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EVALUATION OF SEVERAL METHODS FOR BREAKING DORMANCY OF BITTER VETCH SEEDS (*VICIA ERVILIA* L.)

ABSTRACT

This study analysed the effects of different treatments on breaking dormancy and germination of bitter vetch (*Vicia ervilia* L.) freshly harvested seeds for seeding immediately. Partial scarified seeds (30 seconds with sandpaper) were subjected to different treatments including: GA3 (250, 500 and 750 ppm) ,KNO3 (0.1, 0.2 and 0.3% w/v), cold stratification (2, 4 and 6 days), sulfuric acid (25, 50 and 75 seconds), hot water (90°C; for 2.5 and 5 minutes), hydropriming via seed soaking in distilled water (4 and 8 hours) and mechanical scarification. Among the mentioned treatments, cold stratification for 6 days had a best effect on germination related parameters final germination percentage, mean germination time and vigour index than the other periods. In contrast to scarification with acid and hot water, mechanical scarification improved germination parameters but this effect was lower than the cold stratification. The results suggest that bitter vetch seed has both physical and physiological dormancy.

Key words: bitter vetch, seed dormancy, germination

INTRODUCTION

Vetch species are important forage legumes in the Mediterranean, West Asia, and North Africa regions (Turk, 1999). Bitter vetch (*Vicia ervilia* L.) is adapted to cold and low annual rainfall regions (Samarah *et al.,* 2003). This species can be used for early grazing, straw production, and as a seed crop (Mohamed, 1997; Samarah *et al.,* 2003). Pod shattering has been a major constraint to seed production of bitter vetch (Samarah *et al*., 2003). Delayed seed harvest may increase seed losses due to pod shattering whereas early harvest may influence

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seed germination and dormancy (Samarah *et al.,* 2003). Seed dormancy at harvest may also be influenced by seed desiccation and pre-chilling after harvest. The seed dormancy must be broken to induce germination immediately after harvesting. Various methods depending on the plant species and type of dormancy have been suggested (Koyuncu, 2005). Seeds with physical dormancy remain dormant until some factors render the covering layers permeable to water (Baskin *et al*., 2000). In morpho-physiological dormancy, seeds must be exposed to cold, heat, gibberellic acid (GA3) or chemicals for breaking the dormancy (Otroshy *et al.,* 2009). Some types of seeds with combined dormancy characteristics require two types of treatments for good germination such as cold stratification plus GA3. Incubation of seeds in moist conditions to release dormancy, usually in cold to simulate overwintering is known as stratification (Finkelstein *et al.,* 2008). The effect of GA3 as a germination stimulator is hypothesized to increase with stratification treatment (Yamauchi *et al*., 2004). Stratification also plays an important role in providing the stimulus required to overcome the dormancy. Stratification has been reported to induce an increase in GA3 concentration (Yamauchi *et al*., 2004). Many nitrogen-containing compounds, including NO gas, nitrite (NO_2^-), nitrate (NO_3^-), nitrogen dioxide, ammonium, azide, and cyanide, promote dormancy release and seed germination in many species, possibly as a means of sensing soil N availability (Bethke *et al.,* 2007). Potassium nitrate (KNO_3) is well documented as a compound, which increases the germination of photo-dormant seeds (Shanmugavalli *et al.,* 2007). Many gardeners choose potassium nitrate to break seed dormancy and increase the health of plants. Gibberellins are active as plant hormones and known to stimulate seed germination in a wide range of plant species; the predominant active GA depends on the species (Thomas *et al.,* 2005). Gibberellins stimulate germination by inducing hydrolytic enzymes that weaken the barrier tissues such as the endosperm or seed coat, inducing mobilization of seed storage reserves, and stimulating expansion of the embryo (Bewley and Black, 1994). Aliero (2004) reported that use of hot water, sulfuric acid and sandpaper scarification affected *Parkia biglobosa* seed dormancy-breaking. Farashah *et al.* (2011) noted that enhanced seed germination of *Origanum vulgare* due to seed coat scarification emphasizes that an impermeable covering layer restricts seed germination. It was also detected that scarification stimulates germination, but seed coat can not be the only factor in seed dormancy of this plant. Little information exists about the effect of various treatments on breaking dormancy of bitter vetch seed. Therefore, the objective of this study was to devise an effective method for germination improvement of bitter vetch.

MATERIALS AND METHODS

Site description, plant material and measured traits

This study was done in 2014 at the Department of Agronomy and Plant Breeding, Faculty of Agriculture, Bu-Ali Sina University, Islamic Republic of Iran. Bitter vetch seeds were received from Hamedan province in the west of Iran on July 2014. Measured seed germination parameters were: final germination percentage, mean germination time, vigour index and abnormal seedling.

Seed treatments

All of seeds (control group and treatments) partial scarified with sandpaper for 30 seconds. Details of various treatments applied to break the seed dormancy and improve germination of bitter vetch are presented in Table 1.

Table 1

Details of used treatments to break dormancy of *Vicia ervilia* seed

Treatments	Concentration/ Duration	Method	Remarks
Cold stratification	2, 4 , 6 days	Seeds placed between wet double layered papers at 5 ± 1 °C	
Acid scarification 98 % (v/v)	25, 50, 75 seconds	Using concentrated sulfuric acid (98 % v/v)	Washed with distilled water thoroughly
Scarification by hot water	2.5 , 5 minutes	Using distilled hot water in 90 °C	
Mechanical scarification	Until seeds were scarified	Using sandpaper	
Potassium nitrate (KNO3 w/v)	0.1, 0.2, 0.3% concentration	Seeds soaked for 48 hours at 20 °C	Washed with distilled water thoroughly
Gibberellic acid	250, 500, 750 ppm	Seeds soaked for 48 hours at 20 °C	Washed with distilled water thoroughly
Hydropriming	Distilled water at pH=7	Seeds soaked for 4 and 8 hours at 20°C	

Germination tests

Before keeping the seeds for germination, the seeds were surface-sterilized by soaking in 1 % sodium hypochlorite (NaOCl) for 3 minutes and washed thoroughly with sterilized water. After performing the dormancy breaking treat-

ments, seeds were germinated between Wattman double layered papers (ISTA, 1996) with 15 ml of water in petri dishes (15 cm diameter). These petri dishes contained seeds were placed into plastic bags for maintaining the moisture level. Seeds were allowed to germinate at 20 ± 1 ◦C in the dark condition for 8 days (ISTA, 1996). Germination was considered to have occurred when the radicles were 2 mm long. Germination percentage was recorded every 24 h for 8 days. Mean germination time (MGT) was calculated by following equation (Schelin *et al*., 2003).

$$MGT = \frac{\sum fi \times ni}{N}$$

where *fi*: day during germination period (between 0 and 8 day), ni: number of germinated seeds per day, N: sum of germinated seeds. The seed vigor index (VI) was calculated as following (Rahnama-Ghahfarokhi and Tavakkol-Afshari, 2007).

$$VI = \frac{L_S \times P_g}{100}$$

where *Ls* is the mean of seedling length (mm) and *Pg* is germination percentage.

Statistical analysis

The experiment was laid out in a completely randomized design. Four replications and 100 seeds per replicate were used. Data for germination and abnormal germination percentage were subjected to arcsine transformation before analysis of variance. Statistical analysis was carried out using SAS 9.1 program. Mean comparison was performed with Duncan's test at the 5 % level of probability.

RESULTS

Seed germination parameters increased when seeds imposed to cold stratification, GA3 and mechanical scarification treatments (Table 2, Fig. 1).

Final germination percentage

Germination of bitter vetch was improved significantly in all the cold stratification treatments than the other treatments. The final germination percentage reached to 74.06 % in cold stratification treatment for 6 days (Table 2). Followed by the cold stratification treatment, better performance was also observed in GA3 treatment with 750 ppm and mechanical scarification. The rest of the

treatments were not effective to improve the seed germination percentage of bitter vetch seeds.

Table 2

Effects of treatments on bitter vetch seed germination parameters, Data that do not share the same letters differ significantly at $P < 0.05$ level

Dormancy breaking treatments	FGP [%]	MGT [seed/day]	VI	Abnormal germination [%]
Control	7.00 e,f	41.38 g,h	2.66 g	0 a
Mechanical scarification	58.88 b	17.11 c	13.25 c	3 a
Acid scarification 98% (v/v)				
Sulfuric acid (25 sec)	2.00 f	45.11 h	2.55 g	0 a
Sulfuric acid (50 sec)	2.00 f	45.72 h	2.71 g	0 a
Sulfuric acid (75 sec)	1.00 f	44.12 h	2.75 g	0 a
Scarification by hot water				
90 °C + 2.5 min	2.00 f	38.11 g	4.00 f	7 b
90 °C + 5 min	2.00 f	37.52 g	4.11 f	7 b
Potassium nitrate% (KNO3 w/v)				
0.1	19.11 d	22.11 d	8.22 e	20 c
0.2	11.11 e	27.21 e	4.11 f	18 c
0.3	12.20 e	27.85e	4.22 f	7 b
Cold stratification				
2 days	70.06 a	16.31 c	19.43 b	5 b
4 days	72.00 a	12.51 b	18.21 b	5 b
6 days	74.06 a	9.45 a	24.41 a	3 a
Gibberellic acid				
250 ppm	19.12 d	21.91 d	10.12 d	7 b
500 ppm	27.33 c	21.12 d	11.25 d	5 b
750 ppm	57.21 b	22.45 d	10.22 d	7 b
Hydropriming (hour)				
4	8.00 ef	30.14 f	4.21 f	5 b
8	8.00 ef	31.22 f	4.22 f	5 b

FGP: final germination percentage, MGT: mean germination time, VI: Vigour Index

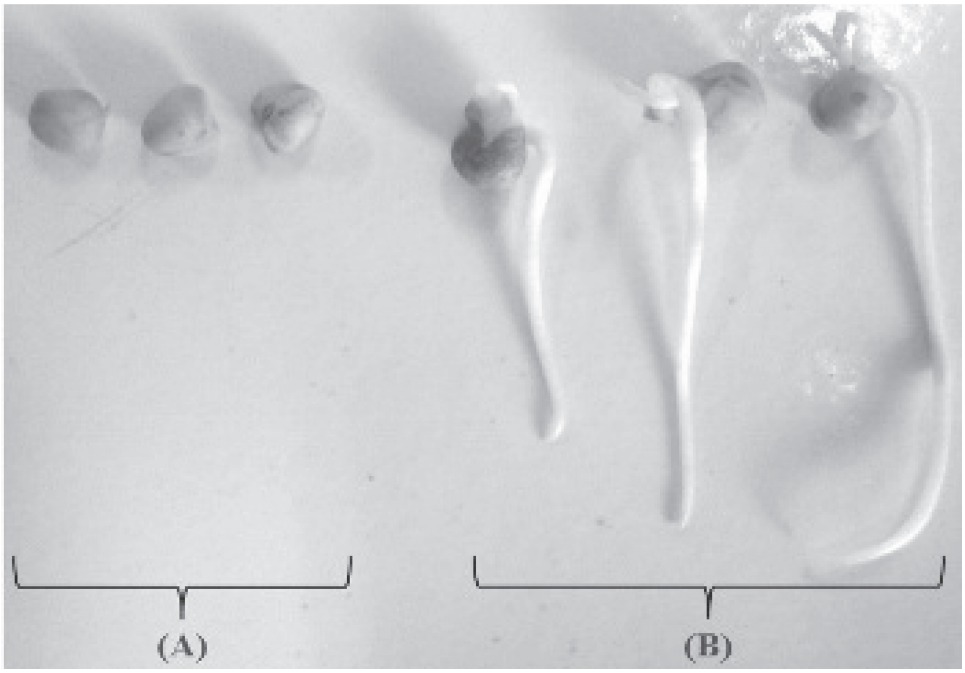

Fig. 1. Comparison of germination between untreated bitter vetch seeds (A) and cold stratification treatment for 6 days (B)

Mean germination time

The mean germination time was significantly lower in cold stratification treatment for 6 days. The cold stratification treatment for 2 and 4 days as well as mechanical scarification also had positive effect on reducing the germination time significantly (Table 2). Rest of the treatments were not effective in reducing the germination time.

Vigour Index

The highest vigour index was recorded in the 6 days cold stratification treatment than the other cold stratification periods and the rest of the other treatments (Table 2). Besides cold stratification treatments, mechanical scarification also improved vigour index in comparison to other treatments. The lowest value of this trait was recorded in sulfuric acid treatments which was at par with the control group. Other treatments including GA3, hydropriming, hot water and KNO_3 resulted in higher vigour index than the control group (Table 2).

Abnormal germination

All the treatments except acid scarification and cold stratification treatment of 6 days resulted in abnormal germination (Table 2).

DISCUSSION

Cold stratification or moist chilling have been widely used as a pre-sowing treatment for breaking seed dormancy and enhancing the maximum rate and germination percentage of dormant seeds of many species (Baskin *et al.,* 1992). Similar to our results, seed germination of *Ferula gummosa* (Rahnama-Ghahfarokhi and Tavakkol-Afshari, 2007) and *Teucrium polium* (Nadjafi *et al.,* 2006) significantly improved at longer periods of prechilling treatment. Chilling has been reported to induce an increase in GA3 concentration and sensitivity (Nadjafi *et al.,* 2006). Eisvand *et al.* (2006) also reported that stratification of imbibed seeds of *Astragalus siliquosus* enhance germination percentage. In contrast to our results, Farashah *et al.* (2011) showed that, with increasing chilling treatment periods beyond 7 days, reduced germination indices as there was no significant difference with control. Stratification led to increased expression of the GA biosynthesis genes GA20ox1 (GIBBERELLIN 20 OXIDASE), GA20ox2, and GA3ox1 and decreased expression of the GA catabolic gene GA2ox2 (Yamauchi *et al.,* 2004). Oh *et al.* (2006) suggested that stratification promotes germination by increasing the potential for bioactive GA accumulation. In our experiment, application of GA3 not stimulated the germination of bitter vetch. Researchers mentioned that the role of gibberellins in dormancy release is controversial. Although GA accumulation is associated with breaking the dormancy and/or germination, GA treatment alone does not stimulate germination in all species (Rouhi *et al.,* 2010). Scarifying seeds with sulfuric acid and hot water did not show any increase in all of the germination parameters as compared to the control, may be due to damage to embryo. Enhancing seed germination parameters due to mechanical scarification emphasizes that an impermeable covering layer restricts bitter vetch seed germination. Our results were consistent with Farashah *et al.* (2011) and in contrast to Aliero (2004). In our study, all of KNO_3 levels improved germination parameters significantly ($P< 0.05$) but these effects not stronger than cold stratification, mechanical scarification and GA3 treatments. Rahnama-Ghahfarokhi and Tavakkol-Afshari (2007) reported that KNO_3 treatment did not stimulate the germination of *Ferula gummosa.* Similar to these results, nitrogenous compounds such as thiourea and KNO_3 were unable to alleviate seed dormancy in *Bonium persicum* (Sharifi and Pouresmael, 2006). Potassium nitrate is being employed for many years, with positive studies beginning in the 1980's but it often increases the germination of photo-dormant seeds (Shanmugavalli *et al.,* 2007). In current experiment, germination parameters did not improved significantly when seeds presoaked in

distilled water. Regarding to enhancing seed germination parameters with mechanical scarification, we think that impermeable seed coat do not allow to seeds for water imbibition sufficiently. The dormancy of bitter vetch seed was broken by some treatments especially with cold stratification and mechanical scarification treatments, but KNO3 levels, hydropriming, scarification with hot water and sulfuric acid had no strong effect. Our results showed that applying cold stratification for 6 days could be the best treatment for bitter vetch dormant seeds (Table 2). Finally, we suggest that bitter vetch has both physical and physiological dormancy because it has impermeable seed coat, but in addition it has a physiological component to their dormancy.

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