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Research Article

Drought Tolerance in Italian Ryegrass is Associated with Genetic Divergence, Water Relation, Photosynthetic Efficiency and Oxidative Stress Responses

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ABSTRACT

Drought stress is a condition that occurs frequently in the field, it reduces of the agricultural yield of field crops. The aim of the study was to screen drought-adapted genotype of Italian rye grass. The experiments were conducted between the two Italian ryegrass (*Lolium multiflorum* L.) cultivars viz. Hwasan (H) and Kowinearly (KE). The plants were exposed to drought for 14 days. The results suggest that the morphological traits and biomass yield of KE significantly affected by drought stress-induced oxidative stress as the hydrogen peroxide (H_2O_2) level was induced, while these parameters were unchanged or less affected in H. Furthermore, the cultivar H showed better adaptation by maintaining several physiological parameter including photosystem-II (Fv/Fm), water use efficiency (WUE) and relative water content (RWC%) level in response to drought stress. These results indicate that the cultivar H shows improved drought tolerance by generic variation, improving photosynthetic efficiency and reducing oxidative stress damages under drought stress. These findings can be useful to the breeder and farmer for improving drought tolerance in Italian rye grass through breeding programs.

(**Key words**: Drought, Italian ryegrass, Oxidative stress, Photosynthetic disturbance, ROS, Water use efficiency)

Ⅰ. INTRODUCTION

Drought stress adversely affects crop growth, phenology, and productivity (Basavaraj et al., 2020). As the dryland areas are increasing gradually, so this problem is predicted to become even more global due to climate change and unsustainable management of the resource, soil, and crop genotypes (Yao et al., 2020). Therefore, discovering the physiological and molecular mechanisms associated with drought stress is important for plant stress tolerance and sustainable crop production. Drought-exposedcrops accumulate the production of reactive oxygen species ($O_2^{\bullet-}$ and H_2O_2), which induce cellular injury by oxidative stress and inhibit the major cellular and metabolic processes in plants (Hussain et al., 2019). These adverse effects of drought stress reduce plant growth, induce wilting, and subsequently result in the death of most crop species. Therefore, uncovering physiological and molecular mechanisms for improving drought tolerance in the plant can be suitable for agricultural production under drought stress.

Different plant traits are considered key indicators for determining drought tolerance efficiency in plants. Drought inhibits plant growth, shoot length, leaf area/size and plant biomass (Pettigrew, 2004). Alterations of several important physiological traits including plant photosynthesis, water use efficiency (WUE), and relative water content (RWC) have been reported during drought stress (Chastain et al., 2016). The reduction of photosynthesis due to drought is an initial indicator of stress perception that has a link to RWC (Sekmen et al., 2014). In addition, the roots are the first sensing organ by which plants sense stress that changes physiological and water potential status (Mahmood et al., 2022). However, root plays a pivotal role in drought stress tolerance.

Several studies have documented that several rye grass species are sensitive to drought stress compared to the legume or other plant species (Rahman et al., 2022; Pornaro et al., 2020). To cope with drought stress, the tolerant genotype exhibits a high tolerance to drought stress by maintaining root growth under drought stress (Mahmood et al., 2022). The regulations of water deficit by the deeper root system, better osmotic potential or increasing photosynthetic water use efficiency are the important traits for tolerant genotype (Matthew et al., 2012). However, the grass species having short root systems are less adapted to

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drought. The geneticists and plant physiologists are positioning to improve plant root systems and other physiological traits under drought stress (Comas et al., 2013). However, a better understanding of plant stress tolerance traits and how different traits are associated with whole plant strategies in increasing drought tolerance without having any quality and yield loss are needed. Italian ryegrass (IRG, *Lolium multiflorum* L.) is an annual rye grass widely cultivated due to its high nutritional value (Pan et al., 2016). IRG is cultivated as for excellent source of ruminant feed including hay and silage production (Ozelcam et al., 2015). However, one of the limitations of IRG is sensitive to drought stress. The potential decrease of production and quality was found to be reduced in IRG under drought stress (Cyriacet al., 2018; Kemesyte et al., 2017). Therefore, improving traits associated with drought tolerance in IRG is essential to stand against drought stress in water-limited cultivated areas globally. The aim of the study was to screen drought tolerant IRG genotype by evaluating genetic divergence, water relation, photosynthetic efficiency and oxidative stress indicators under prolonged drought stress. These traits involving tolerant genotype can be useful to breeder or farmer for improving drought stress tolerance in IRG through breeding programs.

Ⅱ. MATERIALS AND METHODS

1. Plant growth and drought treatment

Italian ryegrass (*Lolium multiflorum* L.) seeds were surface sterilized with 70% ethanol for 5 min before geminated in a germination tray supplemented with distilled water on tissue paper at room temperature. The germinated seeds were then transferred to the hydroponic nutrient solution (Hoagland and Arnon, 1950). The ryegrass seedlings were grown in grow chamber at 25 °C under white fluorescent light (480 μmol m^{-2} s^{-1}) with a 14-h photoperiod and 60–65% relative humidity. Seedlings of two ryegrass cultivars viz. Hwasan (H) and Kowinearly (KE) were transferred to soil pots and maintained growth for 2 weeks then suspended water supply for drought stress. Treatments were maintained for 2 weeks as follows: Hwasan control (H-0); Hwasan 14 d drought (H-DT); Kowinearly control (KE-0); Kowinearly 14 d drought (KE-DT).

2. Analysis of morphological and photosynthetic parameters

Following drought treatments between the two Italian ryegrass growth and its physiological indices were measured. Root and shoot length was measured using a metric scale (cm), and weight (g) was determined by an electronic balance. The maximum yield of photosystem II (Fv/Fm) was determined through a portable fluorometer temperature (PAM 200, Effeltrich, Germany), and plants were adapted for 20 minutes in dark conditions before measurements.

3. Measurement of water use efficiency and relative water content

The relative water use efficiency (WUE%) of crop biomass was determined above ground water component, and daily water consumption (WC). Water consumption was calculated as follows: WC (per L)= daily irrigation− leaching water, while the following formula was used for analysis of WUE% [g FM/L]=biomass/WC×100 and the RWC% was determined following the formula: RWC%=(FW-DW)/(TW-DW) × 100

4. Determination of hydrogen peroxide

Hydrogen peroxide (H_2O_2) was measured using the protocol used previously (Rahman et al., 2014). Briefly, 100 mg of plant sample was mixed properly with potassium phosphate buffer (KP, 50 mM, pH 7.0). The mixture was centrifuged for 20 min at 12,000 rpm then supernatant (0.7 mL) was taken into a new Eppendorf tube and added 0.7 mL of 20%(v/v) H_2SO_4 containing TiCl. The solution mixture was centrifuged at 12,000 rpm for 10 min. Finally, the absorbance of supernatant (1 mL) was read at 410 nm using a spectrophotometer (UV-1650PC, Shimadzu, Japan).

5. Measurement of malondialdehyde(MDA) accumulation

The MDA accumulation was measured according to the protocol used previously (Rahman et al., 2016). Briefly, 100 mg of plant tissue was mixed with 20% (w/v) TCA (trichloroacetic acid). The mixed the solution well and centrifuged it for 15 min at 13000 rpm. Supernatant (0.5 mL) was taken in new Eppendorf tube, and added 0.5 mL TCA containing TBA 0.5% (w/v), and 100 μL BHT (butylated hydroxytoluene) 0.4%(v/v). The solution containing tubes were heated at 95 °C water bath or heating block for 30 min, and then quickly cooled on ice for 5 min.

The homogenate was centrifuged for 15 min at 13,000 rpm, checked the sample OD at 532 nm and 600 nm using the UV-spectrometer (SpectraMAXi3X, San Jose, USA). Finally, subtracted the non-specific absorbance at 600 nm, the MDA content was calculated by considering the extinction coefficient of 155 mM^{-1} cm^{-1}

6. Statistical analysis

The data of physiological parameters were subjected to analysis of variance (ANOVA). The mean differences were measured by Tukey's honest significant difference (HSD). Differences at $p \leq 0.05$ were considered as significant. The software GraphPad Prism 8.4.3 was used for graphical analyses. At least three independent replications were considered for the analysis.

Ⅲ. RESULTS AND DISCUSSION

1. Drought stress-induced morphological variations between the genotypes

A significant phenotypic change was observed between the two Italian ryegrass genotypes (Fig. 1). The Hwasan (H) showed better drought adaptability under prolonged drought stress for 14 days, while Kowinearly (KE) was significantly affected by drought stresses (Fig. 1). The drought stress significantly inhibited root and shoot length in H and KE genotypes, while H showed less drought sensitivity compared to KE (Fig. 2A and B). Furthermore, the effect of drought clearly affected to plant biomass (Fig. 3). The fresh and dry weight of roots and shoots were significantly reduced in KE, while these parameters were found to be less affected by drought in the H genotype (Fig. 3 A-D). The results suggest that the higher drought adaptability of H compared to KE is an important physiological trait that provided better drought tolerance in H. It has been reported that drought tolerant genotypes evolved with elite physiological characteristics including growth rates and root differential growth traits in the plant that greatly enhanced plants capacity to cope with drought stress (Mahmood et al., 2022).

2. Variation of WUE, RWC, and photosynthesis

The water use efficiency (WUE) was increased in under drought treatment in both genotypes, while the H showed higher WUE in response to drought stress than KE (Fig. 4A). In contrast,

Fig. 1. Morphological changes between the two genotypes of Italian ryegrass following drought treatment for 14 days. Hwasan at day 0 (control) (H-0), Hwasan after drought treatment for 14 days (H-DT), Kowinearly at day 0 (control) (KE-0), and Kowinearly after drought treatment for 14 days (KE-DT).

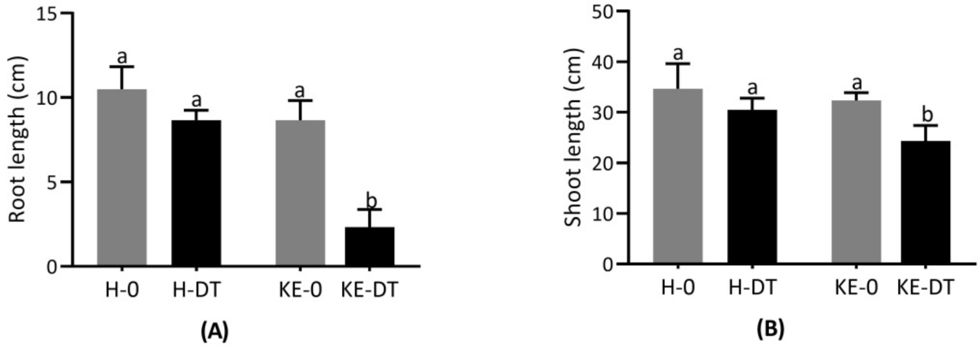

Fig. 2. Effect of drought stress on root length (A), and shoot length (B) in Hwasan at day 0 (control) (H-0), Hwasan after drought treatment for 14 days (H-DT), Kowinearly at day 0 (control) (KE-0), Kowinearly after drought treatment for 14 days (KE-DT). Different letters above the error bar indicate significant differences ($p < 0.05$) among means ± SD of treatments (n = 3).

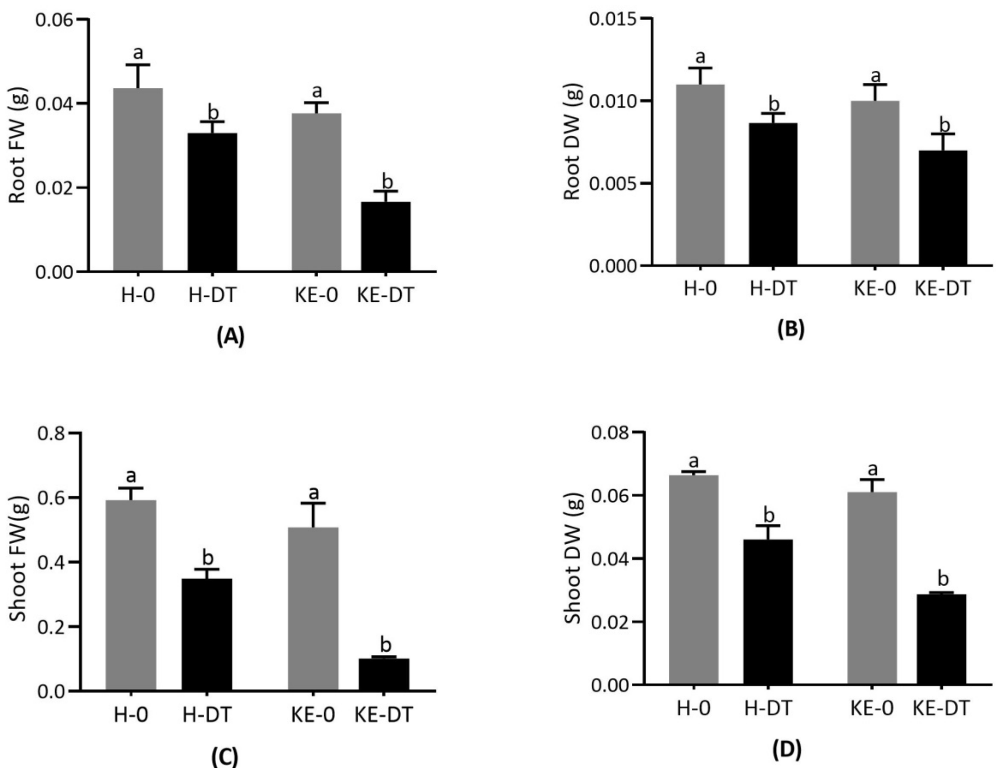

Fig. 3. Effect of drought stress on root fresh weight (A), root dry weight (B), shoot fresh weight (C), and shoot dry weight (D) in Hwasan at day 0 (control) (H-0), Hwasan after drought treatment for 14 days (H-DT), Kowinearly at day 0 (control) (KE-0), Kowinearly after drought treatment for 14 days (KE-DT). Different letters above the error bar indicate significant differences ($p < 0.05$) among means ± SD of treatments (n = 3).

the leaf water content significantly declined in KE relative to Hwasan (Fig. 4B). In this present study, the Fv/Fm ratio was remarkably declined in KE due to prolonged drought stress that lead to photo-inhibition of PSII, which significantly affected the plant growth attributes in IRG. The adverse effect of drought on PSII was greatly adapted in drought tolerant H genotype (Fig. 4C). These results suggest that genotype H improves WUE%, the ratio of biomass production by efficient water use, as well as enhanced photosynthetic assimilation and reduced transpiration. A similar observation was found in perennial ryegrass where the drought tolerance genotype showed a significant difference in intrinsic water use efficiency (WUE) under stress conditions

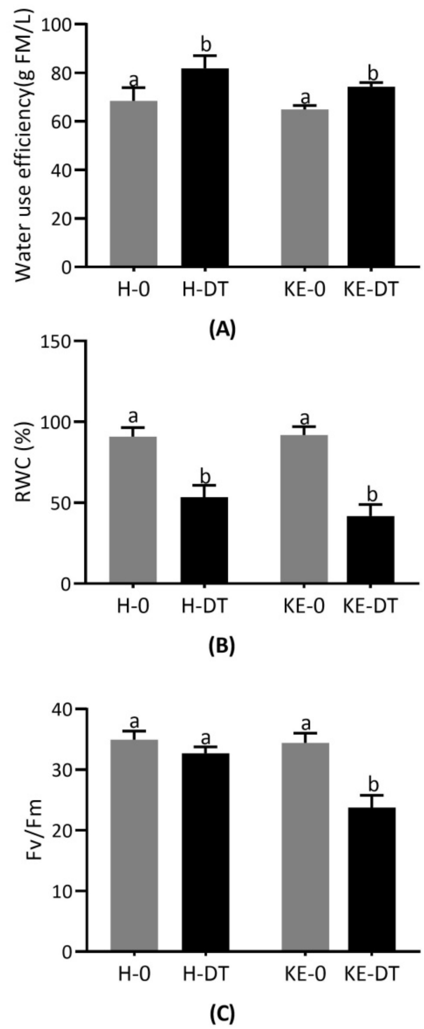

Fig. 4. Effect of drought stress on water use efficiency (A), relative water content (B), and Fv/Fm (C) in Hwasan at day 0 (control) (H-0), Hwasan after drought treatment for 14 days (H-DT), Kowinearly at day 0 (control) (KE-0), Kowinearly after drought treatment for 14 days (KE-DT). Different letters above the error bar indicate significant differences (p 〈 0.05) among means ± SD of treatments (n = 3).

(Westermeier et al., 2018). Drought stress causes photo-inhibition owing to induction of inactive reaction center, reduction of absorption flux, and low transfer of electrons per reaction center, these changes in PSII decline the maximum quantum yield of PSII (Fv/Fm) (Wang et al., 2018). In our present study, the H showed better efficiency in terms of drought tolerance compared to KE. Therefore, the Hwasan could be selected as a low water demand forage crop in water scarcity soils.

3. Regulation of drought-induced oxidative stress indicators

Hydrogen peroxide (H_2O_2) is a potent marker of stress-induced free radicles (Rahman et al., 2021; Raza et al., 2022), and MDA is one of the final products of determining oxidative stress-induced lipid peroxidation in cells (Gaweł et al., 2004). In this present study, the oxidative stress markers levels were regulated by drought stress (Fig. 5). The H_2O_2 accumulation was significantly increased in KE by prolonged drought treatment for 14 days than that of H genotypes (Fig. 5A). The MDA accumulation was showed a similar pattern in response to drought stress, while no comparable variation between the two genotypes in the case of normal water irrigation (Fig. 5B). However, the increase in H_2O_2 and MDA accumulations coincided with IRG plant damages. Accumulation of MDA under stress conditions usually leads to damage to the cell membrane in plants (Gaweł et al., 2004). The abiotic stress-induced oxidative stress injuries,

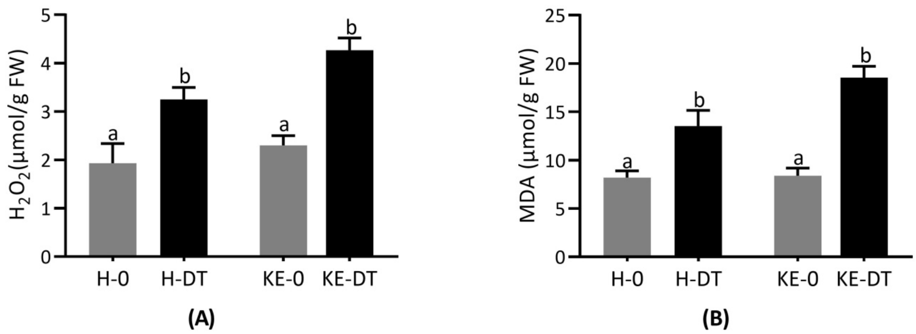

Fig. 5. Effect of drought stress on hydrogen peroxide accumulation (A) and melonaldehyde accumulation (B) in Hwasan at day 0 (control) (H-0), Hwasan after drought treatment for 14 days (H-DT), Kowinearly at day 0 (control) (KE-0), Kowinearly after drought treatment for 14 days (KE-DT). Different letters above the error bar indicate significant differences ($p < 0.05$) among means ± SD of treatments (n = 3).

along with disturbance of morphological and photosynthetic have been reported in several plant species (Haque et al., 2021; Kabir et al., 2021; Rahman et al., 2022). In our current study, low drought-induced oxidative damage occurs in H than KE, which suggests that drought tolerant H genotype has capability to adopt prolonged drought. This information might be useful for the improvement of drought tolerance in IRG and other forage species.

Ⅳ. CONCLUSION

This work provides differential drought tolerance in two Italian ryegrass genotypes viz. Hwasan and Kowinearly. Hwasan showed better adaptation to drought by maintaining plant biomass, WUE and RWC% under drought stress. These results suggest that genotype H improves the ratio of biomass production by efficient water use, as well as enhanced photosynthetic assimilation and reduced transpiration. Therefore, the Hwasan could be selected for cultivation in water scarcity soils as well as for molecular breeding programs.

Ⅴ. ACKNOWLEDGEMENTS

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Ⅵ. REFERENCES

Basavaraj, P.S. and Rane, J. 2020. Avenues to realize potential of phenomics to accelerate crop breeding for heat tolerance. Plant Physiology Reports. 25(4):594-610.

Chastain, D.R., Snider, J.L., Choinski, J.S., Collins, G.D., Perry, C.D. and Whitaker, J. et al. 2016. Leaf ontogeny strongly influences photosynthetic tolerance to drought and high temperature in *Gossypium hirsutum*. Journal of Plant Physiology. 199:18-28.

Comas, L.H., Becker, S.R., Cruz, V.M., Byrne, P.F. and Dierig, D.A. 2013. Root traits contributing to plant productivity under drought. Frontiers in Plant Science. 4:00442.

Cyriac, D., Hofmann, R.W., Stewart, A., Sathish, P., Winefield, C.S. and Moot, D.J. 2018. Intraspecific differences in long-term drought tolerance in perennial ryegrass. PLOS ONE. 13(4):e0194977.

Gaweł, S., Wardas, M., Niedworok, E. and Wardas, P. 2004. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomości Lekarskie. 57(9-10):453-455.

Haque, A.F.M.M., Tasnim, J., El-Shehawi, A.M., Rahman, M.A., Parvez, M.S., Ahmed, M.B. and Kabir, A.H. 2021. The Cd-induced morphological and photosynthetic disruption is related to the reduced Fe status and increased oxidative injuries in sugar beet. Plant Physiology and Biochemistry. 166:448-458.

Hoagland, D.R. and Arnon, D.I. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Statio. 347:32.

Hussain, H.A., Men, S., Hussain, S., Chen, Y., Ali, S., Zhang, S., Zhang, K., Li, Y., Xu, Q., Liao, C. and Wang, L. 2019. Interactive effects of drought and heat stresses on morpho-physiological attributes, yield, nutrient uptake and oxidative status in maize hybrids. Scientific Reports. 9(1):3890.

Kabir, A.H., Das, U., Rahman, M.A. and Lee, K.W. 2021. Silicon induces metallochaperone-driven cadmium binding to the cell wall and restores redox status through elevated glutathione in Cd-stressed sugar beet. Physiologia Plantarum. 173(1):352-368.

Kemesyte, V., Statkeviciute, G. and Brazauskas, G. 2017. Perennial ryegrass yield performance under abiotic stress. Crop Science. 57(4):1935-1940.

Mahmood, T., Iqbal, M.S., Li, H., Nazir, M.F., Khalid, S., Sarfraz, Z., Hu, D., Baojun, C., Geng, X., Tajo, S.M., Dev, W., Iqbal, Z., Zhao, P., Hu, G. and Du, X. 2022. Differential seedling growth and tolerance indices reflect drought tolerance in cotton. BMC Plant Biology 22(1):331.

Matthew, C., Van Der Linden, A., Hussain, S., Easton, H., Hatier, J. and Horne, J. 2012. Which way forward in the quest for drought tolerance in perennial ryegrass? Proceedings of the New Zealand Grassland Association, Gore, New Zealand. pp. 195-200.

Özelçam, H., Kırkpınar, F. and Tan, K. 2015. Chemical composition, in vivo digestibility and metabolizable energy values of caramba (*Lolium multiflorum* cv. caramba) Fresh, Silage and Hay. Asian-Australasian journal of Animal Sciences. 28(10):1427-1432.

Pan, L., Zhang, X., Wang, J., Ma, X., Zhou, M., Huang, L., Nie, G., Wang, P., Yang, Z. and Li, J. 2016. Transcriptional profiles of drought-related genes in modulating metabolic processes and antioxidant defenses in *Lolium multiflorum*. Frontiers in Plant Science. 7:00519.

Pettigrew, W.T. 2004. Moisture deficit effects on cotton lint yield, yield components, and boll distribution. Agronomy Journal. 96:377-83.

Pornaro, C., Serena, M., Macolino, S. and Leinauer, B. 2020. drought stress response of turf-type perennial ryegrass genotypes in a mediterranean environment. Agronomy. 10(11):1810.

Rahman, M.A., Alam, I., Sharmin, S.A., Kabir, A.H., Kim, Y.G., Liu, G. and Lee, B.H. 2021. Physiological and proteomic analyses reveal the protective roles of exogenous hydrogen peroxide in alleviating drought stress in soybean plants. Plant Biotechnology Reports. 15(6):805-818.

Rahman, M.A., Kim, Y.G. and Lee, B.H. 2014. Proteomic response of alfalfa subjected to aluminum (Al) stress at low pH Soil. 2014. Journal of the Korean Society of Grassland and Forage Science. 34(4):262-268.

Rahman, M.A., Kim, Y.G., Alam, I., Liu, G., Lee, H., Lee, J.J. and Lee, B.H. 2016. Proteome analysis of alfalfa roots in response to water deficit stress. Journal of Integrative Agriculture. 15(6):1275-1285.

Rahman, M.A., Woo, J.H., Song, Y., Lee, S.H., Hasan, M.M., Azad, M.A.K. and Lee, K.W. 2022. Heat shock proteins and antioxidant genes involved in heat combined with drought stress responses in perennial rye grass. Life. 12(9):1426.

Raza, A., Salehi, H., Rahman, M.A., Zahid, Z., Haghjou, M.M., Najafi-Kakavand, S., Charagh, S., Osman, H.S., Albaqami, M., Zhuang, Siddique, K.H.M. and Zhuang, W. 2022. Plant hormones and neurotransmitter interactions mediate antioxidant defenses under induced oxidative stress in plants. Frontiers in Plant Science. 13:961872.

Sekmen, A.H., Ozgur, R., Uzilday, B. and Turkan I. 2014. Reactive oxygen species scavenging capacities of cotton (*Gossypium hirsutum)* cultivars under combined drought and heat induced oxidative stress. Environmental Experimental Botany. 99:141-9.

Wang, Z., Li, G., Sun, H., Ma, L., Guo, Y., Zhao, Z., Gao, H. and Mei, L. 2018. Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. Biology Open. 7(11):bio035279.

Westermeier, P., Schäufele, R. and Hartmann, S. 2018. Variation in intrinsic water use efficiency between perennial ryegrass genotypes differing for drought tolerance. In: G. Brazauskas, G. Statkevičiūtė and K. Jonavičienė (Eds.), Breeding grasses and protein crops in the era of genomics. Springer, Cham. pp. 171-175.

Yao, J., Liu, H., Huang, J., Gao, Z., Wang, G., Li, D., Yu, H. and Chen, X. 2020. Accelerated dryland expansion regulates future variability in dryland gross primary production. Nature Communications. 11(1):1665.

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