Seed Quality of Vetch (Vicia sativa) affected by Different Seed Colors and Sizes after Various Storage Periods

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Abstract

In many regions worldwide, vetch is an important forage crops for the production of fibrous feed. Vetch seed available on the south-eastern Europe market often differ in the size, colour and age, hence seed quality and seedling vigour are questionable. Seed samples of two vetch varieties were drawn from three lots during two years. According to these samples, seeds were classified into groups of three sizes and three colours. The effect of the seed size and colour on seed quality and seedling vigour was estimated immediately after harvest, and then nine, 21 and 33 months after harvest. The determined effects of seed size, colour and age, as well as of their interactions on seed quality and seedling vigour were significant. On the other hand, years, varieties, lots and their interactions did not significantly affect seed quality and seedling vigour. There were no significant differences in seed quality and seedling vigour over nine, 21 and 33 months of storage, but the ageing test showed the significant differences among the duration of storage. Germination and vigour of seedlings were significantly higher in large seeds. Pale seeds had better quality after nine and 21 month storage period (time when the seed in the second year after harvesting should be sown), while seed quality was greater in dark seeds after 33 months of storage (time when the seed in the third year after harvesting should be sown). © 2018 Friends Science Publishers

Keywords: Colour; Germination; Seed; Seedling vigour; Size; Vetch

Introduction

The genus Vicia encompasses about 190 species and the most important species of this genus in central and southern Europe as well as in south-western Asia, is common vetch (Vicia sativa), because its cultivation results in high yields of quality feed (Yucel and Avci, 2009; Karagić et al., 2011; Yilmaz et al., 2014). Variation of this species in morphological traits and fodder yield is great (Maxted, 1995 Georgieva, 2018; Mikić et al., 2014; El-Bok et al., 2015). In the feed production, vetch can be grown in the continuous cropping or in a mixture with small grains, which is a cultivation method widely accepted in agronomic practice (Karagić et al., 2011; Yilmaz et al., 2014).

A seed size is an important physical parameter of seed quality. Results on the relationship between the seed size and the initial growth (vigour) of a seedling and their effect on yields obtained in studies carried out with different plant species are very conflicting. For instance, Shahwani (2014) pointed out that yield of large-seeded common wheat is higher by about 10% than in small-seeded common wheat. The higher rice seedling vigour results in higher yields (Khalid et al., 2015). However, according to Munir and Abdel-Rahman (2002), yields of large-seeded vetch are not higher than those of small-seeded vetch.

Seed coat, an outer cover of a seed, differs in colour in many species. The regulation of absorption of water and gasses from the environment during seed germination is one of the most important functions of the seed coat. This is particularly pronounced in the family Fabaceae (Souza and Marcos-Filho, 2001; Van Assche and Vandelooy, 2010; Smýkal et al., 2014) to which vetch belongs. According to Baskin et al. (2000), the seed coat colour is closely related to hard seeds and decreased germination. Vetch seeds with a different seed coat colour differ by their morphology and anatomical structure (Buyukkartal et al., 2013).
Vetch growers in the region of south-eastern Europe come across seeds of different age that are available on the market. Based on this, the point when buying seed is whether attention should be paid to the seed size, colour and age. However, legal regulations concerning placing seeds on the market in the countries of this region specify only germination, which has to be above 75%. Therefore, the aim of this study was: i) to determine effects of the seed size, seed coat colour and seed age on seed quality and seedling vigour and ii) to apply the ageing test and estimate whether the size, colour and age of seeds affect seed viability.

Material and Methods

Experimental Details and Treatments

Seeds of two winter vetch varieties (V. sativa) commercially produced were used in the two-year study (2011–2012) carried out in three locations in eastern Serbia (43°56’- 44°36’ N, 22°18’ - 2°40’ E, 196-306 m asl). The standard cropping practices were applied.

Treatments

After harvest, seed was classified into three sizes - fractions (S): S1 (<4 mm - large seeds), S2 (3.6–4.0 mm - medium large seeds) and S3 (>3.6 mm - small seeds). The ratio established among fractions was 30:40:30% (S1:S2:S3). Moreover, seeds were grouped according to the colour (C) as follows: C1 (dark), C2 (transitional) C3 (pale), with the C1:C2:C3 amounting to 30:45: 25%. Once classified by the size and the colour seed was subjected to testing in two experiments (experiment 1 and 2) immediately after harvest (A0), nine, 21 and 33 months after harvest (A1, A2 and A3, respectively). Nine, 21 and 33 months after harvest correspond to sowing dates in autumn of the same, subsequent and the year following the subsequent year, respectively. The seed was kept in paper bags under storage conditions common for south-eastern Europe (average temperature of 12.4°C and relative air humidity of 73%).

Seed germination (%) and the number of hard seeds (expressed in %) were determined on seeds of different colours (C1, C2, C3) and sizes (S1, S2, S3). Seeds were tested in germination cabinets in sand in the dark and at the temperature of 20°C with pre-chilling at 5°C for seven days. The final count was done on the 14th day (ISTA, 2012.). The tetrazolim test was applied on hard seeds in order to separate dead seeds from hard ones (ISTA, 2008). Seedling vigour was determined in the germinated seeds: embryonic stem length (cm), radicle length (cm) seedling fresh weight (g).

Seeds of different colours (C1, C2, C3) and sizes (S1, S2, S3) were subjected to the ageing test immediately after harvest (A0), and then after nine (A1), 21 (A2) and 33 (A3) months of storage. The ageing test implies the seed subjected to the temperature of 45°C and relative air humidity of 100% for 48 h. According to Samarah (2006), these conditions are optimal for detecting differences in vetch seed viability.

Statistical Analysis

Obtained results were processed by the analysis of variance (ANOVA). The Tukey's Multiple Range test was applied to establish differences among treatments. The coefficient of variation (CV, %) was estimated for each combination of the trait and the treatment. The relationship between traits was established by the Pearson's Correlation Test. Data of germination and dormancy percentages were arcsine transformed [sqr(x/100)] before being subjected to the analysis of variance. The program Minitab 16.1.0 was used for data processing.

Results

Experiment 1

According to the analysis of variance (ANOVA), effects of the variety, lot and the year was not statistically significant (p ≥ 0.05), while the seed size, colour and age, significantly (p ≤ 0.05 to p ≤ 0.001) affected germination, hard seeds and seedling vigour (data not presented). Due to a number of hard seeds (on the average approximately 10% for both S and C) a lower seed germination (87% and for S and for C, respectively) was detected immediately after harvest (storage period - A0). At the same time, germination of large seeds (S1) was higher by 4% than of small seeds (S3). In addition, dependence on the seed coat colour, germination varied by 4% (Table 1a). The number of hard seeds in storage period - A0 differed by 1% and 5% over seeds of different sizes and colours, respectively (Table 1b).

The average germination was not significantly different between nine (A1) and 33 month (A3) old seeds (92% and 94%, respectively for both S and C), but germination of large seeds was higher by 4–6% after each storage period (storage period - A0, A1, A2, A3) (Table 1a). Hard (dormant) seeds reduced in size during the storage (A0 10%, A1 4%, A2 1%) and vanished after 33 months. However, germination was not significantly increased over time because a mild increase in the number of dead seeds was detected (not presented). The difference in hard seeds in dependence on their size was not recorded during seed storage (A0-A3) (Table 1b).

The maximum germination (95%) of dark seeds (C1) was detected after 33 months, while the corresponding value of transitional (C2) and pale (C3) seeds were recorded after three and 21 months, respectively. The significant (p ≤ 0.05) increase in germination (4–5%) of dark seeds (C1) was detected between A1 and A3 periods. On the contrary, germination of pale seeds (C3) was higher by 6% in the period A1 than in the period A3. Dormancy in dark seeds was retained until the end of testing (A0 12%, A1 6%, A2 3%, A3 1%), while there were not dormant pale seeds in the periods A2 and A3 (Table 1).

On the average, the embryonic stem growth from seeds of various sizes in the periods A1 (17.7 cm), A2 (17.4 cm) and A3 (17.2 cm) did not significantly differ, while
stems developed from seeds in A0 (16.6 cm) were significantly \((p \leq 0.05)\) shorter. In all germination times (storage period A0-A3), stems developed from large seeds (S1) were significantly longer than those developed from S2 and S3 seeds (Table 2a). As a rule, the embryonic stem growth from seeds of different colours in the periods A1 (19.6 cm) and A2 (19.8 cm) was significantly greater than the growth in the periods A0 (16.4 cm) and A3 (18.2 cm) (Table 2a). The stems developed from dark seeds (C1) were significantly longer \((p \leq 0.05)\) in the period A3 (21.1 cm) than in the periods A0 and A2 (14.1 and 18.2 cm, respectively) (Table 2a).

On the average in our studies, there were no significant reduction in the radicle length over periods from A1 (13.6 cm) to A3 (12.9 cm). However, the radicle length was significantly shorter (11.1 cm) immediately after harvest (A0), which can be attributed to the effect of seed dormancy (Table 1b). Regardless of seed age, the significantly \((p \leq 0.05)\) longer radicles were determined in the largest seed fraction (S1) than in smaller seed fractions (Table 2b). On the average, for all seed coat colours, the significantly shorter radicle (11.0 cm) was recorded immediately after harvest than in older seeds (on the average about 13.0 cm). The length of radicles developed from dark seeds (C1) was 17.7 cm and 21.7 cm after periods of A0 and A1, respectively, and was significantly shorter than the radicle length of C2 and C3 seeds (Table 2b).

Nonetheless, after the period A3, the radicles developed from dark seeds were significantly longer (23.3 cm) than those developed from C2 and C3 seeds. Tendencies determined in the stem and radicle growth depending on the seed size and colour were similar in seedling weight over the four periods (storage period -A0, A1, A2, A3) (Table 2c).

On the average, for the seed sizes and colours, the greatest correlation between seed germination and seedling vigour \((p \leq 0.001)\) was recorded after nine (A1) and 21 (A2) months. There were no statistically significant dependence between these two parameters in 33-month old seeds (stem \(r=0.23\); radicle \(r=0.23\)) as well as between the stem and the radicle \((r=0.30)\) (Table 3).

**Experiment 2**

The effect of seed size on its viability was not detected in any of storage periods after harvest (A0, A1, A2, A3), but the effect of the colour was significant. Namely, seed viability was significantly greater \((p \leq 0.05)\) in dark seeds (C1) in all periods after harvest. Viability after 21- and 33-month storage (A2 and A3) was significantly greater \((P \leq 0.05)\) in seeds of transitional colours (C2) than in pale seeds (C1). Furthermore, this parameter was greater in dark seeds over all storage periods than in pale seeds.

**Discussion**

In the continental part of south-eastern and central Europe, vetch is harvested during May. After processing and packing, seed is placed on the market and also used for sowing in some of subsequent sowing periods. In principle, seed companies are interested in selling seed as soon as possible, because costs of storage are reduced.
capital turnover is faster (Eric et al., 2007).

However, it often happens that seed is not sold immediately, but it is stored and sold in the subsequent years for greater financial gain. Due to this, seed available on the market is of different age. In addition, this seed differs in the size and the colour (Dokić et al., 2013).

The results from earlier studies related to the effect of the seed size on seed germination and seedling vigour for various agricultural plants are contradictory. For instance, according to Zareian et al. (2013), the Triticum aestivum seed size did not significantly affect germination, while Kakhki et al. (2008) pointed out to a significantly greater germination in large seeds. This indicates that other factors may have an impact on germination in addition to seed size.

Chastin et al. (1995) explained that within large seeds there were a greater store of nutrients, which provided a stronger initial growth of seedlings and better competitiveness in the initial growth resulting in higher yield, which may explain the results obtained in our study (Table 2 and 3).

Generally, the older seed had the shorter radicles in this study, but it is species dependant (Verma et al., 2003; Rajjou et al., 2008; Stanisavljević et al., 2011).

According to Buyukkartal et al. (2013), the anatomical structure of vetch seeds of different colours differs, and it may result in a higher percentage of hard (dormant) seeds in dark seeds than in pale seeds in periods A0 - immediately after harvest and A1 - after nine months. A significant relationship between the seed colour and seed germination as well as between the seed colour and seedling vigour was detected in the forage species Trifolium pratense (Atis et al., 2011).

In the agronomic practice of narrow-rowed forage species, the increased number of seeds per area unit might compensate the reduced seed germination in order to achieve a projected number of plants. Nevertheless, seed germination of species of the genera Festuca, Lolium, Dactylis and Phleum was highly positively correlated (P ≤ 0.001) with vigour of seedlings (Stanisavljević et al., 2010, 2011, 2014).
Generally aging test by applying to the seeds of various conditions storage potential is also affected by agroecological lighter seed coat of seed are characterized by a weaker different colour what we different temperatures (Stanisavljević (2006).

Applied vigour tests by which the differences among seed case of seed production legume) and the yield and quality of fodder or seed in the d the yield and quality of fodder or seed in the decreases in seed viability and storability may be detected even after application to seeds of the leading field crops such as wheat, rice and maize or vegetable species, probably due to the areas on which it is grown or more of the economic value of the seed.

The ageing test performed on seeds is one of the most forage species Reduced vigour of seedlings can hardly be compensated. This problem is particularly pronounced in forage species that are sown in grass-legume mixtures, in which a weaker initial growth of one species results in its weaker competitive ability and dominance of another species. This reflects on both, inability to achieve the projected mixture ratio (grass to legume) and the yield and quality of fodder or seed in the case of seed production (Erić et al., 2007).

The ageing test performed on seeds is one of the most applied vigour tests by which the differences among seed lots may be detected (Marcos Filho, 2015). Results obtained on Vicia seed viability are in accordance with Samarah (2006). Furthermore, the differences in seed viability and storability may be detected even after seeds subjected to different temperatures (Stanisavljević et al., 2013), with what we agree with our results on the assessment of seeds of different colours (Table 4).

In addition to the low-potential storage vetch with lighter seed coat of seed are characterized by a weaker potential for seed yield (Yildiz Tiryaki et al., 2016). Seed storage potential is also affected by agroecological conditions during seed ripening (Li et al., 2017). Generally aging test by applying to the seeds of various legumes it gives important information about the potential for seed storage (Wang et al., 2004), but the application of the test of aging is much smaller compared to the application to seeds of the leading field crops such as wheat, rice and maize or vegetable species, probably due to the areas on which it is grown or more of the economic value of the seed.

### Conclusion

The differences in germination and vigour in nine, 21 and 33 month old seeds were not significant. However, the ageing test shows that viability of seeds stored for 33 months was significantly low, pointing to a reduced sowing potential after this period of storage. Greater seed germination and stronger vigour can be expected from large vetch seeds, which can provide greater competitiveness of vetch in the intercrops with cereal crops and/or weeds. In addition, viability of large seeds was not proven by the ageing test. Pale vetch seeds are more suitable for the establishment of intercrops with cereal crops and/or weeds. In addition to the low-potential storage vetch with lighter seed coat of seed are characterized by a weaker potential for seed yield (Yildiz Tiryaki et al., 2016). Seed storage potential is also affected by agroecological conditions during seed ripening (Li et al., 2017). Generally aging test by applying to the seeds of various

### Table 3: Coefficients of correlation between vetch seed germination and seedling vigour over storage periods (A₀, immediately after harvest; A₁ nine months, A₂ 21 months and A₃ 33 months after harvest) (n=72)

<table>
<thead>
<tr>
<th>Seed age A</th>
<th>Germination % I</th>
<th>Germination % II</th>
<th>Germination % III</th>
<th>Germination % IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₀</td>
<td>I 0.303 **</td>
<td>0.352 **</td>
<td>0.301 *</td>
<td></td>
</tr>
<tr>
<td>A₁</td>
<td>I 0.456 ***</td>
<td>0.601 ***</td>
<td>0.563 *</td>
<td></td>
</tr>
<tr>
<td>A₂</td>
<td>I 0.547 ***</td>
<td>0.526 ***</td>
<td>0.526 *</td>
<td></td>
</tr>
<tr>
<td>A₃</td>
<td>I 0.231 NS</td>
<td>0.229 NS</td>
<td>0.303 *</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: The application of the ageing test on seeds of various sizes and colours immediately after harvest (A₀), nine months (A₁), 21 months (A₂) and 33 months after harvest

<table>
<thead>
<tr>
<th>Trait Fac. / treat. A Seed</th>
<th>A₀ nine</th>
<th>A₁</th>
<th>A₂</th>
<th>A₃</th>
<th>X</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>86 ±0.87</td>
<td>85 ±0.66</td>
<td>84 ±0.81</td>
<td>67 ±1.17</td>
<td>81 * 11.2</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>87 ±0.61</td>
<td>87 ±0.71</td>
<td>85 ±1.07</td>
<td>65 ±1.22</td>
<td>81 * 13.2</td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>85 ±0.89</td>
<td>86 ±0.81</td>
<td>83 ±1.01</td>
<td>65 ±1.45</td>
<td>80 * 12.4</td>
<td></td>
</tr>
<tr>
<td>Pale</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>89 ±0.87</td>
<td>89 ±0.69</td>
<td>88 ±0.87</td>
<td>72 ±1.41</td>
<td>85 * 9.88</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>87 ±0.59</td>
<td>86 ±0.78</td>
<td>84 ±0.87</td>
<td>68 ±1.39</td>
<td>81 b 11.0</td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>85 ±0.61</td>
<td>84 ±0.72</td>
<td>82 c</td>
<td>60 ±1.22</td>
<td>78 c 15.3</td>
<td></td>
</tr>
</tbody>
</table>

Statistical significance level: * P ≤ 0.05, ** P ≤ 0.01, ***P ≤ 0.001, NS non-significant

Reduced vigour of seedlings can hardly be compensated. This problem is particularly pronounced in forage species that are sown in grass-legume mixtures, in which a weaker initial growth of one species results in its weaker competitive ability and dominance of another species. This reflects on both, inability to achieve the projected mixture ratio (grass to legume) and the yield and quality of fodder or seed in the case of seed production (Erić et al., 2007).
the application of the ageing test shows that viability of dark seeds was better than of pale seeds, hence their storability and usability in subsequent sowing periods are greater. Obtained results can be a useful tool in agronomic practice in the selection of seeds for establishing vetch crops, and/or for breeders in development of new varieties.

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