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Original Article

Effect of Priming on Improvement of Deteriorated Seed of *Tanacetum parthenium*

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Abstract

In order to study the effect of of hormo/osmo priming on seed emergence and seedling growth of *Tanacetum parthenium* (L.) Sch.Bip., a factorial experiment was conducted based on completely randomized design with three replications in greenhouse of Research Institute of Forests and Rangeland, Tehran, Iran in 2015. The factors were A) four conservation methods with foure levls including: 10 to 15 years old seeds perserved in active collection (4 °C) and base collection (-18 °C) deteriorated seeds using artificial accelerated ageing (40 °C, 98% of RH for 48 h) and control (two years old seeds in store as normal collection), and the factor B) priming treatments with six levels including: control (no priming), two osmopriming using PEG (-0.3 and -0.6 Mpa), two hormonal priming using Giberlic acid (GA500 and GA1000ppm) and hydropriming (imbibition with distilled water). The results showed that the highest values of emergence percentage, vigor index, root length, seedling length, seedling dry weight were obtained using GA1000 ppm and PEG -0.6 Mpa for seeds preserved in active collection. For seeds preserved in base collection, the effect of osmopriming (PEG -0.6 Mpa) on seedling characteristics were higher than other treatments. Similarly, in artificial accelerated aged seeds, both osmopriming (PEG -0.6, -0.3 Mpa) had significant effect on aged seeds reinvigorate compare than other treatments.

Keywords: Active collection, Artificial accelerated ageing, Base collection, Emergence, Normal collection

Introduction

The genus Tanacetum have 160 species and they scattered in Europe, Asia, North of Africa, and South of America [1]. There are 26 species of Tanacetum in flora of Iran, of which 12 are endemic [2]. Some species of the genus, have traditionally been used in balsams, cosmetics, dyes, insecticides, medicines [3]. They used as anti-helmintic for migraine, neuralgia rheumatism and loss of appetite [4], anti-inflammatory [5], antibacterial [6], antifungal and insecticidal effects [7]. The species of T. parthenium, originated from Asia and the Balkans and it scattered in a large area of Europe and Asia [4]. Some studies have been found that relate to the sesquiterpene, lactone, parthenolide and flavonoids, to the strong biological activity [8].

Seed aging is a natural and irreversible process that emerged during seed storage conditions and it reduced quality, viability and vigor of the seeds. Seed moisture content and temperature affect the speed of the seeds deterioration and increasing both of them lead to seed deterioration occurring more quickly. Seed deterioration of each sample is different among different populations. Each seed sample has special feature of storage conditions [9].

In gene bank managements, seed regeneration of deteriorated accessions is critically important for reinvigorate of the deteriorated seeds. The priming technique has many benefits as dormancy breaking, reduction of germination time, increasing germination uniformity, high seedling performance, highly competitive with weeds and flowering simultaneity [10]. The imbibing of the seeds in water is the simple priming technique before germination processing. Placing of drying seeds on the solutions containing low osmotic materials like polyethylene glycol (PEG), sorbitol,

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manitol, then re-drying of the seeds is nominated as osmo-priming. Any osmotic materials which has negative osmotic potential would have able to cause more water absorption by the seeds. While the metabolic process would be started before germination but the germination and radical emergence would be inhibited [11,12]. The primed seeds which cultivated in fields, have high emergence percentage and also more emergence uniformity. The researcher attributed of high vigor and seedling performance the primed seeds to the more amylase and lipase enzyme activity in the germination process in zinnia seed [13]. They showed that the activity of α-amylase may be potentially useful for the seed industry as a physiological marker of zinnia seed vigor and the effectiveness of osmopriming [13]. In some plant species, sometimes germination drastically reduced, therefore, to reviving and increase germination and seedling growth, some treatments need for breaking dormancy. Priming technique is also one of the methods for enhancement and uniformity of germination of the deteriorated seed [14]. This study was aimed to effect of of hormo/osmo priming on seed emergence and seedling growth of *T. parthenium* that preserved in base and active collections of natural resources gene bank, also improving the aged seed that deteriorated in artificial condition.

Material and Methods

The seeds of five accessions of *T. parthenium* with different derivation, including: West Azarbijan, Yazd, Hamadan, and Gilan province were collected from the country of Iran. A factorial experiment was conducted based on completely randomized design with three replications in greenhouse of Research Institute of Forests and Rangeland, Tehran, Iran in 2015. The expernimental factors were: A) four aged seeds including: two naturally aged seeds preserved in active and basic collection (4 □C and -18 °C, respectively for 10 to 15 years), accelerated aged seeds induced by (98% of relative humidity and temperature of 40 °C for 48 hours) and normal collection seeds (a two year old sample in room temprature), and B) six priming treatments including: no priming (control), osmopriming using PEG6000 (-0.3Mpa, -0.6Mpa), hormonal priming using Gibberellic acid (500 and 1000 ppm) and hydropriming (imbibition with distilled water).
For two levels of osmopriming 27.6g of Ploy Ethylene Glycol 6000 dalton was solved in 200ml of distilled water the solution of PEG–0.3 and -0.6 Mpa were provided [15]. For two levels of hormonal priming, 250mg of Gibberellic acid solved in 250 of distilled water, the concentration of 1000 ppm was obtained. For obtaining of concentration of 500ppm, the 250mL solution of 1000ppm diluted in 250 mL distilled water.

The seeds of accessions were transferred into solution treatments for overnight, then seed samples were dried at room temperature for 24 h. The primed seeds were sown in 2000 mL pots (with ratio 1:1:1 of soil, sand and compost) with three replicates. In each pot 25 seeds were sown in greenhouse of Research Institute of Forests and Rangeland, Tehran, Iran in 2015. The pots were kept in glass house with temperature 20-30 °C in day and 5-12 °C at night using light illumination about 6000-10000 lux and the relative humidity (RH) ranged from 50 to 60%. The data of seedling characteristics, including: emergence percentage, shoot length (mm), root length (mm), seedling length (mm), seedling dry weight (mg) were collected at the end of experiment (the duration of experiment was 45 days). For seedling dry weight five seedlings per pot were taken in each pot, weighted and averaged according to Lekh and Kairwal [16]. The seedlings were then dried in oven for 24 h at 80 °C and weighted as seedling dry weight (mg). The emergence percentage was calculated according to the total number of emerged seedlings in numbering final day [17]. The vigor index was measured according to Abdulbaki and Anderson [18] that their values obtained from following formula.

$$Vi = \frac{\%Gr \times MSH}{100}$$

Where:
Vi = vigor index
%Gr = final germination percentage
MSH = mean seedling height

Statistical Analysis

In analysis of variance the effects of five accessions were merged in the model as completely randomized design with three replications. In the other hand the replications number were 3x5=15. Data were analyzed using SAS software and the differences between treatment means were tested using Duncan's Multiple Range Tests at 1% probability level.

Results and Discussion

The results of the analysis of variance (ANOVA) showed significant effects of aging methods and priming treatments and their interactions for all of the studied traits (Table 1). In a factorial experiment when interaction effect is significant the main effect levels has low priority, however, in the present study the results of the main effects of, aging and priming were summarized in the Table 2 and Table 3.
The results of mean comparisons showed significant differences between four preservation methods for all of traits ($P \leq 0.01$). The higher and lower values of

emergence percentage (89.88 and 10.66%) shoot length (17.90 and 5.23 mm were obtained in control (2 yr old seeds) and accelerated aged seeds, respectively. For emergence percentage, there was no differences between active and base collections (Table 2). For all other traits the higher values were obtained in active store. The mean values of root length and dry weight of the based store ranked in the second place, indicating that seed preserved for 10 to 15 years in based store, produced longer root and higher seedling than conterol and artificial aged seeds (Table 2). The higher values of vigor index (35.53), root length (24.90mm), seedling length (42.26mm) and seedling dry weight (290.7mg) were obtained from active collection (4□C) that were significantly higher than other treatments. The lower values of traits which observed in accelerated aged seeds (Table 3).

Mean comparison at two naturally aging methods (base and active collection) showed that the highest seed emergence traits, including: root length, shoot length, seedling length and seedling dry weight were obtained in both collection indicating the effect of low temperature in keeping seed viability. The seeds preserved in cold store with low humidity and temperature had low metabolic activity and causes delay in seed deterioration. Similar to this research Rincker [19] 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. Farooq [20] reported higher effect of priming on root length in rice. Similar to our result, Sajjadi Jaghoroghi [21] showed that primed seeds with gibberellic acid and potassium nitrate (osmo-priming) improved the germination potential and seedlings growth.

The results of mean comparisons between priming treatments, showed that the higher values of emergence percentage (78.47), vigor index (32.83), root length (22.50mm), seedling length (37.05mm) obtained in Osmo-0.6MP, that were significantly higher than other treatments. The higher values of seedling dry weight (180mg), observed with using of GA1000 ppm that was significantly higher than other treatments (Table 3).

The results of priming by aging interaction effects for emergence percentage, vigor index and root length are presented in Figure 1, and for shoot length, seedling length and seedling dry weight is presented in Figure 2.

The result of priming by aging interaction effect showed the higher values shoot length in active collection using GA1000. For root length and seedling dry weight the higher values were obtained in active collection using PEG -0.6. For other traits such as emergence percentage, seedling length and vigor index, the higher the were obtained in active collection using both GA1000 and PEG -0.6 (Figs 1 and 2).

The results showed that the all seedling traits were significantly lower in accelerated aging test compare with other treatments. These findings are in agreement with reports [22-25] that showed negative effect of aging in relation to seed performance, germination percentage and seedling indices.

Akhter [26] 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 [27]. This result confirmed with the result of Sajjadi Jaghoroghi *et al* [21], who studied the effect of Osmopriming, hydropriming and pre-chilling on seed emergence enhancement and seedling vigor of four medicinal species of Anthemis under greenhouse conditions.

In control seeds (the two years harvested seeds) all of priming treatments except Osmo -0.6MP had significantly increased emergence percentage than control. For naturally aged seed in active collection (4 °C), the higher values of emergence percentage (92-100%) were significantly more by Osmo-0.6MP, GA1000 ppm and hydropriming, while, for naturally aged seed in base collection (-18 °C), higher values of emergence percentage (92-93%) were higher by all levels of Osmo and hormonal priming than other treatments. For accelerated ageing test the higher emergence percentage with average values of 32% were significantly more by Osmo -0.3MP than other treatments (Fig. 1).

In control seeds (Fresh seed), the higher values of vigor indices (38.5 and 34.2) were obtained by hydropriming and GA1000 which were significantly more than other treatment. For naturally aged seed in active collection, the higher values of vigor indices (45 and 52) were significantly more by GA1000 and Osmo-0.6MP, compare with other treatments. For naturally aged seed in base collection the higher values of vigor indices (ranged 30 to 35) were significantly more by all levels of osmo and hormonal priming than other treatments. For accelerated aging test the higher vigor index (7 to 8) were significantly more by osmo and hormonal priming compare with other treatments (Fig. 1). The root and shoot length (52.02, 35.28 mm), were significantly higher by effect of osmo priming (0.6MP) in active collection. The seedling length was significantly more with effect of hormonal priming (GA1000), in active collection. This result proved that Osmo-priming by poly ethylene cause to enhance seedling length. El-Araby and Hejazi [28] stated osmopriming using PEG was to improve germination traits in tomato.

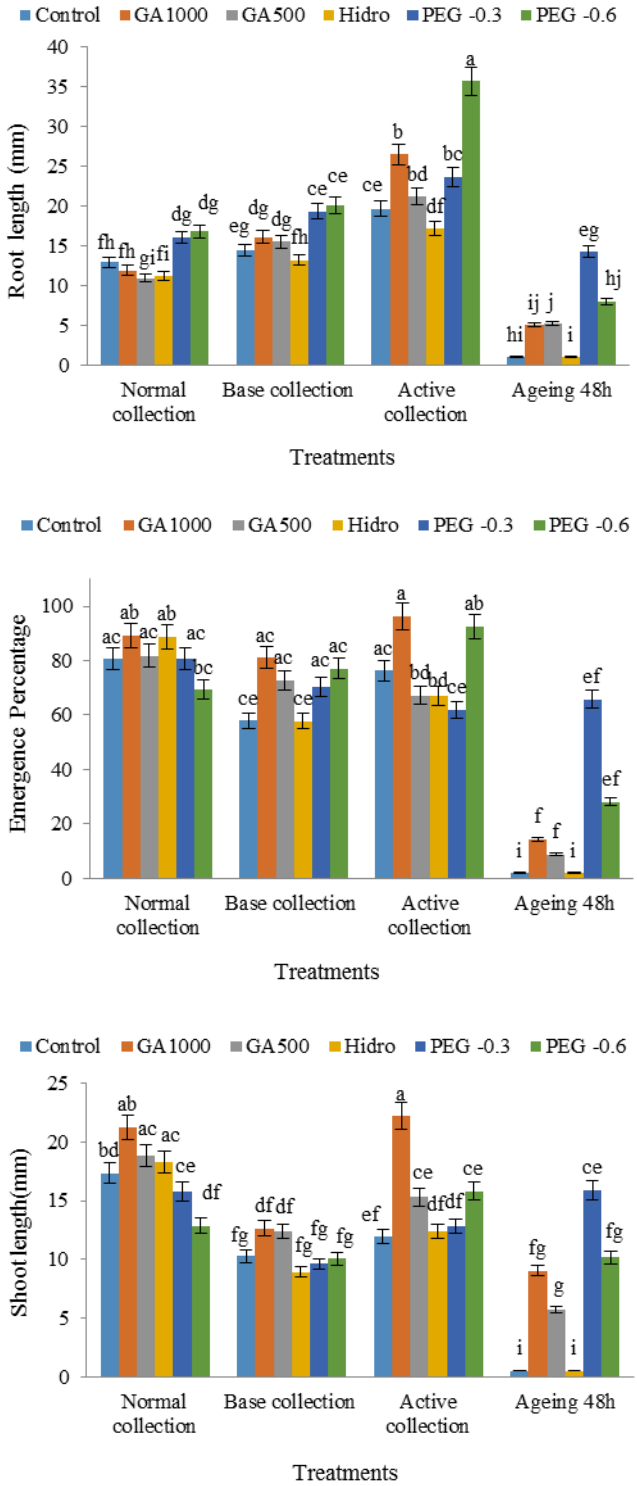

Fig. 1 Priming by conservation interaction effects for emergence percentage, root length, shoot length, of *Taraxacum. parthenium* (L.) Sch.Bip. under greenhouse conditions

Fig. 2 Priming by conservation interaction effects for seedling length,vigor index and dry weight of *Taraxacum. parthenium* (L.) Sch.Bip. under greenhouse conditions

Similar to our research [3] showed that during the 20 years of storing 37 accessions of alfalfa seeds at (-15 °C) with a relative humidity of 60%, the trend of germination decreases was low from 91 to 81%, whereas, in open storage conditions during 10 years Priestley [29] reported the half of the seeds lost their viability. In control seeds, the higher value of root length (16.8 and 18.4 mm) were significantly more by effect of 0.03MP and -0.6MP, than other treatments.

For naturally aged seed in active collection (4 °C), the higher values of root length (33.2 mm) was significantly more by Osmo -0.6MP and similarly in base collection the higher values of root length (24 and 23.5 mm were significantly more by Osmo-0.03 and -0.6MP, compare with other treatments. For accelerated aging test the higher root length (9.43 mm) was significantly more by Osmo -0.3MP (Fig. 1). The reduction of seedling growth

is the trace of seed deterioration which had been reported by many researchers [30,31].

In control seeds (fresh seeds) the higher value of shoot length (20.4 and 20.3 were significantly more by GA1000 and hydropriming compare with other treatments. For naturally aged seed in active collection (4 °C), the higher values of shoot length (22.27 mm) were significantly more by GA1000 ppm than other treatments. Similarly, in base collection the higher values of shoot length (14.6 and 16.3mm) were obtained by GA500 and 1000 ppm. For accelerated aging test the higher seedling length (11.1 mm) was significantly more by Osmo -0.3MP (Fig. 2). Using of the deteriorated seeds, especially in developing countries leaded to retardation of plant growth and finally it causing to reduce of crop yield in field condition [32].

In control seeds (fresh seeds), the higher values of seedling length (33.8 and 32.7mm) were significantly higher by GA1000 and hydropriming compare with other treatments.

For naturally aged seed, the higher seedlings length (45 and 52 mm) were obtained by Osmo-0.6MP and GA1000 ppm, in active collection (4 °C).Similarly, in base collection, the higher values of seedling length (30.9 to 35.1 mm) were significantly more by all levels of Osmo and hormonal priming levels. For accelerated aging test the higher seedling length (20mm) was significantly higher by Osmo -0.3MP (Fig. 2) than other treatments. With reduction of plasma membrane integrity, nucleic acid molecules and reduced enzyme activity are the most important changes in deterioration of the seeds which conserved either by natural and artificial conditions [34].

The higher values of seedling dry weight, in control seeds (173 mg) were significantly higher by hydropriming than other treatments. For naturally aged seed in active collection the higher values of seedling dry weight (280 to 330 mg) were significantly more by all of Osmo and hormonal priming levels than other treatments. Similarly, in base collection the higher values of seedling dry weight (225 and 270mg) were significantly higher by GA1000 ppm and Osmo -0.6MP, than other treatments. For accelerated aging test the higher root length (25.57 mm) was significantly higher by Osmo -0.3MP (Fig. 2) than other treatments. Reduction of plasma membrane integrity, nucleic acid molecules and reduced enzyme activity are the most important changes in deterioration of the seeds which conserved either by natural and artificial conditions [35].

Table 1 Analysis of variance of store condition and seed priming on germination traits of *T. parthenium* (L.) Sch.Bip.

Source of variation	DF	Emergence %	Vigor index	Root length	Shoot length	Seedling length	Seedling dry weight
Aging	3	22719.4**	2901.2**	1318.4**	734.3**	2987.7**	310897.3**
Priming (P)	5	2539.7**	657.6**	314.1**	53.0**	362.1**	14130.0**
Aging *Priming	15	1212.1**	242.7**	54.4**	50.2**	196.8**	19683.6**
Error	48	6.634	1.546	0.418	0.191	1.329	1275.0
C.V		3.61	4.60	3.81	3.08	3.61	22.80

*, **, ^{ns}= Significant at 5%, 1 % and non-significant, respectively.

Table 2 Means comparison of deterioration effects on seedling traits of *T. parthenium* (L.) Sch.Bip. under greenhouse conditions

Aging test	Emergence %	Vigor index	Root Length mm	Shoot length mm	Seedling length mm	Dry Weight mg
Active collection	78.06 b	35.53 a	24.90 a	16.57 b	42.26 a	290.7 a
Base collection	77.22 b	27.82 c	18.57 b	12.82 c	31.65 c	177.6 b
Aging 48 h	10.66 c	6.25 d	3.99 d	5.23 d	11.12 d	12.5 d
Normal collection	89.88 a	28.77 b	14.66 c	17.90 a	33.21 b	84.8 c

Means of column followed with the same letter are not significantly different (P≤0.01).

Table 3 Means comparison of effects priming treatments on seedling traits of *T. parthenium* (L.) Sch.Bip. under greenhouse conditions

Priming Treatments	Emergence %	Vigor index	Root Length mm	Shoot Length mm	Seedling Length mm	Dry Weight mg
Control	60.88 d	20.46 e	15.78 c	14.21 b	30.39 d	141.2 cd
GA500ppm	61.83 d	24.16 d	13.53 e	12.89 c	27.78 e	135.7 d
GA1000ppm	73.00 c	29.16 b	15.41 c	15.56 a	31.19 c	180.0 a
Hydro priming	79.73 a	27.13 c	13.95 d	14.14 b	30.30 d	171.5 ab
Osmo-0.3MP	76.57 b	27.52 c	20.45 b	14.22 b	35.00 b	149.8 c
Osmo-0.6MP	78.47 a	32.83 a	22.50 a	14.15 b	37.04 a	163.4 b

Means of column followed with the same letter are not significantly different (P≤0.01).

These changes resulted in reduced of seed quality, reduction of germination percentage, slower plant growth, increased sensitivity to environmental stresses and are sometimes reduced performance of crop yields.

Conclusions

The findings of this study indicated that the higher percentage of seed emergence and seedling growth were obtained in osmo-priming (PEG Mpa), and hormonal priming (Giberlic acid). It proved that two priming technique including: Osmo-priming using (PEG) and hormonal priming (Giberlic acid) are effective method for improvement of aged seed. The seed which reserved either in base and active collection for 10 to 15 years, they have similar or higher emergence ability and vigor than the fresh seeds that are kept in room temperature (24 °C) for two years as normal collectiion. The higher seedling growth obtained by using osmopriming and hormonal priming. The osmopriming method are also effective in recovery of deteriorated seeds.

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