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Genetic variation and heritability for germination, seed vigour and field emergence in brown and yellow-seeded genotypes of flax

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Abstract

Genetic improvement of seed vigour and emergence of yellow-seeded flax (*Linum usitatissimum* L.) is important to have a good stand establishment, since they are negatively affected by yellow seed colour. This study was conducted to investigate genetic variation, broad-sense heritability and expected genetic response to selection for germination, seed vigour and field emergence. Sixteen genotypes of yellow-seeded flax and 17 of brown-seeded were evaluated for germination at 5°C, 15°C and vigour test (cold test) and also for field emergence in 2005 and 2006. Genetic variation for vigour test and field emergence in yellow-seeded genotypes was much higher than brown-seeded, as shown by their genetic coefficients of variation of 41% *vs.* 17.9% in vigour test, 50.9% *vs.* 3.8% and 34.1% *vs.* 18% for field emergence in 2005 and 2006, respectively. Germination of yellow and brown-seeded genotypes in vigour test varied from 10.7 to 55.3% and 40.7 to 88.7%, respectively. Field emergence ranged from 20 to 217.3 and 35 to 184.3 seedlings/m^2 among yellow-seeded genotypes in 2005 and 2006; however, the corresponding range for brown-seeded genotypes was 266 to 414 and 93.7 to 210 seedlings/m^2. Broad-sense heritability for germination at 5°C and vigour test, days to 50% emergence and seed weight were high (65.9 to 98.4%). The heritabilities for field emergence were low to moderate for brown seeds (6.7% and 51.6%), and moderate to high (56.2 % to 96.8%) for yellow seeds in 2005 and 2006. High estimates of genetic response to selection for seed vigour and field emergence were found in yellow-seeded genotypes, indicating the possibility of genetic improvement of these traits.

Keywords: Heritability; Seed Vigour; Emergence; Seed Colour; Flax

Introduction

High yielding and successful flax production require rapid establishment of uniform plant stands under different environmental conditions. Early planting of field crops confers numerous advantages such as providing suitable temperature conditions at flowering and grain filling, early maturity and disease, pest, and drought avoidance (Tiryaki and Andrews, 2001), however, it may expose the seeds to unfavorable conditions such as low soil temperature, high soil moisture conditions, microbial activity and result in a poor stand in the field. It is expected that high vigour seeds facilitate early seeding (Maluf and Tigchelaar, 1982) and emerge in a wide range of stress and non-stress conditions such as low temperature (AOSA, 1983), since seed vigour has been defined by the Association of Official Seed Analysts (AOSA, 1983) as "those properties which determined the potential of the seed for rapid, uniform emergence, and the development of normal seedlings under a wide range of field conditions.

Temperature is one of the major environmental factors affecting seed germination and seedling emergence in flax (Saeidi and Rowland, 1999a; O'Connor and Gusta, 1994). Yellow seed colour which can be used to visually distinguish the edible-oil flax cultivars from those of brown industrial oil type (Saeidi and Rowland, 1999a) can also negatively affect on seed vigour and emergence of flaxseed (Saeidi and Rowland, 2000, 1999a, 1999b). The soil microorganisms or other soil constituents and/or their interaction with other environmental factors such as low temperature can decrease emergence of yellow-seeded flax (Saeidi and Rowland 2000, 1999b) and it was shown that lower seed yield in yellow-seeded genotypes was associated with lower emergence and poor stands (Reitz et al., 1947).

The strong association between seed colour and each of seed vigour and emergence in flax emphasizes the need to screen and select yellow seeded breeding lines for higher seed vigour and emergence to ensure good stand establishment in the field (Saeidi and Rowland 2000, 1999a, 1999b). Genetic improvement of germination and seedling vigour at low temperature has been successful in other crops such as maize, *Zea mays* L. (McConnell and Gardner, 1979) and alfalfa, *Medica sativa* L. (Klos and Brummer, 2000a, 2000b).

Since, genetic improvement of seed vigour and field emergence, particularly in yellow-seeded cultivars is important in developing new cultivars, the objective of this work was to investigate the genetic variation, to estimate the broad-sense heritability and expected gain from selection for germination at low temperature, seed vigour and field emergence in brown and yellow-seeded genotypes of flax.

Materials and Methods

Laboratory Experiments

Seventeen genotypes of brown and 16 genotypes of yellow-seeded flax were evaluated in germination tests at 5°C and 15°C, and in vigour test. The genotypes were randomly selected from F2.4 populations derived from different crosses between yellow and brown-seeded genotypes to provide near-isogenic populations (Burton, 1966) for seed colour. A randomized complete block design with three replications was used for each experiment in which the experimental unit consisted of 50 seeds in a Petri dish.

In germination tests, the seeds were placed in Petri dishes on two filter papers and incubated in incubators in which the temperature was set at 5°C and 15°C, with an 18-h photoperiod. During the experiment, the seeds moistened once every 2-3 d with distilled water to keep them continuously damp. After 7 and 26 days, germinated seeds (at the dicotyledon stage) were counted at temperature of 15°C and 5°C, respectively and then percentage of germination was calculated.

In vigour test, the seeds were placed in Petri dishes on two filter papers and then lightly sprinkled with screened soil from the field, thus that the seeds could be still visible. The seed were incubated in an incubator with an 18-h photoperiod, for 7 d at 5°C following by 4 d at 20°C (Saeidi and Rowland, 1999a). At the end of experiment, the percentage of germinated seeds (at the dicotyledon stage) was calculated for each experimental unit as an index of seed vigour.

Field Experiment

The field experiments employing a randomized complete block design with three replications were conducted in 2005 and 2006 at the Isfahan University of Technology Research Farm to evaluate field emergence of the same flax genotypes used in germination and vigour tests. Two row plots, each 3m in length and 30cm apart were used in the experiments. Based on the seed weight of the genotypes, approximately the same number of seeds planted for the genotypes (approximately 153 seeds per meter in each row). The seeds were not treated with any fungicides or insecticides. The experiments were planted on 21 and 12 April in 2005 and 2006, respectively. Each plot was seeded by hand at the depth of 2-3 cm on a Silty Clay Loam soil.

Number of days from seeding to initial emergence and 50% emergence for each plot was determined visually as an index for speed of emergence. When almost all the viable seeds were germinated and the seedlings were approximately 5-7 cm length, the number of seedlings in each plot was used to calculate the number of seedlings per square meter.

Data Analysis

Analysis of variance were performed to examine differences among the genotypes and to estimate the variance components, using the general linear model (GLM) of SAS (SAS Institute, Inc. 1989). The variance components, phenotypic and genetic coefficient of variation and correlation, broad-sense heritability and expected gain from selection were computed as suggested by Burton and DeVane (1953) and Miller et al. (1958).

Results and Discussion

No significant difference was found between germination percentage of brown and yellow seeds at 5°C and 15°C; however, yellow-seeded genotypes were significantly later in emergence and had significantly ($P<0.01$) lower vigour and field emergence (Tables 1 and 3). These results were in agreement with those obtained by the others (Saeidi and Rowland, 1999a, 1999b; O'Connor and Gusta, 1994; Culbertson et al., 1960). Phenolic acids (tannins) which are found as pigments in the seed coat of brown seed, but are nearly absent in yellow seed of flax (Freeman, 1995) have antimicrobial properties (Scalbert, 1991) and are most likely the source of the improved seed vigour and emergence in the brown-seeded flax (Saeidi and Rowland, 2000).

Highly significant differences were observed among the genotypes of both brown and yellow-seeded flax for germination at temperature 5°C and vigour test, and also for days to emergence and number of seedlings per m^2 (Table 1). Germination for yellow seeded

Table 1. Range, mean, standard error of mean (SE), coefficient of variation (CV), and *F* values of genotypes for different traits in yellow and brown-seeded genotypes of flax

Trait	Seed Colour	Range	Mean	SE	CV (%)	*F* values of genotypes	#of lines > mean[a]
Germination at 5° C (%)	Yellow	27.3-85.3	48.2a	1.45	20.8	11.27**	5
	Brown	12 -90	56.2a	1.63	20.7	13.65**	11
Germination at 15°C (%)	Yellow	80.7-96.0	88.5a	0.70	5.5	1.92 ns	10
	Brown	81.3-98.0	90.8a	0.72	5.7	3.77**	10
Germination in vigour test (%)	Yellow	10.7-55.3	33.4b	0.54	11.3	40.69**	10
	Brown	40.7-88.7	72.3a	0.40	3.9	62.68**	10
Field emergence in 2005 (Seedlings/m^2)	Yellow	20.0-217.3	110.0b	2.56	16.1	31.34**	8
	Brown	266-414	337.3a	11.33	24.0	1.07 ns	8
Field emergence in 2006 (Seedlings/m^2)	Yellow	35.0-184.3	93.9b	7.52	55.5	2.28*	5
	Brown	93.7-210.0	128.9a	5.44	30.1	2.06*	8
Days to early mergence in 2005	Yellow	6.3-10.0	7.79a	0.12	10.7	4.22**	6
	Brown	8.0-9.0	8.10a	0.05	4.6	1.50 ns	5
Days to early mergence in 2006	Yellow	10-15	12.3a	0.22	12.5	3.94**	8
	Brown	14-18	10.4b	0.18	12.2	5.61**	9
Days to 50 % emergence in 2005	Yellow	11-20	14.6a	0.12	5.7	22.7**	6
	Brown	11-14	11.4b	0.04	2.2	43.4**	3
Days to 50 % emergence in 2006	Yellow	16-22	19.7a	0.19	6.8	4.80**	9
	Brown	14.7-20.3	17.5b	0.23	9.2	2.93**	7
100-Seed weight (g)	Yellow	0.252-0.332	0.302a	0.0004	7.3	7.96**	9
	Brown	0.250-0.355	0.290a	0.0002	5.6	8.00**	8

[a] the number of genotypes with higher mean than the mean of all genotypes.
For each trait, the means followed by the same letter were not significantly different, using the orthogonal contrasts.

genotypes at temperature 5°C and vigour test varied from 27.3 to 85.3% and 10.7 to 55.3%, respectively, while the corresponding ranges for brown-seeded genotypes were 12 to 90% and 40.7 to 88.7%. A wide range of variation for germination at 5°C, field emergence and days to emergence was observed among yellow and brown-seeded genotypes; however, the variation for germination at 15°C was low in both seed colour types (Table 1).

The phenotypic (PCV) and genetic (GCV) coefficients of variation were high for germination at 5°C and vigour test and also for field emergence (Table 2). However, these coefficients were considerably higher in yellow-seeded genotypes than that of brown-seeded for germination in vigour test and field emergence. The differences between the PCV and GCV coefficients were relatively small (Table 2). These results indicated that there was high genetic variation for germination at low temperature, seed vigour and field emergence, particularly in yellow-seeded genotypes and possibility of genetic improvement of these traits by appropriate selection programs (Falconer and Mackay, 1996). In present study, genetic variability was also observed for days to early emergence and 50% emergence, and seed weight in both brown and yellow-seeded genotypes (Tables 1 and 2). Observation of genetic variability in this study agreed with the results of the others who found significant difference among flax genotypes for germination and emergence at low temperature (Saeidi and Rowland, 1999a; O'Connor and Gusta, 1994), seed vigour (Saeidi and Rowland, 1999a) and seedling vigour (Gupta and Basak, 1983), emergence (Saeidi and Rowland, 1999b) and speed of emergence (O'Connor and Gusta, 1994).

Table 2. Estimated variance components, phenotypic (PCV) and genetic (GCV) coefficient of variation, broadsense heritability (H %), and expected gain from selection (GR) and expected gain from selection as a present of mean (GRm) for different traits in yellow and brown-seeded genotypes of flax

Trait	Seed colour	Variance component			PCV (%)	GCV (%)	H (%)	GR	GRm
		σ^2_{Ph}	σ^2_{G}	σ^2 e/r					
Germination at 5°C (%)	Yellow	378.5	344.9	33.6	40.4	38.5	91.1	36.5	75.7
	Brown	618.3	573.0	45.3	44.2	42.6	92.7	47.5	84.5
Germination at 15°C (%)	Yellow	15.0	7.2	7.8	4.4	3.0	48.0	3.8	4.3
	Brown	33.6	24.7	8.9	6.4	5.5	73.5	8.8	9.7
Germination in vigour test (%)	Yellow	191.9	187.2	4.7	41.5	41.0	97.6	27.9	83.5
	Brown	169.3	166.6	2.7	18.0	17.9	98.4	26.4	36.5
Field emergence in 2005 (Seedlings/m^2)	Yellow	3238.2	3134.9	103.3	51.7	50.9	96.8	113.5	103.2
	Brown	2511.3	168.3	2343	14.9	3.8	6.7	6.9	2.1
Field emergence in 2006 (Seedlings/m^2)	Yellow	2068.2	1162.4	905.8	45.5	34.1	56.2	52.7	52.8
	Brown	1038.6	535.9	502.7	25.0	18.0	51.6	34.3	26.7
Days to early mergence in 2005	Yellow	0.98	0.75	0.23	12.7	11.1	76.5	1.6	20.5
	Brown	0.070	0.023	0.047	3.3	1.9	33.2	0.2	2.5
Days to early mergence in 2006	Yellow	3.11	2.32	0.79	14.3	12.4	74.6	2.7	22.0
	Brown	3.01	2.47	0.54	16.6	15.1	82.1	2.9	27.8
Days to 50 % emergence in 2005	Yellow	5.213	4.983	0.230	15.6	15.3	95.6	4.50	30.1
	Brown	0.885	0.865	0.020	8.3	8.2	97.7	1.89	16.6
Days to 50 % emergence in 2006	Yellow	2.88	2.28	0.60	8.6	7.7	79.2	2.8	14.7
	Brown	2.55	1.68	0.87	9.1	7.4	65.9	2.2	12.7
100-Seed weight (g)	Yellow	0.0007	0.0005	0.0002	8.3	7.2	75.0	0.04	13.2
	Brown	0.0008	0.0007	0.0001	9.8	9.2	88.9	0.05	17.3

Estimated broad-sense heritability (H) in yellow-seeded genotypes varied from 48% (for germination at 15°C) to 97.6% (for germination in vigour test) (Table2). In brown seeded genotypes, the heritability ranged from 51.6% (for field emergence) to 98.4% (for germination in vigour test). The estimated heritabilities for germination at 5°C and vigour test were considerably high (91.1% to 98.4%) and for field emergence were low to high (6.7% and 51.6 % for brown-seeded and 96.8% and 56.2% for yellow-seeded genotypes in 2005 and 2006, respectively). Based upon combined analysis of data over years, the estimated heritabilities for field emergence were 46% and 77% in yellow and brown-seeded genotypes, respectively (Table 3). High estimates of broad-sense heritabilities for germination percentage and seedling characters of flax have also been reported by Gupta and Basak (1983). High heritability indicates the more importance of genetic factors in controlling a trait and possibility of its improvement by appropriate selection program. Genetic variation and high heritability are the main requirements of genetic gain from selection (Falconer and Mackay, 1996).

The expected gain from selection (GR) and GRm (GR as percent of mean population) for capability of germination at low temperature (5°C) for brown seeds was higher than that of yellow seeds (Table 2), however, for field emergence, the GR and GRm was much

Table 3. Range, mean, standard error of mean (SE), coefficient of variation (CV) over years, mean of squares of genotype (G) and genotype by year interaction (G×Y), broad sense heritability (H%) and expected gain from selection as a present of mean (GRm) for field emergence (seedlings/m^2), days to early emergence and 50% emergence for yellow and brown-seeded genotypes of flax

Trait	Seed Colour	Range	Mean	SE	CV (%)	Mean squares of genotypes	Mean squares of G×Y	H (%)	GRm
Field emergence	Yellow	57.7-200.8	105.0b	3.96	37.0	10360ns	5559**	46	37.8
	Brown	193.3-399.2	233.1a	6.50	28.0	8078*	2570^{ns}	77	23.5
Days to early emergence	Yellow	8.5-11.8	10.06a	0.13	12.3	5.53ns	6.73**	-	-
	Brown	8.0-11.5	9.28b	0.09	10.1	5.37ns	3.87**	30	5.8
Days to 50% emergence	Yellow	15.5-20.5	17.2a	0.11	6.5	12.43ns	11.85**	4.7	0.81
	Brown	12.8-16.7	14.47b	0.11	8.0	6.15ns	4.18**	32	4.6

For each trait, the means followed by the same letter were not significantly different, using the orthogonal contrast

higher for yellow seeds (Tables 2 and 3). For germination in vigour test, GRm was much higher for yellow seeds than brown seeds (83.5 *vs.* 36.5). These results imply that a large genetic gain is expected by selection for germination at both low temperature and vigour test as well as for field emergence, particularly in yellow-seeded flax genotypes. A high expected genetic gain from selection was also reported for germination and emergence by Cisse and Ejeta (2003) in another crop species. High expected gains from selection (GR and GRm) for days to early emergence and days to 50% emergences (Tables 2 and 3) show that these traits can be effectively improved by selection. Klos and Brummer (2000a) also found that selection for genetic improvement of rapid germination at low temperature has been successful in alfalfa.

In conclusion, the results of this study indicated that yellow seeds were significantly and obviously distinguished from the brown seeds because of their lower germination in vigour test and field emergence, with no significant difference in germination test. Based on these results and findings of the others (O'Connor and Gusta, 1994; Forsyth and Vogel, 1945;), it seems that germination tests under aseptic conditions may not provide useful information to predict field emergence in flax; however, a simple laboratory method like vigour test (cold test) which provides stress conditions from both soil-borne microorganisms and low temperature may be more capable for screening a large number of genotypes in flax breeding programs, particularly in yellow-seeded flax. Genetic variation, high estimates of heritabilities and gain from selection for germination at low temperature (5°C), seed vigour, field emergence and speed of emergence indicates that the attempts to genetically improve these seed quality traits should be successful and optimistic in breeding programs.

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