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 (Havlín 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...
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 photon 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 ZnSO₄ · 7 H₂O) were done 15 days after sowing (18th April and 28th May 2018) according to Table 2.

### Table 1
**Agrochemical properties of soils**

| pH (CaCl₂) | Cw | Clay | Silt | Sand | Plant-available nutrient (mg kg⁻¹) * | Available Zn (mg kg⁻¹) ** |
|------------|----|------|------|------|-------------------------------------|--------------------------|
| 5.7        | 0.80% | 20% | 27%  | 53%  | 113 | 306 | 1766 | 132 | 0.85 |

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

### Table 2
**Variants and their fertilization**

| Variants* | Nutrient doses | zinc (g l⁻¹) ** |
|-----------|----------------|----------------|
| N¹ww      | 0.25           | 0              |
| N¹ + Zn¹ww| 0.25           | 1.25           |
| N¹ + Zn²ww| 0.25           | 2.5            |
| N²ww      | 0.50           | 0              |
| N² + Zn¹ww| 0.50           | 1.25           |
| N² + Zn²ww| 0.50           | 2.5            |
| N¹h       | 0.25           | 0              |
| N¹ + Zn¹h | 0.25           | 1.25           |
| N¹ + Zn²h | 0.25           | 2.5            |
| N²h       | 0.50           | 0              |
| N² + Zn¹h | 0.50           | 1.25           |
| N² + Zn²h | 0.50           | 2.5            |

* irrigation regime: ww – well-watered, h – 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
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 H₂SO₄ and H₂O₂ 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).

### 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 N1ds and N1 + Zn2ds, 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 damages by antioxidant enzymes (Upadhyaya et al., 2013). We statistically evaluated effect of the observed factors; the

| Variants | Irrigation regime | 1st experiment | 2nd experiment | average content |
|----------|------------------|----------------|----------------|----------------|
| N1ww     | well-watered     | 4.34 ±0.15     | 4.83 ±0.40     | 4.59 ±0.19     |
| N1 + Zn1ww | well-watered    | 5.01 ±0.27     | 7.83 ±0.22     | 6.42 ±0.22     |
| N1 + Zn2ww | well-watered    | 4.76 ±0.25     | 5.97 ±0.80     | 5.37 ±0.52     |
| N2ww     | well-watered     | 4.82 ±0.26     | 5.28 ±0.93     | 5.05 ±0.59     |
| N2 + Zn1ww | well-watered    | 2.11 ±0.06     | 2.25 ±0.40     | 2.18 ±0.10     |
| N2 + Zn2ww | well-watered    | 5.58 ±2.68     | 2.54 ±0.41     | 4.06 ±1.17     |
| N1ds     | drought stress   | 6.60 ±0.21     | 7.56 ±0.39     | 7.08 ±0.30     |
| N1 + Zn1ds | drought stress  | 8.10 ±0.18     | 8.04 ±0.12     | 8.07 ±0.15     |
| N1 + Zn2ds | drought stress  | 4.76 ±0.20     | 5.75 ±0.01     | 5.25 ±0.10     |

Table 3 Content of ABA (ng g⁻¹ FW)

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

![Figure 1](http://www.acta.fapz.uniaag.sk)  
**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](http://www.acta.fapz.uniaag.sk)  
**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

| 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 |
| N₁ww     | 9.76 ± 0.39      | 9.86 ± 0.29      | 9.81 ± 0.28     |
| N₁ + Zn₁ww | 10.59 ± 0.32   | 10.10 ± 0.66      | 10.35 ± 0.43    |
| N₁ + Zn₂ww | 9.68 ± 0.24      | 9.89 ± 0.47      | 9.79 ± 0.18     |
| N₂ww     | 10.59 ± 0.20      | 12.42 ± 1.03      | 11.51 ± 0.52    |
| N₂ + Zn₁ww | 11.29 ± 0.20   | 13.00 ± 1.19      | 12.15 ± 0.62    |
| N₂ + Zn₂ww | 11.18 ± 0.30      | 12.84 ± 0.16      | 12.01 ± 0.09    |
| N₁ds     | 6.32 ± 0.42      | 8.03 ± 0.59      | 7.17 ± 0.47     |
| N₁ + Zn₁ds | 7.09 ± 0.05   | 8.94 ± 0.35      | 8.02 ± 0.19     |
| N₁ + Zn₂ds | 6.32 ± 0.52      | 9.21 ± 0.47      | 7.76 ± 0.47     |
| N₂ds     | 8.13 ± 0.28      | 10.68 ± 0.15      | 9.40 ± 0.13     |
| N₂ + Zn₁ds | 8.50 ± 0.23   | 12.19 ± 0.58      | 10.34 ± 0.22    |
| N₂ + Zn₂ds | 8.57 ± 0.38      | 11.50 ± 0.72      | 10.03 ± 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
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).

Acknowledgements

The research was supported by grant no. AF-IGA-2018-tym001.

References

ALVES, A. A. C. and SETTER, T. L. (2000). Response of cassava to water deficit: Leaf area growth and abscisic acid. Crop Science, 40, 131–137. https://doi.org/10.1093/aob/mch179

AMANUEL, B. A. et al. (2015). Forage yield and quality response of annual ryegrass (Lolium multiflorum) to different water and nitrogen levels. African Journal of Range & Forage Science, 32(2), 125–131. https://doi.org/10.2989/10220119.2015.1056228

ASHKAN, A. et al. (2020). Effects of foliar zinc application on yield and oil quality of rapeseed genotypes under drought stress. Journal of Plant Nutrition, 43(11), 1594–1603. https://doi.org/10.1080/01904167.2020.1739299

BANO, A. et al. (2012). Role of abscisic acid and drought stress on the activities of antioxidant enzymes in wheat. Plant, Soil and Environment, 58, 181–185. https://doi.org/10.17221/210/2011-PSE

BOOMINATHAN, P. et al. (2004). Long term transcript accumulation during development of dehydration adaptation in Cicer arietinum. Plant Physiology, 135, 1608–1620. https://doi.org/10.1104/pp.104.03141

BOWEN, H. J. M. (1979). Environmental chemistry of the elements. New York: Academic Press.

BRAMBILLA, D. M. et al. (2012). Impact of nitrogen fertilization on the forage characteristics and beef calf performance on native pasture overseeded with ryegrass. Revista Brasileira de Zootecnia, 41(3), 528–536. https://doi.org/10.1590/S1516-35982012000300008

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

| Variants | Irrigation  | 1st experiment | 2nd experiment | Average content |
|----------|-------------|----------------|----------------|----------------|
|          | regime      | N content (rel. %) | Zn content (mg kg\(^{-1}\)) | N content (rel. %) | Zn content (mg kg\(^{-1}\)) | N content (rel. %) | Zn content (mg kg\(^{-1}\)) |
| N1ww     | well-watered | 4.64b 34.60a | 2.80* | 47.84a | 4.40ab 41.22* |
| N1 + Zn1ww | 4.55b 122.60* | 2.67a | 141.70a | 4.28a | 132.15b |
| N1 + Zn2ww | 4.71b 174.85cd | 2.70a | 227.16e | 4.38ab | 215.05d |
| N2ww     | 5.76d 42.43a | 2.76* | 99.96a | 4.96i | 44.23b |
| N2 + Zn1ww | 5.79d 145.58a | 3.60b | 121.92a | 5.60d | 147.82b |
| N2 + Zn2ww | 5.45d 157.89a | 3.81b | 212.62a | 5.62d | 186.54a |
| N1ds     | drought stress | 4.97a 39.94a | 3.54b | 44.94a | 4.69m | 42.51a |
| N1 + Zn1ds | 4.63b 192.45e | 3.43b | 169.24a | 4.42ab | 182.31a |
| N1 + Zn2ds | 3.72a 315.78f | 3.91b | 294.84a | 4.15a | 313.71f |
| N2ds     | 5.58f 41.24a | 4.65b | 46.21a | 5.67d | 43.71f |
| N2 + Zn1ds | 5.41d 192.57f | 4.65 | 175.72e | 5.62d | 184.90f |
| N2 + Zn2ds | 5.39d 272.80a | 4.85c | 311.86f | 5.73d | 287.91f |

Means sharing the same superscript are not significantly different from each other (P < 0.05) according to LSD test (each column was evaluated separately)
SEED SERVICE. (2020). Characteristics of grasses and their varieties. Seed service. Retrieved October 12, 2020 from https://seedservice.cz/charakteristika-druhu-a-odrud-trav

SEO, P. J. et al. (2009). The MYB96 transcription factor mediates abscisic acid signalling during drought stress response in Arabidopsis. *Plant Physiology*, 151, 275–289. https://doi.org/10.1104/pp.109.144220

SHARP, R. E. (2002). Interaction with ethylene: changing views on the role of abscisic acid in root and shoot growth responses to water stress. *Plant, Cell and Environment*, 25(2), 211–222. https://doi.org/10.1046/j.1365-3040.2002.00798.x

ŠKARPA, P. et al. (2015). Foliar application of zinc reduces the risk of drought stress on poppy (*Papaver somniferum* L.). International Conference on Prosperous Oil Crops, Prague, 123–126.

STATSOFT, Inc. (2013) STATISTICA (data analysis software system), version 12. www.statsoft.com

STEFFENS, J. C. (1990). The heavy metal-binding peptides of plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 41, 553–575. https://doi.org/10.1146/annurev.pp.41.060190.003005

STRIVASTAVA, L. M. (2002). Plant growth and development: Hormones and environment. Academic Press San Diego CA. https://doi.org/10.1093/aob/mcq209

UPADHYAYA, H., DUTTA, B. K. and PANDA, S. K. (2013). Zinc Modulates Drought-Induced Biochemical Damages in Tea (*Camellia sinensis* (L) O Kuntze). *Journal of Agricultural and Food Chemistry*, 61, 6660–6670. https://doi.org/10.1021/jf304254z

VAGHAR, M. S. et al. (2020). Foliar application of iron, zinc, and manganese nano-chelates improves physiological indicators and soybean yield under water deficit stress. *Journal of Plant Nutrition*, 43(18), 2740–2756. https://doi.org/10.1080/01904167.2020.1793180

VELU, G. et al. (2016). Effect of drought and elevated temperature on grain zinc and iron concentrations in CIMMYT spring wheat. *Journal of Cereal Science*, 69, 182–186. https://doi.org/10.1016/j.jcs.2016.03.006

WANG, H. and JIN, J. (2007). Effects of Zinc Deficiency and Drought on Plant Growth and Metabolism of Reactive Oxygen Species in Maize (*Zea mays* L.). *Agricultural Sciences in China*, 6 (8), 988–995. https://doi.org/10.1016/S1671-2927(07)60138-2

WANG, H., LIU, R. L. and JIN, J. Y. (2009). Effects of zinc and soil moisture on photosynthetic rate and chlorophyll fluorescence parameters of maize. *Biologia Plantarum*, 53, 191–194.

XIONG, L. M., SCHUMAKER, K. S. and ZHU, J. K. (2002). Cell signalling during cold, drought, and salt stress. *The Plant Cell*, 14, 165–183. https://doi.org/10.1105/tpc.000596.

YANG, K. Y. et al. (2018). Remodeling of root morphology by CuO and ZnO nanoparticles: effects on drought tolerance for plants colonized by a beneficial pseudomonad. *Botany*, 96, 175–186. https://doi.org/10.1139/cjb-2017-0124

ZHANG, J., ZHANG, X. and LIANG, J. (1995). Exudation rate and hydraulic conductivity of maize roots are enhanced by soil drying and abscisic acid treatment. *New Phytologist*, 131, 329–336. https://doi.org/10.1111/j.1469-8137.1995.tb03068.x