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Determination of Different Priming Applications on *Vicia freyniana* Bronm. Seeds Viability Parametres

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ABSTRACT

Background: Legumes are the second among all the families in terms of varieties and *Vicia* genus is the third genus within the Leguminosae family. The germination and emergence rates decrease due to some reasons such as non-homogeneous genetic structure of seed or effect of environmental factors on it. For this reason, some quality problems occur. Quality enhancing applications are performed for seeds to have the desired quality levels in seeds and eliminate the problems. The aim of the present study was to determine the viability of *Vicia freyniana* Bornm. seeds by different priming treatments. For this purpose, the effect of different chemicals in different doses and periods and at what rate the hot water treatment would affect testa dormancy were studied.

Methods: This study was conducted in Uşak University Agriculture and Natural Sciences Faculty Research laboratory in 2019. An investigation was conducted to determine the effects of the priming treatments (Soaking and imbibation; 2, 4 and 6% doses of NaCl, $CaCl_2$ and KNO_3 salts and 75, 100 *and* 125 ppm doses of GA_3) on seeds of *Vicia freyniana* Bornm. In the study, the germination rate (%), germination speed (day), germination index (day) and EC values (µS/cm g^{-1}) of the *V. freyniana* seeds were determined.

Result: The highest germination rates were obtained from 2% of $CaCl_2$ and 75 ppm of GA_3 treatments and the fastest germination was observed in KNO_3 treatment. From the study, it is concluded that 2% dose of $CaCl_2$ and 75 ppm dose of GA_3 should be preferred in *V. freyniana* seeds and the impregnation technique should be preferred among priming methods due to its high germination performance.

Key words: Germination potential, Germination index, Halopriming, *Vicia freyniana* Bornm.

INTRODUCTION

Turkey is among the richest countries of the world in terms of plant gene pools. Legumes are the second among all the families in terms of varieties and *Vicia* genus is the third genus within the Leguminosae family. There are about 150 species of *Vicia* genus in the world. Totally 59 species, 8 of which are endemic, are included in *Vicia* genus in Turkey. *Vicia freyniana* Bornm. isone of the rare perennial species included in *Vicia* genus. It is a legume endemic to Turkey and it has been identified naturally in a quite wide area in our country (Yüksel and Özçelik, 2015). Alfaalfa species, trefoil and other legumes have impervious testa, gas impermeable testa is included in wild oat grass, coffee, common ash and some pine species. Both hard and soft lines of *Vicia sativa* have similar main testa characteristics; especially both have thickened secondary epidermal walls and layered cell cavities. It has been reported that hilum and micropyle are important structures for water sorption (Rangaswamy and Nandakumar, 1985).

One of the factors preventing germination of seed is the hard seediness that forms due to the chemical change of the layer on the surface of testa and cell wall contacting with air here and forms due to the characteristic of cuticula layer. In the formation of hard testa, the development of seed is as important as the environmental factors effective in ripening period and the post-harvest storage conditions. This impermeability in testa (testa dormancy) may change due to the increase of the proportional humidity and the decrease of ambient temperature (Dübbern De Souza and Marcos-Filho, 2001).

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Also, the germination and emergence rates decrease due to some reasons such as non-homogeneous genetic structure of seed or effect of environmental factors on it. For this reason, some quality problems occur. Quality enhancing applications are performed for seeds to have the desired quality levels in seeds and eliminate the problems. In plant production, firstly seeds should be qualified and then the yield obtained per unit area should be increased in quality cultivation and production (Sivritepe and Şentürk, 2011). Some preliminary pre-cultivation applications are performed before in order for seed to have rapid germination and emergence. Among seed pre-treatment techniques; treating seeds with hard matrices is called as matrix priming;

hydration process with biological compounds is called as biopriming; soaking of seeds into inorganic salt solution is called as halopriming; soaking of seeds into water is called as hydropriming; and soaking of seeds into different organic/ inorganic osmotic solutions is called as osmopriming (Ashraf and Foolad, 2005). By these treatments, metabolites increasing germination are formed with metabolic repair during water intake in seeds whose germination is promoted (Basra *et al*., 2005) so that osmotic balance is set, the time for water intake is decreased and therefore the post-treatment drying time is shortened (Bradford, 1986). In osmotic priming technique, the humidity scope of seeds is increased in liquids the osmotic potential of which are adjusted (Growth-regulating substances, inorganic salts and polyethylene glycol, mannitol, abscisic acid) and kept for a long time without being germinated (Sivritepe, 1999). In addition to these quality enhancing treatments, harvest, drying-processing and storage, seed conditioning and coating treatments may be mentioned. Treatments of seed coating withpriming are the most important seed enhancing treatments (Khiabani, 2014). Apart from these general purposes, it has been determined that priming treatments performed with NaCl increase resistance against salt in sugar beet (Shannon and Grieve, 1999) and corn (Bakht *et al*., 2011). The seeds applied in osmotic conditions germinate in alternative temperatures (Coskun *et al*. 2016) and they resist against extreme conditions (Corbineau and Come, 1990). Priming treatments can be performed to increase the viability and vigor of seeds, it is also important to use several solutions including K^{+2} and Ca^{+2} minerals which has critical importance in terms of plant physiology (Rubio *et al*., 2003). In general, Ca^{+2} is effective in the cell formation and lipid structure formation within plant. On the other hand, K^{+2} has important roles in respiration, photosynthesis, chlorophyll formation, water content of leaves and many other physiological processes in body of plant. However, the most important characteristic of K^{+2} is that it is an activator of the enzymes taking part in formation of peptide bond (Devlin, 1975). Gibberellins have a role in stimulating the enzymes taking part in seed germination phase. Also, in the following phase of germination, gibberellins are transferred to endosperm from embryo and stimulate α-amylase enzyme and have a role in the conversion of starch into sugar for necessary energy (Karakurt *et al*., 2010). Gibberellins are used in general to eliminate the dormancy observed in different periods and parts of plant life and increase the germination rate of seed. Gibberellins are directly applied in seed so that germination is increased. They convert carbohydrates into sugar, promote growth by increasing the plastids in cell wall and decrease the pressure in cell wall. For this reason, as water is taken in cell, cell elongation occurs (Arteca, 1996). It increases the germination rate by reducing dormancy in the seeds of some wild plants.

The aim of the present study was to determine the viability of *Vicia freyniana* Bornm. seeds by different priming treatments. For this purpose, the effect of different chemicals in different doses and periods and at what rate the hot water treatment would affect testa dormancy were studied.

MATERIALS AND METHODS

The study was conducted in Uşak University Agriculture and Natural Sciences Faculty Research laboratory in 2019. Three years old seed lot of *Vicia freyniana* Bornm. species collected in Isparta strikethrough district Fele village was used as plant material. The seeds were kept in refrigerator (4°C) until the tests were performed.

Determination of moisture

For seed moisture, the gravimetric method of International Seed Testing Association (ISTA) was used. Accordingly, 1 gr seed samples with two repetitions were weighed, their initial weights were recorded and all the seed lots were dried at 130°C for 1 hour (High temperature oven method). The seed samples taken out of drying furnaces were kept in a desiccator for about 30 minutes and their final weights were determined. The initial and final weights and the initial moisture amounts (%) were determined.

Moisture amount (%) = ISW- FSW / ISW x 100.
ISW: Initial seed weight.
FSW: Final seed weight.

Priming treatments

1st Halopriming treatment

3-layered blotter was placed in each of 9-cm PE Petri dishes. The seeds were wetted with distilled water (control) and by putting 3 ml of each of 2-4-6% NaCl, $CaCl_2$, KNO_3 and GA_3 solution in 75-100-125 ppm dose and kept at ambient temperature for 1 day.

2nd Halopriming treatment

Seeds were subjected to an application of imbibationin 2% $CaCl_2$, $CaSO_4$ and NaCl solutions (slow water uptake between blotter) and soaking (fast water uptake between blotter) atroom temperature.

Germination test

It continued at 25 (1st halopriming, 2nd halopriming) and 5°C (2nd halopriming) for 10 days between blotter in polyethylene petri dishes after priming treatments. The untreated seeds were not performed were evaluated as control. At the end of the test, the mean germination rate (MGR), mean germination time and germination index (day) (MGT, GI) were determined (ISTA, 1993).

$$MGT = \sum D\,n / \sum n$$

MGT: mean germination time (day).
D: The days as of the start of the test.
n: Number the seeds germinated in D day.

The treatment-related performance changes in *vicia* seeds used in the tests were compared using germination index (GI) formula (Copeland and McDonald, 2001).

$$GI = \sum n / d$$

n: number of normal seedlings obtained on d day.
d: The days from the start of the test.

EC measurement

For the measurement of electrical conductivity of the substances leaking out of seed, the seeds dried until reaching their initial moisture content were subjected to electrical conductivity tests in accordance with ISTA Rules (ISTA, 2012). The measurement was performed by keeping 4x25 seeds in 40 ml of distilled water at 20°C for 1 day.

The data obtained in the study were subjected to analysis of variance in accordance with the randomized parcel experimental designs in JMP 10.0.0 program. Least significant difference tests were used to compare the mean values found to be statistically significant as a result of the analysis of variance (LSD, 0.05% and 0.01%).

RESULTS AND DISCUSSION

It was found that the average moisture contents of *Vicia freyniana* Bornm. seeds were 5.6% and the EC value was 10.56 µS/cm. In the comparison of germination indices, the earliest emergence was provided in 6% NaCl among the different salts applied. The different doses of GA_3 did not affect the germination time differently and statistically non significant (Table 1). Gibberallin applications to seeds also promotes the production of hydrolase enzymes (α-amylase) amounts (Taiz and Zeiger, 1991). Watkinson and Pill (1998) result's support that germination increased with GA_3 application in naturally aged *Sorghastrum nutans* seeds. Table 2 shows the variance analysis results of different germination promoting chemical substances and their doses for mean germination rates and times of *Vicia freyniana* Bornm. seeds. Accordingly, the effect of the chemical applications on MGT caused statistically significant differences at the level of 0.05 and doses caused statistically significant differences at the level of 0.01. The effect of the chemical substance treatments on MGT caused statistically significant differences at the 0.01% level and treatment x dose interaction caused statistically significant differences at the 0.05% level (Table 2). The MGR of *V. freyniana* seeds in the different chemical substances and doses, researched in the study are present in Table 3 and the values of MGT are shown in Table 4. As seen in Table 3, the MGR of *V. freyniana* seeds varied between 16.25% and 23.33% in terms of the treatments. The highest germination rate was observed in $CaCl_2$ and GA_3 treatments, respectively. The highest MGR were determined to be 2% in salts and in 75 ppm dose in GA_3. It was carried out to determine the effect of applying GA_3 to *Dianthus barbatus* L. seeds prior to germination on seed germination in saline conditions. According tor he results, while the highest germination rate of seeds was found in watered with distilled water applied 40 ppm GA_3 pretreatment, the lowest germination rate was determined on seeds pre-treated with distilled water and watered with 100 mM NaCl (Yıldız, 2017).

Table 1: The moisture contents, electrical conductivity (EC, µS/cm) and germination index (GI, day) results of *Vicia freyniana* Bornm. control group seeds.

Germination temperature (°C)	Treatments	GI	EC	Moisture content (%)
	Control	0.9		
	%2 NaCl	1.2		
	%4 NaCl	1.1		
	%6 NaCl	**0.8**		
	%2 $CaCl_2$	1.4		
25°C	%4 $CaCl_2$	1.5	10.56	%5.6
	%6 $CaCl_2$	**1.2**		
	%2 KNO_3	1.5		
	%4 KNO_3	0.8		
	%6 KNO_3	**0.6**		
	75ppm GA_3	**1.1**		
	100ppm GA_3	**1.1**		
	125ppm GA_3	1.3		

Table 2: The results of variance analysis of different treatments and their doses for the MGT and rates of *V. freyniana* seeds.

Variation sources	SD	Mean Squares	
		MGR	MGT
Treatments (T)	3	109.72*	5.94**
Doses (D)	3	472.22**	0.68
T x D	9	50.46	0.97*
Fault	32	25.52	0.44
General	48		
Coefficient Variation (%)		26.35	20.37

*: P≤0.05, **: P≤0.01 indicates significant differences.

Table 3: The mean germination rates of *Vicia freyniana* Bornm. seeds as a result of different halopriming (1st) treatments (%).

Treatments	Doses				
	Control (%0)	%2	%4	%6	Mean
NaCl	11.67	21.67	20.00	18.33	17.92 B
$CaCl_2$	11.67	31.67	30.00	20.00	23.33 A
KNO_3	11.67	28.33	15.00	10.00	16.25 B
	Control (%0)	**75ppm**	**100pmm**	**125ppm**	**Mean**
GA_3	11.67	25.00	18.33	21.67	19.17 AB
Mean	11.67 C	26.67 A	20.83 B	17.50 B	

$LSD_{U(0.05)}$: 4.20, $LSD_{D(0.05)}$: 4.20.

Özden and Demir (2016a) investigated the germination rates in *Solanum torvum* seeds with hot and cold stratification at constant and variable temperatures with GA_3 combinations at different doses. As a result of the study, seed germination did not occur after hot and cold stratification at constant temperatures and the highest germination rate was determined in 15°C-7 day / 30°C-7 day and 1000 ppm GA_3 combinations. In the study conducted by Duclos *et al*., (2014) in 5 different switch grass species, they germinated seeds under different constant (15°C and 3°C-28 days) and variable temperatures and periods to determine the optimum germination temperatures at first. The average highest germination rates among the treatments at variable temperatures for 5 species were obtained to be 65-54% and the highest germination rate at constant temperatures was determined to be 7% in 28-day treatment at 30°. Also, the seeds were inhibited in ABA and GA_3 concentrations at constant (30°C) and variable (30/15°C- 8/16 hours) temperatures and in different doses. The effect of the temperature-hormone and ABA/ GA_3 rate on seed germination was explained with ABA and GA_3 treated at this stage. The increase provided by GA_3 dose on germination was not so effective asvariable temperatures. It was determined that ABA decreased germination in the dormant seeds in all varieties and this decrease was affected by temperature conditions.

The germination rate of the seeds treated (1st halopriming) with different salt solutions in different concentrations increased approximately by 6-20% compared to the control group (except for 6% KNO_3 treatment). The most effective dose on germination performance was found to be 2% and $CaCl_2$ was the prominent salt type (Table 3). When the chemicals included in the test were compared in terms of MGT, the highest germination rates were obtained to be 2.32 days on an average in KNO_3 treatment. The doses did not have a statistically significant effect on the MGT and the germination times varied between 1.60 days and 4.50 days in chemical x dose interaction (Table 4).

With halopriming (1st) applied in different salts and concentrations, the earliness was provided in the germination rate mostly in 2% dose compared to the control group and in seeds treated with KNO_3 and NaCl % 4 among salt types (Table 4). The study was carried out to examine the effects of NaCl primings of perennial reygrass seeds on germination characters of seeds exposed to different NaCl concentrations. As a result, NaCl priming of 15 dS m-1 level may be proposed for perennial ryegrass seeds to increase seedling growth during germination stage under medium salt levels (Tatar, 2018).

Şentürk and Sivritepe (2015) investigated the effect of the priming treatments with NaCl in different dose and periods applied to increase the tolerances of the germination and seedling growth of Dual and Spring pea seeds against salt. As a result of the priming treatments, it was determined that 2-day treatment with 150 mM at 16°C which did not differ from the control group in terms of the normal germination rate, MGT, GI and EC parameters of pea seeds was the most appropriate for the priming applied in pea seeds. As a result, the germination and emergence rates of the KNO_3-treated seeds were found to be statistically higher compared to the control seeds. This effect reached the maximum in KNO_3 treatment after priming and it was stated

Table 4: The results of mean germination rate of *Vicia freyniana* Bornm. seeds as a result of different halopriming (1st) treatments (day).

Treatments	Doses				
	Control	%2	%4	%6	Mean
NaCl	3.50 a-c	3.00 c-e	4.02 a-c	3.28 bc	3.45 B
$CaCl_2$	3.53 a-c	4.27 ab	3.74 a-c	4.50 a	4.01 A
KNO_3	3.57 a-c	1.60 f	2.11 d-f	2.00 ef	2.32 C
	Control (% 0)	75 ppm	100 pmm	125 ppm	Mean
GA_3	3.57 a-c	2.98 c-e	3.05 c-e	3.20 b-d	3.20 B
Mean	3.54	2.96	3.23	3.24	

$LSD_{U(0.05)}$: 0.55, $LSD_{U \times D(0.05)}$: 1.10.

Table 5: The results of the analysis of variance on priming method and chemical treatments.

Variation sources	SD	Mean Squares			
		5°C		25°C	
		MGR	MGT	MGR	MGT
Priming Method (PM)	1	272.03*	417.62**	113.62	2.98
Treatment (T)	3	274.01**	159.28**	28.79	6.93
PM × T	3	58.29	168.04**	129.36*	3.20
Fault	16	35.42	18.67	33.26	2.30
General	23				
Coefficint Variation (%)		45.21	26.18	16.25	15.04

*: P≤0.05, **: P≤0.01 indicates significant differences.

that the priming treatments had the fastest germination time. In the treatment performed with KNO_3 solution with 1% and 2% concentrations in grass seeds, increases were determined in the germination speeds and rates of the seeds, which were dried up to their initial weights, when they were planted on the ground (Duman *et al*., 2012).

Table 5 shows the results of the analysis of variance performed with the data obtained the second halopriming treatment included in the study. Accordingly, priming method caused significant differences on the MGR at the level of 0.05 and on the MGT at the level of 0.01 in the germination tests performed at 5°C. The chemicals applied caused statistically significant differences on both MGR and MGT at the level of 0.01. In the tests performed at 25°C, priming method x treatment interaction was found to be significant at the level of 0.05. In MGR results, at 5°C were examined after halopriming (2nd) treatment applied to *Vicia freyniana* Bornm. seeds as soaking and imbibation, the salt solution applied at low temperature did not have a positive effect on germination performance compared to the control. However, in the comparison of the halopriming methods, the slow water intake of testa at 5°C with imbibation method was more effective compared to soaking at radicle emergence (Table 6).

When the mean germination speeds at 5°C were compared after the halopriming (2nd) treatment applied to *Vicia freyniana* Bornm. seeds as immersion and impregnation, radicle emergence happened faster with soaking method compared to halopriming methods (Table 6). Although it varied based on the method among the salt solutions, the earliest germination was observed in the seed group treated by $CaCl_2$ (Table 7). Results of MGR at 25°C were examined after the halopriming (2nd) treatment applied as soakingand imbibation, no increase was determined in the germination performance with soaking method compared to the control and more germination of approximately 7% (except for $CaCl_2$) was observed with imbibation. When the mean values were examined, no significant difference was found among the salt solutions in terms of the effect on germination compared to the control (Table 8). Tilaki *et al.* (2010) reported that the increase in germination and vigor index after NaCl pretreatment may result from an increase in cell division.

When the results of mean germination speed at 25°C after the halopriming (2nd) treatment applied as soaking and imbibation were examined, there was no significant difference between soaking and imbibation methods and germination was late compared to the control (Table 9). Three different priming treatments containing PEG, KNO_3 and KH_2PO_4 in different concentrations were applied to newly harvested cockscomb seeds for one week and substances such as MeJA, ACC and BAP were added. As a result, the highest germination rates were obtained by adding growth regulating substances into PEG, KNO_3 and KH_2PO_4 solutions (Tiryaki *et al*., 2005). The variety of priming methods was found to be statistically significant at low temperature (5°C) in terms of mean germination rate and time (Table 10).

According to the results obtained from halopriming (1st)

Table 6: The mean germination rate results of *Vicia freyniana* Bornm. seeds at 5°C after halopriming (2nd) applied as soaking and imbibation (%).

Treatments	Priming Method		
	Soaking	Imbibation	Mean
%2 NaCl	2.67	5.33	4.00 B+
%2 $CaCl_2$	4.44	6.67	3.33 B
%2 $CaSO_4$	5.33	5.33	5.33 B
Control	13.33	18.67	16.00 A
Mean	5.33 B+	9.00 A	

$LSD_{PY(0.05)}$: 5.15, $LSD_{U(0.05)}$: 7.28.

*: The data subjected to arcsine transformation were used in the analysis of variance and the values in the table are the actual values.

+: The differences between the mean values indicated with similar capital letters in the same row and column are insignificant.

Table 7: The mean germination speed results of *Vicia freyniana* Bornm. seeds at 5°C after halopriming (2nd) treatment applied as soaking and imbibation (day).

Treatments	Priming method		
	Soaking	Imbibation	Mean
%2 NaCl	4.17 bx	12.83 a	8.50 B
%2 $CaCl_2$	8.88 b	13.17 a	6.58 B
%2 $CaSO_4$	13.00 a	12.67 a	12.83 A
Control	12.43 a	12.87a	12.65 A
Mean	7.40 B	12.88 A	

$LSD_{PY(0.05)}$: 3.73, $LSD_{U(0.05)}$: 5.29, $LSD_{PY\ x\ U(0.05)}$: 7.48.

x: + : The differences between the mean values indicated with the same lower case letter are insignificant.

Table 8: The mean germination rates of *Vicia freyniana* Bornm. seeds at 25°C after halopriming applied as soaking and imbibation (%).

Treatments	Priming Method		
	Soaking	Imbibation	Mean
%2 NaCl	30.67 bcx	36.00 ac	33.33
%2 $CaCl_2$	41.33 ab	21.33 c	31.33
%2 $CaSO_4$	29.33 bc	36.00 ac	32.67
Control	49.33 a	29.33 bc	39.33
Mean	37.67	30.66	

$LSD_{PY\ x\ U(0.05)}$: 9.98.

Table 9: The mean germination speed results of *Vicia freyniana* Bornm. seeds at 25°C after halopriming applied as soaking and imbibation (day).

Treatments	Priming Method		
	Soaking	Imbibation	Mean
%2 NaCl	3.00	3.73	3.37
%2 $CaCl_2$	3.60	3.20	3.40
%2 $CaSO_4$	3.90	3.23	3.57
Control	2.82	1.70	2.26
Mean	3.33	2.97	

*: P≤0.05, **: P≤0.01 indicates significant differences.

Table 10: The results of the analysis of variance on priming treatments and methods.

Variation sources	SD	Mean Squares			
		5°C		25°C	
		MGR	MGT	MGR	MGT
Priming Method (PM)	1	272.03*	417.62**	113.62	2.98
Treatment (T)	3	274.01**	159.28**	28.79	6.93
PM x T	3	58.29	168.04**	129.36*	3.20
Fault	16	35.42	18.67	33.26	2.30
General	23				
Coefficient variation (%)		45.21	26.18	16.25	15.04

treatment, $CaCl_2$ and GA_3 treatments in terms of applied chemicals gave the the highest germination rates and these values were found to be very significantly higher compared to the control. In terms of dosages, the highest values were obtained at 2 ppm GA_3 and 75 ppm for salt applications. In terms of MGT, KNO_3 treatment allowed the germination time of seeds to decrease. According to the results obtained in halopriming (2nd) treatment, the germination rates determined in the tests performed at 5°C was quite lower compared to the tests performed at 25°C and increasing germination temperature allowed the germination period to shorten. The imbibation technique, which is one of priming techniques, was found to have more successful results at low temperatures compared to soaking technique.

CONCLUSION

When the results obtained in the study were examined together, it was concluded that 2% dose of $CaCl_2$, 75 ppm dose of GA_3 and the imbibation technique from priming methods should be preferred in *Vicia freyniana* seeds should be preferred due to its high germination performance.

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