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Enhancement of seed germination in three grass species using chemical and temperature treatments

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Abstract

Seeds of three forage plant species, cocksfoot, tall fescue and perennial ryegrass with good amount of dormancy were chemically treated (25%, 50%, 75% and 98% H_2SO_4) for different durations and exposed to different temperatures (40°C, 50°C, 60°C, 70°C, 80°C and 90°C) for varying periods with an aim to decrease seed dormancy and increase seed germination. Three groups of seeds based on after ripening period of 0, 3 and 8 months were subjected to these treatments. Immediately after harvest, germination of cocksfoot and tall fescue seeds increased by 24%, but only 13% in perennial ryegrass. Three months after harvest it was possible to increase germination by 20% (cocksfoot), 18% (tall fescue) and 6% (perennial ryegrass). Eight months after harvest it was still possible to increase seed germination of cocksfoot and tall fescue by 4-5% whereas, in ryegrass dormancy was completely lost after 8 months storage

Keywords: Dormancy, Forage grass seed, Germination, Sulphuric acid, Temperature

Introduction

Tall fescue and perennial ryegrass are grassland species intensively cultivated for fodder (Katoch *et al*., 2012) but also used for special purposes: sports fields, parks, home gardens, soil conservation, etc. On the other hand, cocksfoot is mainly used in combination with forage legumes to produce fodder and with a greater importance in arid regions. Seeds of higher germination and seedlings with a strong vigour are necessary for sowing of these species (Stanisavljeviæ *et al*., 2011, 2014). However, dormancy is expressed in seeds of grasses immediately after harvesting or dispersal (Simpson, 1990; Stanisavljeviæ *et al*., 2010, 2011; Verma *et al*., 2014). Seed dormancy is undesirable either when establishing lawns, or for use in agriculture and horticulture due to subsequent germination and poor competence of seedlings with already developed seedlings from previously germinated seeds. Nevertheless, seed dormancy and delayed germination can be advantageous under natural conditions as it provides germination and the initial growth of seedlings in the period with favourable climatic conditions (Bewley, 1997).

The degree of seed dormancy is determined by complex physiological and biochemical processes (Finch-Savage and Leubner, 2006) that primarily depend on the genetic origin (Adkins *et al*., 2002) and the environment during seed maturation (Boyce *et al*., 1976). However, post-harvest treatments (chemical substances, temperatures, electromagnetic waves, etc.) can also affect seed dormancy of forage crops (Stanisavljeviæ *et al*., 2012, 2014; Vinod *et al*., 2014). This paper presents results obtained on the dormancy level and germination percentage of seeds, as well as on seedling vigour of three forage species (cocksfoot, tall fescue and perennial ryegrass) during three periods that coincided with the period immediately after harvest, autumn sowing in the same year and spring sowing in the succeeding year. The objective of the study was to determine the optimum application of chemical (H_2SO_4) and/or temperature treatments for the increasing the seed germination during these periods.

Materials and Methods

Seeds of six cultivars or populations of cocksfoot (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) were used in the experiments in locations in Serbia and Bosnia and Herzegovina. Seed samples were drawn from designated plants in the same location during three years (2011-

2013). After harvesting and drying of seeds to the moisture content of approximately 12%, the 1000-seed weight was established.

The analysis of seed germination and dormancy was performed in three periods: immediately after seed harvesting (C1), three months after seed harvesting (C2) and eight months after seed harvesting (C3). Seed in paper bags was stored in the conventional store room (average temperature of 12.4^{o}C and air relative humidity of 73%) until analysis. Prior to each period of analysis (C1–C3) seed was exposed to the chemical treatment with H_2SO_4 in the following concentrations: 98%, 75%, 50% and 25% for 30, 20 and 10 min for each concentration [A1–A12]. Afterwards, seed was rinsed under tap water for 10 min, dried and tested. In another experiment, seed was exposed to various temperatures: 90°C, 80°C, 70°C, 60°C, 50°C and 40°C for 90, 60 and 30 min [A13–A30] at each temperature.

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The germination test with 4 x 100 seeds was done on filter paper under alternate temperatures of 20/30°C (dark/light). Germination of tall fescue and perennial ryegrass seeds was determined on the 14th day and of cocksfoot seeds on the 21st day as per ISTA Rules (2003). The seed was considered to be germinated if the seedling is normal with 5 mm root and shoot. The tetrazolium test was applied to some seeds to identify dormant and dead seeds. Seeds are placed in a tetrazolium solution (1%) for a period of 18 h at 30°C as indicated in ISTA Handbook (2008).

Collected data were statistically analysed using the analysis of variance (ANOVA) appropriate for the complete randomised block design. The mean of six genotypes for each species was used in statistical analyses. The *LSD* multiple range test was used to detect significant differences among means at the 5% level of probability. Data of germinating and dormancy percentage were arcsine transformed prior to the analysis. The correlation between traits was established employing the Pearson's Correlation Test.

Results and Discussion

The differences in 1000-seed weight in cultivars and populations (hereinafter referred to as genotypes) over locations were amounted to 0.98–1.10 g in cocksfoot, 2.09–2.28 g tall fescue and 2.39–2.50 g in perennial ryegrass and was not statistically significant (LSD test; P>0.05). Further, the correlation between 1000-seed weight and germination was not significant in any of the species (P>0.05). Therefore, it can be concluded that differences in 1000-seed weight did not affect obtained results over treatments.

The analysis of variance (F test) showed that seed treatments (by chemicals or temperature) and the after ripening period significantly ($P \leq 0.01$) affected germination and dormancy in all three species, while genotypes and years had no significant effect on these traits in any of the species (data not shown). Consequently, only interactions between seed treatments and the after ripening period was significant ($P \leq 0.01$) among the second and third level of interactions for all species. Hence, the analysis of germination and dormancy data combined for seed treatment levels and the after ripening period for each species (Tables 1–3).

In the period immediately after harvest, seed dormancy and germination in cocksfoot control plants (A0) were 26% and 70%, respectively. Treatments with 98% H_2SO_4 (A1–A6) completely broke seed dormancy, but they also ruined seed germination (A1–A3). The exposure of cocksfoot seeds to temperatures of 90^{o}C (A13–A15) and 80°C (A16) also entirely broke seed dormancy, and increased germination by 18% (A13) to 23% (A16). The treatment A16 (exposure of seeds to the temperature of 80°C for 90 min) was the most suitable for treating cocksfoot seeds immediately after harvest, because dormancy was totally broken, and seed germination amounted to 93%. Absolutely the highest germination (94%) in cocksfoot seeds was recorded in the treatment with 50% H_2SO_4 for 30 min (A7), while seed dormancy was only 2%. Seed dormancy and germination in tall fescue immediately after harvest were 28% and 71%, respectively. All applied treatments (A1–A5, A13–A15) decreased or totally broke seed dormancy in tall fescue and at the same time increased seed germination (all but A1–A3). The germination increase was the greatest when seeds had been treated with 50% H_2SO_4 for 30 min (A7) and 20 min (A8) (24% and 23%, respectively). At the same time, seed dormancy amounted to 2–4%. The treatment A5 can also be considered successful because germination amounted to 93%, while seed dormancy was completely broken. Out of three species, the highest initial (A0) germination (80%) and the lowest seed dormancy (18%) were recorded in seeds of perennial ryegrass. As with the two previous species, treatments A1–A3 completely broke seed dormancy, but they also destroyed seed germination. The majority of treatments did not significantly affect the germination increase in perennial ryegrass seeds. However, germination upon

Table 1. Effects of chemical and temperature treatments (mean value ± SEM) applied immediately after sampling in June on seed germination (G) and dormancy (D)[1].

Treatment (A)		Cocksfoot		Tall fescue		Perennial ryegrass	
		G%	D %	G %	D%	G%	D %
Control	A0	70 ± 0.98^{h}	26 ± 1.12^{a}	71 ± 0.99^{e}	28±0.89a	$80\pm1.85^{d-f}$	18 ± 1.01^{a}
H_2SO_4 98%	30' - A1	0 ± 0.00^{i}	0 ± 0.00^{j}	0 ± 0.00^{f}	0 ± 0.00^{k}	0 ± 0.00^{h}	0 ± 0.00^{k}
	20' - A2	0 ± 0.00^{i}	0 ± 0.00^{j}	0 ± 0.00^{f}	0 ± 0.00^{k}	0 ± 0.00^{h}	0 ± 0.00^{k}
	10' - A3	2 ± 0.89^{i}	0 ± 0.00^{j}	5 ± 2.08^{f}	0 ± 0.00^{k}	0 ± 0.00^{h}	0 ± 0.00^{k}
H_2SO_4 75%	30' - A4	71 ± 1.12^{h}	0 ± 0.00^{j}	$78\pm1.11^{c-e}$	0 ± 0.00^{k}	62 ± 0.91^{g}	0 ± 0.00^{k}
	20' - A5	$74\pm1.08^{f-h}$	0 ± 0.00^{j}	$93\pm0.75^{a-c}$	0 ± 0.00^{k}	$65\pm1.03^{f-g}$	0 ± 0.00^{k}
	10' - A6	$85\pm1.08^{a-g}$	0 ± 0.00^{j}	$88\pm1.04^{a-d}$	$5\pm1.05^{g-j}$	$66\pm1.03^{e-g}$	0 ± 0.00^{k}
H_2SO_4 50%	30' - A7	94 ± 0.58^{a}	$2\pm0.68^{i-j}$	95 ± 0.62^{a}	$2\pm0.91^{i-k}$	$88\pm0.71^{a-c}$	0 ± 0.00^{k}
	20' - A8	$90\pm1.01^{a-c}$	$6\pm0,92^{g-j}$	$94\pm0.59^{a-b}$	$4\pm0.93^{h-k}$	$92\pm0.75^{a-b}$	$2\pm1.17^{i-k}$
	10' - A9	$89\pm0.78^{a-d}$	$8\pm1.08^{f-g}$	$92\pm1.01^{a-c}$	$6\pm0.94^{f-i}$	$91\pm0.73^{a-c}$	$4\pm0.14^{h-k}$
H_2SO_4 25%	30' - A10	93 ± 0.63^{a}	$3\pm0.85^{h-j}$	$80\pm1.08^{a-e}$	$15\pm1.13^{c-d}$	93 ± 0.56^{a}	$3\pm0.13^{i-k}$
	20' - A11	$79\pm1.35^{b-h}$	$18\pm0.95^{b-d}$	$78\pm1.54^{c-e}$	$18\pm1.14^{b-c}$	$85\pm0.73^{a-d}$	$10\pm0.82^{d-g}$
	10' - A12	$77\pm1.06^{d-h}$	$20\pm0.99^{a-d}$	$76\pm0.95^{d-e}$	$19\pm1.01^{b-c}$	$86\pm0.83^{a-d}$	13 ± 0.74^{b}
T^{o}C 90	90' - A13	$88\pm0.94^{a-f}$	0 ± 0.00^{j}	$81\pm1.03^{a-e}$	0 ± 0.00^{k}	$77\pm0.89^{c-f}$	0 ± 0.00^{k}
	60' - A14	$90\pm0.99^{a-c}$	0 ± 0.00^{j}	$88\pm0.98^{a-d}$	0 ± 0.00^{k}	$82\pm0.71^{a-d}$	0 ± 0.00^{k}
	30' - A15	$91\pm0.88^{a-b}$	0 ± 0.00^{j}	$92\pm0.05^{a-c}$	0 ± 0.00^{k}	$84\pm0.76^{a-d}$	0 ± 0.00^{k}
T^{o}C 80	90' - A16	93 ± 0.62^{a}	0 ± 0.00^{j}	$92\pm0.52^{a-c}$	0 ± 0.00^{k}	$88\pm0.74^{a-c}$	1 ± 1.13^{k}
	60' - A17	$88\pm1.14^{a-e}$	$5\pm0-59^{g-j}$	$90\pm0.52^{a-d}$	$2\pm0.82^{i-k}$	$86\pm0.88^{a-d}$	$3\pm1.01^{i-k}$
	30' - A18	$85\pm0.87^{a-g}$	$7\pm1.11^{g-i}$	$88\pm1.19^{a-d}$	$4\pm0.92^{h-k}$	$85\pm0.82^{a-d}$	$4\pm0.84^{h-k}$
T^{o}C 70	90' - A19	$88\pm0.96^{a-e}$	$8\pm0.79^{g-i}$	$89\pm1.09^{a-d}$	$3\pm0.92^{h-k}$	$86\pm0.79^{a-d}$	$6\pm0.92^{g-j}$
	60' - A20	$83\pm1.14^{a-g}$	$10\pm 0.98^{f-g}$	$87\pm0.95^{a-d}$	$6\pm0.93^{f-i}$	$84\pm0.77^{a-d}$	$7\pm1.13^{f-i}$
	30' - A21	$80\pm0.79^{b-h}$	$15\pm0.87^{d-f}$	$86\pm0.95^{a-e}$	$9\pm0.96^{f-g}$	$83\pm0.71^{a-d}$	$7\pm1.21^{f-i}$
T^{o}C 60	90' - A22	$82\pm1.23^{a-h}$	$16\pm0.86^{d-e}$	$87\pm0.96^{a-d}$	$8\pm0.95^{e-h}$	$86\pm0.79^{a-d}$	$8\pm1.32^{f-g}$
	60' - A23	$80\pm0.95^{b-h}$	$17\pm0.98^{c-d}$	$85\pm0.92^{a-e}$	$10\pm1.01^{e-f}$	$84\pm0.82^{a-d}$	$9\pm0.84^{f-g}$
	30' - A24	$78\pm1.13^{c-h}$	$19\pm0.52^{b-d}$	$84\pm0.84^{a-e}$	$12\pm0.98^{d-e}$	$82\pm0.96^{a-d}$	$10\pm1.24^{d-g}$
T^{o}C 50	90' - A25	$79\pm1.52^{b-c}$	$16\pm1.06^{d-e}$	$83\pm1.11^{a-e}$	$17\pm.0.95^{b-c}$	$81\pm0.99^{a-d}$	$11\pm1.11^{c-f}$
	60' - A26	$77\pm0.97^{e-h}$	$19\pm0.89^{b-d}$	$80\pm0.59^{a-e}$	$19\pm0.45^{b-c}$	$80\pm1.06^{b-c}$	$13\pm1.10^{b-e}$
	30' - A27	$76\pm0.87^{e-h}$	$21\pm1.05^{a-c}$	$78\pm1.05^{c-e}$	20 ± 0.35^{b}	$79\pm0.88^{c-d}$	$15\pm1.42^{a-c}$
T^{o}C 40	90' - A28	$78\pm1.45^{c-h}$	$20\pm1.12^{a-d}$	$79\pm0.61^{b-c}$	$18\pm0.98^{b-c}$	$80\pm0.89^{b-c}$	$14\pm0.61^{a-d}$
	60' - A29	$76\pm1.36^{e-h}$	$23\pm0.99^{a-c}$	$77\pm0.56^{c-e}$	20 ± 0.98^{b}	$79\pm0.96^{c-d}$	$16\pm1.11^{a-b}$
	30' - A30	$74\pm1.21^{f-h}$	$25\pm1.21^{a-b}$	$75\pm0.78^{d-e}$	23 ± 0.95^{b}	$77\pm1.02^{c-f}$	$17\pm1.42^{a-b}$

[1]Different letters within the same column denote significant differences (*LSD*, $P\leq0.05$). Values are mean ± standard error of the mean

the application of the three treatments (A8–10) exceeded 90% (92–94%), and at the same time seed dormancy ranged from 2% to 4%.

In the region of south-eastern and central Europe, as well as, in regions up to 500 m above sea level, seeds of cocksfoot, tall fescue and perennial ryegrass mature in June, this coincides with harvest. Seeds of these species can be used in agronomic practice immediately after harvest to establish lawns in horticultural purposes. In order to have intensive cropping practice, it is necessary to have seed of high germination, *i.e.* reduced dormancy. According to Adkins *et al.* (2002) seed dormancy in grasses can be based on tissues surrounding the embryo, then on mechanical barriers that prevent embryo enlargement and on prevention of germination inhibitors. Dormancy may be present in the embryo and can be a result of enzymes producing gene depressions and affecting the embryo (Dyer *et al.*, 1993). Our results show that a significant increase in germination and a reduction in dormancy of seeds of observed species were possible immediately after harvest if the short-duration treatment (10–30 min) with 50% H_2SO_4 was applied.

Seed dormancy in cocksfoot, tall fescue and perennial ryegrass was reduced by 2%, 8% and 8%, respectively, three months after harvest, which coincided with the period of autumn sowing (September). At the same time, germination in cocksfoot, tall fescue and perennial ryegrass was increased by 3%, 4% and 6%, respectively

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Table 2. Effects of chemical and temperature treatments (mean value ± SEM) applied three months after sampling in June on seed germination (G) and dormancy (D)[1].

Treatment (A)		Cocksfoot		Tall fescue		Perennial ryegrass	
		G%	D %	G %	D%	G%	D %
Control	A0	73±1.23^{c}	24±1.24^{a}	75±1.31^{b}	20±1.24^{a}	86±1.28^{ab}	10^{a}±1.05
H_2SO_4 98%	30' - A1	0±0.00^{d}	0±0.00^{j}	0±0.00^{c}	0±0.00^{j}	0±0.00^{e}	0±0.00^{g}
	20' - A2	0±0.00^{d}	0±0.00^{j}	0±0.00^{c}	0±0.00^{j}	0±0.00^{e}	0±0.00^{g}
	10' - A3	0±0.00^{d}	0±0.00^{j}	7±0.89^{c}	0±0.00^{j}	0±0.00^{e}	0±0.00^{g}
H_2SO_4 75%	30' - A4	70±1.41^{c}	0±0.00^{j}	71±1.47^{b}	0±0.00^{j}	53±1.16^{d}	0±0.00^{g}
	20' - A5	72±1.09^{b-c}	0±0.00^{j}	90±0.67^{a}	0±0.00^{j}	59±1.41^{cd}	0±0.00^{g}
	10' - A6	81±1.14^{a-c}	0±0.00^{j}	93±0.49^{a}	0±0.00^{j}	60±0.23^{cd}	0±0.00^{g}
H_2SO_4 50%	30' - A7	92±0.71^{a}	0±0.00^{j}	93±0.51^{a}	0±0.00^{j}	82±1.14^{ab}	0±0.00^{g}
	20' - A8	93±0.58^{a}	0±0.00^{j}	93±0.65^{a}	0±0.00^{j}	90±1-05^{ab}	0±0.00^{g}
	10' - A9	91±0.69^{a}	5±1.04^{g-i}	93±0.54^{a}	0±0.00^{j}	91±0.62a	0±0.00^{g}
H_2SO_4 25%	30' - A10	92±0.48^{a}	1±0.98^{i-j}	89±0.96^{a-b}	4±0.07^{f-i}	92±0.61a	0±0.00^{g}
	20' - A11	90±0.55^{a}	9±1.21^{d-g}	85±0.98^{a-b}	9±1.12^{d-d}	90±0.78^{ab}	3±1.16^{d-g}
	10' - A12	86±0.87^{a-c}	12±b-d	80±1.28^{a-b}	11±1.13^{b}	90±1.01^{ab}	5±1.28^{b-e}
T°C 90	90' - A13	85±0.94^{a-c}	0±0.00^{j}	75±1.25^{a-b}	0±0.00^{j}	69±1.15^{b-d}	0±0.00^{g}
	60' - A14	88±1.19^{a-b}	0±0.00^{j}	85±1.08^{a-b}	0±0.00^{j}	72±1.08^{a-d}	0±0.00^{g}
	30' - A15	90±0.49^{a}	0±0.00^{j}	88±1.25^{a-b}	0±0.00^{j}	77±0.96^{a-c}	0±0.00^{g}
T°C 80	90' - A16	91±0.52^{a}	0±0.00^{j}	90±0.87^{a}	0±0.00^{j}	85±0.79^{a-b}	0±0.00^{g}
	60' - A17	92±0.47^{a}	2±0.92^{h-j}	91±0.71^{a}	0±0.00^{j}	86±0.87^{a-b}	0±0.00^{g}
	30' - A18	90±0.78^{a}	4±1.06^{g-j}	90±0.49^{a}	0±0.00^{j}	86±1.12^{a-b}	0±0.00^{g}
T°C 70	90' - A19	90±0.56^{a}	4±0.87^{g-j}	90±0-62^{a}	0±0.00^{j}	88±1.05^{a-b}	0±0.00^{g}
	60' - A20	88±0.78^{a-b}	6±0.98^{f-h}	90±0.56^{a}	2±0.98^{h-j}	87±1.56^{ab}	1±0.92^{f-g}
	30' - A21	86±0.89^{a-c}	7±0.29^{e-g}	88±1.25^{ab}	3±0.98^{g-j}	90±0.98^{ab}	2±0.89^{e-g}
T°C 60	90' - A22	89±1.12^{a-b}	6±1.56^{f-h}	90±0.62^{a}	1±0.87^{i-j}	91±0.68a	1±1.56^{f-g}
	60' - A23	88±1.12^{a-b}	8±0.98^{d-g}	90±0.58^{a}	5±0.89^{e-h}	90±0.69^{ab}	2±0.89^{e-g}
	30' - A24	82±0.99^{a-c}	10±1.26^{c-f}	87±0.95^{a-b}	7±0.89^{c-g}	87±1.36^{ab}	4±1.05^{c-f}
T°C 50	90' - A25	87±0.88^{a-c}	10±0.98^{c-f}	89±1.56^{a-b}	7±1.05^{c-g}	89±1.25^{ab}	3±1.25^{d-g}
	60' - A26	85±0.98^{a-c}	11±1.25^{b-e}	88±1.41^{a-b}	8±0.96^{b-e}	86±1.16^{ab}	5±0.89^{b-e}
	30' - A27	83±1.25^{a-c}	13±0.97^{b-c}	85±0.89^{a-b}	9±0.95^{b-d}	85±1.23^{ab}	6±1.02^{b-c}
T°C 40	90' - A28	86±1.04^{a-c}	12±0.98^{b-d}	88±1.25^{a-b}	6±1.05^{d-g}	87±0.87^{ab}	4±1.26^{c-f}
	60' - A29	84±1.23^{a-c}	14±1.35^{b-c}	86±1.05^{a-b}	9±1.12^{b-d}	86±1.56^{ab}	6±0.98^{b-c}
	30' - A30	81±0.78^{a-c}	15±1.52^{b}	85±0.89^{a-b}	10±1.45^{b-c}	84±0.89^{ab}	8±1.12^{a-b}

[1]Different letters within the same column denote significant differences (*LSD*, $P \leq 0.05$). Values are mean ± standard error of the mean

(Table 2). Germination below 75% can be a problem of meeting regulations for moving seed in trade (Canode *et al*., 1963; Stanisavljeviæ*et al*., 2010). Poor germination is often attributed to weaker seedling vigour (Bretagnolle *et al*., 1995; Stanisavljeviæ*et al*., 2011, 2014). Therefore, it is very important to increase seed germination of forage grasses intended for autumn sowing as it, in comparison to spring sowing in the succeeding year, provides much higher forage yields. In seed production, the majority of grasses sown in spring will not yield. Even in establishing lawns in horticultural purposes, autumn sowing is much more advantageous than spring sowing in the succeeding year (Salehi and Khosh-Khui, 2004).

In cocksfoot, all temperature treatments resulted in increased seed germination and reduced seed dormancy. Nonetheless, when seed was exposed to lower temperatures (40 and 50°C) dormancy was 10% or above. The maximum germination (92%) in cocksfoot was achieved at 80°C for 60 min (A17). Chemical treatments were more successful in breaking cocksfoot seed dormancy. The highest germination of 92–93%, with the simultaneous dormancy breaking, was obtained with 50% H_2SO_4 for 30 min (A7) and 20 min (A8). However, the difference in seed germination when the best chemical and temperature treatment had been applied was only 1%. Similar results were gained in tall fescue where the most successful treatment was with 25% and

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50% H_2SO_4 (A6–A9) at which the germination amounted to 93%, while seed dormancy was completely broken. Maximum germination (91%) was recorded in temperature treatments (A19) and without dormant seeds. The lowest seed dormancy of all perennial ryegrass genotypes was recorded in this period. The highest seed germination (91%) of this species was recorded at a somewhat lower concentration of H_2SO_4 (25%) than the concentration which was optimal for cocksfoot and tall fescue (50%). The same germination percentage in perennial ryegrass seeds was achieved when seeds were exposed to the temperature of 60°C for 90 min (A22), but dormancy was not totally broken.

In contrast to the application of chemical and temperature treatments immediately and three months after harvest, there was no statistically significant germination increase in either of the species when these treatments had been applied eight months after harvest (Table 3). The seed germination eight months after harvest amounted to 89%, 90% and 93% in cocksfoot, tall fescue and perennial ryegrass, respectively. There were no dormant seeds in perennial ryegrass, while seed dormancy in remaining two species amounted to 3–4%. After the application of different treatments, maximum germination of 94% was obtained in cocksfoot and tall fescue by using temperature treatment (40°C for 30 min).

Table 3. Effects of chemical and temperature treatments (mean value ± SEM) applied eight months after sampling in June on seed germination (G) and dormancy (D)[1].

Treatment (A)		Cocksfoot		Tall fescue		Perennial ryegrass	
		G%	D %	G %	D%	G%	D %
Control	A0	89±0.97^{a}	4±1.05^{a}	90±0.58^{a}	3±0.89^{a}	93±0.65^{a}	0±0.00^{a}
H_2SO_4 98%	30' - A1	0±0.00^{d}	0±0.00^{c}	0±0.00^{d}	0±0.00^{d}	0±0.00^{h}	0±0.00^{a}
	20' - A2	0±0.00^{d}	0±0.00^{c}	0±0.00^{d}	0±0.00^{d}	0±0.00^{h}	0±0.00^{a}
	10' - A3	0±0.00^{d}	0±0.00^{c}	0±0.00^{d}	0±0.00^{d}	0±0.00^{h}	0±0.00^{a}
H_2SO_4 75%	30' - A4	67±1.34^{c}	0±0.00^{c}	65±1.48^{b-c}	0±0.00^{d}	47±1.39^{g}	0±0.00^{a}
	20' - A5	69±1.09^{b-c}	0±0.00^{c}	83±1.19^{a-c}	0±0.00^{d}	50±1.32^{g}	0±0.00^{a}
	10' - A6	77±1.23^{a-c}	0±0.00^{c}	86±1.25^{a-c}	0±0.00^{d}	52±1.26^{f-g}	0±0.00^{a}
H_2SO_4 50%	30' - A7	86±0.98^{a-c}	0±0.00^{c}	85±1.31^{a-c}	0±0.00^{d}	72±1.09^{b-e}	0±0.00^{a}
	20' - A8	90±0.78^{a}	0±0.00^{c}	86±1.25^{a-c}	0±0.00^{d}	78±1.06^{a-e}	0±0.00^{a}
	10' - A9	92±0.71^{a}	1±1.08^{bc}	88±1.12^{a-b}	0±0.00^{d}	89±0.78^{ab}	0±0.00^{a}
H_2SO_4 25%	30' - A10	89±0.89^{a}	0±0.00^{c}	88±1.09^{a-b}	0±0.00^{d}	82±0.98^{a-d}	0±0.00^{a}
	20' - A11	92±0.59^{a}	1±0.87^{bc}	90±0.62^{a}	0±0.00^{d}	85±0.98^{a-c}	0±0.00^{a}
	10' - A12	93±0.59^{a}	2±0.77^{b}	92±0.56^{a}	2±0.99^{b}	91±0.64^{ab}	0±0.00^{a}
T°C 90	90' - A13	82±0.98^{a-c}	0±0.00^{c}	70±1,08^{a-c}	0±0.00^{d}	58±1.54^{e-g}	0±0.00^{a}
	60' - A14	85±1.23^{a-c}	0±0.00^{c}	82±1,06^{a-c}	0±0.00^{d}	63±1.29^{d-g}	0±0.00^{a}
	30' - A15	88±1.36^{a-b}	0±0.00^{c}	83±1.45^{a-c}	0±0.00^{d}	67±1.36^{c-g}	0±0.00^{a}
T°C 80	90' - A16	89±0.99^{a}	0±0.00^{c}	75±1.32^{a-c}	0±0.00^{d}	76±1.41^{a-e}	0±0.00^{a}
	60' - A17	90±0.87^{a}	0±0.00^{c}	84±0.98^{a-c}	0±0.00^{d}	78±1.23^{a-e}	0±0.00^{a}
	30' - A18	90±0.98a	0±0.00^{c}	85±0.89^{a-c}	0±0.00^{d}	79±1.31^{a-d}	0±0.00^{a}
T°C 70	90' - A19	89±1.08^{a}	0±0.00^{c}	77±0.89^{a-c}	0±0.00^{d}	78±1.26^{a-e}	0±0.00^{a}
	60' - A20	89±1.15^{a}	0±0.00^{c}	85±0.98^{a-c}	0±0.00^{d}	80±1.12^{a-d}	0±0.00^{a}
	30' - A21	90±0.98^{a}	0±0.00^{c}	86±1.09^{a-c}	0±0.00^{d}	81±1.09^{a-d}	0±0.00^{a}
T°C 60	90' - A22	90±0.89a	0±0.00^{c}	80±1.12^{a-c}	0±0.00^{d}	80±1.56^{a-d}	0±0.00^{a}
	60' - A23	91±0.98^{a}	0±0.00^{c}	87±1.05^{a-c}	0±0.00^{d}	83±1.56^{a-d}	0±0.00^{a}
	30' - A24	92±0.63^{a}	0±0.00^{c}	90±0.63^{a}	0±0.00^{d}	85±1.26^{a-c}	0±0.00^{a}
T°C 50	90' - A25	89±1.09^{a}	0±0.00^{c}	89±0.57^{a}	0±0.00^{d}	84±1.19^{a-c}	0±0.00^{a}
	60' - A26	90±0.61^{a}	0±0.00^{c}	91±0.61^{a}	0±0.00^{d}	87±1.23^{a-c}	0±0.00^{a}
	30' - A27	93±0.49^{a}	1±1.01^{bc}	93±0.59^{a}	1±0.89^{c}	91±1.02^{a-b}	0±0.00^{a}
T°C 40	90' - A28	90±0.55^{a}	0±0.00^{c}	90±0.64^{a}	0±0.00^{d}	88±0.78^{ab}	0±0.00^{a}
	60' - A29	93±0.59^{a}	1±0.73^{bc}	92±0.58^{a}	0±0.00^{d}	91±0.89^{a-b}	0±0.00^{a}
	30' - A30	94±0.61^{a}	2±1.05^{b}	94±0.56^{a}	1±1.09^{c}	93±0,58^{a}	0±0.00^{a}

[1]Different letters within the same column denote significant differences (*LSD*, $P \leq 0.05$). Values are mean ± standard error of the mean.

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Generally, the best results in these two species were achieved at lower either temperatures (40°C and 50°C) or concentrations H_2SO_4 (25%), which is in accordance with results obtained by Fumagalli and Freire (2007) on seeds of beard grass (*Brachiaria brizantha*). Since there were no dormant seeds in perennial ryegrass, the application of chemical and temperature treatments adversely affected germination, and therefore their application is not recommended on seeds of this species after eight months ofharvest. Spring sowing of forage and ornamental grasses in March of the following year coincides with the eighth month after harvest in continental regions of central, south and eastern Europe. Sowing in this period ensures that seedlings are free from freezing, the amount of precipitation is reasonably sufficient and there are no danger form stronger droughts until seedlings are rooted.

The previous studies have showed that it was possible to overcome seed dormancy and increase seed germination of grasses by the application of chemical and temperature treatments. At the same time, it was observed that species differently responded to their effects. For instance, a significant ($P \leq 0.05$) increase in germination of beard grass (*Brachiaria brizantha*) seeds in relation to control has been achieved by the application of 98% H_2SO_4 for 5–10 min (Lacerda *et al*., 2010), while Garcia and Cicero (1992) have recommended a mixture of H_2SO_4 and KNO_3 (0.2%) for 15 min in order to reduce seed dormancy in the same species. On the other hand, Martins and Silva (2001) have recommended that beard grass seeds would be exposed to 85°C for 10–15 min. In order to optimally increase seed germination of Bermuda grass (*Cynodon dactylon*), red fescue (*Festuca rubra*) and meadow grass (*Poa pratensis*) it is necessary to apply 50%, 25% and 50% H_2SO_4 for 25, 15 and 15-25 min, respectively (Salehi and Khosh-Khui, 2005). Although seed germination in the majority of grasses increases with lower concentrations (25–50%) of H_2SO_4, there are also species such as African foxtail grass (*Cenchrus ciliaris*) in which the highest seed germination is achieved when 100% H_2SO_4 was applied for 4 min (Bhattarai *et al*., 2008). As expected, germination was positively and significantly ($P \leq 0.01$) correlated with root length, shoot length and seedling biomass in all three species. According to Verma *et al*. (2014), seeds with strong vigour are characterised with rapid and uniform germination during the crop establishment and it is a prerequisite to obtain high yields.

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Conclusion

Seed germination of cocksfoot and tall fescue can be increased, by varying exposure to temperature or sulphuric concentration, up to eight months after harvest. This coincides with spring sowing in the succeeding year. In a case of perennial ryegrass there were not a single treatment resulted in the seed germination increase after three months, which coincides with the period of autumn sowing.

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