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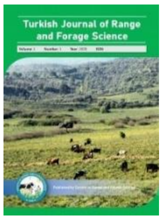

Seed Dormancy and Germination in F_5 Strains from *Vicia sativa* subsp. *sativa* x *Vicia sativa* subsp. *macrocarpa*

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A B S T R A C T

The objectives of this study were to determine the seed dormancy and germination characteristics of F_5 strains from common vetch x big seeded vetch hybrids. White flowered common vetch accessions and red-flowered accessions were crossed and a total 24 high forage and seed yielding strains were developed from F_5 generation of different hybrid combinations. Standard germination tests were performed at a temperature of 20°C, without light, for 14 days. The seeds were subjected to (a) no treatment (control); (b) chilling; (c) mechanical scarification; and (d) mechanical scarification + chilling. The first counts were taken on day-5 and the final counts were made on day-14.

Hard seed percentages were very low in the tested common vetch cultivars and strains. Chilling slightly increased the germination rate in some accessions. Big seeded vetch seeds showed very high hard seed contents with the germination percentage of Ericek strain were only 5-15% and almost nil in ICARDA-5283. Big seeded vetch seeds required scarification + prechilling treatments to overcome seed dormancy. The strains differed in the persistence of hard seed in all hybrid combinations. Untreated control seeds of some strains had very high germination rates. Contrarily, some hybrid seeds required prechilling and/or scarification treatments to induce germination.

1. Introduction

Vicia sativa L. is a genetically and phenotypically variable genus comprised of several subspecies, and known as a *Vicia sativa* complex. Common vetch (*Vicia sativa* subsp. *sativa* L.) is widespread around many parts of the world.

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It is commonly grown winter cover crops, or green manure, and is also used as past pasture, silage, and hay (Seymour et al., 2002; Uzun et al., 2011). Big seeded vetch (subsp. macrocarpa) is late maturing vigorous subspecies with limited agronomic uses as a fodder. It has a small number of pods per plant, but it seeds are very large (Berger et al., 2002; Van de Wouw et al., 2003). Its hard seed coat and physiological dormancy did not allow high germination during the one-year period after harvest (Uzun et al., 2013). Shattering is also a major problem in seed production of big seeded vetch. Most species of *Vicia* have an impermeable seed coat that imposes a physical exogenous dormancy (Elkins et al., 1966; Mosjidis and Zhang, 1995; Ortega-Olivencia and Devesa, 1997). In our previous studies, the *Vicia sativa* subsp. *macrocarpa* seeds had very low final germination rates (2-4%) throughout the 12 months periods in two experimental years. Mechanical scarification did not enhance the germination and chilling slightly increased. The germination rates maximized 74% in the seeds subjected to both scarification and chilling treatments (Uzun et al., 2013). Mechanical scarification improved the germination rate in several *Medicago* and *Trifolium* species (Aydin and Uzun, 2001; Can et al., 2009; Khaef et al., 2011).

Hard seeds have a survival advantage than soft seeds, but it causes problems in the short term rotations. Hard seed content results in poor stand establishment because of reduced germination and non-uniformed seedling emergence. Therefore, hard seeds require dormancy-breaking treatments before using in short term crop rotations for successful stand establishment. The treatments to break the seed dormancy are tedious and time-consuming applications. This problem is overcome by developing the cultivars which have a very high percentage of soft seeds. Contrarily, hard seeded annual Medicago and Trifolium species have superior self-reseeding characteristics in the dryland rangelands. The plants can regenerate in later years from residual hard seeds. Hard seeded vetches with pod-shattering traits may re-establish themselves by natural reseeding. Limited information is available in the published literature about the seed dormancy characteristics of common vetch x big seeded vetch hybrids. This present study was conducted to assess the dormancy characteristics of selected high yielding strains *of Vicia sativa* subsp. *sativa* x *Vicia sativa* subsp. *macrocarpa* hybrids, and to determine the effects of some pretreatments on the release of seed dormancy.

2. Materials and Method

Two white flowered common vetch (*Vicia sativa subsp. sativa* L.) accessions (W-1 and Soner) and two red-flowered big seeded vetch (*Vicia sativa* subsp. *macrocarpa* (Moris) Archang.) accessions (Ericek and ICARDA-5283) were crossed in 2009-2010 growing season under greenhouse conditions. White-flowered sativa accessions were used as a female and purple-flowered macrocarpa accessions were used as a male in the hybridization studies. Purple flower color is completely dominant to white (Donnelly and Clark, 1962; Chowdhury et al., 2004). Therefore, hybrid plants were easily detected by purple flowers during the flowering stage. F1 and F2 plants were grown in the greenhouse conditions. Selections were started in F3 generation in field conditions and continued in F4 and F5 generations for high seed and forage yields. The research material of this study consisted of the 13 strains from W-1 x Ericek hybrid, 9 strains from W-1 x Soner hybrid and 2 strains from W-1 x ICARDA 5283 hybrid selected for high seed and forage yields. The fall-seeded strains were grown under rain-fed conditions of Uludag University research plots in Bursa, Turkey. No fertilizers or chemicals were applied. The seeds of all strains were harvested in June 2016, threshed and cleaned by hand. The seeds were stored in paper bags at room temperature (20-21°C) during the experimental period.

Germination studies of each hybrid combination were done separately in the October – December period of 2016. Standard germination tests (ISTA, 2007) were performed using a completely randomized block design. Two replications of 50 seeds for each treatment were placed on blotter paper in 9-cm-diameter Petri dishes in a germination chamber at a temperature of 20°C, without light, for 14 days. First counts were taken on day-5 and final counts were made on day-14. The seeds were subjected to (a) no treatment (control); (b) chilling; (c) mechanical scarification; and (d) mechanical scarification + chilling, as applied in our previous study (Uzun et al., 2013).

For each test, analysis of variance (ANOVA) was performed separately with the statistical package JMP 5.0.1 (SAS, 1989-2002). Statistically significant differences among the mean values were determined with the least significant difference (LSD) test at the 0.05 level.

3. Results and Discussion

According to analysis of variance, genotypes, treatments and genotype x treatments were statistically significant at 0.01 level in all tests. The results of analysis of variance and LSD values of the treatments were summarized in Table 1.

In close agreement with previous studies (Sattell et al., 1998; Samarah et al., 2004; Uzun et al., 2013), hard seed percentages were very low in tested common vetch cultivars and strains. Particularly W-1 strain showed very high germination (95 – 100%) in the tests. Germination rate was 79% in untreated normal seeds and reach 99% after chilling period in cv. Soner seeds. Big seeded vetch was very hard in all tests. The germination percentage of Ericek strain at 14 days after planting was only 5-15% in the two tests. No germination was obtained in ICARDA-5283 normal seeds. This finding was consistent with our previous study (Uzun et al., 2013). The scarification treatment did not improve seed germination in both stains of big seeded vetch. The chilled seeds of big seeded vetch germinated to a higher percentage, 47-57% in Ericek strain and 47% in ICARDA-5283, at 14 days. If scarified seeds are subsequently subjected to the chilling period, the final germination rates were reached

82-92% in Ericek and 75% in ICARDA-5283. This result clearly showed that dormancy cannot be broken by scarification or chilling treatment only, but big seeded vetch seeds required scarification + prechilling treatments to overcome seed dormancy largely (Table 2, 3, 4).

In W-1 x Ericek hybrids, untreated control seeds of several strains (6a1, 6b, 6c, and 8a1) had more than 75% germination at after 5 days and 89% at after 14 days. The other strains had very high percentage of hard seeds, some of the hybrid strains (1, 10, 11, 2a1, 2b, 5b1 and 5b2) showed less than 50% germination at after 14 days. After scarification, 84-97% of the seed germinated, and after scarification + chilling nearly all seeds germinated in those strains (Table 2).

Table 1. Results of variance analysis of germination speed and rates with lsd values in the tests

Source	df	Germination speed		Germination rate	
		F values	Lsd (0.05)	F values	Lsd (0.05)
W-1 x Ericek hybrids					
Genotypes (G)	14	**	8.52	**	6.94
Blocks (B)	3	**	4.40	**	3.58
G x B Interaction	42	**	17.04	**	13.88
Soner x Ericek Hybrids					
Genotypes (G)	10	**	10.53	**	9.34
Blocks (B)	3	**	6.35	**	5.63
G x B Interaction	30	**	21.06	**	18.68
W-1 x ICARDA-5283 Hybrids					
Genotypes (G)	3	**	10.3	**	9.2
Blocks (B)	3	**	10.2	**	9.3
G x B Interaction	9	**	20.4	**	18.3

df: degree of freedom, **: F-test significant at 0.01 level,

Table 2. Germination speed (after five days) and germination rates (after 14 days) of common vetch W-1 and big vetch Ericek hybrids (%).

Genotypes	Germination speed (%)					Germination rate (%)				
	N*	S	C	S + C	Average	N	S	C	S + C	Average
1	6	59	11	86	40.5 d**	40	84	32	91	61.8 ef
10	13	44	17	90	41.0 d	27	78	32	95	58.0 efg
11	36	89	16	96	59.3 c	44	97	18	98	64.3 e
2a1	4	48	9	84	36.3 de	13	80	13	94	50.0 h
2b	5	41	2	65	28.3 e	27	87	17	80	52.8 gh
5a	55	72	70	77	68.3 b	74	77	77	86	78.5 d
5b1	12	77	84	94	66.8 bc	24	91	86	94	73.8 d
5b2	7	26	6	92	32.8 de	35	84	15	95	57.3 fg
6a1	86	99	92	93	92.5 a	96	99	95	100	97.5 a
6b	75	85	86	99	86.3 b	97	99	97	100	98.3 a
6c	84	80	83	92	84.8 a	90	90	88	94	90.5 bc
8a1	90	89	73	97	87.3 a	90	95	86	98	92.3 abc
8b1	78	72	72	74	74.0 b	89	92	76	87	86.0 c
W-1	82	86	91	91	87.5 a	95	93	97	94	94.8 ab
Ericek	2	8	23	84	29.3 e	15	21	57	92	46.3h
Average	42.3 D	65.9 B	48.0 C	87.6 A		57.1 C	86.7 B	56.7 C	93.2 A	

*: N: untreated control, S: scarification, C: chilling, S+C: scarification + chilling

**: The percentages within germination speed and rates that are followed by the same letter are not significantly different at the 0.05 level using the LSD test.

Table 3. Germination speed (after five days) and germination rates (after 14 days) of common vetch Soner and big vetch Ericek hybrids (%).

Genotypes	Germination speed (%)					Germination rate (%)				
	N*	S	C	S + C	Average	N	S	C	S + C	Average
1	16	30	48	72	41.5 fg**	33	40	70	79	55.5 ef
2b	24	32	66	86	52.0 def	33	51	83	92	61.8 cde
2c	13	28	44	69	38.5 g	23	44	50	70	48.8 f
2d1	28	35	53	94	52.5 de	51	50	79	98	69.5 bc
3a	39	50	93	95	69.3 ab	47	55	97	96	73.8 bc
3b	39	32	81	90	60.5 bcd	47	40	88	91	66.5 cd
3c	58	47	79	81	66.3 abc	74	59	89	88	77.5 b
3d	23	17	93	63	49 efg	44	27	66	98	58.8 de
4b	25	39	86	74	56 cde	44	46	96	80	66.5 cd
Soner	45	69	94	98	76.5 a	79	89	99	99	91.5 a
Ericek	1	1	22	69	23.5 h	5	2	47	82	34.0 g
Average	28.3 C	34.6 C	69.0 B	81.0 A		43.6 B	45.7 B	81.5 A	85.5 A	

*: N: untreated control, S: scarification, C: chilling, S+C: scarification + chilling

**: The percentages within germination speed and rates that are followed by the same letter are not significantly different at the 0.05 level using the LSD test.

Untreated normal seeds of most strains in Soner x Ericek hybrids showed very low germination speed and germination rates (Table 3). No soft seeded strain was detected in this hybrid combination. Chilling treatments significantly increased germination. Some strains (3a, 3b and 4b) had more than 80% germination after chilling treatments at after 5 days and more than 90% after 14 days. Scarification + chilling treatment increased the germination rate slightly in some strains but there was no significant difference between the average values of two treatments.

Scarification did not affect the germination rate of big seeded vetch strain ICARDA-5283 with completely no germination (Table 4). Chilling treatment and scarification + chilling treatment resulted in final germination rate of 47% and 75%, respectively. Germination speed and rates of the hybrids were intermediate between the two parents. Chilling treatment alone and scarification + chilling treatment showed the same final germination rate in the hybrids (91 and 95%).

The results of these experiments showed that seeds the hybrid strains of all combinations exhibited different levels of dormancy. Some strains produced more than 79% soft seeds. The normal seeds of some strains had very low germination rates. Germination pretreatments to break hard-seed dormancy in *Vicia sativa* subsp. *macrocarpa* parents and hybrid strains significantly improved germination. In general, the germination speed and rate of the scarified seeds increased compared to the untreated normal seeds but were lower than the germination of the scarified + chilled seeds. This indicated that dormancy was not imposed only by the impermeable seed coat.

Table 4. Germination speed (after five days) and germination rates (after 14 days) of common vetch W-1 and big vetch ICARDA 5283 hybrids (%).

Genotypes	Germination speed (%)					Germination rate (%)				
	N*	S	C	S + C	Average	N	S	C	S + C	Average
1	7	23	73	72	43.8 b**	28	28	79	91	56.5 b
2	21	26	58	87	48.0 b	28	33	84	95	60.0 b
W-1	94	84	81	89	87.0 a	100	90	96	88	93.5 a
5283	0	0	9	63	18.0 c	0	0	47	75	30.5 c
Average	30.5 C	33.3 C	55.3 B	77.8 A		39.0 B	37.8 B	79.5 A	84.3 A	

*: N: untreated control, S: scarification, C: chilling, S+C: scarification + chilling

**: The percentages within germination speed and rates that are followed by the same letter are not significantly different at the 0.05 level using the LSD test.

As indicated in our previous study (Uzun et al., 2013), the seeds of *Vicia sativa* subsp. *macrocarpa* parents and some hybrid strains possessed physiological dormancy rather than just physical dormancy, scarification alone did not allow high germination. The combined scarification and chilling treatment was a suitable method to release the seeds from dormancy.

The experiments clearly showed that both soft seeded and hard seeded strains can be developed from *Vicia sativa* subsp. *sativa* x *Vicia sativa* subsp. *macrocarpa* hybrids. The hybrids combined with the high hay and seed yields with the soft seed are suitable for use in the crop rotations, without the risk of a weed problem in the following crops. Contrarily, several strains in this study had very high percentage of hard seeds and some of them showed severe pod-shattering during seed harvest. Those characteristics permit natural reseeding and persist continuously in the pastures grazed properly. It is well known that self-regenerating and hard seeded annual legume species are widely grown in dryland pastures in some parts of Australia and New Zealand. Subterranean clover (*Trifolium subterraneum* L.) and annual medics (*Medicago* spp.) are the most successful species in those regions. However, new species could be considered in the future to overcome the constraints of existing species (Nichols, et al., 2012). Most cultivated *Vicia* species used as forage crops are not suitable for self-regenerating pastures. However, Christiansen et al. (1996) indicated that the hard seeded subterranean vetch (*Vicia sativa* ssp. *amphicarpa*) compares favorably with the annual *Medicago* spp. in most respects, and it has great potential for pasture improvement in dry areas. High hard seed content and pod shattering characteristics of *Vicia sativa* subsp. *sativa* x *Vicia sativa* subsp. *macrocarpa* hybrids make them suitable for self-regenerating pasture systems in those regions. Certainly, further breeding and selection activities will be included greater hardseededness, pod shattering and high forage yielding for pastures of low-rainfall regions, and tested under grazing conditions.

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