Effects of storage duration and hydro-priming on seed germination and vigour of Common vetch

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Abstract

Karta K. Kalsa, Tomer R.P.S. & Bekele Abebie 2011. Effects of storage duration and hydro-priming on seed germination and vigour of Common vetch. *Journal of Science and Development* 1(1), 65-73.

The possibility for prolonged ambient seed storage, and the role of seed hydro-priming were studied in Common vetch (*Vicia sativa* L.) in the laboratory and greenhouse. Seeds stored for zero, one, two, and three years under ambient conditions at Kulumsa, Ethiopia, were soaked in distilled water for 24 h at 20±1°C and subsequently surface-dried at room temperature for *ca*. 6 h. Part of the seeds from the four storage durations was maintained unprimed, and was compared with hydro-primed seeds. Effects of ambient storage duration and hydro-priming, including their interactions, were significant (P<0.01) for all parameters considered. There was no significant reduction in germination percentage, speed of germination, and emergence index of seeds stored for up to two years, as compared to freshly harvested seeds. Despite its negative influence on germination percentage and some vigour parameters that depend on seed age, hydro-priming improved the speed of germination in all age groups, root length and Vigour Index-I of aged seed lots, and emergence index for zero and one-year storage duration. Therefore, Common vetch seeds can be stored under the ambient conditions of a tropical highland environment for about two years without significant loss in germination percentage and emergence index. The positive influences of hydro-priming on speed of germination and emergence index could be an opportunity to be considered in over-sowing studies with vetch on native pastures.

Keywords: Emergence, over-sowing, *Vicia sativa* L., tropical. ISSN 2222-5722.

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Introduction

Common vetch (*Vicia sativa* L.) is an annual legume commonly cultivated in the semiarid regions of Mediterranean countries. Varieties with forage production potential in pure stands and in association with small cereals, and for over-sowing of native pastures, are being promoted for use in the highlands of Ethiopia (KARC, 2007). Seed production of vetches in Ethiopia is limited, mainly because of fluctuating demand, which necessitates unforeseen long-term seed storage under adverse conditions.

Effects of adverse storage conditions on the seed vigour of various crop species have been well documented (Hopkinson & English, 2005; Ouzouline *et al.*, 2009). When stored under prevailing temperature and humidity conditions, seeds of many plant species lose viability and vigour within a short time, except for legume seeds with impermeable seed coats (Cupic *et al.*, 2005). Vigour loss is associated with biochemical losses associated with seed ageing (Murthy *et al.*, 2003). However, published data on the viability and vigour of Common vetch seeds after prolonged storage are scarce (Pita *et al.*, 2005).

Hydro-priming is one of the techniques used to improve the germination and vigour of seeds (Harris, 1996; Harris *et al.*, 1999). Hydro-priming improved the field performance of barley and chickpea (Rashid *et al.*, 2006; Ghassemi-Golezami *et al.*, 2008). However, Abush Tesfaye & Modi (2009) reported that hydro-priming negatively influences the normal germination of dry bean. Dehydration damage and loss of nutrients from large-seeded legumes accounted for the low performance of hydro-primed seeds. On the other hand, hydro-priming is reported to improve seed and seedling performance in wheat (Giri & Schellinger, 2003), sunflower (Hussain *et al.*, 2006), maize (Dezfuli *et al.*, 2008), and soybean (Mohammadi, 2009), whereas reports are rarely available on the role of hydro-priming on seed vigour of forage species, vetch seeds in particular. Therefore, the present study was carried out to investigate the effects of prolonged storage under the ambient conditions of a tropical highland environment, and to evaluate the potential role of hydro-priming in the germination and vigour of Common vetch seeds for over-sowing of native pastures.

Materials and Methods

Two sets of experiments were carried out in 2009, under laboratory and greenhouse conditions at Kulumsa Agricultural Research Centre, Ethiopia. The first set involved the testing of germination percentage and seedling growth (root length, shoot length, seedling dry mass); the second set involved the testing of speed of germination and emergence index. A 4×2 factorial combination, comprising four storage durations and two priming treatments, was laid out in a completely randomised design with four replications.

Seeds of Common vetch (*Vicia sativa* L.), variety IG-62786, produced at Kulumsa Agricultural Research Centre, Ethiopia, in the years 2008, 2007, 2006, and 2005, were stored for zero, one, two, and three years, respectively, under ambient conditions at temperatures of 6–28 °C, and relative humidity of 40–

85%. Seed moisture content at harvest of the four seed lots was between 10–11%.

In the laboratory, seeds were hydro-primed by complete-immersion soaking in distilled water, and incubated for 24 h at 20±1°C. The seeds then were surface-dried on blotting paper for *ca*. 6 h at room temperature. Part of a seed lot was kept unprimed. Note that the hydro-priming treatment was performed separately for the two sets of experiments, but with the same procedure.

The germination percentage was evaluated by taking 400 seeds (in four replications) per treatment, according to the ISTA (2005) rules. Seeds were placed in plastic boxes filled (up to 3 cm) with moistened fine sand (0.05–0.85 mm), and the boxes were placed in a cement-concrete germination room until the final counts were taken after 14 days. The temperature of the room was adjusted to 20±1°C.

After the final count in the standard germination test, seedling growth rate was assessed by measuring root and shoot lengths and seedling dry mass, on 10 normal seedlings per replicate randomly taken from the standard germination test. The means were computed.

Ten randomly selected seedlings were washed with running tap water, cut free from their cotyledons, placed in envelopes, then oven-dried at 80±1°C for 24 h, for determination of seedling dry mass, as suggested by Fiala (1987). Since the seedlings were obtained from the standard germination test, their age was 14 days after planting.

The speed of germination was measured in four replicates of 25 seeds from each treatment. Seeds were placed on double-layered Whatman #101 filter paper in 90-mm diameter Petri dishes, and kept in an incubator at 20±1°C for 20 days, until no further germination took place. Each day, normally germinated seeds (with radicles emerged more than 5 mm out of the seed coat) were removed, until seed germination had ceased. An index was calculated as follows:

Speed of Germination = $\Sigma(G_t / D_t)$,

where G_t is the number of germinated seeds on day t and D_t is the number of days after planting when the germinated seeds were counted.

The emergence index was recorded in a greenhouse, on seedlings grown in pots filled with untreated agricultural soil. To simulate field conditions, no temperature or humidity control was applied in the greenhouse during the study period. From every replicate of a treatment, 25 seeds were placed in rows, and covered with 3 cm of soil. The soil was then packed gently and pots were placed in a watering bath, to supply water from below the pots whenever needed. This was intended to avoid disturbing seed placement by watering from above. Daily emergence was counted, and continued until constant readings were obtained. The emergence index was calculated as $\Sigma (E_t / D_t)$, where E_t is the number of seedlings emerged, D_t is the number of days after planting when the seedlings were counted (Yang *et al.,* 2005).

Percentage values were arcsin transformed and analysed by means of the SAS Systems for Windows, Version 9 (SAS Institute Inc., 2002).

Results and Discussion

With one exception, germination percentage, root length, Vigour Index-I, seedling dry mass, Vigour Index-II, shoot length, speed of germination, and emergence index of Common vetch seeds were significantly (P<0.01) influenced by duration of storage, hydro-priming, and by their interaction (Table 1). The effect of hydro-priming on Vigour Index-I was non-significant. Since the interaction effects were highly significant for all traits investigated, the results and discussion are mainly based on the interactions.

Germination percentage

Mean germination percentage of unprimed seeds remained between 93–100 % for the first three seed-storage durations (Figure 1). The maximum germination percentage was recorded in unprimed seeds after one year's storage. The increase in germination percentage of Common vetch seeds with increased seed age could be attributed to the seed-coat characteristics of legumes. It is well documented that the exposure of legume seeds to adverse storage conditions can contribute to the softening of the impermeable seed coat, and improve the germination potential of seeds by enhancing gas and water uptake (Cupic *et al*., 2005). These authors reported an improved germination percentage in alfalfa seeds after four years' storage under ambient conditions. The present study has shown that storage of Common vetch seeds for up to two years under ambient conditions in a tropical highland environment may not adversely affect germination percentage.

The effect of hydro-priming on germination percentage was significantly negative for

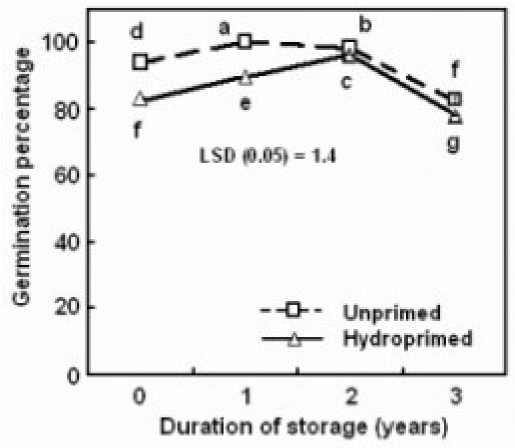

Figure 1. Germination percentage (%) of Common vetch seeds as affected by duration of seed storage and hydro-priming; means ±SE. Different letters show a significant difference at 0.05 level.

Table 1. *Analysis of variance for effects of duration of seed storage and hydro-priming on germination and vigour of Common vetch seeds*

Sources of Variation	DF	Germination (%)	Root length (cm)	Vigour Index-I	Shoot length (cm)	Seedling dry mass (mg)	Vigour Index-II	Speed of Germination	Emergence Index
Duration of storage	3	454.0**	4.7**	11035.4**	46.6**	0.2**	5256.7*	10.9**	69.1**
Hydro-priming	1	406.1**	2.2**	864.2ns	66.5**	0.5**	10099.8	92.3**	68.2**
Interaction	3	39.2**	7.1**	80881.9**	218.5**	0.3**	3536.8*	3.9**	28.9**
Error	24	0.90	0.20	1416.60	0.95	0.01	69.9	0.29	3.12
CV %		**2.2**	**5.1**	**4.9**	**5.7**	**4.6**	**4.8**	**6.0**	**7.1**
Mean		**90.1(67.4)**	**8.6**	**772.8**	**17.1**	**1.9**	**174.9**	**10.0**	**24.8**
R^2		**0.99**	**0.89**	**0.89**	**0.97**	**0.91**	**0.96**	**0.95**	**0.83**

Note: *Significant at 0.05 level; **Significant at 0.01 level; ns non-significant.

seeds from all storage durations (Figure 1). The reduction in germination percentage of hydro-primed seeds could be attributed to dehydration damage and nutrient leakage. When hydro-primed seeds were surface-dried for nearly 6 h at room temperature, dehydration damage to the embryo column may have occurred, hence there was an increase in the number of abnormal seedlings at the expense of normal germination. This result is in agreement with that of Abush Tesfaye & Modi (2009), where dehydration treatment of hydro-primed seeds reduced the percentage of normal germination in dry bean.

Seedling root length and vigour

The mean root length of unprimed seeds decreased as the duration of storage increased, up to two years (Figure 2). The superior root growth of unprimed, freshly harvested seeds could indicate that they have better initial nutrient reserves (proteins, lipids, starch) which, through storage under adverse conditions, were gradually depleted in the older seed lots. Earlier reports have shown that storage under adverse conditions could cause depletion of important nutrient reserves (Murthy *et al*., 2003). The root length of unprimed seeds from the lot stored for three years was greater, but still was inferior to that of freshly harvested seeds.

Hydro-priming remarkably improved the root length of seedlings from one-, two- and three-year-old seed lots (Figure 1). Previous studies in *Zea mays* L. indicated positive influences of hydro-priming on radicle length (Dezfuli *et al*., 2008). Better performance in root length of hydroprimed seeds could be attributed to earlier mobilisation of major nutrient reserves. Freshly harvested seeds, however, were negatively influenced by hydro-priming; this could be due to nutrient leakage, *cf.* Abush Tesfaye & Modi (2009).

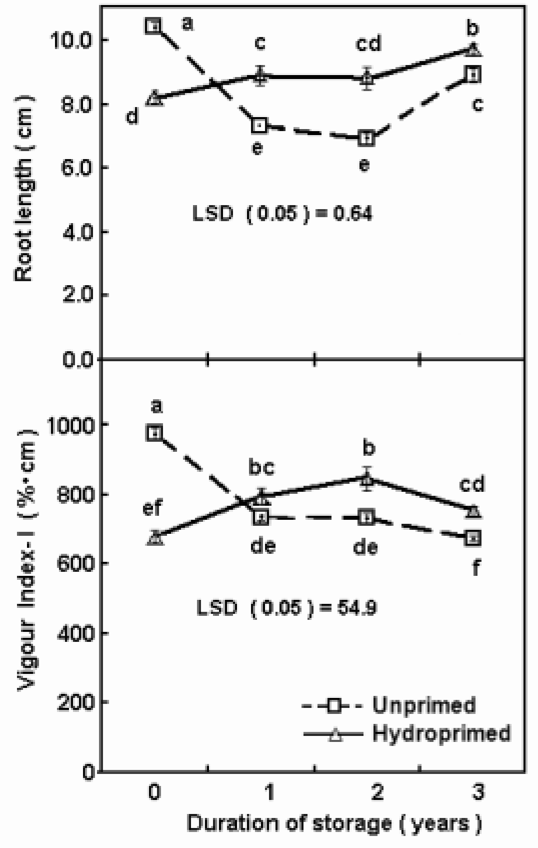

Figure 2. Root length and Vigour Index-I of Common vetch seeds as affected by duration of seed storage and hydro-priming; means ±SE. Different letters show a significant difference at 0.05 level.

Vigour Index-I (multiple of root length and germination percentage) indicated that unprimed, freshly harvested seeds were more vigorous at all durations of storage, than the remaining seed lots, including hydro-primed seeds (Figure 2). The vigour of unprimed seeds declined as seed age increased, up to three years. Murthy *et al*. (2003) reported a decline in vigour of *Vigna radiata* (L.) Wilczek as the seed-storage period increased from zero to several days. These authors indicated that loss of seed vigour was associated with biochemical deterioration during seed ageing. Hydro-priming improved seed vigour in older seed lots, indicating that there was faster rehabilitation of intracellular structures after the hydro-priming treatment.

Seedling dry mass and seedling vigour

Seedling dry mass of unprimed controls significantly decreased as seed storage duration increased (Figure 3). Vigour Index-II (multiple of seedling dry mass and germination percentage) results, however, indicated that unprimed seeds at storage durations of zero and one year were equally vigorous. Our result agrees with that of Makawi & van Gastel (2006), who reported a reduction in seedling dry mass after different periods of accelerated ageing treatment on seeds of different varieties of lentil (*Lens culinaris* Medikus). In our study, except for seeds stored for two years, hydro-priming negatively influenced seedling dry mass and seedling vigour at all storage durations.

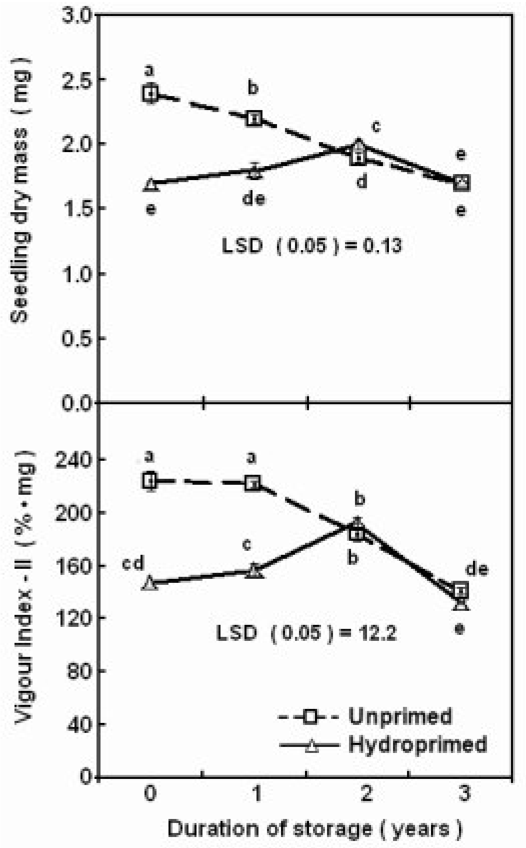

Figure 3. Seedling dry mass and Vigour Index-II of Common vetch seeds as affected by duration of seed storage and hydro-priming; means ±SE. Different letters show a significant difference at 0.05 level.

Shoot length

Shoot length of unprimed seeds was considerably higher for seed lots stored for one and two years, as compared to freshly harvested and three-year-old seed lots (Figure 4). This could be due to a higher germination capacity of the stored seed lots, which resulted in normal seedlings with longer shoots. Hydro-primed seeds of freshly harvested lots showed a significant improvement in shoot length, as compared to the unprimed control. Shoot length of seeds stored for one year was negatively influenced by hydro-priming.

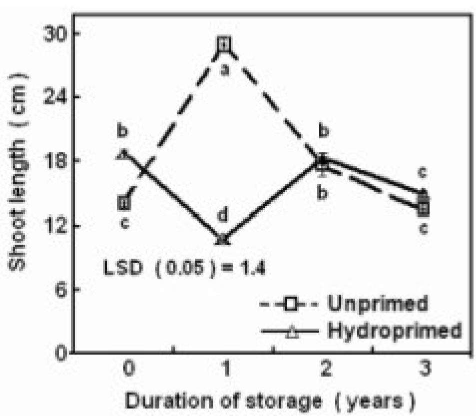

Figure 4. Seedling shoot length of Common vetch seeds as affected by duration of seed storage and hydro-primingmeans ±SE. Different letters show a significant difference at 0.05 level.

Speed of germination and emergence index

Speed of germination of unprimed seeds was not affected by storing seeds for up to two years under ambient conditions, whereas emergence index increased as seed-storage

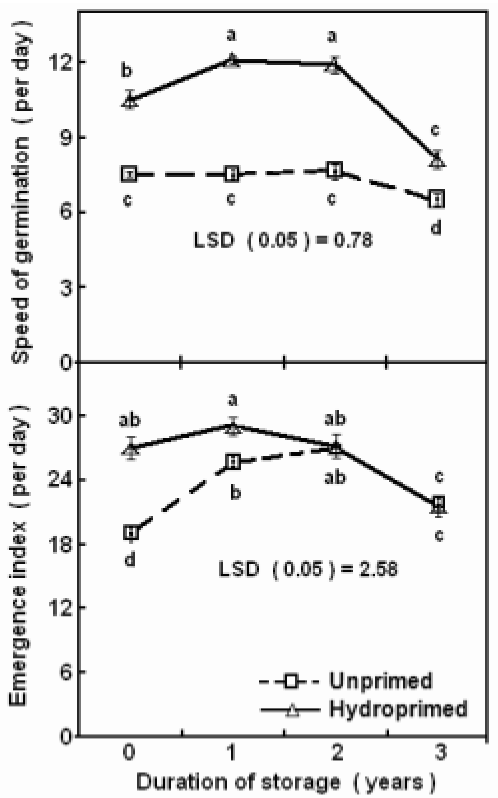

Figure 5. Speed of germination and seedling emergence index of Common vetch seeds as affected by duration of seed storage and hydro-priming; means ±SE. Different letters show a significant difference at 0.05 level.

duration increased, up to two years (Figure 5). Speed of germination, unlike germination percentage, seedling dry mass and shoot length, was significantly improved by hydro-priming at all durations of storage. For zero and one year's storage, emergence index was also improved by the hydro-priming of Common vetch seeds. Our results agree with those of Giri & Shillinger (2003) and Mohammadi (2009), who reported that hydro-priming significantly improved seedling emergence in wheat, and speed of germination in soybean. A faster rate of germination of hydro-primed seeds could be attributed to enhanced repair of the intra-cellular architecture, and mobilisation of hydrolytic enzymes required for visible germination to occur (Bewley, 1997).

Association of traits

The relationship between emergence index and vigour parameters such as speed of germination, Vigour Index-I and root length, was significant (Table 2). Germination percentage was positively related to emergence index, but the association was not statistically significant. The associations of speed of germination and root length with emergence index were strong (r = 0.70, and r = –0.65, respectively). Our results concerning the strong and positive association of emergence index with speed of germination is in agreement with the findings of Wang *et al*. (2004) for Purple vetch (*Vicia benghalensis* L.) and alfalfa (*Medicago sativa* L.). Those authors also reported a negative relationship between field emergence and root length, but it was weak and not statistically significant. The strong and negative association of root length with seedling emergence index observed in our study is, however, not tenable and calls for further investigation.

In conclusion, there was no significant reduction in germination percentage, speed of germination, and emergence index of Common vetch seeds stored for up to two years, as compared to freshly harvested seeds. Despite its negative influence on germination percentage and some vigour parameters that depend on seed age, hydro-priming improved the speed of germination of all age groups, root length and Vigour Index-I of aged seed lots, and emergence index at zero and one-year storage durations. Therefore, Common vetch seeds can be stored under the ambient conditions of a tropical highland environment for about two years without significant loss of germination percentage and emergence index. Since hydro-priming improved emergence index at the zero and one-year storage durations, this finding can be considered in the context of over-sowing of native pastures,

Table 2. *Pearson correlation coefficients of germination percentage, root length, Vigour Index-I, seedling dry mass, Vigour Index-II, shoot length, speed of germination, and emergence index of Common vetch seeds*

Parameter	Germination (%)	Root length (cm)	Vigour Index-I	Seedling dry mass (mg)	Vigour Index-II	Shoot length (cm)	Speed of Germination	Emergence Index
GP †	1.00							
RL	−0.57**	1.00						
VI_I	0.06	0.76**	1.00					
DW	0.67**	0.14	0.66**	1.00				
VI_II	0.85**	−0.11	0.52**	0.95**	1.00			
SL	0.70**	−0.61**	−0.31	0.42*	0.51**	1.00		
GS	−0.11	0.06	0.11	−0.21	−0.14	−0.20	1.00	
EI	0.28	−0.65**	−0.47**	−0.32	−0.09	0.20	0.70**	1.00

Note: *Significant at 0.05 level; **Significant at 0.01 level. †GP = Germination percentage, RL = Root length (cm), Vigour Index-I, DW = Seedling dry mass (mg), Vigour Index-II, SL = Shoot length (cm), GS = Speed of germination, EI = Emergence Index.

after further investigation of its applicability under harsher field conditions.

Our study has, however, several limitations that call for further investigation. First, the study involved a single variety, and lacks information on genotypic effects on responses to seed ageing, as well as to hydro-priming treatment. Moreover, the hydro-priming treatment was based only on a 24-h duration; shorter durations may need to be evaluated.

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