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Full Length Research Paper

Influence of seed priming on seed germination and vigor traits of *Vicia villosa* ssp. *dasycarpa* (Ten.)

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Laboratory and greenhouse experiments were conducted to investigate the effects of hydro- and osmotic priming on seed germination and vigor traits of two seed lots of wooly pod vetch (*Vicia villosa ssp. dasycarpa* Ten.). Factorial combinations of two seed lots (two periods of storage) and six levels of priming treatment (unprimed control, distilled water (hydro-primed), 0.155, 0.310, 0.465 and 0.620 M KNO_3) were laid out in a completely randomized design with four replications. Seeds were primed for 24 h at temperature of 20 ± 1°C. The effects of seed lot, priming treatment, and their interactions were significant at ($P<0.05$) for seed germination and vigour traits. Seed priming significantly improved all traits investigated in both seed lots with the exception of root length, seedling dry weight, and vigor index-II (germination percentage × seedling dry weight) of stored seeds. Both seed lots achieved significantly higher germination percentage at 0.155 M KNO_3, compared to unprimed controls and hydro-primed seeds. However, hydro-priming showed an advantage over unprimed seeds and osmo-primed seeds in most of the traits studied. Generally, the present study has shown that seed priming (hydro- and osmotic priming) has the potential to improve seed germination and vigor traits in wooly pod vetch.

Key words: Wooly pod vetch, ambient storage, osmo-priming, hydro-priming.

INTRODUCTION

Wooly pod vetch (*Vicia villosa* ssp. *dasycarpa* Ten.) is a widely utilized forage legume in the highlands of Ethiopia in monoculture, in mixture with oat (*Avena sativa*), or for over-sowing of natural pastures. Wooly pod vetch varieties, however, are hard seeded (10 to 70% dormancy) and can occur as voluntary weeds with seeds breaking dormancy following crops for many years (Matic et al., 2008). Moreover, its initial growth is poor and in early growth stages, it is a poor competitor with weeds. Minimizing of hard seed (dormancy) proportion and enhancing of germination and seedling growth in wooly pod vetch could, therefore, result in improved establishment rate and competitiveness to weeds, and reduced risk of voluntary weeds in subsequent crops.

Reports indicated that seed priming (pre-sowing treatment that involves the controlled hydration of seeds is sufficient to allow pre-germination metabolic events to take place but insufficient to allow radicle protrusion through the seed coat) can improve seed and seedling performance in several species. Hydro-priming (soaking seeds in water) and osmo-priming (soaking seed in chemicals that lower osmotic potential in the seed environment) are commonly used to improve seed performance in various cultivated crops. Arin et al. (2011) reported that osmotic priming with potassium salts improved seedling emergence in onion. Mohammadi and Amiri (2010) reported that seed priming has improved seed performance of canola (*Brassica napus* L.). Umair et al. (2010) reported that osmotic priming of mung bean (*Vigna radiate*) seeds in potassium salts has improved seed vigor. Mohammadi (2009) reported that seeds primed with KNO_3 showed highest values of germination percentage, seedling dry weight, and germination rate in late-spring seeded soybean (*Glycine max* L.).

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Nascimento et al. (2004) reported that germination rate and germination percentage at different temperature regimes were increased by priming muskmelon (*Cucumis melo* L.) seeds with KNO_3. Hydro-priming also resulted in lower time taken to 50% germination, higher germination rate, vigor index and final germination percentage in maize (Dezfuli et al., 2008). Hydro-priming hairy vetch (*Vicia villosa* Roth.) seeds resulted in improved germination rate and seedling emergence from compacted soil (Snapp et al., 2008). On the other hand, Mohammadi and Amiri (2010) indicated that canola seeds primed with KNO_3 showed better vigor performance than those primed with distilled water.

Prolonged seed storage period under adverse conditions (high temperature and relative humidity) is reported to have a positive influence on seed germination and emergence of several species with impermeable seed coat. Storing *Vicia dasycarpa, Trifolium tembense*, and *Vicia sativa* seeds for two to five years under ambient temperature and relative humidity has rather improved germination percentage, germination rate, and seedling emergence (Tsegahun et al., 2009; Kalsa et al., 2011). Moreover, Čupić (2005) found in alfalfa (*Medicago sativa* L.) that the share of hard seed was decreased by storage time, temperature and air moisture changes; and therefore, directly kept the level of seed germination in a longer period of storage. Kalsa et al. (2011) found that there is a significant interaction between hydro-priming treatment and seed age of common vetch. However, published data on effects of seed priming, seed age and their interactions on seed germination and vigor of wooly pod vetch are limited. Hence, the present study was conducted to evaluate the effect of seed priming and seed age on germination and vigor of wooly pod vetch seeds.

MATERIALS AND METHODS

Laboratory and greenhouse experiments were conducted at Kulumsa Agricultural Research Centre, Ethiopia, to determine the effects of seed priming on seed germination and vigor traits in wooly pod vetch. A 2 × 6 factorial combination, comprising of two seed lots (two periods of storage) and five priming treatments was laid out in a completely randomized design with four replications. Seed lots of wooly pod vetch (*V. villosa ssp. dasycarpa* Ten.) variety Kauhak (which was obtained from the International Center for Agricultural Research in Dry Areas, ICARDA) were produced at Kulumsa Agricultural Research Centre, Ethiopia, in the years 2007, and 2008. Seeds from 2007 harvest were stored for one year under ambient conditions (Figure 1) whereas; the 2008 harvests were used as fresh seed without storage. Seed moisture content at harvest of the two seed lots ranged from 10 to 11%.

In the laboratory, seeds were primed with distilled water, 0.155, 0.310, 0.465, 0.620 M KNO_3 by complete immersion soaking, and incubated for 24 h at 20 ± 1°C under dark condition. The seeds were then washed with distilled water and surface-dried on blotting paper for Ca. 6 h at room temperature. Part of a seed lot was kept unprimed. For every treatment combination, the germination percentage was evaluated by taking 400 seeds (100 seeds in each replication), according to the ISTA (2005) rules. After six hours of surface drying, seeds were placed in plastic boxes filled (up to 3 cm) with moistened fine sand (0.05 to 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 was assessed by measuring root length and seedling dry weight on 10 normal seedlings randomly taken per replicate from the standard germination test. For root length and seedling dry weight, means were computed based on ten seedlings. Root length was measured from tip of the tap root to the joining point of the cotyledon.

After measuring of root length, the seedlings were cut free from their cotyledons, placed in envelopes, then oven-dried at 80 ± 1°C for 24 h for determination of seedling dry weight, as suggested by Fiala (1987). Since the seedlings were obtained from the standard germination test, age of the seedlings was 14 days after planting.

The germination rate 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:

Germination Rate = $\sum(G_t/D_t)$, 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.
Seedling vigor indices were calculated as follows:

Vigor Index-I = Standard Germination (%) × Seedling Root length (cm);

Vigor Index-II = Standard Germination (%) × Seedling dry weight (mg).

The emergence index and days required for 50% emergence were recorded in a greenhouse on seedlings grown in pots filled with untreated agricultural (clay loam) soil to simulate field conditions. Temperature and humidity control facilities in the greenhouse were not operational during the study period. In each replication, 25 seeds were placed at equal level and covered with a 3 cm thick layer of soil. The soil was then compacted gently and pots were placed in a watered bath to supply water from below. Daily seedling emergence was counted, and continued until constant readings were obtained. The emergence index was calculated as:

Emergence Index = $\sum(E_t/D_t)$,

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 arc-sin transformed and analysis of variance was carried out using the Statistical Analysis Software for Windows, Version 9 (SAS Institute Inc, 2002). Multiple comparisons on main effects as well as, their interactions were performed using least square means with Turkey's adjustment.

RESULTS

Results showed that effects of priming treatment, seed age, and their interactions were significant at (P<0.05) for all traits investigated (Tables 1 and 2). Because of the significant interactions, the two seed ages were analyzed

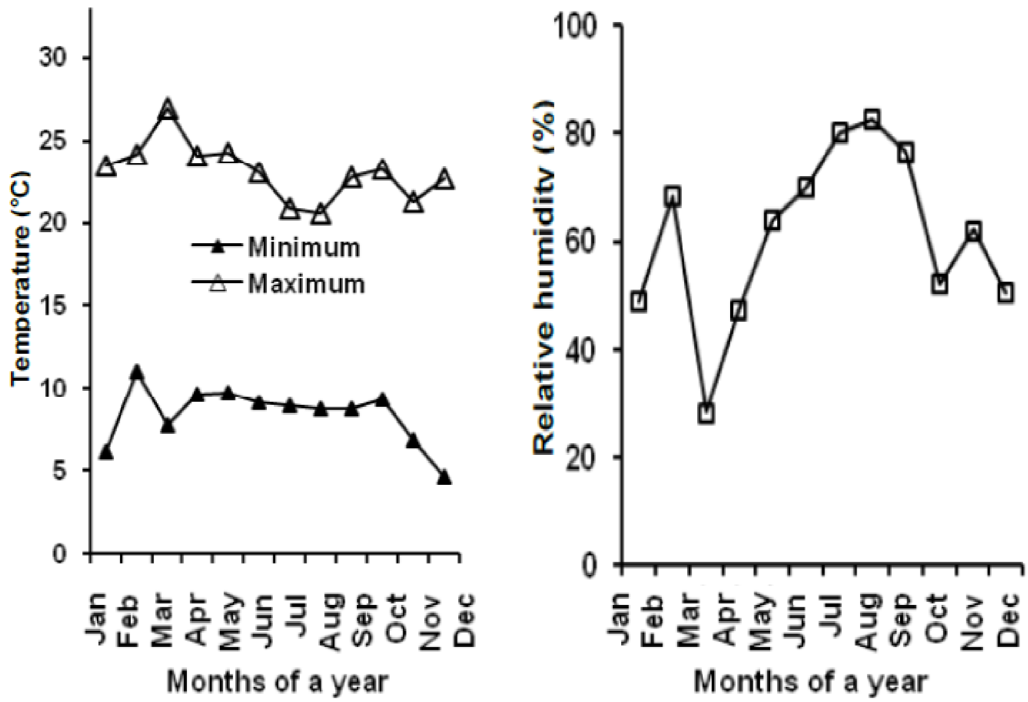

Figure 1. Temperature and relative humidity of open air at Kulumsa during the one year period of seed storage.

Table 1. Analysis of variance for germination percentage, hard seed percentage, germination rate, root length, and seedling dry weight of wooly pod vetch seeds.

Sources of variation	DF	Germination percentage	Hard seed percentage	Germination rate (per day)	Root length (cm)	Seedling dry weight (mg)
Seed lot (L)	1	7146.8**	1576.2**	377.1**	24.3**	12.1**
Priming (P)	5	173.9**	39.8**	28.2**	6.7**	15.1**
L*P	5	124.5**	10.3**	7.6**	6.8**	4.9*
Error	36	4.3	0.5	0.13	0.44	1.43
R^2		0.98	0.98	0.99	0.85	0.68
CV		3.8	9.4	6.1	6.7	7.2
Mean		54.4	7.9	5.9	9.9	16.5

*, ** Effect is significant at α = 0.05 and α = 0.01 levels of significance, respectively.

Table 2. Analysis of variance for vigor indices, seedling emergence and electrolyte leakage of wooly pod vetch seeds.

Sources of variation	DF	Vigor index-I (cm. %)	Vigor index-II (mg. %)	Emergence index	Days to 50% emergence	Electrical conductivity ($\mu Scm^{-1}g^{-1}$)
Seed lot (L)	1	308078.7**	1155071.1**	579.4**	150.5**	52381.1**
Priming (P)	5	45119.4**	91427.2**	75.7**	3.2**	73898.0**
L*P	5	39182.4**	57742.7**	89.7**	19.2**	19427.0**
Error	36	1697.3	9057.3	1.6	0.31	97.1
R^2		0.92	0.85	0.96	0.96	0.99
CV (%)		7.8	7.3	8.4	6.5	5.9
Mean		531.1	1298.1	14.7	8.6	167.6

*, ** Effect is significant at α=0.05 and α = 0.01 levels of significance, respectively.

Table 3. Means± SE for period of seed storage x priming treatment effects on germination percentage and hard seed percentage of wooly pod vetch seeds.

Priming treatments	Germination percentage		Hard seed percentage	
	Lot-1 (Stored)	Lot-2 (fresh)	Lot-1 (stored)	Lot-2 (Fresh)
Unprimed	88.0 ± 0.4 (61.7)[†c]	58.3 ± 1.5 (35.6)[f]	9.3 ± 0.3 (5.3)[c]	31.0 ± 1.1 (18.2)[a]
Distilled water	94.3 ± 1.2 (70.8)[b]	63.8 ± 0.6 (39.6)[e]	3.8 ± 0.3 (2.1)[d]	19.5 ± 0.6 (11.2)[b]
0.155 M KNO_3	95.3 ± 0.5 (72.3)[a]	73.0 ± 1.1 (46.9)[d]	2.3 ± 0.3 (1.3)[d]	21.8 ± 0.6 (12.6)[b]
0.310 M KNO_3	97.0 ± 0.4 (76.0)[a]	67.5 ± 1.5 (42.5)[e]	3.0 ± 0.0 (1.7)[d]	29.0 ± 0.8 (16.9)[a]
0.465 M KNO_3	89.0 ± 0.9 (62.9)[c]	71.8 ± 1.3 (55.9)[d]	3.0 ± 0.0 (1.7)[d]	19.3 ± 0.3 (11.1)[b]
0.620 M KNO_3	83.0 ± 0.4 (56.1)[c]	68.0 ± 1.2 (42.9)[e]	1.0 ± 0.0 (0.6)[d]	20.0 ± 1.1 (11.5)[b]
Mean	91.1 ± 1.0 (66.6)	67.0 ± 1.1 (42.2)	3.7 ± 0.5 (2.1	23.5 ± 1.1 (13.6)

Means under same parameter sharing letters are not significantly different at α=0.05. [†]Numbers in bracket are arcsine transformed values.

separately.

Seed germination percentage and hard seed proportion

There was a significant effect of seed lot (as explained by period of seed storage) and priming treatment on germination percentage and hard seed proportion. The mean germination percentage was higher for seeds stored for one year under ambient conditions (Table 3). Seed priming improved performance of germination percentage of both seed lots as compared to their untreated controls (Table 3). In the stored seed lot, maximum gains in germination percentage over the control were about 6, 7 and 9% due to priming seeds with water, 0.155 M and 0.310 M KNO_3, respectively. Further increase in concentration of KNO_3 tended to decrease in germination percentage. There was about 5 to 14% increase in germination percentage by priming freshly harvested seeds with water or KNO_3. Highest percentage of 97% for stored seeds was obtained by priming with 0.310 M KNO_3 while the highest 73% germination of freshly harvested seeds was obtained by priming with 0.155 M KNO_3. Our results also showed that the proportion of hard seed of the lot stored for one year was lower than that of freshly harvested seeds (Table 3). As compared to controls, seed priming was significantly decreased hard seed proportion in both seed lots except for freshly harvested seeds at 0.310 M KNO_3.

Germination rate, root length and seedling dry weight

The effects of seed age, seed priming and their interaction were significant on germination rate root length, and seedling dry weight (Table 1). Germination rate of unprimed seeds was faster in stored seeds than in freshly harvested seeds while there was no significant effect on root length and seedling dry weight (Table 4). Priming in water or 0.155 M KNO_3 increased germination rate by about 100% in stored seeds and by about 180 to 250% in freshly harvested seeds. The effect of priming treatment was non-significant on both root length and seedling dry weight of stored seeds. However, both root length and seedling dry weight of fresh seeds were significantly improved by priming with KNO_3 solutions (Table 4). Priming fresh seeds with 0.155M KNO_3 has increased root length by about 43% while seedling dry weight of the same lot was improved by about 3 to 4 mg by priming with 0.310 to 0.620 M KNO_3 solutions.

Seedling vigor index

The effects of period of seed storage, seed priming and their interaction were significant on seedling Vigor indices I and II (Table 1). Observations from unprimed seeds indicated that stored seeds have significantly better seedling vigor indices as compared to that of fresh seeds (Table 5). Storing wooly pod vetch seeds for one year improved vigor index I and II by 42 and 57%, respectively. Seed priming with water or lower concentrations of KNO_3 significantly improved vigor index I of stored seeds. Vigor index II of fresh seeds was significantly improved by priming with higher concentrations of KNO_3.

Seedling emergence

Both emergence index and days to 50% emergence were significantly influenced by seed priming depending on the period of seed storage. As observed on unprimed controls, stored seeds exhibited higher emergence index and required shorter days to achieve 50% emergence (Table 6). The highest emergence index of stored seeds was recorded by seeds primed with water, followed by those primed with 0.310 M KNO_3. Priming stored seeds with water significantly decreased number of days required for 50% emergence as compared to unprimed seeds. Maximum emergence indices of the younger seed

Table 4. Means ± SE for period of seed storage x priming treatment effects on germination rate, root length, and seedling dry weight of wooly pod vetch seeds.

Priming treatments	Germination rate (per day)		Root length (cm)		Seedling dry weight (mg)	
	Lot-1 (stored)	Lot-2 (fresh)	Lot-1 (stored)	Lot-2 (fresh)	Lot-1 (stored)	Lot-2 (fresh)
Unprimed	5.7 ± 0.3^{d}	1.2 ± 0.0^{g}	8.6 ± 0.3^{c}	8.7 ± 0.2^{c}	15.6 ± 0.8^{bc}	15.1 ± 0.6^{c}
Distilled Water	11.8 ± 0.2^{a}	3.4 ± 0.1^{e}	9.7 ± 0.3^{bc}	8.2 ± 0.3^{c}	15.7 ± 0.6^{bc}	15.3 ± 0.6^{c}
0.155 M KNO_3	11.4 ± 0.3^{a}	4.2 ± 0.2^{e}	9.0 ± 0.3^{bc}	12.5 ± 0.4^{a}	15.4 ± 0.8^{c}	15.3 ± 0.6^{c}
0.310 M KNO_3	10.5 ± 0.2^{b}	4.2 ± 0.1^{e}	9.8 ± 0.4^{bc}	11.5 ± 0.4^{a}	15.3 ± 0.6^{c}	18.4 ± 0.4^{a}
0.465 M KNO_3	6.9 ± 0.1^{c}	3.3 ± 0.1^{e}	8.7 ± 0.5^{c}	10.6 ± 0.4^{bc}	16.0 ± 0.3^{bc}	18.4 ± 0.5^{a}
0.620 M KNO_3	6.2 ± 0.2^{c}	2.4 ± 0.1^{f}	9.1 ± 0.2^{bc}	12.0 ± 0.2^{ab}	17.9 ± 0.7^{abc}	19.6 ± 0.2^{a}
Mean	8.7 ± 0.5	3.3 ± 0.2	9.1 ± 0.2	10.6 ± 0.4	16.0 ± 0.3	17.0 ± 0.4

Means under same column sharing letters are not significantly different at α = 0.05.

Table 5. Means ± SE for period of seed storage x priming treatment effects on seedling vigor indices of wooly pod vetch seeds.

Priming treatments	Vigor index-I (cm. %)		Vigor index-II (mg. %)	
	Lot-1 (stored)	Lot-2 (fresh)	Lot-1 (stored)	Lot-2 (fresh)
Unprimed	532.1 ± 19.6^{c}	309.5 ± 13.5^{d}	1374.4 ± 70.0^{ab}	876.3 ± 30.9^{c}
Distilled water	684.5 ± 28.9^{ab}	324.8 ± 13.4^{d}	1480.9 ± 52.4^{a}	977.2 ± 41.5c
0.155 M KNO_3	650.6 ± 16.7^{ab}	584.3 ± 11.9^{bc}	1463.9 ± 71.9^{a}	1111.7 ± 28.1^{bc}
0.310 M KNO_3	743.8 ± 29.8^{a}	488.9 ± 26.8^{c}	1486.2 ± 60.2^{a}	1240.4 ± 16.3^{b}
0.465 M KNO_3	546.3 ± 29.4^{c}	485.6 ± 11.9^{c}	1426.2 ± 32.0^{a}	1321.0 ± 37.5^{ab}
0.620 M KNO_3	510.2 ± 15.7^{c}	513.1 ± 15.3^{c}	1488.0 ± 52.4^{a}	1331.7 ± 42.6^{ab}
Mean	611.3 ± 20.1	451.0 ± 21.7	1453.3 ± 22.6	1143.0 ± 37.8

Means under same column sharing letters are not significantly different at α = 0.05 level of significance.

Table 6. Means ± SE for period of seed storage x priming treatment effects on seedling emergence index, days to 50% emergence, and seed electrolyte leakage of wooly pod vetch seeds.

Priming treatments	Emergence index		Days to 50% emergence		Electrical conductivity ($\mu Scm^{-1}g^{-1}$)	
	Lot-1 (stored)	Lot-2 (fresh)	Lot-1 (stored)	Lot-2 (fresh)	Lot-1 (stored)	Lot-2 (fresh)
Unprimed	14.3 ± 0.6^{c}	9.0 ± 0.4^{e}	7 ± 0^{cd}	11 ± 0^{ab}	59.4 ± 3.5^{g}	146.3 ± 8.0^{e}
Distilled Water	25.7 ± 0.8^{a}	12.6 ± 0.5^{cd}	5 ± 0^{d}	10 ± 0^{b}	52.3 ± 0.7^{g}	36.5 ± 1.5^{g}
0.155 M KNO_3	21.2 ± 1.2^{b}	9.1±0.4^{e}	6 ± 0^{d}	12 ± 10^{a}	131.0 ± 2.3^{e}	85.9 ± 2.6^{f}
0.310 M KNO_3	23.9 ± 0.7a^{b}	11.0 ± 0.1^{de}	6 ± 0^{d}	10 ± 0^{b}	248.8 ± 5.8^{b}	163.4 ± 3.3^{de}
0.465 M KNO_3	12.5 ± 0.8^{cd}	13.3 ± 0.4^{cd}	10 ± 0^{b}	8 ± 0^{c}	360.0 ± 7.3^{a}	171.4 ± 1.3^{d}
0.620 M KNO_3	11.6 ± 0.5^{cde}	12.5 ± 0.3^{cd}	7 ± 0^{cd}	11 ± 1^{b}	352.3 ± 8.7^{a}	203.9 ± 5.0^{c}
Mean	18.2 ± 1.2	11.3 ± 0.4	6.8 ± 0.4	10 ± 0	200.6 ± 26.7	134.6 ± 11.9

Means under same column sharing letters are not significantly different at α = 0.05 level of significance.

lot were recorded by priming seeds with distilled water or 0.465 and 0.620 M KNO_3 (Table 6). Moreover, priming freshly harvested seeds with distilled water and 0.465 M KNO_3 has decreased days to 50% emergence by about 1 and 3 days, respectively, as compared to the unprimed seeds.

Relationship of seedling emergence with germination rate and vigor indices

Linear regression analysis indicated that both emergence index and days to 50% emergence have strong and statistically significant relationships with germination rate,

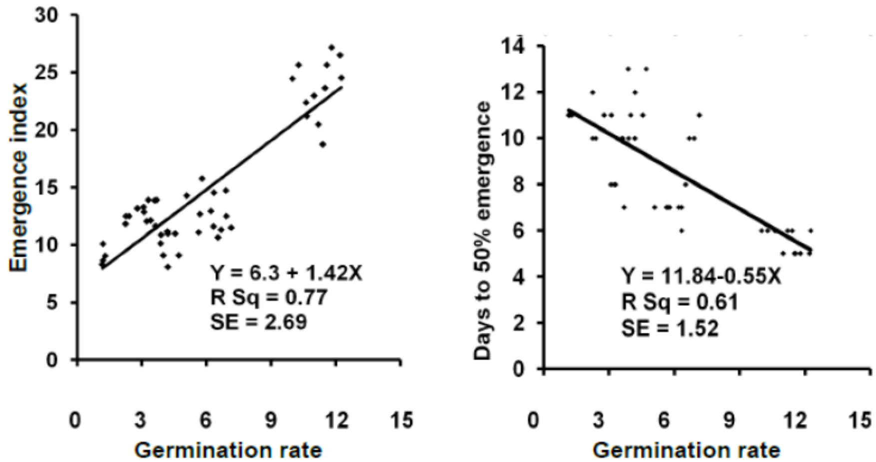

Figure 2. Relationship of seedling emergence with germination rate (seeds per day) of wooly pod vetch seeds.

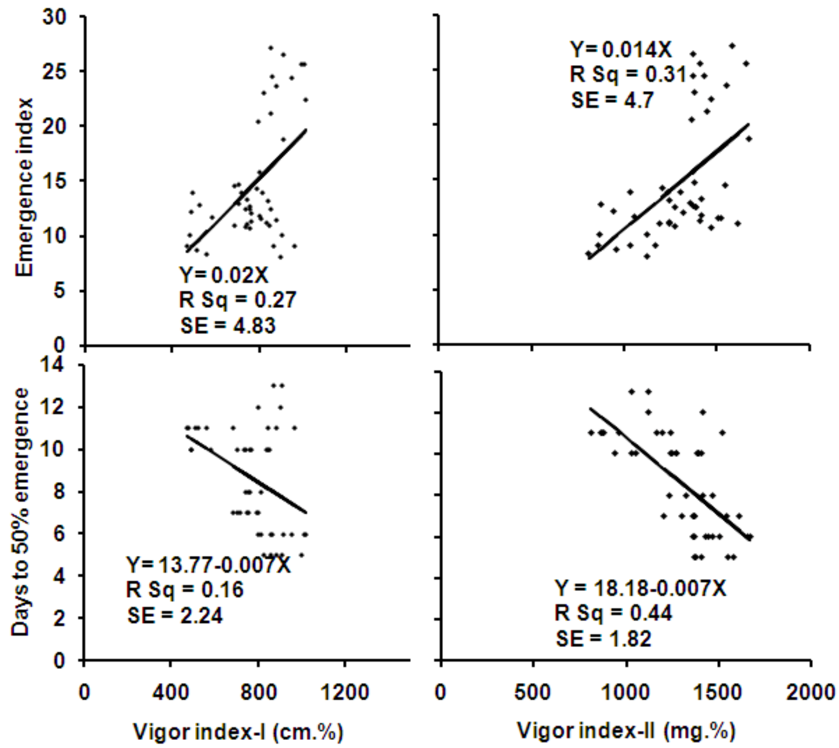

Figure 1. Relationship between seedling emergence with vigor index I and vigor index-II of wooly pod vetch seeds.

vigor index I and II. Germination rate exhibited positive relationship with emergence index and opposite association with days to 50% seedling emergence (Figure 2). Germination rate explained about 77% of variations in emergence index and 61% of variations in days to 50% emergence, with standard errors of 2.69 and 1.52, respectively.

Both vigor index I and II had exhibited a positive linear relationship with seedling emergence index while their associations were negative with days to 50% emergence (Figure 3). Vigor index I explained 27% of variation in emergence index while 31% of variation in the same trait was explained by vigor index II. Vigor index II had also explained bout 44 of variation in days to 50% emergence with a standard error of 1.82 (Figure 3).

DISCUSSION

There was a positive relationship of seed age and seed germination and vigor of wooly pod vetch up to one year of storage. Increase in germination percentage and seedling vigor in older seeds is due to gradual decrease in the proportion of hard seeds through storage time. Such a gradual decrease in the proportion of hard seeds by storage time and an increase in germination in older seeds of alfalfa were also reported by other workers (Čupić et al., 2005).

Improvement in germination traits of wooly pod vetch seeds due to priming is attributed to removal of inhibitory substances in seed coats through hydro-priming or enhanced release of ethylene within embryonic tissues in the presence of imbibed NO_3^{-1} ion (Benech-Arnold et al., 2000; Varier et al., 2010). Better seedling vigor traits of primed seeds is a combined effect of better status of nutrient reserves in freshly harvested seeds (Murthy et al., 2003), increased seed nitrate level (Benech-Arnold et al., 2000), enhanced permeability of loosened seed coat to water and oxygen (Baskin et al., 2000), and earlier nutrient mobilization (Bewley, 1997).

Mohammadi (2009) reported a significant improvement in seedling dry weight of soybean seeds primed with potassium nitrate. In this study, priming freshly harvested seeds with 0.620 M KNO_3 improved seedling dry weight by about 50 mg and vigor index-II by about 1136% mg over the unprimed control (Tables 4 and 5). Continuous increase in seedling dry weight with KNO_3 concentration is attributed to the positive influence of increased seed nitrate level (Benech-Arnold et al., 2000).

The reason for increase in emergence index and decreased in time for 50% emergence is due to faster seed germination as a result of loosening of seed coats through storage (Čupić et al., 2005), and washing out of inhibitory substances in the seed coat during priming (Leadem, 1997; Baskin et al., 2000; Moïse et al., 2005).

In conclusion, the present study has indicated that seed priming improves seed germination and seedling vigor traits of wooly pod vetch. Hydro-priming is more economical for older seeds while osmo-priming at 0.155 M KNO_3 is recommendable for freshly harvested wooly pod vetch seeds. Moreover, storing wooly pod vetch seeds before sowing is helpful to reduce hard seed (dormancy) proportion. The greater germination due to priming may be statistically significant, but this can be solved by small increases in seeding densities. In contrast, reductions in dormancy appear to result in large decreases in the remnant seed that can be weedy in subsequent years.

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