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INFLUENCE OF SEED PRE-TREATMENT AND TEMPERATURES DURING GERMINATION OF FOUR SEED LOTS OF *FESTULOLIUM*
UTICAJ PRED TRETMANA NA SEMENU I TEMPERATURE U KLIJALIŠTU NA KLIJAVOST ČETIRI PARTIJA SEMENA FESTULOLIUMA

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ABSTRACT

A species called Festulolium was created in the middle of the last century by crossing two genuses: Lolium and Festuca. This was followed by a period of research on Festulolium from the aspect of genetics and breeding, seed production, cultivation, technology and use. We conducted various pre-treatment impact studies on Festulolium seeds: i) cooling at 5-7 ^oC for 3 days, ii) application of KNO_3 on seed germination medium, and the third pre-treatment was control, without pre-treatment (iii). After pre-treatment (i, ii, iii), seed germination have examined at the following temperature regimes (provided by the seed quality regulations): 20 ^oC (dark); alternately 20^{o}C/ 30^{o}C (light /dark); and 30 ^oC (dark). The tests were performed on four seed lots of the variety Peron, in four replicates. The germination temperature of 30 ^oC reduced germination and increased dead seed and abnormal germination in all pre-treatments. The variant without the application of pre-treatment had a significantly lower overall germination in the assessment of Festulolium seed quality. Seed lots showed a high influence on the Festulolium seed quality.

***Key words**: Festulolium, pre-treatment, seed quality.*

REZIME

Vrsta pod nazivom Festulolium nastala je sredinom prošlog veka ukrštanjem dva roda: Lolium i Festuca. Usledio je period istraživanja Festulolijuma sa aspekta genetike i oplemenjivanja, proizvodnje semena, tehnologije gajenja i upotrebe. U istraživanima je prikazan uticaja pre tretmana na seme Festuloliuma: i) hlađenje na 5-7 ^oC tokom 3 dana, ii) nanošenje KNO_3 na podlogu za klijanje semena, i treći predtretman (iii) je bio kontrolni, bez prethodnog tretmana . Nakon predtretmana (i, ii, iii), klijavost semena je ispitana na sledećim temperaturnim režimima: 20 ^oC/ mrak; naizmenično 20/30 (svetlo /mrak), i 30 ^oC/ mrak. Ispitivanja su obavljena u četiri ponavljanja, korišćeno seme je sorte Peron. Temperatura za klijanje od 30 ^oC uticala je na smanjenje klijavosti i povećanje mrtvog semena i nenormalnih klijavaca na svim primenjenim pred tretmaniam. Varijanta bez primene pred-tretmana je uticala na značajno nižu ukupnu klijavost u oceni kvaliteta semena Festuloliuma. Partije semena su pokazale visok uticaj na određivanje kvaliteta semena Festuloliuma

***Ključne reči:** Festuloliuma, kvalitet semena, pred-tretmani.*

INTRODUCTION

In the seventies of the last century, intensive work began on the possibilities of interbreeding. The species from the genus *Lolium* were crossed with the species from the genus *Festuca*, thus *Festulolium* was formed. However, in Europe, the cultivation of new species on larger areas started after 2010 (*Østrem et al., 2013*). Such studies have shown that *Festulolium* has a higher potential for green fodder, dry matter and seed yields compared to its parents (*L. multiflorum* and *F. pratense*) (*Akgun et al., 2008*). *Festulolium* also showed good adaptability to the external environment conditions (*Abdelhalim 2016; Humphreys et al., 2013, 2014*). Studies by *Deleuran et al., (2010)* gives optimal cultivation technologies for the production of *Festulolium* seeds, but there is a lack of research and many unknowns about seed storage and aging (*Stanisavljević et al., 2020*).

Seed quality control in Serbia is done according to the rulebook on seed quality, "*Official Gazette of SFRY, 1987 (47/87) and supplement to 34/2013*" which, after amendments, is harmonized with the ISTA rules (*ISTA 2021*). But, there are no protocols for *Festulolium* seed quality in this rule book. In such cases, the generally accepted rule is that the analysis is performed as the most related species. In this case, they are *Lolium multiflorum* and *Festuca pratense* as the parent species. However, the germination energy reading for *L. multiflorum* is on the fifth day after placement in the hatchery, and for the *F. pratense* it is after seven days. Also, the minimum germination for placing seeds on the market is 70% for the *L. multiflorum* seeds, and the 75% for the *F. Pratense* seeds.

Due to these problems above for the *Festulolium* seed test procedure and to complete our knowledge, we decided to examine: the effect of seed pre-treatment for this species; then, the effect of after each of these pre-treatments on the germination of seeds in the seed hatchery at a constant temperature; and the effect on germination energy, total germination, abnormal seedlings, and dead seeds.

MATERIJAL AND METHODS

The material for this research was four seed lots of the *Festulolium*, Perun variety. After application of seed pre-treatment to interrupt seed dormancy, trials were conducted in four replicates. Three pre-treatments were aplicated: i) seeds were placed in KNO_3 solution, ii) seeds were cooled to a temperature of about 5 ^oC for three days before placement seeds in the hatchery and, iii) without treatment application - Ø. Then the seeds from each pre-treatment were placed in the hatchery at three temperature regimes: I) 20 /30 ^oC (dark/light), II) 20 ^oC dark, III) 30 ^oC dark. Germination energy was read after the fifth and seventh days, and total germination, abnormal seedlings, and

dead seeds were read after the fourteenth day (%). All analyzes were performed in four replicates. The obtained results were processed by analysis of variance ANOVA. For the significance between mid-treatments, Tukey's Multiple Range test was applied. For the variability of values, the coefficient of variation (CV%) was applied. The standard error of the mean (± s.e.m) was applied for the deviation from the obtained mean values. The transformation of the obtained values was performed for the value expressed in percent, (√x / 100). The analysis was performed in the statistical program Minitab Inc., version 16.1.0, free version.

RESULTS AND DISSCUSSION

The accepted definition of seed vitality is the sum of seed properties that represent seed potential for germination in different conditions (*Perry 1980*), which determines the complex interaction of genetic and environmental factors (*Hodgkin and Hegarty 1978; Finch-Savage and Bassel 2016*); cultivation technologies (*Bewley et al., 2013*); and storage period (*Stanisavljević et al., 2020, 2019, 2016*). This also explains the significant influence of the seed lot for seed quality in our tests (Tables 1 to 4).

Table 1. Seed germination energy (%) on the 5th and 7th day, on four seed lots of Festulolium and three temperatures after application of different pre-treatments on the seed

Pre-treatment	°C	Seed lot								$\overline{X}$
		I		II		III		IV		
		5th day	7th day	5th day	7th day	5th day	7th day	5th day	7th day	
KNO_3	20/30	72±0.34aC	73±0.29aC	81±0.55aB	82±0.48aB	65±0.22aD	66±0.26aD	91±0.74aA	91±0.81aA	78
	20	70±0.45aC	72±0.61aC	79±0.74aB	82±0.19aB	66±0.29aD	67±0.34aD	92±0.63aA	92±0.79aA	78
	30	55±0.63bC	57±0.82bC	71±0.63bB	73±0.38bB	52±0.19bC	53±0.33bC	88±0.25bA	88±0.49bA	67
$\overline{X}$		66	67	77	79	61	62	90	90	
CV %, KNO_3		14.1	13.3	6.87	6.58	12.8	12.6	2.30	2.30	
Cooling (5-7°C)	20/30	70±0.34aC	72±0.31aC	78±0.65aB	80±0.77aB	63±0.81aD	63±0.45aD	91±0.63aA	92±0.31Aa	76
	20	71±0.71aC	71±0.47aC	80±0.55aB	81±0.63aB	62±0.23aD	63±0.33aD	90±0.50aA	91±0.68aA	76
	30	62±0.23bC	63±0.60bC	79±0.69bB	71±0.41bB	54±0.51bD	56±0.41bD	87±0.19aA	87±0.60aA	70
$\overline{X}$		68	69	79	81	60	61	89	90	
CV %, cooling		7.29	7.18	1.27	0.72	8.27	6.66	2.33	2.94	
Ø	20/30	67±0.69aB	68±0.49aB	70±0.53aAB	72±0.69aAB	58±0.18aB	60±0.45aB	88±0.25aA	88±0.28aA	71
	20	66±0.42aAB	69±0.30aAB	71±0.62aAB	73±0.55aAB	59±0.39aB	60±0.38aB	87±0.72aA	89±0.47aA	72
	30	58±0.66bAB	59±0.28bAB	62±0.19bAB	64±0.64bAB	50±0.45bB	52±0.33bB	83±0.29bA	83±0.38bA	64
$\overline{X}$, Ø		64	65	68	70	56	57	86	87	
CV %		7.75	8.43	7.29	7.08	8.86	8.06	3.08	3.71	
$\overline{X}$ g., KNO_3+cool.+ Ø		66	67	75	75	59	60	89	89	
CV % g., KNO_3+cool.+ Ø		9.23	8.92	8.57	8.20	9.80	8.98	3.15	3.28	

For the column, different small letters (a, b… x), significant effect, P ≤ 0.05; Tukey's Multiple Range test, for the row different capital letters (A, B... x), significant effect, P ≤ 0.05; Tukey's Multiple Range test, ± s.e.m, standard error of the mean

Table 2. Total germination (%) of four seed lots of Festulolium seeds at three temperatures after application of different pre-treatments on seeds

Pre-treatment	Temperature °C	Seed lot				$\overline{X}$
		I	II	III	IV	
KNO_3	20-30	75±0.39aC	84±0.44aB	68±0.65aD	92±0.60aA	80
	20	74±0.32aC	85±0.52aB	69±0.79aD	93±0.32aA	80
	30	62±0.54bC	76±0.62bB	55±0.18bD	89±0.25bA	71
$\overline{X}$		70	82	64	91	-
CV %, KNO_3		10.29	6.05	12.20	2.279	-
Cooling (5-7°C)	20-30	76±0.60aC	82±0.17aB	66±0.55aD	92±0.62aA	79
	20	75±0.39aC	83±0.42aB	67±0.78aD	93±0.99aA	80
	30	65±0.17bC	74±0.66bB	59±0.52bD	88±0.65bA	72
$\overline{X}$		72	80	64	91	-
CV %, Ph		8.448	6.192	6.811	2.907	-
No pre-treatment Ø	20-30	72±0.31bB	74±0.69aB	63±0.43aC	90±0.75aA	75
	20	81±0.91aB	74±0.64aC	64±0.54aD	90±0.28aA	77
	30	62±0.32cB	68±0.15bB	54±0.53bC	85±0.32bA	67
$\overline{X}$, Ø		72	72	60	88	-
CV %		13.26	4.811	9.129	3.268	-
$\overline{X}$ g., KNO_3+cooling+Ø		71	78	63	90	-
CV % g., KNO_3+cooling+Ø		10.76	7.915	9.442	3.066	-

For the column different small letters (a, b… x), significant effect, P ≤ 0.05; Tukey's Multiple Range test, for the row different capital letters (A, B... x), significant effect, P ≤ 0.05; Tukey's Multiple Range test, ± s.e.m, standard error of the mean

According to our seed quality regulations (*Official Gazette of SFRY, 47/87, supplement to 34/2013*), *Festulolium* does not exist as a species, so based on practice, the assessment of germination energy was read on the 5th and on the 7th day, and total germination was read on the 14th day.

After all pre-treatments on seeds (KNO_3, cooling, Ø) and after all temperatures in the hatchery (20/30°C, 20 °C, 30 °C) no statistically significant difference was found for germination energy on the 5th or 7th day, in none of the four examined seed lots (Table 1). But as expected, there was a trend of higher values read after the seventh day.

Before treatments with KNO3 and pre-cooling on seeds, *F. pratensis* and *L. multiflorum* were recommended according to the current regulations on seed quality, which in these studies proved to be realistic for assessing the quality of *Festulolium* seeds. On the other hand, the variant of quality testing without pre-treatment (Ø) affected significantly lower germination energy, total germination and more dead seeds and abnormal seedlings regardless of the temperature in the hatchery (Tables 1 to 4). They pointed out the significant influence of temperature on the germination of fodder plant seeds: *Vivanco et al., (2021), Milivojević et al., (2018), Butler et al (2017), Zhou et al (2013)*.

In all tested seed lots and in all pre-treatments the lowest obtained value of germination energy was at a temperature of 30 °C dark. These values were always statistically lower ($P \leq 0.05$) compared to the values obtained at an alternating temperature of 20/30 °C (dark/light); (Table 1.).

A statistically significant difference for germination energy (on the 5th and 7th day) was not found between the tests at 20/30 °C and 20 °C regardless of the applied pre-treatment (Table 1).

Seed germination is affected by a number of factors, such as the period of seed storage (*Stanisavljević et al., 2019, 2020*). The most important factors for seed germination are also the temperature and relative humidity in the storage (*Viera et al., 2001*).

In our tests after the applied pre-treatments KNO_3 and pre-cooling the total germination of the four tested seed lots, was 80 or 79% after germination at 20/30 (dark/light), 30 °C (dark) or 20 °C (dark), which indicates that these two pre-treatments on seed are valid for assessing total germination of *Festulolium*. On the other hand, regardless of the pre-treatment, germination at 30 °C was lower by 9% after pre-treatment with KNO_3, by 7 to 8% after pre-treatment with pre-cooling and by 80 to 10% after the variant without pre-treatment (Table 2).

On the seed lot with the highest germination (seed lot IV) the applied temperatures caused the lowest variability for germination on pre-treatment with KNO_3 (CV% = 2.279); CV% = 2.907 on pre-treatment with cooling; and CV% = 3.268 on pre-treatment Ø). On the other hand, the highest variability was in seed lot I after pre-treatment cooling and Ø (CV% = 8.448 and CV% = 13.26) and lot III after pre-treatment with KNO_3 (CV% = 12.20).

Table 3. Abnormal seedlings (%) on four seed lots of Festulolium and three temperatures after application of various pre-treatments on seeds

Pre-treatment	Temperature °C	Seed lot				$\overline{X}$
		I	II	III	IV	
KNO_3	20-30	5±0.17aB	4±0.64aB	7±0.42aA	2±0.34aC	5
	20	7±0.23bB	4±0.19aC	9±0.72bA	2±0.60aD	6
	30	10±0.87cB	7b±0.64C	14±0.24cA	4b±0.16D	9
$\overline{X}$		7	5	10	3	-
CV %, KNO_3		34.32	34.64	36.06	43.30	-
Cooling (5-7°C)	20-30	7±0.29cB	4±0.45bC	12±0.77aA	2±0.39bD	6
	20	9±0.46bA	4±0.53bB	8±0.20bA	2±0.63bC	6
	30	10±0.14aA	7±0.29aB	11±0.44aA	4±0.41aC	8
$\overline{X}$		9	5	10	3	-
CV %, cooling		17.63	34.64	20.15	43.30	-
No pre-treatment Ø	20-30	11±0.19bB	9±0.45aC	13±0.32bA	3±0.75bD	9
	20	6±0.63cC	10±0.62aB	13±0.87bA	4±0.52abD	8
	30	15±0.29aA	10±0.86aB	16±0.45aA	5±0.54aC	15
$\overline{X}$, Ø		11	10	14	4	-
CV %		42.27	5.970	12.37	25.00	-
$\overline{X}$ g., KNO3+cooling+Ø		9	7	11	3	-
CV % g., KNO_3+Ph+Ø		34.42	40.44	25.89	37.50	-

For the column different small letters (a, b… x), significant effect, $P \leq 0.05$; Tukey's Multiple Range test, for the row different capital letters (A, B... x), significant effect, $P \leq 0.05$; Tukey's Multiple Range test, ± s.e.m, standard error of the mean

Abnormal seedlings are not capable for normal growth in the field and do not give a normally developed plant on which there is a high correlation (*Knežević et al., 2019*). Therefore, when assessing the quality of seeds, abnormal seedlings do not enter the germinated seeds that will give a normally developed plant in the field. In the category of abnormal seedlings are seedlings that lack one or more of its essential seedling structures; this can be a root, shoot or final bud. It is very important to know what caused the abnormalities because they have a significant impact on the use value of the seed.

According to *Vujaković and Jovičić (2011)*, atypical seedlings do not show the potential to develop into a normal plant in good soil and favourable conditions of humidity, temperature and light. In this group is the next types of seedlings: deformed, broken, cotyledons erupt before the primary root from the seedling, consists of two connected seedlings, rotten due to primary infection or if the primary root is stunted, thickened, lagging, missing, broken, split from the top, narrowed, spindle-shaped twisted, trapped in the seedling, with

negative geotropism, vitreous and rotten due to primary infection.

In our tests with applied pre-treatments, abnormal seedlings occurred at a germination temperature of 30 °C, which was higher than other applied temperatures (by 4-5% at pre-treatment with KNO_3; by 2%, at pre-treatment with cooling and by 6-7% on the pre-treatment variant Ø). Taking into account all pre-treatments, temperatures for germination and seed lots, the share of abnormal seedlings ranged from 2% to 16%, which had an impact on seed germination (Tables 2 and 3).

After all pre-treatments, the influence of temperature on all seed lots showed high variability for the presence of abnormal seedlings (from CV% = 5.970 pre-treatment Ø on the seed lot II, and CV% = 36.06, before treatment KNO3 on the seed lot III).

Table 4. Dead seeds (%) of four batches of Festulolium, three temperatures after application of various pre-treatments on seeds

Pre-treatment	Temperature °C	Seed lot				$\overline{X}$
		I	II	III	IV	
KNO_3	20-30	20±0.63bB	12±0.45bC	25±0.18bA	6±0.74aD	16
	20	19±0.54bA	11±0.69bB	22±0.24cA	2±0.85bC	14
	30	28±0.69aB	17±0.47aC	31±0.67aA	7±0.29aD	21
$\overline{X}$		22	13	26	5	-
CV %, KNO_3		22.09	24.11	17.63	52.92	-
Cooling (5-7°C)	20-30	17±0.14bB	14±0.54bC	22±0.62cA	6±0.74bD	15
	20	16±0.61bB	13±0.18bC	25±0.69bA	5±0.82cD	15
	30	25±0.45aB	19±0.25aC	30±0.63aA	8±0.92aD	21
$\overline{X}$		19	15	26	6	-
CV %, cooling		25.51	20.96	15.75	24.12	-
No pre-treatment Ø	20-30	17±0.23bB	17±0.71abB	25±0.41abA	7±0.49bC	17
	20	13±0.53cC	16±0.42bB	23±0.62bA	3±0.19cC	14
	30	23±0.18aB	22±0.61aB	28±0.29aA	10±0.63aC	21
$\overline{X}$, Ø		18	18	25	7	-
CV %		28.49	17.53	9.93	52.68	-
$\overline{X}$ g., KNO_3+cooling+Ø		18	17	25	7	-
CV % g., KNO_3+cooling+Ø		24.20	18.24	10.60	39.71	-

For the column different small letters (a, b… x), significant effect, P ≤ 0.05; Tukey's Multiple Range test, for the row different capital letters (A, B... x), significant effect, P ≤ 0.05; Tukey's Multiple Range test, ± s.e.m, standard error of the mean

In the analysis of seed quality, normal seedlings (germinated seeds) + abnormal seedlings + dead seeds together make up 100% of the seeds for quality assessment (Tables 2 to 4). Dead seed is the part of the seed that does not develop a germ during germination. In our research, a significant increase in dead seed was influenced by the germination temperature of 30 °C in all seed lots and after all treatments (Table 4). This, together with abnormal seedlings, affected the reduction of total germination (Table 2 to 4).

On the other hand, in most seed lots between the temperature of 20/30 °C and 30 °C, regardless of the pre-treatment, the difference was minimal (Table 4).

CONCLUSION

Although *Festulolium* is relatively new cultivated species, it is not a mistake to apply the rulebook on seed quality for *F. pratensis* or *L. multiflorum* as its close relatives. This conclusion is indicated by these tests, where the difference in germination energy between 0 and 3 days of reading is 0-3%. Pre-treatments of seeds with KNO_3 or cooling give a uniform quality assessment; while in the non-pre-treatment variant (Ø) there is significantly lower germination energy and total germination. At the temperature in the hatchery 20/30 °C (dark/light) or 20 °C dark, the examined seed quality parameters are similar, while the temperature of 30 °C affects a significant increase in the share of dead seeds and abnormal seedlings, which causes significantly reduced total germination. Seed lots are the main carriers of seed quality.

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