Low temperature and hardening effects on photosynthetic apparatus efficiency and survival of forage grass varieties

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ABSTRACT

Freezing tolerance is essential for perennial plants and ability to adapt to extreme temperature is crucial for their survival in many environments. Freezing tolerance of hardened and unhardened plants of *Dactylis glomerata* and *Lolium perenne* varieties was probed by their quantum photosynthetic efficiency using the chlorophyll fluorescence technique. Quantum yield of photosystem II (PSII) electron transport (Φ_{PSII}), maximal (F_m') and steady-state (F_s) chlorophyll fluorescence yields of light-adapted samples were measured. Φ_{PSII} depended on developmental phase, temperature and hardening process. A clear decline in PSII activity, especially after –10°C application was observed. Plant hardening during emergence phase had a positive impact on PSII activity, especially after –5°C application. After 72 h of –5°C temperature treatment, hardened plants showed quicker recovery of their photosynthetic apparatus (0.527–0.697) as compared to unhardened ones (0.224–0.330). Stress temperature of –10°C caused irreversible changes of photosynthetic apparatus of hardened and unhardened plants independently of growth phases (0.003–0.014). Φ_{PSII} and F_m' parameters were strongly correlated with shoots survival under stress. Our results suggest that perennial plants' hardening allows them to survive low temperatures due *inter alia* enhancing their photosynthetic machinery performance.

Keywords: chlorophyll fluorescence; *Dactylis glomerata*; freezing; frost acclimation; *Lolium perenne*

The increasing need for sustainable agricultural grassland requires grass species and varieties characterized a reliable extended season of growth, combined with good winter survival to ensure sward longevity. Perennial ryegrass (*Lolium perenne* L.) is the most important grass species of temperate climate regions and their diploid varieties characterize significantly greater cold tolerance than autotetraploid ones (Sugiyama 1998). Cocksfoot (*Dactylis glomerata* L.) is also widely grown forage grass in temperate climate countries in Europe and is considered as overwintering and resistant to unfavourable growing conditions (Lemežiene et al. 2004, Borawska-Jarmułowicz et al. 2010).

Low temperature during winter is a significant factor determining geographical distribution of temperate perennial grass species and this could be contributed to the negative impact of low temperature on photosynthetic apparatus functioning (Kalaji et al. 2011, Borawska-Jarmułowicz et al. 2014) and plants' capability for acclimation (Harrison et al. 1997).

Chlorophyll fluorescence measurements allow evaluating physiological status of plants based on detection changes in some PSII components, electron transport chain components, and light-dependent photochemical reactions. It is a very sensitive tool that allows sensing changes in general bioenergetic status of plants' photosynthetic apparatus (Borawska-Jarmułowicz et al. 2014).

In the present study, the effect of freezing stress on physiological status and survival of hardened and unhardened several forage varieties of *Dactylis glomerata* and *Lolium perenne* is investigated.

MATERIAL AND METHODS

The study was performed on seedlings of Polish forage varieties of *Dactylis glomerata* (L.) – Amera, Amila and *Lolium perenne* (L.) – Diament and Gagat. Pot experiment was conducted under controlled conditions in plant growth chambers (Phytotron – BLOCK a.s.,Valašské Meziříčí, Czech Republic) at the Warsaw University of Life Science-SGGW. Fifty seeds of each varieties were sown per pot (18 cm diameter and 15 cm height) in three replications, filled with 3.5 kg mineral brown soil of 70% capillary water capacity (soil water regime was controlled by weighing each pot daily and restoring soil water content). Plants were thinned 30 days after sowing, leaving 30 plants per pot. Conditions in growth chamber were as follows: humidity of air 65%, temperature 17°C/10°C (day/night) at 12 h photoperiod with light intensity 1000 µmol photons/m^2/s.

Concentrations of available soil phosphorus (66 mg/kg) was low, potassium (133 mg/kg) and magnesium (64 mg/kg) were medium and soil pH_{KCl} was 5.1. Fertilizer was applied as follows: N – 1.039 g, P – 0.268 g, K – 0.162 g per pot once before sowing. At emergence and tillering phases (45 and 74 days after sowing, respectively) plants were exposed to low temperature of either –5°C or –10°C for 24 h. Two different ways of low temperature treatment were applied: plants were hardened or unhardened before freezing. Hardening of plants was carried out in a low temperature over 72 h. Temperature was gradually reduced over 12 h from 16°C to 5°C and then maintained for 24 h. Over the next 12 h temperature decreased to 0°C and maintained for 24 h. Unhardened plants were immediately subjected to stress temperatures. After 24 h of low temperature treatment all pots with frozen plants were placed to a growth chamber for one week.

Chlorophyll fluorescence parameters: quantum yield of photosystem II (PSII) electron transport (Φ_{PSII}), maximal (F_m') and steady-state (F_s) chlorophyll fluorescence yields of light-adapted samples were measured using a fluorometer FMS-2 (Hansatech Ltd., Kings Lynn, UK). The experiment was conducted at emergence and tillering phases on 10 randomly selected plants from each pot (in 3 replications for each variety) on fully developed leaves. Measurements were performed once before temperature stress (control), twice during hardening (after 5°C and 0°C) and 3 times after the application of temperature stress of –5°C or –10°C (directly, after 24 and 72 h). After one week of low temperature stress application survival rate of shoots (percentage share of live tillers visible) and root/shoot ratio (R/S) were registered.

Experimental data were analysed statistically using multifactor analysis of variance. Significance of differences between means were determined using the Tukey's test and significance level $P < 0.05$* and $P < 0.01$**. Relations between some selected fluorescence parameters, morphological traits and survival of shoots were assessed by a correlation analysis.

RESULTS AND DISCUSSION

Quantum efficiency of photosystem PSII of *D. glomerata* and *L. perenne* varieties depended on developmental phase, temperature and way of low temperature application (hardened and unhardened plants) (Tables 1 and 2, Figure 1). During emergence phase, average value of Φ_{PSII} of control plants of investigated varieties (maintained in a growth chamber) ranged from 0.738 to 0.757. Plants subjected to low temperature stress showed a clear decline in PSII activity, especially after application of –10°C (mean 0.199). Plants' hardening had a positive impact on the activity of photosystem, especially after –5°C application. The average value of measured parameter after temperature stress was higher in hardened plants (0.527 cv. Diament – 0.697 cv. Amera) in comparison to unhardened (differed from 0.224 to 0.330). Φ_{PSII} measured directly after temperature –5°C application was at the same level, regardless of plants' hardening. However next measurements after 24 and 72 h from freezing temperature, revealed a positive effect of hardening on measured parameter. It indicates that hardened plants started more quickly to rebuild their photosynthetic apparatus than unhardened plants. Finally after 72 h from –5°C temperature treatment potential quantum efficiency reached values similar to control (0.527–0.697), especially in plants of cv. Amera of *D. glomerata*. These results indicated to PSII regeneration of investigated grass varieties. After low temperature application Φ_{PSII} values also increased in not hardened plants however, they reached only about half of those recorded for control plants (0.224–0.330). Plants treated with stress temperature of –10°C regardless of variety and hardening indicated very low values of potential quantum efficiency Φ_{PSII} not only after 24 h, but as well as after 72 h (0.003–0.014).

Table 1. Effects of freezing temperature (–5°C and –10°C) after 72 h and low temperature treatment (I – unhardened; II – hardened) on quantum yield of PSII electron transport (ΦPSII), maximal (F_m') and steady-state (F_s) chlorophyll fluorescence yields of light-adapted samples, survival of shoots (%), and root/shoot ratio (R/S) of *Dactylis glomerata* and *Lollium perenne* varieties at emergence and tillering phases. Chlorophyll fluorescence parameters are shown as relative units

Variety	Temperature (°C)	Low temperature treatment									
		Φ_{PSII}		survival		R/S ratio		F_s		F_m'	
		I	II	I	II	I	II	I	II	I	II
Emergence phase											
Amera	–5	0.240^a	0.697^b	14.23^a	71.63^b	2.072^a	2.190^a	323.97^a	399.93^b	556.20^a	1468.47^b
	–10	0.012^a	0.011^a	0.00^a	0.00^a	0.853^a	1.228^a	286.80^a	235.63^a	283.93^a	234.20^a
Amila	–5	0.330^a	0.585^b	17.53^a	43.17^b	4.689^a	4.044^a	304.60^a	350.80^a	599.97^a	1140.30^b
	–10	0.011^a	0.007^a	0.00^a	0.00^a	1.741^a	2.182^a	219.47^a	203.40^a	221.60^a	200.57^a
Diament	–5	0.224^a	0.527^a	9.10^a	44.53^b	3.301^a	4.223^a	213.97^a	301.90^b	373.80^a	789.47^b
	–10	0.005^a	0.004^a	0.00^a	0.00^a	1.043^a	1.575^a	240.70^a	239.90^a	236.43^a	234.13^a
Gagat	–5	0.244^a	0.559^b	20.60^a	43.20^b	1.918^a	3.485^b	237.13^a	415.90^b	395.63^a	1272.27^b
	–10	0.014^a	0.014^a	0.00^a	0.00^a	1.715^a	1.668^a	234.90^a	214.77^a	233.43^a	214.97^a
$HSD_{0.01}$		0.12**		15.52**		1.46**		66.73**		316.29**	
Tillering phase											
Amera	–5	0.099^a	0.377^b	38.03^a	40.90^a	0.775^a	1.536^b	384.60^a	463.83^a	502.17^a	887.70^b
	–10	0.008^a	0.013^a	0.00^a	0.00^a	0.674^a	0.571^a	285.87^a	335.80^a	284.73^a	341.00^a
Amila	–5	0.079^a	0.399^b	25.84^a	66.07^b	1.371^a	2.384^b	345.17^a	373.00^a	430.87^a	858.53^b
	–10	0.009^a	0.008^a	0.00^a	0.00^a	0.493^a	0.507^a	251.60^a	303.73^a	250.90^a	304.67^a
Diament	–5	0.162^a	0.363^b	10.23^a	25.37^b	1.652^a	1.643^a	329.13^a	313.70^a	504.30^a	633.57^a
	–10	0.003^a	0.007^a	0.00^a	0.00^a	0.617^a	0.740^a	316.33^a	228.67^a	298.07^a	218.87^a
Gagat	–5	0.042^a	0.325^b	7.17^a	42.13^b	1.012^a	0.864^a	317.63^a	628.40^b	334.73^a	1098.60^b
	–10	0.003^a	0.005^a	0.00^a	0.00^a	0.942^a	0.649^a	262.00^a	275.80^a	259.00^a	274.43^a
$HSD_{0.01}$		0.08**		8.89**		0.50**		91.05**		215.45**	

Average values followed by same letter in lines for low temperature applications (unhardened and hardened) create homologous groups at 0.01 probability level; $^{**}P \leq 0.01$

During tillering phase, similarly to emergence phase, reduction of Φ_{PSII} values after low temperatures stress was observed (0.615–0.705). However, these values were lower than those recorded in emergence phase, regardless of temperature and conditions of low temperature applications. Plants subjected to –5°C temperature irrespective of cold-acclimation (due hardening), showed a decrease of Φ_{PSII} values directly after stress (about 0.6 relative units). Simultaneously, obtained results indicated that, hardened plants of all varieties regenerated better after 72 h from freezing in comparison with plants that were not hardened. It was evidenced by higher values of potential photosynthetic efficiency (mean 0.366 and 0.059, respectively). Stress temperature of –10°C, as well as in emergence phase, caused irreversible changes of assimilation apparatus of plants, both hardened and unhardened. Φ_{PSII} values of plants subjected to this temperature were very low, not only immediately after stress (0.004–0.067), but also after 72 h (0.003–0.009).

A strong correlation (r = 0.848) was noted between Φ_{PSII} parameter values after 72 h of low temperatures (–5°C and –10°C) and survival of shoots estimated one week after thermal stress in all investigated varieties (Figure 2). Similar results

Table 2. Homogeneous groups of factor levels and interactions according to ANOVA table analysis showing significance of obtained results relevant to the studied varieties, freezing temperatures, conditions of low temperature treatment, and developmental phases

Factor	Factor level	Φ_{PSII}	Survival	R/S ratio	F_s	F_m'
Variety A	Amera	0.182	20.60^{b}	1.24^{a}	339.55^{b}	569.80^{b}
	Amila	0.179 NS*	19.08^{ab}	2.18^{b}	293.97^{ab}	500.93^{ab}
	Diament	0.162	11.15^{a}	1.85^{ab}	273.04^{ab}	411.08^{a}
	Gagat	0.151	14.14^{ab}	1.53^{ab}	323.32^{ab}	510.38^{ab}
Temperature B	–5	0.33^{b}	32.48^{b}	2.32^{b}	356.48^{b}	740.41^{b}
	–10	0.01^{a}	0.00^{a}	1.08^{a}	258.46^{ab}	255.68^{a}
Low temparature treatment C	unhardened	0.09^{a}	8.92^{a}	1.55 NS*	284.62^{a}	360.36^{a}
	hardened	0.24^{b}	23.6^{b}	1.84	330.32^{b}	635.73^{b}
Phase D	emergence	0.22^{b}	16.50 NS*	2.37^{b}	276.49^{a}	528.46 NS*
	tillering	0.12^{a}	15.98	1.03^{a}	338.45^{b}	467.63
	interactions	BC**	AB**	AB**	AB**	BC**
		BD**	BC**	BD**	AC**	BD**
			ACD**	BCD**	BC**	
			ABCD**		ABC**	

*$P \leq 0.05$; **$P \leq 0.01$. Averages (mean values) followed by the same letter in columns create homologous groups at 0.01 probability levels; NS – not significant

were found for F_m' parameter. At the same time survival of shoots varied in emergence and as well as in tillering phases, depending on low temperature and hardening treatment (Tables 1 and 2). Hardening of plants had a significant impact on their survival after exposure to –5°C temperature in both developmental phases. A comprehensive study of frost resistance of meadow fescue (*Festuca pratensis* Huds.) presented by Rapacz et al. (2000) reported that cold acclimation at 5°C during 4 weeks resulted in increase in frost resistance of their leaves (expressed in lethal temperature LT50). A significantly higher survival rate of cv. Amera variety at emergence phase (about 72%) than survival of remaining varieties (43.2–44.5%) was observed. Moreover, shoot survival of hardened plants was worse in tillering than in emergence phase for most varieties. Only cv. Amila variety had a better survival rate (by 23%) in tillering phase compared to emergence phase. Moreover, Amila characterized significantly higher survival of shoots than other varieties. Among *L. perenne* varieties, a significantly better survival was observed in cv. Gagat variety. It seems that each variety has its own threshold of this low temperature (–5°C) at each growth stage. These results correspond with those of Harrison et al. (1997) who showed a sharp decline in photosynthetic efficiency in unhardened material of *L. perenne* varieties and observed that apical meristems die after exposure to temperatures below –5°C.

After –10°C application during emergence and tillering phases no recovery was observed (plants did not survived) for all studied varieties, independently of the use of hardening. Harrison et al. (1997) noticed that in hardened material of *L. perenne* varieties recovery of photosynthetic efficiency was observed in plants frozen to as low as –11°C. According to Eagles et al. (1993) improved survival of whole plant of *L. perenne* was associated with ability to produce viable regrowth from tillers after freezing to lower temperatures.

Φ_{PSII} parameter value after 72 h of low temperatures (–5°C and –10°C) was clearly correlated not only to survival of shoots (0.848), but also to R/S ratio ($r = 0.571$) (Figure 2). Higher values of R/S were found in emergence phase (2.37) than in tillering (1.03) and in plants treated with temperature –5° C (2.32) than –10°C (1.08), independent of other factors (Tables 1 and 2). Hardening plants in emergence phase sig-

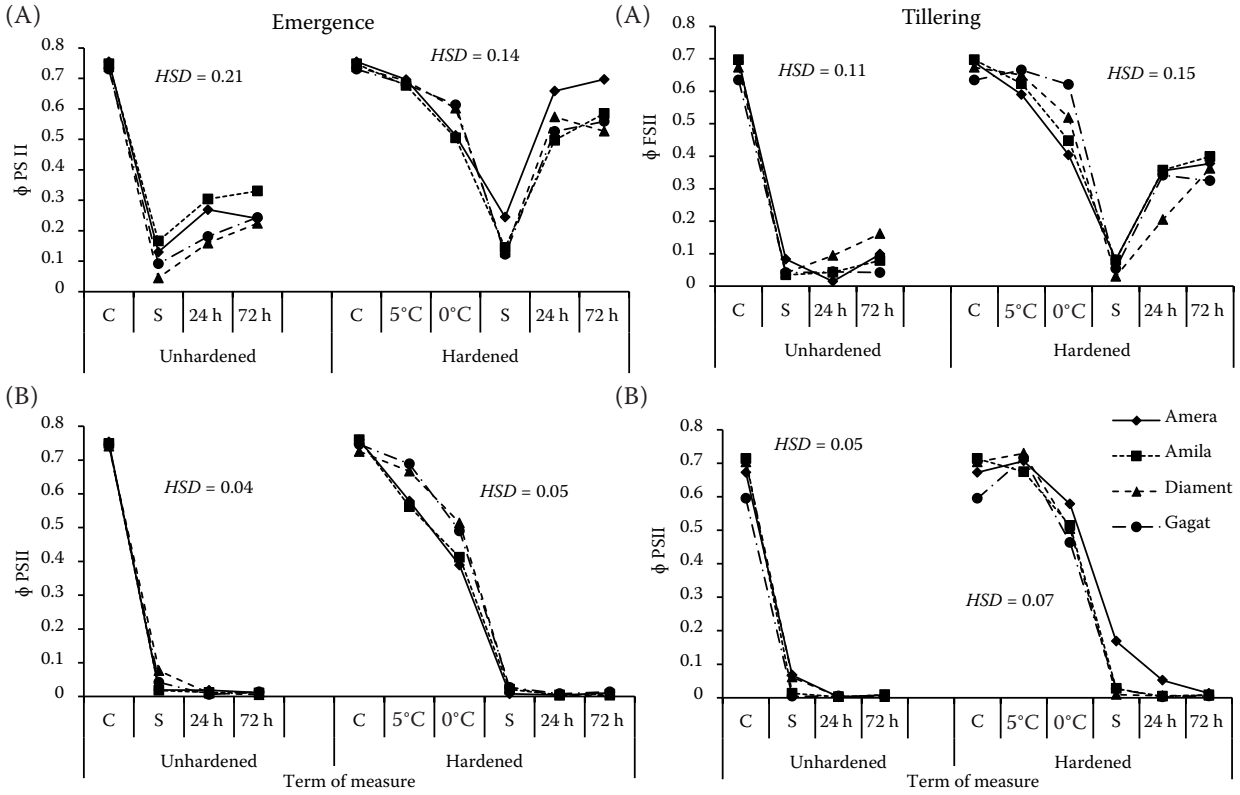

Figure 1. Changes of the values of quantum yield of PSII (Φ_{PSII}), in leaves of *Dactylis glomerata* and *Lollium perenne* varieties treated with low temperature (A) –5°C; (B) –10°C at emergence and tillering phases with two different conditions of low temperature treatment (unhardened and hardened) in following measurements' terms: C – control, during hardening after 5°C and 0°C; S – directly after stress, after 24 and 72 h

nificantly increased R/S ratio (about 45%) only in plants of *L. perenne* cv. Gagat treated with –5°C while in tillering phase only in *D. glomerata* Amila and Amera varieties (by 42.5% and 49.5%, respectively).

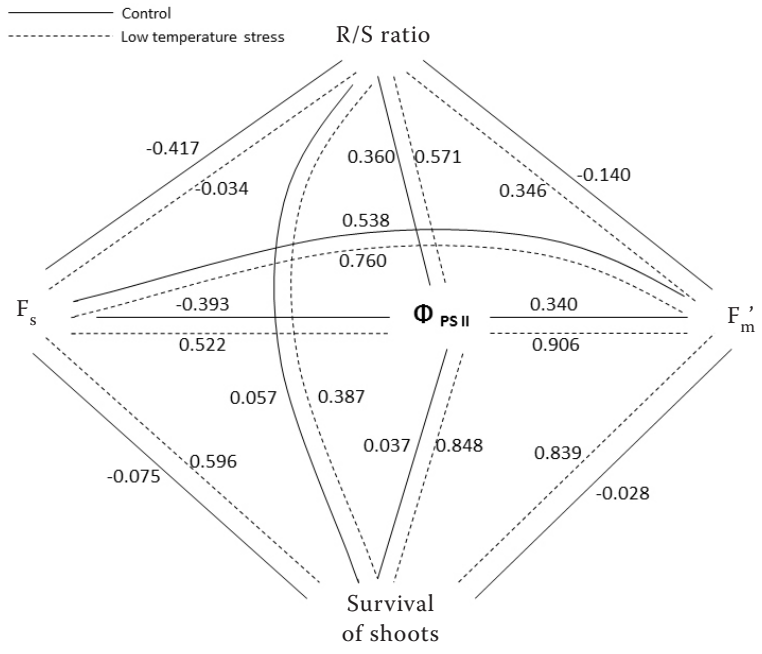

Figure 2. Correlation coefficients (r) between selected fluorescence parameters, morphological traits and survival of shoots (correlations significant at $P < 0.05$). R/S – root/shoot ratio; Φ_{PSII} – quantum yield of photosystem II (PSII) electron transport; maximal (F_m') and steady-state (F_s) chlorophyll fluorescence yields of light-adapted samples were measured

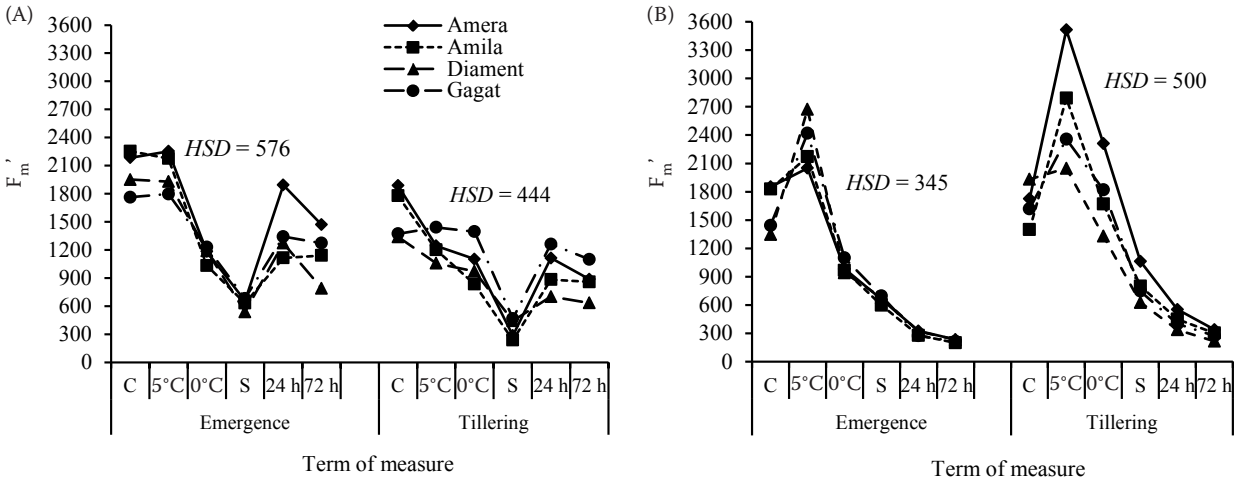

Figure 3. Changes of values of maximum fluorescence parameter (F_m') in leaves of *Dactylis glomerata* and *Lollium perenne* varieties treated with low temperature: (A) –5°C; (B) –10°C during hardening of plants at emergence and tillering phases at following terms of measurements: C – control, during hardening after 5°C and 0°C; S – directly after stress, after 24 and 72 h

A significant differences in F_s parameter values for all estimated varieties after 72 h of stress, depending on used parameters, were observed (Tables 1, 2). Higher values of steady-state fluorescence F_s were observed at tillering than at emergence phase. A significant effect of hardening was also shown on F_s values. Hardening of plants increased the values of parameter F_s under stressful temperature –5°C, especially at emergence stage, but had no effect under temperature of –10°C. There was a positive correlation after application of low temperature stress (–5°C and –10°C) between steady-state fluorescence values F_s and Φ_{PSII} (r = 0.522) and as well as survival of shoots (r = 0.596). On the other hand, a negative relationship between F_s values and R/S ratio was noted (Figure 2).

Maximum fluorescence values (F_m') varied depending on applied stress temperature and hardening treatment (Figure 3, Tables 1 and 2). Values of F_m' parameter after 72 h following the end of freezing period at –5°C and –10°C were reduced compared to control parameters. At the same time value of F_m' parameter after –5°C application was significantly higher compared to temperature –10°C treatment (740.41 and 255.68, respectively). Hardening of plants had a significant impact on F_m'. It was observed that hardened plants of all *D. glomerata* and *L. perenne* varieties in emergence as well as tillering phases after –5°C temperature stress were characterized by higher values of this parameter in comparison to unhardened plants. At emergence phase the greatest difference of F_m' parameter was noticed at cv. Amera of *D. glomerata*, while at cv. Diament of *L. perenne* it was smallest. In tillering phase, it was cv. Gagat of *L. perenne* that the most clearly responded to hardening (difference of F_m' parameter values between hardened and unhardened plants was ca. 760). Statistical analysis showed also highly significant positive correlation between F_m' and survival of shoots (r = 0.839) and also parameter of Φ_{PSII} (r = 0.906) (Figure 2).

A comparison of correlation coefficients diagram among all studied parameters (photosynthetic activity/efficiency, R/S ratio and shoots survival) permits to analyse different strategies used by analysed grasses under control and low temperature stress conditions. When grown without stress, grasses did not show any significant relationships between any of traits pairs (Figure 2). There was also no clear indication showing that, photosynthetic efficiency of plants is responsible for plant development or growth. Noteworthy are nearly nulled correlation coefficients for shoot survival and R/S, Φ_{PSII}, F_m' and F_s, respectively. Thus, under optimal temperature neither survival of shoots nor R/S ratio were determined by any individual processes of analysed photosynthetic efficiency.

Similarly to Kalaji and Pietkiewicz (1993) work on salinity stress and barley, freezing stress forced grasses to strengthen their regulation of photosynthetic productivity processes to withstand unfavourable conditions and assure plants survival. This was clearly verified by reinforcing relationships among almost all studied indicators/parameters (more significant correlation coefficient). Low

temperature stress (–5°C and –10°C) increased the significance of positive relationships: F_m' *vs* Φ_{PSII}, Φ_{PSII} *vs* shoots survival, F_m' *vs* F_s, Φ_{PSII} *vs* survival and R/S ratio *vs* Φ_{PSII}. Simultaneously it changed the direction of other relationships from insignificant negative to significant positive ones: F_m' *vs* survival, F_s *vs* Φ_{PSII} and F_s *vs* survival, and F_m' *vs* R/S ratio. The only negative correlation maintained, but at a much higher level, was F_s *vs* R/S. Thus, under stress conditions the strategy of tested grasses is clearly to enhance/shift the photosynthetic efficiency performance towards assurance of maximum high possible level of shoots survival.

In conclusions, our results indicate, that hardening of plants had a positive impact on activity of photosystem II (Φ_{PSII}). Hardened plants more quickly started to rebuild their photosynthetic apparatus after freezing stress as compared to unhardened ones. Φ_{PSII} and F_m parameters were strongly correlated with shoots survival under stress.

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