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Original Paper

The effect of zinc application on annual ryegrass (*Lolium multiflorum*) under drought stress

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The aim of the vegetation pot experiment observed the effect of zinc foliar application in combination with nitrogen fertilization on annual ryegrass plant ability to deal with drought stress. Experiment with two irrigation regimes was established and the rate of drought stress was evaluated by determination of abscisic acid (ABA) content in above-ground part of ryegrass, dry matter weight and nutrient content in plant were also determined. Foliar Zn application had statistically significant effect (P <0.05) on decrease of ABA content in comparison to variants without Zn fertilization which implies that zinc contributed to better plant dealing with drought stress. Foliar application of higher Zn dose (1.25 g l^{-1}) under drought stress was found the strongest decreased of ABA in comparison with variant without Zn fertilization (about 50.1%). Zinc supported also the growth of above-ground part regardless to irrigation, we found the highest dry matter weight on variant with higher nitrogen dose (0.5 g pot^{-1}) and higher Zn dose (1.25 g l^{-1}). Graded levels of zinc application significantly effected of Zn content in plant.

Keywords: annual ryegrass, foliar zinc application, drought stress, abscisic acid content

1 Introduction

Annual ryegrass (*Lolium multiflorum*) is a water demanding species cultivated mainly for fodder or as a catch crop in the Czech Republic. Ryegrass growth and productivity is influenced mainly by drought and the lack of water leads to reduction of yield. One of possible ways how to contribute to plant resistance to drought is the application of microelements, especially of zinc (Zn). Many authors stated that zinc plays an essential role in alleviating drought stress (Karim et al., 2012, Ma et al., 2017, Hussain et al., 2020). Zn application raises plant resistance to drought stress (Vaghar et al., 2020) and it contributes to the stability of photosynthetic activity under the lack of water (Wang et al., 2009). The role of zinc in plant is not completely described, nevertheless the positive effect of Zn to plants water management is mentioned by Karim et al. (2012) and Sadoogh et al. (2014). Zinc is vital for normal growth and development of plants, it is a part of proteins and an activator of many enzymes (Upadhyaya et al., 2013), especially of enzymes that involve plants growth (Havlin et al., 1999). Zinc is necessary for structural and catalytic components of proteins and enzymes, many protein sequences also contain Zn-binding structural domains (Steffens, 1990; Clarke and Berg, 1998). The level of Zn in plant has an impact on stomatal conductance which is reduced in case of zinc deficiency (Khan et al., 2004). Zinc also affects dry matter accumulation (Upadhyaya et al., 2013) and it plays an important role in a plant's defence against reactive oxygen species, whose occurrence increases under stress conditions (Cakmak, 2000).

Hormonal and osmotic regulation is one of plants physiological defence systems against the lack of water (Huang et al., 2014). Drought stress signals are primarily perceived by root system which is exposed to reduced water content in soil. Many plant hormones participate

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in root growth under drought, especially abscisic acid (ABA) has a special role in plant response to drought stress (Seo et al., 2009). Not only root reaction to drought are involved by ABA, but ABA also regulates stomatal aperture under drought stress (Stristava, 2002). ABA has multiple roles in plant, it involves in abscission of leaves and fruits and inhibits germination. This hormone also plays an important role in water management in plant, it participates in osmotic regulation (Procházka et al., 1998). Many authors (Ivanovic et al., 1992; Conti et al., 1994; Cao et al., 2000) described that concentrations of ABA in shoot and root tissues under water-limited conditions are increased, but also other abiotic stress such as salinity or extreme temperature lead to ABA accumulation in plants (Xiong et al., 2002). Accumulated ABA protects plants from drought stress by inducing stomata closure which leads to lower water losses by transpiration (Li et al., 2000) and improves hydraulic conductance for water movement from root to leaves (Zhang et al., 1995). ABA is also responsible for inhibition of leaf growth and due to this helps plants to reduce transpiration during water stress (Alves and Setter, 2000). Sharp (2002) mentioned that high ABA concentrations in water stressed plants correlate with growth inhibition.

The objective of this study was to determine the effect of foliar application of zinc on ryegrass under water stress and evaluated ABA content in above-ground mass as a rate of drought impact on plant.

2 Material and methods

The vegetation pots experiment was established in the growth chambers (PlantMaster, CLF Plant Climatics GmbH, Germany) of the Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriSciences, Mendel University in Brno. This experiment was performed twice under the same conditions and with the same methods used. Annual ryegrass variety Jivet was sown in 4th April and 14th May 2018 (1 g seeds per pot). Jivet is early Czech variety resistant to lodging and it has a huge root system which improves soil structures. It is ideal for cultivation in humid areas (Seed service, 2020). Pots were filled with 6 kg of arenic chernozem soil. The soil was removed from upper layer of topsoil (locality Žabčice, South Moravia, 49.0193672 N, 16.5925011 E), it was subsequently dried and homogenised. The selected agrochemical properties of soils are shown in Table 1.

Plants were cultivated in controlled temperature, humidity and light mode (12 h day length, photosynthetic photo flux density of 350 µmol m^{-2} s^{-1}, temperature of 23/18 °C (day/night) and relative humidity of 55/70%). Plants were divided in two groups (irrigation regime) 15 days after sowing (18th April and 28th May 2018), first group was well-watered (100% irrigation doses), and second group was stressed by drought (50% irrigation doses). Soil application of nitrogen (solution of ammonium nitrate) and foliar application of zinc (solution of $ZnSO_4 \cdot 7\ H_2O$) were done 15 days after sowing (18th April and 28th May 2018) according to Table 2.

Table 2 Variants and their fertilization

Variants*		Nutrient doses	
		nitrogen (g pot^{-1})	zinc (g l^{-1}) **
1	N1^{ww}	0.25	0
2	N1 + Zn1^{ww}	0.25	1.25
3	N1 + Zn2^{ww}	0.25	2.5
4	N2^{ww}	0.50	0
5	N2 + Zn1^{ww}	0.50	1.25
6	N2 + Zn2^{ww}	0.50	2.5
7	N1^{ds}	0.25	0
8	N1 + Zn1^{ds}	0.25	1.25
9	N1 + Zn2^{ds}	0.25	2.5
10	N2^{ds}	0.50	0
11	N2 + Zn1^{ds}	0.50	1.25
12	N2 + Zn2^{ds}	0.50	2.5

* irrigation regime: ^{ww} – well-watered, ^{ds} – drought stress, ** 5 ml zinc solution per pot

All variants were conducted in four repetitions (pots). Sampling of above-ground mass of ryegrass was performed 30 days after sowing (3rd May and 12th June 2018). Plant samples from both experiments were analysed equally. The content of abscisic acid was evaluated in homogenous sample of ryegrass above-ground mass. The samples of 1 g plant biomass were taken from each variant in two repetition and homogenized by using mortar and pestle with sea sand and distilled water in 1 : 10 ratio. Homogenized samples were put into freezer for 14 days. After that the extraction of ABA into water was

Table 1 Agrochemical properties of soils

pH ($CaCl_2$)	C_{ox}	Clay	Silt	Sand	Plant-available nutrient (mg kg^{-1}) *				Available Zn (mg kg^{-1}) **
					P	K	Ca	Mg	
5.7	0.80%	20%	27%	53%	113	306	1766	132	0.85

* Mehlich 3 (Jones, 1990), ** Lindsay and Norvell (1978)

done by using shaker for 12 hours in dark condition and temperature of 12 °C. These samples were centrifuged for 8 minutes at 5,000 rpm. ABA content was determined by RIA method (radioimmunoassay) in separated supernatant. This sensitive method uses radiolabeled molecules for measuring concentration of ABA in ng per g of fresh weight (FW). The main components are antigen MAC 252, marked radioligand 3H-ABA, ABA in analyzed samples and ABA standards of known concentration for creating calibration curve (Quarrie et al., 1988). The content of ABA was measured using spectrophotometer PACKARD 2900 TR (PerkinElmer, USA) and the results were determined by program Securia PACKARD. ml

Plant samples were dried to constant weight at the temperature of 60 °C, dry matter weight and the contents of nitrogen and zinc in above-ground plant mass was determined. Plant samples were crushed in a grinder and homogenized after weighing. The resultant of crushed plant mass was mineralized using a mixture of H_2SO_4 and H_2O_2 in microwave system Milestone Ethos 1 (Milestone, Italy) according to Zbíral et al. (2005). Method of Kjeldahl was used for determination of nitrogen (N) content, it was determined colorimetrically using Unicam 8,625 UV/Vis spectrometer (ATI Unicam, UK). Zinc (Zn) content was determined by using Atomic Absorption Spectrophotometry (AAS) in ContrAA 700 instrument (Analytik Jena AG, Germany). Statistical evaluation of monitored parameters was performed by Statistica 12 CZ program (StatSoft, 2013). ANOVA analysis of variance and follow-up tests according to Fisher (LSD test) at 95% (P <0.05) level of significance were used and the results were expressed as a mean ± standard error (SE).

3 Results and discussion

Results in Figure 1 show higher ABA content in most variants under drought stress in compare to well-watered, we found significant differences between both irrigation regime in N1, N2, N1 + Zn1, N2 + Zn2 variants. For example, ABA content is about 87% higher on well-watered variant N1 than N1 stressed by drought. This trend corresponds to the information that ABA is enhanced during water stress which is reported by many authors (Pospíšilová, 2003; Boominathan et al., 2004; Bano et al., 2012; Karim and Rahman, 2015; Yang et al., 2018). Zinc application had an effect on decreasing drought stress in plants which had lower ABA content in biomass. Application of higher Zn dose (Zn2) seems to be the most suitable for better plant holding with drought stress, the level of ABA is getting closer to ABA content under well-water conditions.

The significantly highest differences between ABA content in drought stressed plants were found on variants N1^{ds} and N1 + Zn2^{ds}, ABA content was decreased on average by about 50.1% thanks to Zn application (Table 3). Ren et al. (2017) also observed ABA content increase from ~2.93 ng g^{-1} FW (well-watered) to ~251.97 ng g^{-1} FW (soil water content reduction to 25%) in the *Festuca elata* leaves. The role of Zn in plant during drought is not fully understood, but it involves the elevation of water use efficiency (Karim et al., 2012), transpiration rate changes and the leaf osmotic potential (Sadoogh et al., 2014) and it also participates in modulating biochemical dtamages by antioxidant enzymes (Upadhyaya et al., 2013). We statistically evaluated effect of the observed factors; the

Table 3 Content of ABA (ng g^{-1} FW)

Variants	Irrigation regime	Content of ABA (ng g^{-1} FW)		
		1st experiment	2nd experiment	average content
N1^{ww}	well-watered	4.34^{ab} ±0.15	4.83^{cd} ±0.40	4.59^{b} ±0.19
N1 + Zn1^{ww}		5.01^{b} ±0.27	7.83f ±0.22	6.42^{cd} ±0.22
N1 + Zn2^{ww}		4.76^{b} ±0.25	5.97^{de} ±0.80	5.37^{bc} ±0.52
N2^{ww}		4.82^{b} ±0.26	5.28^{cd} ±0.93	5.05^{b} ±0.59
N2 + Zn1^{ww}		2.11^{a} ±0.06	2.25^{a} ±0.40	2.18^{a} ±0.17
N2 + Zn2^{ww}		5.58^{b} ±2.68	2.54^{ab} ±0.41	4.06^{b} ±1.17
N1^{ds}	drought stress	9.15^{d} ±0.27	8.01f ±0.60	8.58f ±0.17
N1 + Zn1^{ds}		8.23^{cd} ±0.13	8.69f ±0.22	8.46f ±0.14
N1 + Zn2^{ds}		4.35^{ab} ±0.36	4.09^{bc} ±1.13	4.22^{b} ±0.51
N2^{ds}		8.10^{cd} ±0.18	8.04f ±0.12	8.07^{e}f ±0.15
N2 + Zn1^{ds}		6.60^{bc} ±0.21	7.56^{e}f ±0.39	7.08^{de} ±0.30
N2 + Zn2^{ds}		4.76^{b} ±0.20	5.75^{cd} ±0.01	5.25^{bc} ±0.10

means sharing the same superscript are not significantly different from each other (P <0.05) according to LSD test (each column was evaluated separately)

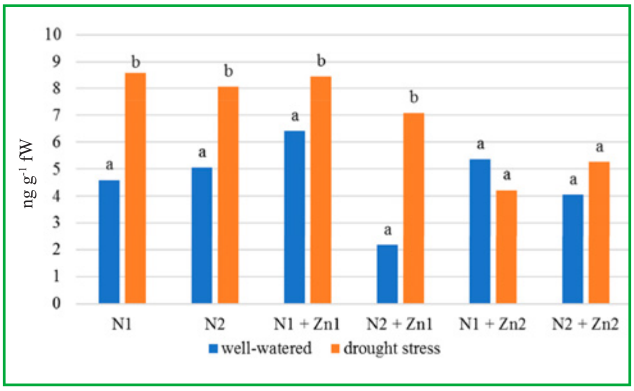

Figure 1 Effect of irrigation regime on average ABA content in ryegrass biomass
statistically evaluated within irrigation regime (well-watered, drought stress), same letters are not significantly different (*P* <0.05) according to LSD test

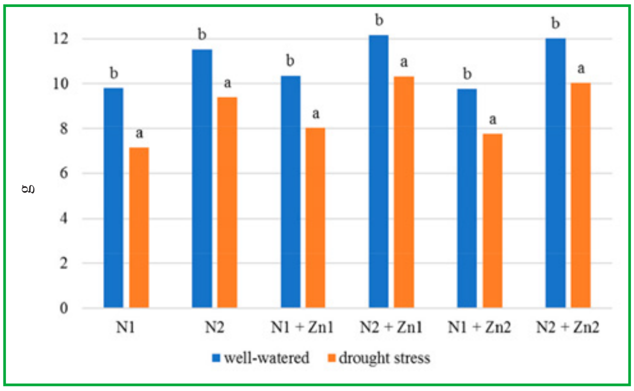

Figure 2 Effect of irrigation regime on dry matter weight of ryegrass above-ground biomass
statistically evaluated within irrigation regime (well-watered, drought stress), same letters are not significantly different (*P* <0.05) according to LSD test

total variability of the ABA content was affected by factor "irrigation regime" in the amount of 46.0%, factor "zinc application" in the amount of 11.3% and factor "nitrogen fertilization" in the amount of 8.6% in average of both experiments.

It is obvious from Figure 2, that dry matter weight of plants above-ground was decreased in dry condition regardless of the type of fertilization. The graded doses of nitrogen increased the dry matter weight. The same effect of higher N doses on ryegrass was described by Brambilla et al. (2012), Amanuel et al. (2015) and Cinar et al. (2020). Zinc application under drought conditions would have positive effect on crop yield and quality (Monjezi and Hassanzadehdelouei, 2013, Škarpa et al., 2015, Ashkiani et al., 2020). In our experiment, dry matter weight was higher after both Zn doses in contrast to variants without Zn. Contrary to results of ABA content, where application of higher dose of zinc showed better plant endurance to stress, dry matter weight seems to be better after application of lower Zn dose (1.25 g l^{-1} Zn).

The highest dry matter weight from well-watered variants was found on variant N2 + Zn1. The same type of fertilization showed the highest dry matter weight also in plants under drought stress (Table 4). The dry matter weight of variant N2 + Zn1 was about 5.6% higher (well-watered) and 10% higher (drought stress) than dry matter weight of variants N2. Positive effect of Zn application on ryegrass dry matter production was also

Table 4 Dry matter weight (g pot^{-1}) of above-ground part of ryegrass

Variants	Irrigation regime	Dry matter weight (g pot^{-1})		
		1st experiment	2nd experiment	average content
$N1^{ww}$	well-watered	9.76^{cd} ±0.39	9.86^{bc} ±0.29	9.81^{b} ±0.28
$N1 + Zn1^{ww}$		10.59^{de} ±0.32	10.10^{bc} ±0.66	10.35^{b} ±0.43
$N1 + Zn2^{ww}$		9.68^{c} ±0.24	9.89^{bc} ±0.47	9.79^{b} ±0.18
$N2^{ww}$		10.59^{de} ±0.20	12.42^{de} ±1.03	11.51^{c} ±0.52
$N2 + Zn1^{ww}$		11.29^{e} ±0.20	13.00^{e} ±1.19	12.15^{c} ±0.62
$N2 + Zn2^{ww}$		11.18^{e} ±0.30	12.84^{e} ±0.16	12.01^{c} ±0.09
$N1^{ds}$	drought stress	6.32^{a} ±0.42	8.03^{a} ±0.59	7.17^{a} ±0.47
$N1 + Zn1^{ds}$		7.09^{a} ±0.05	8.94^{ab} ±0.35	8.02^{a} ±0.19
$N1 + Zn2^{ds}$		6.32^{a} ±0.52	9.21^{ab} ±0.47	7.76^{a} ±0.47
$N2^{ds}$		8.13^{b} ±0.28	10.68^{bcd} ±0.15	9.40^{b} ±0.13
$N2 + Zn1^{ds}$		8.50^{b} ±0.23	12.19^{de} ±0.58	10.34^{b} ±0.22
$N2 + Zn2^{ds}$		8.57^{b} ±0.38	11.50^{cde} ±0.72	10.03^{b} ±0.47

means sharing the same superscript are not significantly different from each other (*P* <0.05) according to LSD test (each column was evaluated separately

Table 5 Content of nitrogen and zinc in above-ground part of ryegrass

Variants	Irrigation regime	1st experiment		2nd experiment		Average content	
		N content (rel. %)	Zn content (mg kg^{-1})	N content (rel. %)	Zn content (mg kg^{-1})	N content (rel. %)	Zn content (mg kg^{-1})
N1^{ww}	well-watered	4.64^{b}	34.60^{a}	2.80^{a}	47.84^{a}	4.40^{ab}	41.22^{a}
N1 + Zn1^{ww}		4.55^{b}	122.60^{b}	2.67^{a}	141.70^{bc}	4.28^{a}	132.15^{b}
N1 + Zn2^{ww}		4.71^{b}	174.85^{cd}	2.70^{a}	227.16^{e}	4.38^{ab}	215.05^{d}
N2^{ww}		5.76^{d}	42.43^{a}	2.76^{a}	99.96^{ab}	4.96^{c}	44.23^{a}
N2 + Zn1^{ww}		5.79^{d}	145.58^{bc}	3.60^{b}	121.92^{bc}	5.60^{d}	147.82^{b}
N2 + Zn2^{ww}		5.45^{cd}	157.89^{c}	3.81^{b}	212.62^{de}	5.62^{d}	186.54^{c}
N1^{ds}	drought stress	4.97^{bc}	39.94^{a}	3.54^{b}	44.94^{a}	4.69^{bc}	42.51^{a}
N1 + Zn1^{ds}		4.63^{b}	192.45^{d}	3.43^{b}	169.24^{cd}	4.42^{ab}	182.31^{c}
N1 + Zn2^{ds}		3.72^{a}	315.78f	3.91^{b}	294.84f	4.15^{a}	313.71f
N2^{ds}		5.58^{d}	41.24^{a}	4.65^{c}	46.21^{a}	5.67^{d}	43.71^{a}
N2 + Zn1^{ds}		5.41^{cd}	192.57^{d}	4.65^{c}	175.72^{bce}	5.62^{d}	184.90^{c}
N2 + Zn2^{ds}		5.39^{cd}	272.80^{e}	4.85^{c}	311.86f	5.73^{d}	287.91^{e}

means sharing the same superscript are not significantly different from each other (P <0.05) according to LSD test (each column was evaluated separately)

described by Kremper and Seres (2010). Grzebisz et al. (2008) observed the same effect on maize, Mathpal et al. (2015) on wheat and Poblaciones et al. (2017) on *Lolium rigidum* and *Trifolium subterraneum*.

Zinc treatment affected its content in annual ryegrass (*Lolium multiflorum*), which was significantly enhanced on all variants with microelement application (Table 5). Bowen (1979) states that the range of normal Zn concentration in plants growing on unpolluted soil is 20–400 mg kg^{-1} of dry weight. We determined higher Zn content on variants under drought stress in compare to well-watered variants, the similar trend was observed for maize (Wang and Jing 2007), sorghum (Dimkpa et al., 2019) and wheat (Velu et al., 2016).

4 Conclusions

According to ABA content in plants as drought stress marker, foliar application of zinc improved annual ryegrass (*Lolium multiflorum*) reaction to drought stress. The combination of lower N dose with higher Zn dose (variant N1 + Zn2) appears as the most suitable for supporting the ryegrass in dealing with drought stress. Foliar Zn application contributed to increase of dry matter weight of plant regardless to drought stress, the highest dry matter weight was found on variant fertilized with higher N dose with combination of lower Zn dose (variant N2 + Zn1).

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