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Influence of Nano-Priming on Festuca ovina Seed Germination and Early Seedling Traits under Drought Stress, in Laboratory Condition

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ABSTRACT

Aims Germination stage is one of the most sensitive stages to drought stresses and if the plant is able to tolerate stresses in this stage, it can pass the later growth stages. Priming could improve germination of seeds under stress.

Materials & Methods Experiment was carried out using a randomized complete block design. 25 seeds were placed in petri dish under drought stress with polyethylene glycol 6000 in three levels of 0, -6 and -12 bar. After 14 days, it was found that the germination percent in these seeds was low (>40%). Then nano priming was used to improve seed germination attributes. Treatments were; control, silver nanoparticles with the concentrations of 25, 50 and 75%.

Findings Data analysis of variance indicated that influence of nano priming, drought stress, and their interaction was significant on root and shoot length, wet and dry weight, vigor index, allometric coefficient and mean germination time ($p \leq 0.01$). Results showed that PEG stress had a negative effect on seeds germination. And an increase of silver nanoparticles concentration improves F. ovina seed germination and seedling traits. The maximum of GP (86%), SG (6N/D), Vi (5), AC (6) and MGT (7.08d) were recorded for seeds nano primed in the stress of 0 level.

Conclusion Nano-priming is an impressive technique to the betterment of seedlings germination and growth of F. ovina. In the most studied indices, nano-priming 75% had the greatest influence. Before planting to restore of rangelands, to promote the establishment and growth of planted F. ovina it is recommended to prime seeds with nano-silver particles.

Keywords Nanoparticles; Polyethylene Glycol; Rangeland Species; Vigor Index

CITATION LINKS

[1] Effect of water stress on seed germination ... [2] IStudy of yield and yield components of Iranian ... [3] Effects of different drought and salinity levels on seed ... [4] Effects of drought stress on Artemisia sieberi Besser ... [5] Meta-analysis of seed priming effects on seed germination, seedling ... [6] Responses of seed germination, seedling growth, and ... [7] Comparison of three priming techniques for onion seed lots ... [8] Effects of seed priming with plant growth promoting ... [9] Effect of pre-sowing seed treatments on germination traits and ... [10] Broader societal issues of ... [11] Nanotechnology in agricultural diseases and food ... [12] Effect of nano silver on seed germination and seedling growth ... [13] Effects of nanopriming and bioprimingon on growth ... [14] Effects of silica and silver nanoparticles on seed germination ... [15] Effect of different treatments on improving seed germination ... [16] Flora iranica: facts and figures and a list of publications ... [17] Presentation of some important plant species suitable ... [18] Evaluation of seedling vigor of hydro and matriprimed ... [19] The effect of osmopriming and hormopriyming on germination and early growth of Festuca ... [20] Seed treatment with nano-iron (III) oxide enhances germination, seeding growth ... [21] Effect of seed priming with zinc sulfate on germination characteristics and seedling ... [22] The osmotic potential of polyethylene glycol ... [23] Effect of halopriming on germination, morphological and ... [24] The effect of N-Si on tomato seed germination under ... [25] Germination of salt tolerant shrub Suaeda fruticosa from Pakistan ... [26] The quantification of ageing and survival in ... [27] Priming effect of rice seeds on seedling establishment under adverse soil ... [28] Growth analysis of weed and crop species with reference to seed ... [29] Viability and leaching of sugard from germinating ... [30] Review of data analysis methods for seed ... [31] The effect of priming treatments on salinity tolerance of Festuca arundinacea ... [32] Environmental effects of nanosilver: impact on castor ... [33] Germination, vigour and dehydrogenase activity of naturally aged ... [34] The effect of hydropriming and halopriming on germination and early growth ... [35] Role of nano-SiO2 in germination of tomato ... [36] Effect of Seed Priming on Plant Growth and Bulb Yield ... [37] Effects of seed priming methods on gemination parameters ...

1- Introduction

One of the important problems for plants is drought, which causes delay of germination, reduces shoots growth and also decreases the production of dry matter. Water stress affects physiology, morphology, and anatomy of plants and causes reduction of aerial part growth, decrease in dry biomass and delay of germination [1]. In fact, abiotic stresses (Salinity, drought, and extreme temperatures) represent serious limitations to plants and their yields. Drought stress occurs when water gained by the plant is lower than the lost water it can be due to excessive loss of water or decreased absorption or both of them [2]. Also, drought stress significantly affected germination and enhance of drought level strongly reduce seedling growth and seed germination [3].

As plant life will begin from seed germination, success in this stage has a critical role in life succession and preservation of the species especially rangeland ecosystems [4]. So, the germination stage is one of the most sensitive stages to salinity and drought stresses and if the plant is able to tolerate stresses in this stage, it can pass the later growth stages. In this regard, the use of different methods for increasing the speed and power of germination is very necessary [5]. Priming is a pre-treatment of seed sowing that imparts it to a determined solution that allows partial hydration but not germination [6]. In priming, seed hydration attains the second stage of sorption but does not allow radicle protrusion via the coat of seed [7]. Priming could improve germination and early growth of seeds under stress conditions compared to unprimed seeds [8]. Hydropriming also has more impact than halopriming on percentage and speed if germination of *Pinus eldarica* [9].

Today, nanotechnology has become an engaged field of interdisciplinary researches. It extensively uses in various fields like medicine, pharmaceuticals, electronics, and agriculture. Nanoparticles (NPs) are molecular or atomic aggregates at 1 to 100nm dimensions [10], that their physicochemical characteristics can strongly modify compared to the bulk materials. Different forms of silver such as silver salt, silicate and water-soluble polymer to radioactive rays are a stimulator of plant-growth [11]. Silver nanoparticles have a second application after carbon nanotubes in the nano world and their use is ever increasing [12]. Recent improvements in the use of nanotechnology in agriculture and natural resources are widely grown. Nano pricing and biopriming effects on growth characteristics of Onobrychis sativa showed that the highest stem length and allometric coefficient were observed in the nanosilicate 1000 ppm and the highest germination percentage and germination velocity were observed in the nano-titanium 1000 ppm [13]. Higher values of Thymus kotschyanus seed germination traits were observed in priming with nano-silver 20% [14].

According to the problems of germination in some plants and also overgrazing the rate of forage production is greatly reduced [15]. Thus, to take the advantages of such plants, it is necessary to identify and remove barriers of germination and establishment of suitable plants [15]. *Festuca ovina* L. belongs to the geramineae family (Poaceae). It is a perennial plant, bunch grass and needle-shaped leaves (3-25cm), with 45-72cm tall. The root system such as a grid network is retained all small and large particles in the soil and it is very effective in erosion control, especially in steep areas. It is also palatable as an important source of forage is alpine meadows [16, 17]. Thus, according to the mentioned characteristics and protection and forage value of this species, can be used in the rehabilitation and development of many alpine meadows used as temporary and mixed cultivations. Also, the amount and uniformity of germination and seedling emergence improve. For these reasons priming treatments in normal conditions and under stress are used [18]. For this purpose, nanoparticles that applied less in range science were used. Whether these materials will have a positive impact on seed germination or not?

2- Objective

One of the simple techniques for increasing the seedling emergence rate, recovery power and seedling establishment, and therefore improves the efficiency of the plant in the field, is an adopted strategy as seed priming. Hence, this study was conducted to comprehend the role of silver nanoparticles as nano priming (That it is a new method of priming) in seed germination of *F. ovina*.

3- Materials and Methods

3-1- Study area: The present experiment was carried out using randomized complete block design with four replications [14], at the Rangeland laboratory of the Faculty of Agricultural and Natural Resources in the University of Mohaghegh Ardabili, Ardabil, Iran. Ardabil city is the capital of the Ardabil province in the northwestern of Iran.

3-2- Seed materials, priming and drought stress and experiment method: In this research, at first step to check the quality and germination percent of *F. ovina*, 25 intact seeds [19] were placed in petri dish under drought stress with Polyethylene Glycol (PEG); then, germination test was performed with the distilled water. After 14 days, it was found that the germination percent in the intact seeds was low (>40%). For this reason, the treatment of seed priming and different concentrations of nanoparticles were used to improve germination attributes. Treatments were; control (Distilled water), and silver nanoparticles with the concentrations of 25, 50 and 75% [20]. Drought stress was applied using polyethylene glycol 6000 in three levels of 0, -6 and -12 bar [21] (0, 218 and 316 grams of PEG per thousand ml of sterile distilled water, respectively [22]). Twenty-five seeds were put in each Petri dish. Seeds were collected from Sabalan mountain rangelands in Ardabil province. First, the seeds were deodorized with sodium hypochlorite 10% for 30 seconds, then were rinsed with distilled water [14]. Then the petri dishes were divided and the amount of 3ml of different solutions of PEG was added to the petri dish related to the same concentration. The petri dishes were transferred to the germinator with an average temperature of 25±1°C for 8/16 hours' darkness and light respectively. After the petri dishes were came out from the germinator, priming the seeds was performed. Seeds for the treatments of nanoparticles were placed in different nano-silver solutions for 24 hours, and after washing seeds with distilled water, they were sown on filter paper in petri dishes and were watered with 10ml of distilled water [24].

3-3- Seeds germination and studied characteristics: Seeds germination was beginning from the fourth day after cultivation and germinated seeds were counted on the same day and recorded. Exiting of 2mm root length was germination criterion [23]. Seed counting continued for 14 days [24]. On the last day, the shoot and root of seedlings were measured using a ruler. Germination percentage (GP), speed of germination (SG), mean germination time (MGT), root length (RL), shoots length (SL), wet (WW) and dry weight (DW), allometric coefficient (AC) and vigor index (Vi) were measured in this study. Seedling fresh and dry weight were recorded using a digital scale and other indices are presented in Table 1.

3-4- Statistical Analysis: General Linear Model (Multivariate tests) was used for statistical analysis and then Tukey test was used to examine the differences between treatments. Before carrying out the analysis, the normality and homogeneity of data were evaluated by Kolmogorov-Smirnov's and Leven's tests, respectively. Data were analyzed using SPSS 22 software.

Table 1) The method of calculating seed germination and seedling growth of *F. ovina*

Studied indices	Calculation formula	Unit of measurement	Used References
Germination Percent	$GP = \frac{Ni}{N} * 100$	%	[25]
Speed of Germination	$S = \frac{ni}{di}$	number/day	[25]
Mean Germination Time	$\text{MGT} = \frac{\sum \text{ni} * \text{di}}{\text{N}}$	day	[26]
Root Length	Ruler	cm	[27]
Shoot Length	Ruler	cm	[27]
Wet Weight	Precision scale	g	[28]
Dry Weight	Precision scale	g	[28]
Vigor index	$Vi = (Rl + Sl) * GP$	-	[29]
Allometric Coefficient	$Ac = \frac{Sl}{Rl}$	-	[30]

N: Total number of seeds and Ni: germinated seeds at the end of counting days, ni: germinated seeds per day and di: counting day

4- Findings

Data analysis of variance indicated that influence of nano priming, drought stress, and their interaction was significant on root and shoot length, wet and dry weight, vigor index, allometric coefficient and mean germination time ($p \leq 0.01$). Also, characters of germination percent and speed of germination significantly impressed by priming treatment and its interplay with drought stress ($p \leq 0.01$), while stress effect on these traits was not significant (Table 2).

Nano priming treatments did not have significant effects on root length, shoots length, wet and dry weight of *F. ovina* seeds. But these treatments improved the speed of germination, percentage of germination, mean germination time, allometric coefficient and vigor index (Table 3). According to this table data, priming in the drought level of 0 improved germinations of seeds and other studied indices compare with control and these by values have fallen by increasing drought levels. In all the selected parameters, silver nanoparticles with 75% concentration showed a higher value in comparison with the 25 and 50% concentrations. In fact, with increasing concentration of nanoparticles, studied Indices have enhanced. Maximum of GP (86 %), SG (6 N/D), Vi (5), AC (6) and MGT (7.08 d) were recorded for seeds nano primed in drought stress of 0 levels, followed by nano primed seeds with 50% concentration and control. The lowest value at all studied indicators belongs to drought stress of -12 bar. Although drought stress significantly increases MGT for both primed and control seeds, the primed seeds had significantly lower MGT in 0 bar level of stress compared to unprimed seeds (Table 3).

Table 2) Analysis of variance of the influences of nano priming and drought stress on the tested properties in *F. ovina*

Source of Variance	df	Mean of Square								
		Germination Percent (%)	Speed of Germination (N/D)	Root Length (cm)	Shoot Length (cm)	Wet Weight (g)	Dry Weight (g)	Vigor index	Allometric Coefficient	Mean Germination Time (d)
Drought Stress	2	280.00^{ns}	2.00^{ns}	0.00^{**}	0.00^{**}	0.01^{**}	0.01^{**}	0.00^{**}	4.00^{**}	0.00^{**}
Priming Treatment	3	7962.00^{**}	30.00^{**}	2.00^{**}	1.06^{**}	0.06^{**}	0.09^{**}	35.00^{**}	18.00^{**}	11.00^{**}
Stress * Treatment	6	75.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	4.00^{**}	1.00^{**}
Error	36	63.00	0.00	0.08	0.00	0.00	0.01	1.00	0.00	0.00

ns and **: at the 0.01 level of probability; non-significant and significant, respectively.

Table 3) Influence of nano priming treatments on seedling growth and germination of *F. ovina*

Mean Germination Time (day)	Allometric Coefficient	Vigor index	Dry Weight (gr)	Wet Weight (gr)	Shoot Length (cm)	Root Length (cm)	Speed of Germination (N/D)	Germination Percent (%)	drouth stress (bar)	nano priming concentrations (%)
$3^{d}\pm0.44$	$2^{d}\pm0.15$	$1^{d}\pm0.19$	$8^{a}\pm0.04$	$8^{a}\pm0.04$	$4^{a}\pm0.17$	$1^{ab}\pm0.16$	$\pm0.642^{d}$	$\pm1\ 39^{bc}$		Control
$4^{c}\pm1.28$	$5^{b}\pm0.43$	$1^{d}\pm0.14$	$8^{a}\pm0.05$	$8^{a}\pm0.11$	$4^{a}\pm0.25$	$1^{bcd}\pm0.12$	$2^{d}\pm0.70$	$24^{c}\pm6.80$	0	
$5^{b}\pm0.87$	$6^{a}\pm0.36$	$2^{c}\pm0.31$	$8^{a}\pm0.04$	$8^{a}\pm0.03$	$3^{a}\pm0.18$	$1^{bcd}\pm0.30$	$3^{cd}\pm0.36$	$40^{bc}\pm2.50$	-6	25
$5^{b}\pm0.66$	$3^{c}\pm0.54$	$1^{d}\pm0.21$	$8^{a}\pm0.01$	$8^{a}\pm0.00$	$3^{a}\pm0.45$	$1^{bcd}\pm0.16$	$2^{d}\pm0.16$	$23^{c}\pm4.32$	-12	
$6^{ab}\pm0.12$	$3^{c}\pm0.18$	$2^{c}\pm0.26$	$8^{a}\pm0.06$	$8^{a}\pm0.04$	$4^{a}\pm0.17$	$1^{cd}\pm0.05$	$4^{bcd}\pm0.30$	$46^{b}\pm2.58$	0	
$6^{ab}\pm0.21$	$2^{d}\pm0.11$	$3^{b}\pm0.16$	$8^{a}\pm0.03$	$8^{a}\pm0.03$	$3^{a}\pm0.12$	$1^{ab}\pm0.06$	$4^{abc}\pm0.22$	$46^{b}\pm2.58$	-6	50
$7^{a}\pm1.01$	$3^{c}\pm0.59$	$2^{c}\pm0.26$	$8^{a}\pm0.01$	$8^{a}\pm0.01$	$3^{a}\pm0.23$	$0^{d}\pm0.03$	$3^{cd}\pm0.24$	$43^{b}\pm3.65$	-12	
$7^{a}\pm0.25$	$3^{c}\pm0.24$	$5^{a}\pm0.22$	$8^{a}\pm0.05$	$8^{a}\pm0.05$	$4^{a}\pm0.07$	$1^{abc}\pm0.10$	$5^{ab}\pm0.34$	$84^{ab}\pm4.63$	0	
$6^{ab}\pm0.65$	$4^{bc}\pm0.22$	$4^{a}\pm0.23$	$8^{a}\pm0.03$	$8^{a}\pm0.02$	$4^{a}\pm0.17$	$1^{ab}\pm0.05$	$6^{a}\pm0.11$	$86^{a}\pm3.65$	-6	75
$5^{b}\pm0.38$	$3^{c}\pm0.14$	$3^{b}\pm0.34$	$8^{a}\pm0.11$	$8^{a}\pm0.01$	$3^{a}\pm0.21$	$0^{cd}\pm0.03$	$5^{ab}\pm0.21$	$76^{ab}\pm1.54$	-12	

Numbers with different letters in each column show significant difference ($p< 0.05$) and the same letters show not significant difference.

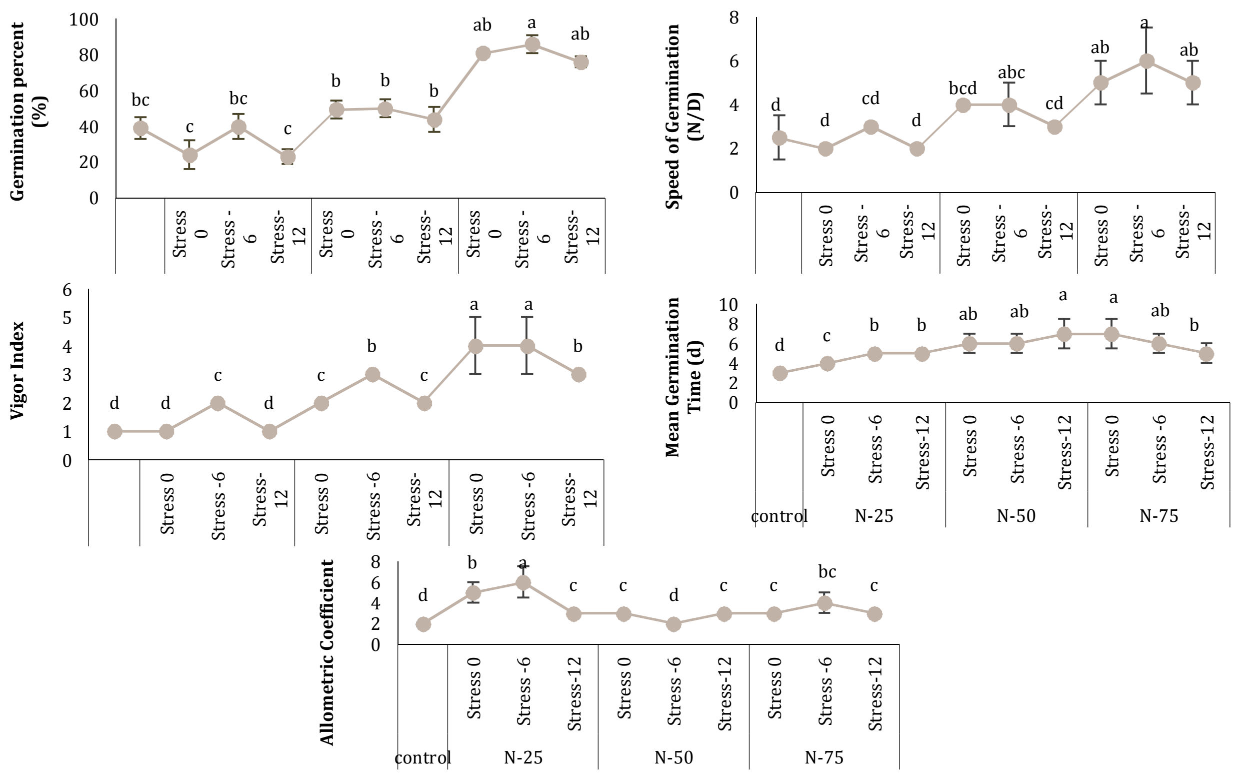

Diagram 1) Change trend in percent of germination, germination speed, mean time of germination, vigor index and allometric coefficient under drought stress and effect of nano-silver concentrations and different letters in show significant difference

In most treatments with increased levels of PEG stress, we have seen a decline in values of seed indices, while with the rise of nano-silver concentration, seed germination traits had an increasing trend (Diagram 1).

5- Discussion

5-1- Percentage and speed of germination: In this study, seed priming in treatments without drought stress have been displayed as a prosperous strategy for the *F. ovina* seed germination improve. The positive effect of seed priming with silver nanoparticles was found with a higher percentage of germination and germination speed in primed seeds as compared to non-primed seeds. Drought stress affects germination by limiting water absorption by seed, by transferring seed reserves or by directly influencing on organic structure and protein synthesis of embryo. A similar result was shown in the use of silver nanoparticles significantly improved germination of Fenugreek seed, percent of germination, mean time of germination, seed germination index, vigor index, fresh and dry weight of seedling due to nanoparticles is related to their penetration into seeds [12]. Sorghum seed priming treatment with nano-iron oxide showed an increase of germination, seeding growth and salinity tolerance at 500mg/L n-Fe2O3 [20].

5-2- Mean germination time: Seed priming in this research, in treatments without drought stress had the highest effect on the mean time of germination factor of *F. ovina* seeds and reduced it. Seed priming of *F. ovina* with a NaCl solution with EC 45 dS/m decreased mean germination time [31]. Nano silver solution showed better function in improving germination than a fungicide. AgNPs even in the greatest concentration did not disturb the seed germination and growth of *Ricinus communis* [32]. The great effects of N-Si to improve stress on tomato seed germination and MGT had low reduction under NaCl by N- Si application in their study [21].

5-3- Vigor index: *F. ovina* seeds vigor index decreased with increasing stress level. The high concentration of PEG solution prevents water absorption and free radical production of oxygen, and damage to the cell membrane and changes in enzyme activity and ultimately reduces seed germination and emergence of seedlings [33]. Vigor and germination index in hydro primed seeds under osmotic potentials were more than haloprimed or untreated seeds [34]. Moreover, SiO2 nanoparticles in tomato seed germination (*Lycopersicum esculentum*) and reported that usage of SiO2 nanoparticles with 8 ppm level to be the best treatment to increase seed germination and vigor index [35]. Also, seed priming with GA3 has shown better effect in improving all the growth characters and bulb vigor in primed seed lots over the control in Onion [36].

5-4- Allometric Coefficient: As shown in results, an increase in nano-silver concentration markedly improved the allometric coefficient of *F. ovina*. Water uptake by seed in stress reduces the secretion of hormones and enzymes activity and as a result of disruption to seedling growth (including shoot length). The small particle size of n-Fe2O3 may lead to a higher bioavailability of iron to seeds and can increase the motion and uptake of water, nutrients, and oxygen via pores in the seed coat [20]. In the study of priming on germination indicators of Ajowan seed (*Carum copticum* L.*)*, polyethylene glycol -4 bar reduced significantly allometric coefficient compared to the control treatment that due to the reduction in the shoot length [37].

6- Conclusion

In this research, PEG stress had a negative effect on seed germination. This is probably due to limited available oxygen in germinating seeds by polyethylene glycol. Increase of *F. ovina* seed germination using nanoparticles probably due to extremely small dimensions of this materials and subsequent quickly and easily influence of these substances within the seed. In conclusion, seed nano-priming is an impressive technique to the betterment of seedlings growth and seed germination of *F. ovina*., and in the most studied indices, nano-priming with a concentration of 75% had the greatest influence.

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Ethical permissions: This study was conducted at the rangeland laboratory of the faculty of agricultural and natural resources in the University of Mohaghegh Ardabili as the first author Ph.D seminar. The university was allowed to carry out experiments related to this study in a laboratory in the form of a seminar.

Conflict of Interest: The authors have no conflict of interest.

Authors' Contribution: Abbasi Khalaki M. (First author), Methodologist/Original researcher/ Statistical analyst (50%); Ghorbani A. (Second author), Introduction author/ Discussion author (30%); Dadjou F. (Third author), Assistant researcher (20%)

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