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CHANGES IN HARDSEEDEDNESS AND OTHER CHARACTERISTICS OF *TRIFOLIUM RESUPINATUM* VAR. *TYPICUM* FIORI ET PAOL. (*FABACEAE*) SEEDS STORED IN UNCONTROLLED CONDITIONS

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SUMMARY

This research was carried out at Analyses Laboratory of field Crops Department in Agricultural Faculty, Namik Kemal University, Tekirdag, Turkey. The germination experiments, which were set up as completely randomized blocks with three replicates, were performed from seed harvest to end in 15 day intervals. In the experiment, the hardseededness (%), dead seed (%), germination speed (%) and vigor (%) ratios in Trifolium resupinatum var. typicum Fiori et Paol. which has different seed color such as yellow, red, green and mixed were determined and their variations in a year were examined. It was determined that seed color and time after harvest could affect all characteristics found out. While the highest hardseededness was determining green seeds (68.4%), the highest dead seed ratio was 2.4% in red seeds. The germination speed was 31.0% in yellow seed and their vigor was found high (38.9%). While the highest hard seed ratio was found in 10^{th} (94.3%) germination period, the highest dead seed ratio was 3.1% in 18^{th} germination period. The highest germination speed and vigor were counted in 21^{st} – 26^{th} period and the lowest values were obtained from 10^{th} period.

Key-words: germination, hardseededness, seed, Trifolium resupinatum L.

INTRODUCTION

High quality seed is the basis of higher agricultural productivity. Quality in seeds embraces all the physical, biological, pathological, and genetic attributes which contribute to the final yield of a crop. Such seeds are specifically bred and genetically pure; they are free from disease, vigorous, and high in germination percentage (Basra, 1995).

Germination as used here includes the imbibitions of water and the rupture of the seed coat by the extruding radicle as proposed by Black (1959). The inability of a viable seed to germinate under conditions normally considered favorable for the purpose, namely requisite, water, temperature, and oxygen is termed dormancy. The two major classes of dormancy are seed coat-imposed dormancy and embryo dormancy (Basu, 1995). Hardseededness may prevent germination by preventing water uptake [for example, alfalfa (*Medicago* sp.) and clover (*Trifolium* sp.) species] or gas exchange [for example, coffee (*Gymnocladus* sp.) and ash tree (*Fraxinus* sp.) species], mechanically restraining the growth of the embryo, chemically inhibiting germination, or acting as a barrier to photoreception (Basu, 1995; Soya and Geren, 1999; Tekeli and Ates, 2006a). This causes hardseededness so that germination of a seedlot may extend over months or years. Hardseededness can also occur after harvest, during storage. Genetic factors and environmental conditions during plant growth determine the maximum proportion of hard seeds produced by the plant while genotype controls the rate of natural permeability increase of these seeds (Tinius, 1991; Souza and Marcos-Filho, 2001). Hardseededness is naturally overcome, as water and gasses are gradually allowed to penetrate the seed via one or several ways. Depending on the species and variety these pathways are: epidermal cracks or pores, strophiole, hilum, micropyle, chalasa, raphe or the epidermis, which becomes more permeable as the seed ages (Yakar and Bilge, 1987; Peske and Pereira,

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1993; Souza and Marcos-Filho, 2001). Besides, hardseededness can be broken by thinning or breaking the seed coat. Three common scarification techniques are used to soften or break the seed coat. These are mechanical, chemical and heat scarification (Tarawali et al., 1995). Fifteen angiosperm families with species producing hardseededness are cited in numerous relevant reviews (Egley, 1989; Thanos et al., 1992; Bozcuk, 1995; Doran, 1997; Souza and Marcos-Filho, 2001). Among these families is the *Fabaceae*, of which most species produce hard seeds. Nevertheless, the seed coat of many clover species is hard and impervious to water. Variation in hardseededness will occur within a clover species, between varieties of the same species.

The Persian clover (*T. resupinatum* L.) is a native of Anatolia, Greece, Bulgaria, Portugal, Iran, Iraq and Afghanistan. It was cultivated for forage during the 19th century. Persian clover varieties have been used as hay, green manure, self-regenerating grassland and grain production in subtropical climates (Ates and Tekeli, 2004). Orak (2000), Tekeli et al. (2003) have outlined the high feeding value and various roles of Persian clover in Mediterranean countries, southern Australia and America. There are three main subspecies of *T. resupinatum*: *majus*, *typicum* and *resupinatum* (Tekeli and Ates, 2006b). The Persian clover subspecies show considerable variation in seed characteristics.

The objective of this research* was to determine the changes in some characteristics (hardseededness, germination speed, germination vigor and dead seed ratios) of *T. resupinatum* var. *typicum* Fiori et Paol. seeds stored in uncontrolled conditions.

MATERIAL AND METHODS

This study was carried out in the Analyses Laboratory of Field Crops Department at the Agricultural Faculty, Namik Kemal University, Turkey. In the experiment, the hardseededness ratio (%), the dead seed ratio (%, rotten seed after germination), germination speed (%, third day after germination) (Ellis et al., 1985; Tekeli and Ates, 2006a), germination vigor (%, seven day after germination) in *T. resupinatum* var. *typicum* Fiori et Paol. which has different seed color such as yellow, green, red and mixed were determined and their variations in a year were examined. Germination experiments set up as completely randomized blocks with three replicates were performed from seed harvest to end by 15 day intervals. Seeds surface was sterilized with 2% sodium hypochlorite for 15 minute. One hundred seeds of each color were placed on towel paper in 14x21 cm plastic germination dishes containing 50 mL distilled water. The seeds were incubated in the darkness at 20±1. Germination counts were made daily. Each germination experiment ended after 7 days of incubation (Ellis et al., 1985; Tekeli and Ates, 2006a).

Germination data were analyzed using the TARIST statistical program. The same program was used for comparison of the means.

RESULTS AND DISCUSSION

The results of the research are shown in Tables 1-2 and Figures 1-4. In *T. resupinatum* var. *typicum* Fiori et Paol., germination occurred within one day and the highest hardseededness ratio (94.3%) occurred in 10th germination period ($P<0.01$). The hardseededness was reduced to 13-26 periods. The lowest hardseededness ratios (22.3-26.3%) occurred in 21st – 26th germination period ($P<0.01$) (Table 1 and Figure 1). However, the lowest hardseededness ratio (57.0%) was obtained from red color seeds, while the maximum dead seed ratio (2.4%) was from the red color seeds ($P<0.01$) (Table 1 and Figure 2). The temperature and humidity of seed storage facility have a pronounced effect on hardseededness. Levels and the amount of fluctuation of temperature and humidity both play a critical role in breaking hardseededness. The hardseededness of many pasture legumes are softened during the summer and autumn under natural conditions by a combination of high and fluctuating temperatures (Norman et al., 2002). Besides, variation in hardseededness in seeds is influenced by uncontrolled storage at subtropical regions (Tekeli and Ates, 2006b). Nair et al. (2004) found a hardseedednes ratio of 37.43% (5 months after storage) for balansa clover (*T. michelianum* Savi.). Similar results were obtained in *Stylosanthes hamata* cv. Verano and *T. resupinatum* var. *majus* Boiss. for changes of hardseededness

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(Argel and Humphreys, 1983; Tekeli and Ates, 2006a). The effect of storage time on alfalfa (*Medicago sativa* L.) seed quality was investigated by Čupić et al. (2005); they reported that hard seed content decreased significantly to 2.9%, and decreasing tendency continued without differences between 2, 4 and 8 years of storage.

Table 1. The hardseededness and dead seed ratios of *Trifolium resupinatum* var. *typicum* Fiori et Paol. seeds
Tablica 1. Odnosi čvrstoće i klijavosti sjemena Trifolium resupinatum var. typicum Fiori et Paol.

Seed Color Periods[1] *Boja sjemena Razdoblja*[1]	Hardseededness - *Čvrstoća* (%)					Dead Seed - *Neklijavo sjeme* (%)				
	Yellow *Žuto*	Green *Zeleno*	Red *Crveno*	Mixture *Miješano*	Average *Prosjek*	Yellow *Žuto*	Green *Zeleno*	Red *Crveno*	Mixture *Miješano*	Average *Prosjek*
1	92.3	94.3	82.3	86.3	88.8 **bc**	0.3	0.2	0.7	0.9	0.5 **hi**
2	90.3	93.3	83.6	87.3	88.6 **bc**	0.2	0.1	1.2	0.2	0.4 **hi**
3	90.3	92.0	83.6	86.0	88.0 **c**	0.2	0.2	0.4	0.4	0.3 **hi**
4	85.6	92.0	90.6	94.3	90.8 **abc**	0.3	0.1	1.1	0.1	0.4 **hi**
5	94.0	93.3	90.3	93.6	92.8 **abc**	0.1	0.3	0.8	0.2	0.3 **hi**
6	95.0	96.3	89.0	92.3	93.1 **ab**	0.2	0.3	0.6	0.7	0.4 **hi**
7	90.3	97.0	91.0	84.0	90.5 **abc**	0.2	0	0.6	0.6	0.3 **hi**
8	96.0	92.0	82.0	90.6	90.0 **abc**	0	0.2	1.7	0.6	0.6 **ghi**
9	94.3	97.0	87.0	89.6	92.0 **abc**	0.1	0.1	0.7	0.1	0.3 **i**
10	97.6	96.3	89.6	93.6	94.3 **a**	0.2	0.2	0.9	0.6	0.5 **hi**
11	85.3	95.3	85.7	90.3	89.1 **bc**	0.3	0.1	0.8	0.8	0.5 **hi**
12	93.0	93.0	83.3	85.3	88.6 **bc**	0.4	0.3	2.3	1.2	1.1 **efghi**
13	78.7	81.0	76.0	76.0	78.0 **d**	0.6	0.8	0.7	1.0	1.3 **defghi**
14	66.7	66.7	65.3	59.3	64.5 **e**	1.0	1.2	3.7	2.8	2.3 **abcd**
15	49.0	47.7	40.7	44.7	45.5 **f**	0.3	1.3	3.9	1.7	1.8 **bcdef**
16	32.3	49.3	37.0	34.7	38.3 **g**	0.3	0.3	6.5	0.9	2.0 **abcde**
17	28.0	45.3	25.0	27.3	31.4 **h**	0.4	0.4	6.8	1.2	2.2 **abcd**
18	20.0	50.3	28.7	27.7	32.5 **h**	2.1	0.3	8.1	1.8	3.1 **a**
19	26.3	47.3	29.3	23.7	31.6 **h**	0.8	1.3	6.8	0.7	2.5 **abc**
20	29.3	45.0	28.0	36.3	34.6 **gh**	0.4	0.6	5.2	4.5	2.7 **ab**
21	18.7	33.0	18.0	27.3	24.2 **i**	0.2	0.5	2.5	2.3	1.4 **cdefgh**
22	17.3	33.3	18.7	20.0	22.3 **i**	0.6	1.1	0.6	0.9	0.8 **fghi**
23	21.3	35.7	22.3	22.0	25.3 **i**	0.6	0.7	1.0	0.5	0.7 **fghi**
24	21.3	42.3	17.7	24.0	26.3 **i**	0.6	1.0	1.0	0.7	0.8 **fghi**
25	18.7	32.7	19.7	27.3	24.6 **i**	0.7	1.2	2.0	2.7	1.6 **bcdefg**
26	18.0	33.3	18.7	20.7	22.6 **i**	0.6	1.1	0.6	0.4	0.7 **fghi**
Average	59.6 **b**	68.4 **a**	57.0 **c**	59.4 **b**		0.5 **c**	0.5 **c**	2.4 **a**	1.1 **b**	
LSD	Periods-*Razdoblja*: 4.991** Color-*Boja*: 1.958**					Periods-*Razdoblja*: 1.109** Color-*Boja*: 0.435**				

**: Significant at the 0.01 probability level, 1: Every 15 days – ***Značajno na 0,01 razini vjerojatnosti, 1: Svakih 15 dana*

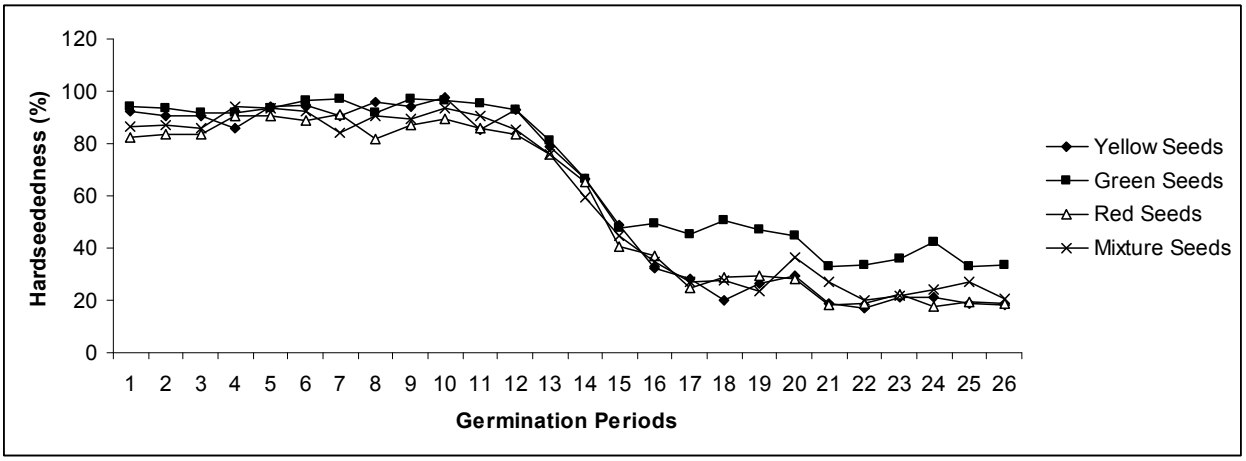

Figure 1. Changes of the hardseededness ratios of the different colour seeds in germination periods
Slika 1. Promjene čvrstoće sjemena različitih boja u vrijeme klijanja

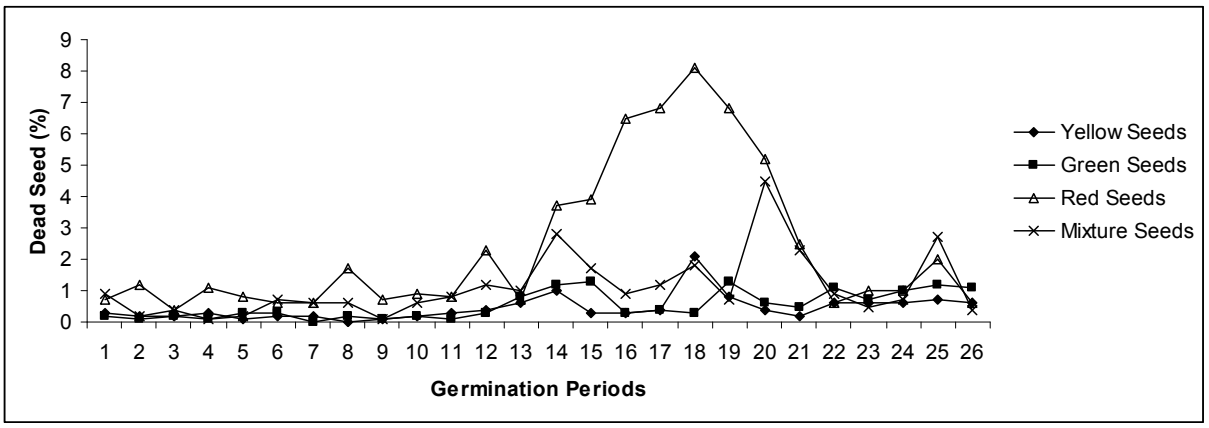

Figure 2. Changes of the dead seed ratios of the different color seeds in germination periods
Slika 2. Promjene klijavosti sjemena različitih boja u vrijeme klijanja

Table 2. The germination speed and germination vigor ratios of *Trifolium resupinatum* var. *typicum* Fiori et Paol. Seeds
Tablica 2. Omjer brzine i energije klijanja sjemena Trifolium resupinatum var. typicum Fiori et Paol.

Seed Color Periods[1] *Boja sjemena Razdoblja[1]*	Germination Speed – *Brzina klijanja* (%)					Germination Vigor – *Energija klijanja* (%)				
	Yellow Žuto	Green Zeleno	Red Crveno	Mixture Miješano	Average Prosjek	Yellow Žuto	Green Zeleno	Red Crveno	Mixture Miješano	Average Prosjek
1	0.6	2.3	7.0	6.3	4.0 **g**	6.6	5.0	15.6	11.0	9.5 **gh**
2	7.3	2.7	9.7	8.7	7.1 **fg**	9.0	6.3	12.6	12.0	10.0 **gh**
3	8.0	4.6	9.0	9.0	7.6 **fg**	9.0	7.3	15.0	12.6	11.0 **g**
4	9.6	7.3	4.3	5.3	6.6 **fg**	13.3	7.6	5.3	5.3	7.9 **ghi**
5	1.6	1.6	6.0	3.3	3.1 **g**	5.6	5.6	7.3	5.6	6.0 **ghi**
6	2.0	0.6	8.0	2.6	3.3 **g**	4.3	2.6	9.3	5.6	5.5 **hi**
7	1.3	0.6	4.0	8.3	3.5 **g**	9.0	3.0	7.0	14.0	8.2 **ghi**
8	1.3	5.0	9.0	5.0	5.1 **fg**	4.0	7.3	13.3	7.6	8.0 **ghi**
9	1.3	0	7.3	5.3	3.5 **g**	5.3	2.6	10.6	10.0	7.1 **ghi**
10	1.0	1.6	5.0	2.3	2.5 **g**	1.6	3.0	7.3	4.3	4.0 **i**
11	10.3	4.0	8.0	4.3	6.6 **fg**	13.6	4.3	11.6	8.0	9.4 **gh**
12	4.6	3.3	6.3	8.0	5.5 **fg**	5.6	6.0	9.6	11.0	8.0 **ghi**
13	14.0	8.6	6.3	10.6	9.9 **f**	19.3	16.6	15.0	21.0	18.0 **f**
14	21.3	20.3	15.3	18.3	18.8 **e**	28.3	29.6	23.3	32.3	28.4 **e**
15	27.0	26.6	26.3	25.3	26.3 **d**	50.0	48.3	44.3	50.3	48.2 **d**
16	38.6	27.6	33.3	32.3	33.0 **c**	66.6	49.6	43.3	62.3	55.5 **c**
17	39.6	28.6	31.6	38.0	34.5 **c**	70.6	53.3	54.6	69.0	61.9 **b**
18	45.6	33.0	25.6	35.6	35.0 **c**	73.6	45.3	47.0	56.6	58.1 **bc**
19	44.0	33.3	31.3	39.3	37.0 **c**	72.6	48.6	50.0	73.0	61.0 **b**
20	69.0	45.0	44.3	49.6	52.0 **b**	69.3	53.0	56.3	53.3	58.0 **bc**
21	79.0	65.3	74.0	63.0	70.3 **a**	80.6	65.3	74.3	65.6	71.5 **a**
22	75.6	62.3	77.3	75.3	72.6 **a**	80.6	63.3	79.3	77.0	75.0 **a**
23	76.6	60.3	73.3	76.0	71.6 **a**	76.6	62.0	74.6	76.3	72.4 **a**
24	74.0	51.6	78.6	67.6	68.0 **a**	76.6	54.6	79.3	73.6	71.0 **a**
25	78.0	63.6	74.0	63.0	69.6 **a**	79.0	63.6	74.3	64.6	70.4 **a**
26	75.0	62.3	77.3	75.3	72.5 **a**	80.0	63.3	79.3	79.3	75.5 **a**
Average	31.0 **a**	23.9 **c**	29.0**ab**	28.4 **b**		38.9 **a**	29.9 **c**	35.3 **b**	37.3 **ab**	
LSD	Periods-*Razdoblja*: 5.199** Color-*Boja*: 2.039**					Periods-Razdoblja: 5.157 ** Color-*Boja*: 2.023**				

**: Significant at the 0.01 probability level, 1: Every 15 days - ***Značajno na 0,01 razini vjerojatnosti, 1: Svakih 15 dana*

As seen in Table 2, the effect of the time on the germination speed and vigor were all significant ($P<0.01$). The highest germination speed (68.0-72.6%) and vigor (70.4-75.5%) were determined in 21st – 26th germination period and lowest values were obtained from 10th germination period (2.5%, 4.0%, respectively) (Table 2, Figures 3-4). The yellow seeds exhibited higher ratios ($P<0.01$) than the other seeds for germination speed (31.0%) and vigor (38.9%) (Table 2, Figures 3-4). Small grain seed lots

with nearly identical percentages, planted in nearly identical field conditions, can have very different emergence rates and final plant stands. This variation in field performance can be attributed to several events including differences in seed vigor (Spears, 2004). However, germination speed can be different between species and varieties too. Beside temperature, Seed testa is also effective on the germination speed of seeds. Andrade et al. (2002) studied the effect of temperature on percentage of germination of canistel seeds (*Pouteria campechiana*) and they reported that the best temperature for canistel germination is 30 °C, where 89.4% of the seeds germinated, with a higher germination speed, which allowed it to reach the maximum germination in the shortest time (9^{th} week). Balkaya (2004) stated that the effect of temperature on germination speed was much more important than the other possible effective parameters since 83% to 99% of the variation in germination speed was explained by temperature depending on the plant species and varieties. Changes in hardseededness traits of Persian clover (*T. resupinatum* var. *majus* Boiss.) seed was investigated by Tekeli and Ates (2006a). They reported that the germination speed and vigor ratios ranged from 94.8% to 97.9% and 95.4-98.5% respectively, in different seed colors.

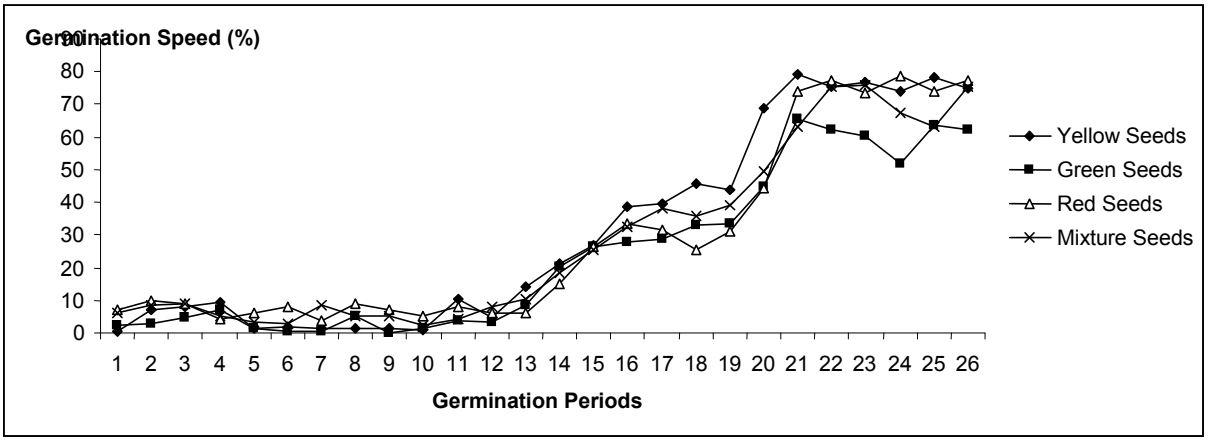

Figure 3. Changes of the germination speed of the different color seeds in germination periods
Slika 3. Promjene brzine klijanja sjemena različitih boja u vrijeme klijanja

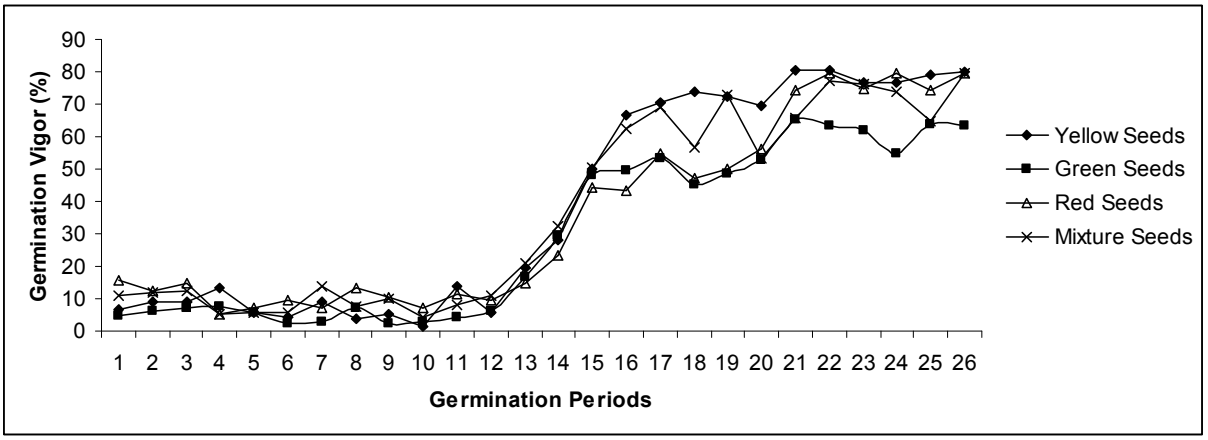

Figure 4. Changes of the germination vigor of the different color seeds in germination periods
Slika 4. Promjene energije klijanja sjemena različitih boja u vrijeme klijanja

CONCLUSION

The red color seeds displayed lower levels of hardseededness than other seeds in uncontrolled storage of subtropical environment. However, the variability within each seeds will enable hard seed germplasm to be selected for cultivar release in current and future breeding programs.

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PROMJENE U ČVRSTOĆI I DRUGIM KARAKTERISTIKAMA *TRIFOLIUM RESUPINATUM* VAR. *TYPICUM* FIORI ET PAOL. (*FABACEAE*) SJEMENA USKLADIŠTENOM U NEKONTROLIRANIM UVJETIMA

SAŽETAK

Ovo se istraživanje provodilo u Laboratoriju Zavoda za analize poljskih usjeva na Poljoprivrednom fakultetu, Namik Kemal Sveučilišta u Tekirdagu, Turska. Pokusi s klijanjem, postavljeni po potpuno slučajnom rasporedu u tri ponavljanja, trajali su od skupljanja sjemena do kraja u vremenskim intervalima od 15 dana. U ovom pokusu utvrđivala se čvrstoća sjemena (%), klijavost sjemena (%), brzina klijanja (%) i vigor (%) u Trifolium resupinatum var. typicum Fiori et Paol. koji ima raznobojno sjeme (žuto, crveno, zeleno i miješano), kao i njihove varijacije tijekom godine. Utvrđeno je da boja sjemena, kao i vrijeme nakon žetve, mogu utjecati na sve karakteristike sjemena. Dok je najveća čvrstoća utvrđena kod zelenog sjemena (68,4%), najveći udio neklijavog sjemena (2,4%) imalo je crveno sjeme. Brzina klijanja žutog sjemenu bila je 31,0%, dok je vigor bio visok (38,9%). Najveća čvrstoća sjemena utvrđena je u 10. razdoblju klijanja (94.3%), a najviše neklijavog sjemena (3.1%) bilo je u 18. razdoblju klijanja. Najveća brzina klijanja, kao i vigor, utvrđeni su od 21. do 26. razdoblja, a najniže vrijednosti dobivene su u 10. razdoblju.

Ključne riječi: klijanje, čvrstoća sjemena, sjeme, Trifolium resupinatum L.

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