

Research Article

# Comparative Study of Drought Stress Responses on Several Italian Ryegrass Varieties

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## ABSTRACT

Italian ryegrass (*Lolium multiflorum* Lam.) is one of the most widely grown winter forage crops in Korea, but its yields are known to be greatly affected by drought that occur frequently in spring. This study aimed to compare the growth and tolerance response characteristics to drought stress in several Italian ryegrass varieties cultivated in Korea. Twenty-day-old Italian ryegrass was subjected to drought treatment for 4 days, and then the growth and physiological responses of the plants were compared. Drought stress reduced leaf length, fresh weight, and dry weight in all Italian ryegrass varieties compared to the control. In addition, chlorophyll content was significantly decreased in all varieties treated with drought stress, but Fv/Fm was significantly decreased only in Winter hawk. For H<sub>2</sub>O<sub>2</sub> and malondialdehyde (MDA) contents, Winter hawk showed the highest increase and New dawn showed the least increase. In terms of relative water content (RWC), New dawn showed the least decrease and Winter hawk showed the greatest decrease. These results indicate that New dawn is relatively drought-tolerant and Winter hawk is a drought-sensitive variety, indicating that each variety of Italian ryegrass has different drought tolerance mechanisms, which may provide basic insight for the development of tolerant varieties in the future.

(Key words: Drought stress, Italian ryegrass, Tolerance)

## I. INTRODUCTION

Italian ryegrass (*Lolium multiflorum* Lam.) accounts for approximately 81% of domestic winter forage crop production and has high feed value and livestock palatability. Among the various climatic factors that reduce Italian ryegrass yields, spring drought is known to be the major factors (Kim et al., 2016; Kim and Sung, 2021). Drought causes substantial damage to crop growth and productivity, and the frequency and extent of drought damage are increasing worldwide due to climate change (Wu et al., 2022). A decrease in soil moisture due to drought causes water deficiency in plants. Drought reduces the chlorophyll content and quantum yield of photosystem II (PSII) of plants and increases reactive oxygen species, causing oxidative stress in the cell (Wu et al., 2022). Drought tolerance in plants is related to the scavenging of reactive oxygen species and lipid peroxidation and maintenance of water content, which is reported to differ between varieties (Zhang et al., 2019). In

addition, drought tolerance in crop plants is related to growth and yield. In this study, we investigated how much the drought tolerance in Italian ryegrass differs depending on the Italian ryegrass variety, and our results can be provided as basic data for developing drought-tolerant varieties in preparation for future climate change.

## II. MATERIALS AND METHODS

### 1. Plants growth conditions and drought treatment

Seeds of 4 varieties of Italian ryegrass, Florida 80, Kowinearly, New dawn, and Winter hawk seeds were sown in pots filled with sand and horticultural media at a ratio of 1:3 (v/v), then irrigated with distilled water. After thinning out 7-day-old Italian ryegrass, it was irrigated with 1/2 × Hoagland solution (PhytoTechLABS, USA) for growth of 6 seedlings. Twenty-day-old Italian ryegrass was grouped as a control

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(irrigated distilled water) and a drought treatment (no irrigation) and then subjected to drought stress for four days. The Italian ryegrass plants were grown under controlled conditions at a humidity of 55%, temperature of 23°C, photoperiod of 16/8 (light/dark) hours, and light intensity of 450  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

## 2. Measurement of relative water content

Relative water content (RWC) was measured by the previous method (Gao et al., 2019) after measuring the fresh weight (FW), turgor weight (TW), and dry weight (DW) of plants. Relative water content (%) =  $(FW - DW)/(TW - DW) \times 100$

## 3. Measurement of chlorophyll content and Fv/Fm

Chlorophyll content was measured in fully developed leaves of Italian ryegrass using a chlorophyll content meter (Hansatech Instrument Ltd, UK) (Kim et al., 2022). The maximum quantum yield of PSII (Fv/Fm) was measured using Handy PEA (Hansatech Instrument Ltd, UK) after acclimating fully developed leaves to the dark conditions for 20 min.

## 4. H<sub>2</sub>O<sub>2</sub> and MDA measurements

The H<sub>2</sub>O<sub>2</sub> content was measured by extracting Italian ryegrass leaves grind with liquid nitrogen with 100 mM KP buffer (pH 7.0) and then reacting with 20% H<sub>2</sub>SO<sub>4</sub> containing 0.1% TiCl<sub>4</sub>. And then the absorbance was measured at 410 nm. H<sub>2</sub>O<sub>2</sub> content was calculated using an extinction coefficient of 0.28  $\mu\text{mol}^{-1} \text{cm}^{-1}$  (Lee et al., 2022). Malondialdehyde (MDA) content was measured by extracting leaves grind with liquid nitrogen with 1% trichloroacetic acid and then reacting with 20% trichloroacetic acid containing 0.5% thiobarbituric acid at 95°C. And then the absorbance was measured at 532 and 600 nm. MDA content was calculated using an extinction coefficient of 155  $\text{mM}^{-1} \text{cm}^{-1}$  (Tirani and Haghjou, 2019).

## 5. Statistical analysis

Statistical analysis was conducted using IBM SPSS statistics (IBM SPSS statistic for window, version 25.0, Armonk, NY, USA) through one-way ANOVA, and significance was tested at the 5% level through the Duncan post hoc test.

## III. RESULTS AND DISCUSSION

A decrease in soil moisture due to drought stress causes water deficiency in plants. Drought tolerance in plants is associated with their ability to retain water (Kudo et al., 2023). We conducted a comparative study using 4 varieties of Italian ryegrass to determine how drought stress tolerance and growth characteristics are different among varieties under drought stress condition. Italian ryegrass was subjected to drought stress for 4 days and their phenotypic changes were compared. Winter hawk showed the most wilted phenotype, by contrast, the other three cultivars showed similarly less wilting (Fig. 1).

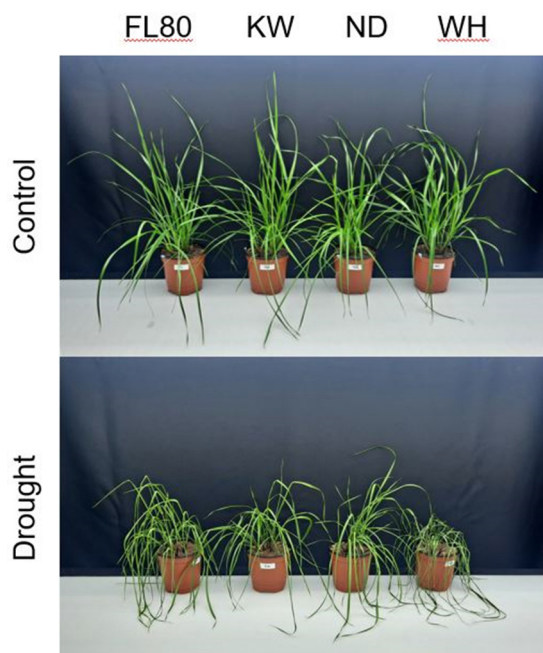


Fig. 1. Phenotypic change in Italian ryegrass varieties after drought stress treatment. Twenty-day-old Italian ryegrass subjected to watering (control) or drought for 4 days. IRG, Italian ryegrass; FL80, Florida 80; KW, Kowinearly; ND, New dawn; WH, Winter hawk.

In addition, when comparing the changes in shoot length of the plants after drought stress treatment, no significant difference was observed among all varieties. However, significant decreases of fresh and dry weight of the shoots were observed in all varieties after drought treatment, in which Winter hawk showed the highest decrease (Fig. 2).

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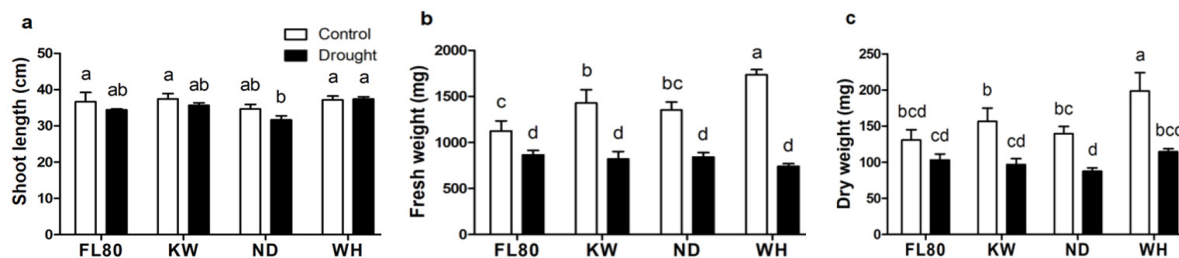


Fig. 2. Changes of plant growth in Italian ryegrass after drought stress treatment. (a) Shoot length. (b) Shoot fresh weight. (c) Shoot dry weight. Twenty-day-old Italian ryegrass was subjected to watering (control) or drought stress for 4 days. Different letter indicate significant differences ( $p < 0.05$ ) between all treatments (Means  $\pm$  SE;  $n = 4$ ).

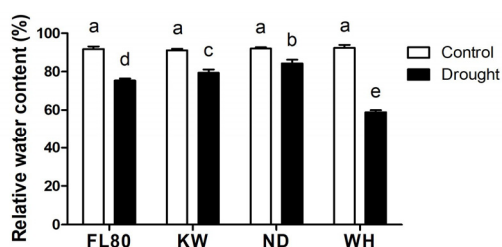


Fig. 3. Change of relative water content in Italian ryegrass after drought stress treatment. Twenty-day-old Italian ryegrass was subjected to watering (control) or drought stress for 4 days. Different letter indicate significant differences ( $p < 0.05$ ) between all treatments (Means  $\pm$  SE;  $n = 4$ ).

As is known, relative water content (RWC) is an important indicator of water status in plants and has been proposed as an integrated measure of drought stress tolerance in plants (Blum, 1999). Drought stress treatment significantly decreased the RWC of all Italian ryegrass varieties, in which Winter hawk and New dawn showed the greatest decrease and the least decrease, respectively (Fig. 3). These results suggest that Winter

hawk is the most sensitive and New dawn is the most tolerant variety to drought stress. Similarly, it has been reported that higher RWC values after drought stress treatment indicate higher drought tolerance in wheat (Larbi, 2004) or other crops (Lafitte, 2002).

Chlorophyll content or chlorophyll fluorescence techniques, mainly the ratio  $F_v/F_m$ , estimate the maximum photochemical efficiency of PSII, and have been widely used as powerful tool to detect damages in the photosynthetic apparatus due to abiotic stresses (Botyanszka et al., 2020). Chlorophyll fluorescence has also been commonly used as a tool to detect genotypic differences in response to drought stress (Jumrani et al., 2019). When the 20 days-old Italian ryegrass was subjected to drought for 4 days, chlorophyll content of all varieties was significantly decreased compared to the control (Fig. 4). However, in the case of  $F_v/F_m$ , only Winter hawk showed a significant decrease after drought stress treatment, and no difference was observed in all other varieties. These results suggest that Winter hawk has greater damage to PSII due to drought stress than other varieties. Meanwhile, under drought stress, chlorophyll content decreased significantly compared to

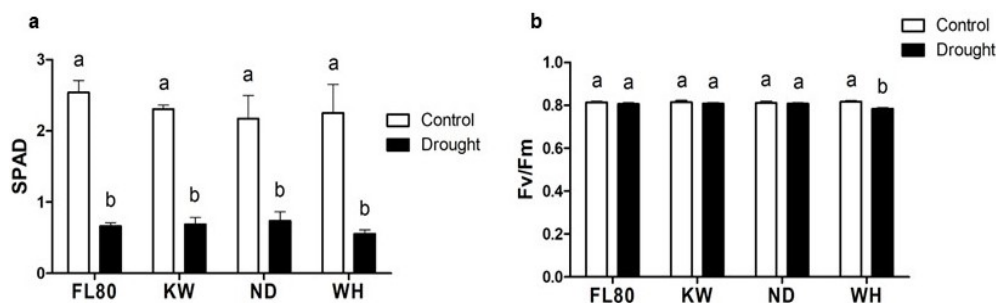


Fig. 4. Change of chlorophyll content and the maximum quantum yield of PSII ( $F_v/F_m$ ) in Italian ryegrass after drought stress treatment. (a) Shoot SPAD reading. (b) Shoot  $F_v/F_m$ . Twenty-day-old Italian ryegrass subjected to watering (control) or drought for 4 days. Different letter indicate significant differences ( $p < 0.05$ ) between all treatments (Means  $\pm$  SE;  $n = 3$ ).

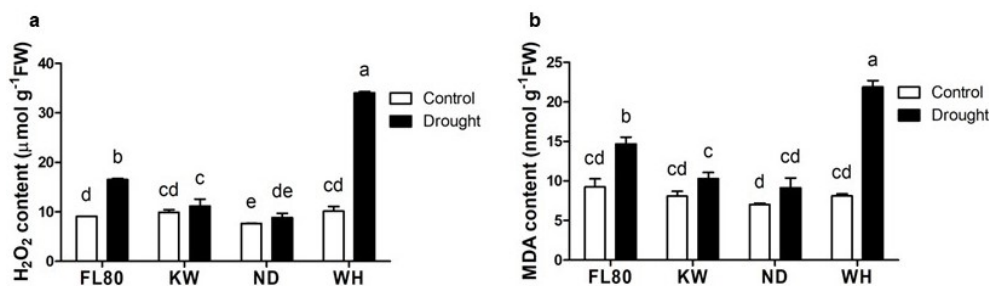


Fig. 5. Change of H<sub>2</sub>O<sub>2</sub> and MDA content in Italian ryegrass after drought stress treatment. (a) Shoot H<sub>2</sub>O<sub>2</sub> content. (b) Shoot MDA content. Twenty-day-old Italian ryegrass was subjected to watering (control) or drought stress for 4 days. Different letter indicate significant differences ( $p < 0.05$ ) between all treatments (Means  $\pm$  SE; n =3).

the control, but the decrease in Fv/Fm was not so significant, in which a similar phenomenon was observed in sweet cherries (Jalili et al., 2023).

When plants are damaged caused by abiotic stress including drought, oxidative stress damage increased by reactive oxygen species increases (Wu et al., 2022). Drought tolerance of plants was related to the alleviation of oxidative stress caused by drought (Zhang et al., 2019). We compared the H<sub>2</sub>O<sub>2</sub> and MDA contents in all varieties after drought stress treatment. As a result, the H<sub>2</sub>O<sub>2</sub> and MDA contents in the drought stress treatment group of all varieties increased compared to the control, and the Winter hawk and New dawn showed the highest and the least increase, respectively, compared to other varieties (Fig. 5). Among the 13 upland rice varieties, the most drought-tolerant variety showed the MDA was least increase compared to the other varieties (Zu et al., 2017). Thus these results suggest that Winter hawk is a drought-sensitive and New dawn is a more tolerant variety compared to other varieties.

#### IV. CONCLUSIONS

As a result of comparing the drought stress response characteristics of four Italian ryegrass varieties, Winter hawk showed the greatest decrease in growth and dry weight compared to other varieties after drought treatment. The Fv/Fm greatest decreased, while H<sub>2</sub>O<sub>2</sub> and MDA contents increased the highest in winter hawk. On the other hand, New dawn showed the opposite results. These results suggest that Italian ryegrass has different drought stress tolerance mechanisms for each variety, which may provide basic insight for the development of tolerant varieties in the future.

#### V. ACKNOWLEDGMENTS

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