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EFFECTS OF SEED COAT COLOUR ON THE SEED QUALITY AND INITIAL SEEDLING GROWTH OF RED CLOVER CULTIVARS (*Trifolium pratense*)

UTICAJ BOJE SEMENNE OBLIČI NA KVALITET SEMENA I POČETNI PORAST KLIJANACA SVETLA CRVENE DETELINE (*Trifolium pratense*)

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ABSTRACT

The objective of this study was to investigate the influence of seed coat colour on the seed quality of four red clover cultivars (germination, dormancy (hard seeds) and initial seedling growth (vigour)) produced in Serbia over a period of two years. The seeds analyzed were first separated visually into bright, dark and mixed seed groups, followed by subsequent digital colour measurements. The results obtained showed that the seed coat colour of red clover could be considered a good indicator of seed quality and seedling growth ability. The results furthermore showed that bright-coloured seeds of red clover cultivars indicated increased vigour and seed quality compared to other colours. The impact of the production area and year on the seed quality parameters was inconclusive. A high variability in hard (CV = 22.22 %) and dead seeds (CV = 40.18 %) was recorded between different seed lots of red clover. A lower variability was found in the germination parameter of bright- and mixed-coloured seeds (CV = 4.53 % and CV = 8.53 %). The seed coat colour could be an important factor in determining the quality of red clover seeds, potentially increasing germination simply by removing dark-coloured seeds.

Keywords: vigour, red clover, seed coat colour, seed quality.

REZIME

Cilj istraživanja bio je da se ispita uticaj boje semena četiri sorte crvene deteline proizvedene u Srbiji tokom dve godine na njihov kvalitet (kljanje, dormantnost (tvrdo seme), mrtvo seme i početni porast kljanaca (vigor)). U ovom istraživanju su prvo vizuelno razdvojena semena po boji, a zatim su razdvojena digitalnim kolor separatorom na svetla, tamna i mešovita. Rezultati su pokazali da boja semena crvene deteline može biti dobar pokazatelj kvaliteta semena i početnog porasta kljanaca. Rezultati su pokazali da su svetlo obojena semena sorte crvene deteline imala snažniji vigor i bolji kvalitet od drugih boja. Nije bilo jasnog zaključka o uticaju regiona i godine na praćene parametre kvaliteta semena. Zabeležena je visoka varijabilnost za tvrda (CV = 22,22%) i mrtva semena (CV = 40,18%) sorti crvene deteline. Za kljanje je zabeležena manja varijabilnost svetlih i mešovitih semena (CV = 4,53% i CV = 8,53%). Boja semena može biti značajan faktor kvaliteta semena crvene deteline i zbog toga je moguće povećati kljavost uklanjanjem tamno obojenih semena.

Ključne reči: vigor, crvena detelina, boja semenjače, kvalitet semena.

INTRODUCTION

The red clover (*Trifolium pratense* L.) is one of the main forage species found natively in the temperate regions of Southern Europe and Southern Eurasia (Taylor and Quesenberry, 1996; Algan and Buyukkartal, 2000; Herrmann et al., 2006). Although Mediterranean in origin, it is widely adapted to many climatic conditions around the world (Taylor and Smith, 1979). The red clover has a high nutritive value, and it is of immense importance to the environment and soil due to its ability to fix atmospheric nitrogen. Furthermore, it is an important component of grass-legume mixtures used to achieve high-quality forage production, particularly high-quality silage (Dias et al., 2008; Knežević, 2013; Knežević et al., 2014). From a botanical perspective, the red clover is a perennial species which generally persists in pastures two to three years under normal agricultural conditions (Ulloa et al., 2003). This annual legume can be used in various manners such as renovation, pasture improvement, erosion control or soil restoration programs. Harvesting and seed processing of forage legumes is highly important for producing high-quality seeds (Đokić et al., 2012; 2015).

Many studies have demonstrated that seed colour influences water uptake (Powell et al., 1986), gas diffusion, seed dormancy (Baskin et al., 2000), seed quality, germination and seedling emergence (Mavi, 2010) in some crop plants, owing to colour pigments located in the seed coat (Powell et al., 1986; Abdullah et al., 1991). The seed coat allows the passage of water and gasses to the tissues, and it features a varying colour. Differences in the seed colour within a species are associated with harvesting seeds in different developmental stages of fruit and some genetic differences. In red clovers, the seed colour is determined by two loci. If two loci are homozygous and recessive, the colour will be yellow. If two loci are heterozygote and dominant, the seed colour will be light purple. If two loci are homozygous and dominant, the seed colour will be purple (Bortnem and Boe, 2003). The formation of brown and red colour in red clover seed lots is a consequence of seed aging.

Although such variations in the colour of clover seeds have been acknowledged by researchers for more than 100 years (Brown and Hillman, 1906), the specialist literature dealing with the effect of red clover seed colour on the seed quality is fairly scarce.

The objective of this study was to determine a relationship between the seed coat colour, seed quality and initial seedling growth of the selected red clover cultivars.

MATERIALS AND METHODS

Over a period of two years (2015 and 2016), the experiment was conducted under laboratory conditions, including seeds of four diploid red clover cultivars (K-39, Sana, K-17, and Una), i.e. the second cut of the cultivars grown in different locations in Serbia. After harvesting, the seeds were dried to a moisture content of 12 % and separated into three colour groupings: dark, mixed and bright. The relationship (%) between the colours of the seed coat of each cultivar (Table 1) was established thereafter on the basis of the following parameters: germination, hard or dormant seeds, dead seeds and initial seedling growth. The analysis of seed germination was performed two months after the harvest, which corresponds to the fall planting period (September to October).

A germination test with 4x100 seeds was carried out in two plastic pots, using filter paper at a temperature of 20 °C (in the dark). The germination was recorded on the 10th day in accordance with the ISTA rules (ISTA, 2016). The Tetrazolium Chloride (TZ) test was applied on hard seeds in order to separate dead seeds from the hard ones (ISTA 2008). The initial growth of seedlings was determined in germinating seeds by measuring the following parameters: shoot length (cm), root length (cm) and seedling weight (g).

The data collated were analyzed by the analysis of variance (ANOVA – F test) adapted to a randomized block design, whereas the evaluation of difference significance was performed by the mean Tukey test method. Tukey's multiple range tests were applied to establish differences between the treatments. The coefficient of variation (CV, %) was calculated and the relationship between the traits was established by the Pearson's correlation test (r). The program Minitab 16.1.0 (statistics software package) was used for statistical analysis. The results obtained are presented in Table 1, 2 and 3.

RESULTS AND DISCUSSION

The cultivars analyzed showed a significant variability in the seed colour (dark CV = 15.89 %, mixed CV = 15.89 %, bright CV = 12.76 %) (Table 1).

Table 1. Relation (%) between the seed colour of the tested red clover cultivars

Cultivar Colour	K-39	Sana	K-17	Una	CV (%)
Dark (1)	23 %	25 %	21 %	17 %	15.89
Bright (2)	55 %	46 %	54 %	63 %	12.76
Mixed (3)	22 %	31 %	25 %	20 %	15.89

Using the F-test, it was determined that the year of the experiment had no significant effects on the tested parameters (results are shown in average values), whereas the colour and the cultivar were greatly affected ($P \leq 0.05$ or $P \leq 0.01$).

Visually inspected and separated seeds were subjected to digital colour measurements in order to perform colour classification. The statistical analysis showed that the seed coat colour was significantly different. As expected, the germination values were the highest for bright seeds and the lowest for dark seeds, i.e. 57 % for black-coloured and 78 % for bright-coloured seeds (Table 2). The findings are in accordance with those of Atis et al. (2010) who confirmed that the brown-coloured seed lots had the lowest total germination percentage (58 %), whereas the yellow-coloured seed lots had the highest total germination (99 %). The total germination percentages for mixed-coloured

seeds were between the dark and bright-coloured lots. In like fashion, the values recorded for hard seeds were the highest (28–44 %) in the dark-coloured seed lots, whereas the lowest values (18 %) were in bright seeds. The highest CV (56.77 %) was calculated for dead seeds in the cultivar K-39. The initial seedling growth of the tested red clover cultivars was significantly different according to each coat colour: the root was 1.8 cm in bright-coloured seeds compared to 1.4 cm in dark seeds, whereas the shoot was 4.3 cm in bright-coloured seeds compared to 3.1 cm in dark seeds. This was confirmed by another study which showed that brown (dark) and mixed-coloured seed lots indicated lower vigour than yellow (bright)-coloured seed lots (Atis et al., 2010).

The ANOVA showed that the seedling weights significantly changed relative to the change in the colour of red clover seed lots. The lowest seedlings weight of red clovers was recorded in dark-coloured seed lots (1.176 g), whereas the highest seedling weight of red clovers was recorded in bright-coloured seed lots (1.605 g) (Table 2).

Table 2. Effects of different seed coat colour of red clover cultivars on the seed quality and initial seedling growth (Note: Lower case letters in the bars indicate the statistical significance between the seed colour means, whereas different numbers indicate the statistical significance ($p < 0.05$) between different seed colour means of the tested red clover cultivars)

Cultivars of red clovers					
	K-39	Sana	K-17	Una	CV%
a) Germination					
Dark	61 ^c	51 ^c	56 ^c	57 ^c	7.3
Bright	80 ^a	75 ^a	81 ^a	78 ^a	3.4
Mixed	70 ^b	67 ^b	71 ^b	70 ^b	2.5
CV%	13.5	19.0	18.2	15.5	
b) Hard seed					
Dark	33 ^a	45 ^a	39 ^a	38 ^a	12.7
Bright	18 ^c	18 ^c	14 ^c	18 ^c	11.8
Mixed	26 ^b	27 ^b	23 ^b	25 ^b	6.8
CV%	29.2	45.8	49.9	37.6	
c) Dead seed					
Dark	6 ^a	4 ^b	5 ^a	5 ^a	16.3
Bright	2 ^b	7 ^a	5 ^a	4 ^b	46.3
Mixed	4 ^b	6 ^{ab}	6 ^b	5 ^a	18.2
CV%	50.0	26.9	10.8	12.4	
d) Root					
Dark	1.4 ^a	1.3 ^b	1.2 ^b	1.7 ^a	15.4
Bright	1.6 ^a	2.1 ^a	1.6 ^a	1.8 ^a	13.3
Mixed	1.3 ^a	1.4 ^b	1.2 ^b	1.5 ^a	9.6
CV%	10.7	27.2	17.3	9.2	
e) Shoot					
Dark	3.5 ^a	2.8 ^b	3.6 ^{ab}	2.6 ^c	15.9
Bright	4.5 ^a	4.2 ^a	4.5 ^a	4.0 ^a	5.7
Mixed	4.1 ^a	3.6 ^{ab}	4.2 ^a	3.4 ^b	10.1
CV%	12.5	19.9	11.2	21.1	
f) Seedling weight					
Dark	1.158 ^b	1.035 ^b	1.282 ^c	1.229 ^c	9.1
Bright	1.395 ^a	1.417 ^a	1.932 ^a	1.677 ^a	15.7
Mixed	1.317 ^{ab}	1.432 ^a	1.427 ^b	1.411 ^b	3.8
CV%	9.4	17.4	22.1	15.6	

Using the Pearson's correlation test (r), the most significant correlation between germination and dead seeds ($P \leq 0.001$) was recorded in dark-coloured seeds, whereas a negative correlation was determined between germination and hard seeds. A significant relationship between germination and hard seeds was

determined ($P \leq 0.01$) in mixed-coloured seeds, whereas the correlation value was not significant in bright-coloured seeds (in contrast with dark- and mixed-coloured seeds which indicated a more significant correlation) (Table 3).

Table 3. The correlation coefficient (r) of the tested red clover parameters and seed coat colours

Seed coat colour	Parameter	Germ.	Hard seed	Dead seed	Root	Shoot	Seedling weight
Dark	Germination %	-	-0.999***	0.993***	0.119 ns	0.483 ns	0.507 ns
Mixed	Germination %	-	-0.845**	-0.302 ns	0.333 ns	0.523 ns	-0.271 ns
Bright	Germination %	-	-0.629 ns	-0.666 ns	0.195 ns	0.667 ns	0.562 ns

Statistical significance level: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; NS = not

significant

Differences in the seed coat colour within red clover lots can be visually identified and easily discerned using a digital colour meter (Table 1). Therefore, the colour evaluation in this study was performed using a digital colour meter. The seed lots were classified according to the seed coat colour using the image analysis method in flax (Dana and Ivo, 2008) and *Ambrosia trifida* (Sako et al., 2001) species. Our research showed that this method can be used to classify red clover seed lots according to the seed coat colour. Seed colour is an important distinguishing feature between hard-seeded and soft-seeded lines (Juan et al., 1994; Brochmann, 1992). The hard seeds of *Vicia sativa* are smaller and lighter than the soft seeds. The soft seeds are light brown in colour, whereas the hard seeds are black, indicating certain degree of chemical or mechanical differences (Büyükkartal et al., 2013). Differences in seed colour also refer to differences in the amount of colour pigments in the seed coat. The water uptake and tolerance of seeds to excessive water were closely associated with the seed colour in some rape species, and coloured seeds showed a slow water uptake, low electrical conductivity and high tolerance to excessive water (Zhang et al., 2008). The seed coat protects seeds from water penetration in the initial germination stage. Additionally, the seed coat does not allow the passage of water and/or oxygen, thus hindering the overall germination (Mohamed-Yassen et al., 1994). Seed coat colour affects the water uptake in many legume species, especially in bright-coloured varieties and cultivars which absorb water faster than those of darker colour and have a lower germination percentage (Powell et al., 1986). The red clover seed lots ought to be harvested in a timely manner in order to minimise the adverse effects of seed coat on the quality of seeds.

CONCLUSION

In the present study, seeds of four red clover cultivars were examined. The results obtained showed that the colour of red clover seeds can be a good indicator of the seed quality and initial seedling growth. Light-coloured cultivars of red clover seeds indicated stronger vigour and were of better quality than those of other colours. The seed coat colour could be an important factor in determining the quality of red clover seeds, potentially increasing germination simply by removing dark-coloured seeds. Differences in the seed colour of red clovers can be used as seed quality markers. Moreover, dark-coloured seeds are readily separated in seed lots using digital colour measuring equipment.

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