 f	84.67 de	4.33 c
	Control	96.89 ab	83.50 d	45.50 ij	40.61 de	86.11 kl	45.33 k	9.55 f	71.67 ef	4.46 c
Baneh-2	GA250ppm	72.80 cd	106.01 b	107.62 a	47.76 cd	155.38 a	80.00 cd	14.87 ef	123.38 b	4.42 c
	GA500ppm	56.67 ef	74.70 de	98.43 bc	42.63 de	139.9 b-d	67.00 ef	19.54 de	128.33 ab	3.74 d
	PEG-0.3MP	66.67 de	97.81 bc	100.82 b	48.60 cd	149.41 b	87.33 b	11.15 ef	86.53 de	3.92 d
Zanjan	Control	100.00 a	97.89 bc	51.56 i	46.33 d	97.89 jk	54.22 i	11.53 ef	66.47 f	4.83 c
	GA250ppm	78.33 cd	119.46 a	100.62 b	54.66 b	155.28 a	75.75 de	16.57 e	81.13 de	2.55 e
	GA500ppm	45.57 fg	51.91 f	86.01 d	36.93 f	136.72 de	92.25 a	29.67 b-d	108.33 c	2.30 e
	PEG-0.3MP	79.33 cd	117.01 a	91.29 cd	59.58 a	150.88 ab	86.00 bc	14.87 ef	64.27 f	1.67 f

Means with the same letter are not significantly different (P=0.01)

Although the involvement of protective enzymes like SOD or peroxidase is likely to be probable, our data demonstrated that catalase is a key enzyme for seed repair against ageing ROS-induced damage during priming treatment.

Conclusion

Present study showed that osmo-priming and hormonal priming is useful methods to improve quality of deteriorated and old seeds of *A. vermicularis*. The responses of different accessions of *A. vermicularis* to aging and priming treatments were significantly different. Catalase is a key enzyme for seed repair against ageing ROS-induced damage during priming treatment.

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