VARIABILITY AND CORRELATIVE INTERDEPENDENCE OF RED CLOVER AND ITALIAN RYEGRASS SEED QUALITY DEPENDING ON VARIETIES DURING THE MULTI-YEAR STORAGE PERIOD

VARIJABILNOST I KORELATIVNA MEĐUZAVISNOST KVALITETA SEMENA CRVENE DATELINE I ITALIJANSKOG LJULJA ZAVISNO OD SORTI TOKOM VIŠEGODIŠNJEG PERIODA SKLADIŠTENJA

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ABSTRACT

Seed quality is crucial for achieving the desired number of plants in the mixture, as well as the ratio of grass-legume components. Seeds of red clover and Italian ryegrass can be placed on the market with germination of 70 % and more. In this experiment, the seeds of six varieties of red clover and four varieties of Italian lily were examined. Seeds up to four years of age were tested. Seed quality was examined by monitoring the following parameters: germination energy, amount of hard-dormant seed, total germination and amount of abnormal seedlings. The tested varieties of red clover and Italian ryegrass seeds showed the best quality after one and two years of storage. After four years of storing seeds, out of six tested varieties of red clover, two did not meet the criteria for marketing in Serbia, according to the current rulebook on seed quality. In the case of Italian ryegrass, out of four tested varieties, two did not meet the criteria for placing seeds on the market.

Keywords: Italian ryegrass, red clover, seed quality.

INTRODUCTION

According to many kinds of research and practical experiences, red clover (Trifolium pratense L.) and Italian ryegrass (Lolium multiflorum L.) are some of the most productive grass-leguminous mixtures for fodder production on more fertile soils (Tomić et al. 2012; Camlin et al 1983).

Both species can also be grown as pure crops. However, sowing and growing in a mixture with legumes is better, because of the well-known benefits: legumes are nitrogen fixers, and grasses have pronounced nitrogen requirements (Knežević and Stanisavljević 2018). Studies have shown that these species grown in a mixture have a positive effect on soil biogenicity and weed reduction in relation to the cultivation of these forage plants in pure culture (Sleugh et al. 2000).

The initial crop density in all plants is achieved by the amount of germinating seeds in the crop establishment. To achieve the optimal ratio between legumes and grasses in the mixture, it is important to correctly determine the number of seeds for sowing. In production, the ratio in the mixture determines the yield and quality of feed. Namely, it is generally known that the legume component is of better quality and that the grass component is more productive. There is no doubt that seed germination is the most important indicator of seed quality.
and crop establishment success. And the goal is to maintain high-quality seed germination for as long as possible during the seed storage period. But the reality is that the seed reaches maximum germination during storage and then follows the period of seed aging, which inevitably leads to a decrease in germination, which is different for each species (Kandil et al. 2013; Desheva, et al. 2017). Of course, for preserving germination, ambient conditions in which the seed is stored are also of high importance.

The aim of these tests was to examine the quality of seeds during the year of harvesting and the next four years of storage through indicators: germination energy, hard-dormant seeds, total germination and abnormal seedlings. Seeds of six varieties of red clover, and the seeds of three varieties and one population of the Italian ryegrass were used in the experiment.

**MATERIAL AND METHODS**

After harvesting 2016, the seeds of 6 red clover varieties (K-17, Viola, Kolubara, Nike, Una, and Sana) were taken and 4 varieties of Italian ryegrass (K-13, Aubade, Draga, and population P1). Seeds were collected from commercial production or experiments from the second increase in red clover and from the first increase in Italian ryegrass. In all cases, the seeds were collected from Serbia. After drying the seeds to humidity lower than 12 %, the seeds were placed in a two-layer paper package and stored in the seed warehouse, the ambient conditions of which are shown in Table 1.

The next parameters of the seed quality were examined: germination energy, hard-dormant seed, total germination and abnormal germination.

The test was performed in March (the time of spring sowing period) on seed stored for one year (Y1); two years (Y2); three years (Y3); and four years (Y4).

In the year of harvest (Y0) red clover seed was stored for 1.5 months, and the Italian ryegrass seed was stored for 4 months (due to different times of harvest). The testing time was during September on seeds from the year of harvest (Y0), autumn sowing period.

Seed quality determination was done in accordance with the rulebook on seed quality testing of the (Official Gazette of SFRY, 1987), which is harmonized with ISTA Rules (ISTA, 2020).

For statistical analysis, Tukey’s multiple-range F test was applied and the coefficient of variation was used to test for the effects of the treatments. The standard error of the mean was calculated to indicate variation around the mean. Data for germination and dormancy percentages were arcsine transformed before being subjected to analysis of variance [sqrt(x/100)]. The program Minitab, version 19.1.0 (Minitab Inc., State College, PA, USA) was used to process data (free version).

**RESULTS AND DISCUSSION**

In the year Y0, the high presence of hard seeds caused lower germination energy on both tested species (Tables 2 and 3). However, factor variety influenced differently on germination energy of red clover seed (Viola 60% to Una 70%), with the variability of all tested varieties (CV = 5.44 %). The variety influenced the germination energy of the Italian ryegrass differently: Draga 65% to Aubada 73 %, with the variability of all examined varieties (CV = 5.08 %). Total germination behaved similarly to germination energy (Tables 2 and 3).

In legumes, hard seeds are characterized as seeds that cannot receive water and gases (Matilla, et al. 2005; Baskin, et al. 1998), and these seeds germinate subsequently when the seeds become permeable. In other words, seedling developed from a hard seed (which sprouts later than the classic ones) cannot produce a new plant (Bass et al. 1988). Seeds from Y0 had lower germination energy (67 % for all varieties), with a difference of 10% (Viola 60 % to Una 70 %), and variability (CV = 5.44%). On seeds from Y0, abnormal seedlings ranged between 1% and 2% (Table 2).

All investigated varieties met the condition for placing on the market (according to the given criterion of total germination of 70 % and more percent). But the difference between the varieties in the total germination was 8%. (Viola 70 %, Nike 78 %; Table 2). In Y0 year, the lowest germination of red clover cultivars had the Viola (70 %) and the Nilke had the highest (78 %) with the variability coefficient of 3.87 %. The average germination of ryegrass was 77 %, with CV = 1.69 %. The lowest germination on Italian ryegrass had the variety K-13 (75 %), and the highest Draga (78 %). Given that red clover and Italian ryegrass are mainly grown in a mixture (Tomić et al. 2004) and in accordance with the current regulations on seed quality, the seeds from the year of harvest (Y0) could be used for sowing in compliance with legal regulations.

For the professional aspect, it can successfully be used to establish a mixture of red clover and Italian ryegrass. In the Y0 were found 1 % to 2 % of abnormal seedlings in the red clover seeds, and 2 % to 4% of abnormal seedlings on Italian ryegrass seeds (Tables 2 and 3).

Italian ryegrass for seed production is mostly harvested in May. If the seeds were used in the same year for sowing (autumn sowing period), then it would take about 4 months from harvesting to sowing. In such a short period after harvesting, fodder grass seeds are characterized by a high presence of dormant seeds (Stanisavljević et al. 2011, 2017, 2020). Seed quality tests showed seed dormancy, of all varieties (17.3 % from Y0). The examined varieties diverged by 4 % (15 % of P1 populations, 19 % of Aubada). The coefficient of variation, depending on the tested varieties, was CV = 9.90 %.

In general, in the year Y0, there was a high presence of hard seeds in all tested varieties of red clover (17.8%). However, the examined varieties differed by 5% (K-14 15% to Kolubara and Nike by 20% each). A statistically significant difference (p< 0.05) was found between these varieties, with a coefficient of variation CV = 11.4% (Table 2).

**Table 1. Average monthly (I-XII) temperatures (°C) and humidity (PH) in the warehouse during storage of seeds for steam for five years (average Y0 – Y4).**

| Month | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII |
|-------|---|----|-----|----|---|----|-----|------|----|---|----|-----|
| Temperature °C | 2.2 | 6.3 | 9.3 | 11.4 | 13.8 | 20.5 | 22.7 | 23.7 | 12.9 | 11.2 | 6.1 | 3.2 |
| Relative humidity- PH, % | 81.3 | 79.3 | 78.5 | 66.3 | 65.9 | 63.9 | 64.6 | 68.7 | 74.7 | 76.7 | 77.6 | 79.6 |
### Table 2. Seed quality of red clover cultivars during the five-year period (Y0 to Y4).

| Year | K-17 | Viola | Kolubara | Nike | Una | Sana | $\bar{X}$ | CV% |
|------|------|-------|----------|------|-----|------|-------|-----|
|      | Germination energy % | Germination energy % | Germination energy % | Germination energy % | Germination energy % | Germination energy % | Germination energy % | Germination energy % |
| Y0   | 69.0±0.01 b A | 60.0±0.31 b B | 67.0±0.61 b AB | 69.0±0.31 b A | 70.0±0.19 b A | 68.0±0.64 b A | 67.0±0.64 b A | 5.44 |
| Y1   | 82.0±0.33 a B | 85.0±0.31 a AB | 80.0±0.53 a B | 85.0±0.24 a AB | 90.0±0.31 a A | 86.0±0.60 a AB | 85.0±0.60 a AB | 4.07 |
| Y2   | 89.0±0.33 a A | 86.0±0.19 a B | 86.0±0.43 a B | 90.0±0.41 a A | 88.0±0.4 a A | 87.0±0.55 a AB | 88.0±0.55 a AB | 1.86 |
| Y3   | 76.0±0.33 a B | 80.0±0.43 a AB | 80.0±0.44 a AB | 74.0±0.62 b B | 83.0±0.24 a A | 87.0±0.49 b B | 79.0±0.49 b B | 5.25 |
| Y4   | 65.0±0.29 b B | 78.0±0.43 a AB | 69.0±0.40 b B | 73.0±0.94 b AB | 74.0±0.49 b AB | 70.0±0.33 b AB | 72.0±0.33 b AB | 6.30 |

#### Abnormal seedlings %

| Year | Y0 | Y1 | Y2 | Y3 | Y4 | $\bar{X}$ | CV% |
|------|----|----|----|----|----|-------|-----|
|      | 16.0±0.24 b B | 2.0±0.61 b A | 2.0±0.66 b A | 2.0±0.66 b A | 2.0±0.66 b A | 1.7±0.66 b A | 3.0 |

#### Hard - dormant seed %

| Year | Y0 | Y1 | Y2 | Y3 | Y4 | $\bar{X}$ | CV% |
|------|----|----|----|----|----|-------|-----|
|      | 15.0±0.41 a B | 16.0±0.41 a B | 16.0±0.41 a B | 16.0±0.41 a B | 16.0±0.41 a B | 17.0±0.41 a B | 11.4 |

#### Total germination %

| Year | Y0 | Y1 | Y2 | Y3 | Y4 | $\bar{X}$ | CV% |
|------|----|----|----|----|----|-------|-----|
|      | 77.0±0.48 b A | 70.0±1.04 c B | 74.0±0.50 c AB | 78.0±0.50 c AB | 75.0±0.14 c AB | 73.0±0.24 c AB | 75.0±0.24 c AB | 3.87 |

During the examination of seeds for the germination energy and total germination, no statistically significant difference (p ≥0.05) was found in red clover between Y1 year and Y2 year. In Italian ryegrass, a significant difference was found for total germination (p≤ 0.05) only in the P1 population. On the other hand, the influence of red clover varieties on germination energy and total germination was significant both in Y1 and Y2. In Italian ryegrass, the influence of variety was significant (p≤ 0.05) only for Y1 for total germination, while for germination energy in Y1 and Y2 and total germination in Y2 not significant (p≤ 0.05). Red clover seeds from these two years are also of the highest quality for total germination (average values for Y1 was 90%, and for Y2 was 91%). For Italian ryegrass, the average values of total germination were 91% for Y1 and 90% for Y2. In both years (Y1 and Y2) red clover varieties Una and Nike showed the highest germination energy (90% each) while for total germination Una had the highest germination in Y1 (93%). The K-17 variety showed the highest germination on seeds from Y2. As expected, starting from Y0 to Y2, there is an obvious trend of a decrease in hard seed in red clover and dormant seed in Italian ryegrass (Tables 2 and 3). For the same period, in both tested varieties, there is no clear conclusion about abnormal seedlings in either species.

Red clover and Italian ryegrass seeds from Y3 and Y4 had reduced quality (Tables 2 to 5). On all examined varieties of red clover, the total germination of Y3 seeds significantly decreased in relation to the maximum achieved. On the seeds of Italian ryegrass, only varieties K-13 aged Y3 had no significantly lower germination compared to the maximum, while the others also had significantly lower total germination.

On seeds from Y4, the total germination on red clover varieties indicates that two of them that had lower germination of 70% did not meet the condition of placing seeds on the market (K-17, germination 67% and Nike germination 55%). Also, in the case of Italian ryegrass, one variety and one population had not germination for placing seeds on the market (Draga - 69%...
and P1 - 67%). In the Y3 and the Y4, factor variety had a significant impact on differences in germination energy of red clover, and also for abnormal seedlings and total germination. The same can be concluded for the seeds of Italian ryegrass.

According to Tomić (1989), without data in which conditions the seeds were stored, after four years of storage, the total germination of red clover seeds ranged from 71 to 80% for different varieties. Total germination of the different varieties of Italian ryegrass was 60 to 67%.

The conditions that prevail in the seed storage, primarily humidity and temperature, are of high importance for preserving the quality of seeds, to which seeds of each species react differently (Ellis and Roberts 1981). The conditions in which our seeds were stored (Table 1) during the test are typical for seed warehouses in which there is no artificial regulation of humidity and temperature.

Tables 4 and 5 show the correlation between the examined traits. The coefficients of simple correlations (r) on red clover seed were determined by the highest positive correlation (p ≤ 0.01) between total germination and germination energy on varieties K-17, Una and Sana. Also, significant (p ≤ 0.05) but negative and lower interdependence was found between total germination and germination energy for Kolubara variety; as well as between total germination and abnormal seedlings on Nike variety and between abnormal seedlings and hard seed on K-17 variety (Table 4). Highly significant (p ≤ 0.001) and positive interdependence was found between the total germination and germination energy in the Italian ryegrass on the varieties K-13 and Aubada. Also, on the population P1 and variety Draga, a significant (p ≤ 0.01 and p ≤ 0.05) interdependence was found between total germination and germination energy.

On the other hand, a negative and significant (p ≤ 0.05) interdependence was found between the total germination of abnormal seedlings (Table 5).
CONCLUSION

After all years of storage, the seeds of the tested fodder species of Red clover and Italian ryegrass showed high compatibility for the quality of seeds. Seeds had the highest quality of both species after storage of one (Y1) and two years (Y2). After four years of storage, the quality of seeds, above all, the total germination is about 70%, which is the limit for placing seeds on the market. The influence of variety was significant for seed germination after all periods of red clover storage (Y0 to Y4). For Italian ryegrass seeds after storage for two years (Y2), the varieties differed in total germination by only 2% and the influence of the variety was not significant. On the tested seed of red clover cultivars, stored for four years (Y4), the influence of the variety was significant. On the Italian ryegrass seed stored for four years, two varieties had higher germination of 70% and two had lower than 70%. Varieties with better preservation of total germination during the storage period could be stored for a longer period.
ACKNOWLEDGMENT: We thank the Ministry of Education, Science and Technological Development and Ministry of Innovations and Technological Development, Republic of Serbia, for financial support of this research, Contract No. 451-03-9/2021-14/200010.

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Received: 20. 02. 2021. Accepted: 29. 03. 2021.