

Article

Seed Hydropriming Improves Drought Tolerance in Tall Fescue Associated with Changes in Osmotic Adjustment, Ion Regulation, and Photosynthetic Stability

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Abstract

Drought limits sustainable forage production in arid and semi-arid regions, where poor crop establishment is common, especially under inadequate irrigation. To address this challenge, research has turned to simple, rapid solutions that improve crop performance under variable conditions. Among green technologies, seed priming has gained attention as an eco-friendly and cost-effective approach. This study evaluates the effectiveness of seed hydropriming in alleviating the effects of water-deficit stress (40% of field capacity) on tall fescue (*Festuca arundinacea* Schreb.) at the seedling stage. Without priming, drought stress reduced root and shoot growth, tissue hydration, and nutrient uptake. By contrast, hydropriming improved drought tolerance by improving relative water content and ion supply and sustaining photosynthetic activity. These effects were accompanied by greater accumulation of proline and soluble sugars. Hence, (i) a regulated supply with essential mineral nutrients, (ii) an osmotic adjustment using the absorbed ions (such as K^+ , Ca^{2+} , and Mg^{2+}) in the vacuole and the accumulated osmotica (such as proline and soluble sugars) in the cytoplasm, and (iii) a maintained photosynthetic machinery together with improved root development are associated with enhanced drought tolerance in *F. arundinacea* plants issued from hydroprimed seeds. It seems that seed priming emerges more and more as a practical, low-cost technique to enhance drought resilience and support sustainable forage production under limited water conditions.

Keywords: *Festuca arundinacea* Schreb.; plants; JIP test parameters; photosynthetic pigments; proline; total sugars



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1. Introduction

Drought or water deficit is a critical and complex threat affecting the globe and during the last decades it has become more and more frequent and with higher magnitude, the trend being expected to extend due to climate change [1]. Drylands are estimated to cover about 41% of the whole land of the globe and are expected to degrade more and more areas under the current and predicted climate change circumstances [2]. Drought is among the major causes of the decrease in crop yields and, thereby, a threat to food security [3]. Indeed, drought can interfere with a variety of physiological processes, inducing disturbances in

mineral nutrition, damages to cell components, a decrease in photosynthetic activity, etc., which are all able to affect crop yields and quality [4]. Therefore, the selection of drought-tolerant plant species and crop varieties is urgently needed [5].

Tall fescue (*Festuca arundinacea* Schreb.) is often considered a grass with higher tolerance to drought and other environmental stresses compared to other cool-season grasses and that requires only minor maintenance [6]. The culture of cultivar 'Fawn' succeeded in one of the arid regions, Saudi Arabia [7]. Although its growth was significantly decreased when grown under increasing water-deficit levels, it showed constant water productivity regardless of irrigation treatment, in contrast to Tekapo orchard grass, which exhibited much higher water productivity at 0.75 and 1.2 standard crop evapotranspiration (ET_c) than at 0.3 and 0.65 ET_c [8].

Seed germination is a critical phase of the life cycle of a plant, and consequently, improving this physiological process is highly important to ensure not only better crop establishment but also higher aptitude to tolerate environmental stresses [9]. Seed priming is an emerging technique with a multitude of methods commonly used in agriculture and restoration that aims to improve seed germination and seedling performance [10] as well as plant tolerance to environmental stresses [11]. It is a physiological approach based on controlling seed hydration and drying that stimulates the metabolic processes of the pre-germinative phase to ensure rapid and uniform germination [12]. The efficiency of seed priming depends on plant species/genotype, the quality of seeds themselves, as well as the method used for priming (including its conditions such as time, aeration, and temperature) [13]. In the literature, seed priming was applied to tall fescue with the aim to improve its germination and seedling establishment under both non-stress and stress conditions [14–16].

Recently, Ellouzi et al. (2024) [11] found in a preliminary work that the application of hydropriming to seeds of the tall fescue 'Fawn' alleviated the detrimental effects of a subsequent drought stress on plant growth, but despite evidence that seed hydropriming can improve drought tolerance in tall fescue, the underlying physiological mechanisms remain poorly understood, particularly during early seedling development. Therefore, the present study aims to elucidate how hydropriming enhances drought tolerance in the 'Fawn' cultivar by examining its effects on osmotic adjustment, ion homeostasis, and photosynthetic performance under water-deficit conditions.

2. Materials and Methods

2.1. Seed Hydropriming, Plant Culture, Treatments, and Harvest

Tall fescue 'Fawn' seeds were hydroprimed by soaking them in distilled water for 20 h at room temperature and then air-drying them until sowing. Sixteen plastic pots of 1 kg size were used for plant growth. Seeds from the primed and unprimed lots were sown on sandy soil (20 seeds per pot) and irrigated with clean water for 30 d at 100% field capacity. After germination and seedling establishment, ten uniform seedlings were kept per pot with two lots: 8 pots containing seedlings obtained from unprimed seeds and 8 other pots containing seedlings issued from hydroprimed seeds. On the 30th day after sowing, each lot of plants was divided into two sub-lots: the first irrigated at 100% of the field capacity (control) and the second irrigated at 40% of the field capacity (drought stress), both using clean water. The four treatments considered were as follows:

- ✓ UPC: Plants obtained from unprimed seeds and irrigated at 100% of the field capacity.
- ✓ HPC: Plants obtained from hydroprimed seeds and irrigated at 100% of the field capacity.
- ✓ UPS: Plants obtained from unprimed seeds and irrigated at 40% of the field capacity.

- ✓ HPS: Plants obtained from hydroprimed seeds and irrigated at 40% of the field capacity.

The experiment was performed in a completely randomized design and the treatments lasted 17 d. Plant culture was performed under sunlight conditions (a day/night temperature regime of 20–25 °C/12–15 °C, a relative humidity of 55–60%, and a photoperiod of 10 h). On the harvest day, fully expanded leaves were dark-adapted for 30 min before the measurements of JIP test parameters using FluorPen (Table 1). Then, the plants were harvested and cut into shoots and roots and washed with distilled water, then dried with filter paper and weighed fresh.

Table 1. Summary of formulae and definitions of some JIP test parameters (chlorophyll *a* fluorescence parameters derived from OJIP transient curve).

Formulae	Definitions
$F_v/F_m = (F_m - F_o)/F_m$	Maximum quantum efficiency of PSII photochemistry in the dark-adapted state
$F_v/F_o = (F_m - F_o)/F_o$	Indicator of the potential activity of PSII and the efficiency of energy conversion at the reaction center level
$ABS/RC = (M_o/V_j) (1/\phi P_o)$	Apparent antenna size of PSII reaction centers (i.e., energy absorbed per active reaction center)
$TR_o/RC = M_o/V_j$	Energy trapping per reaction center: reduction in Pheophytin and Q_A
$\Psi_{E_o} = 1 - V_j$	Electron transport probability beyond Q_A
$ET_o/RC = (M_o/V_j) \Psi_{E_o}$	Electron transport per reaction center beyond Q_A
Dl_o/RC	Dissipated energy flux per RC

Chlorophyll fluorescence was measured using the OJIP transient analysis, which reflects the polyphasic rise in fluorescence (O–J–I–P) and provides insight into the structure and function of photosystem II (PSII). The JIP test was applied to derive key parameters such as maximum quantum efficiency of PSII (F_v/F_m), absorption per reaction center (ABS/RC), trapped energy flux (TR_o/RC), and dissipated energy (Dl_o/RC). These parameters allow assessment of energy absorption, trapping, and dissipation processes, and were selected to evaluate the effects of hydropriming on photosynthetic performance under drought stress.

2.2. Photosynthetic Pigment Analysis

The same fully expanded leaves used for the JIP test measurements were used for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoid (Car) assays according to the slightly modified method of Lichtenthaler and Buschmann [17]. Leaf disk samples (100 mg) were kept in 5 mL acetone (pure solvent) in the dark at 4 °C for 96 h. Then, pigment extracts were read at 470, 645, and 662 nm using a UV-vis spectrophotometer and their concentrations were calculated according to the following equations:

$$\text{Chl } a \text{ (}\mu\text{g. mL}^{-1}\text{)} = 11.24 \times A_{662} - 2.04 \times A_{645}$$

$$\text{Chl } b \text{ (}\mu\text{g. mL}^{-1}\text{)} = 20.13 \times A_{645} - 4.19 \times A_{662}$$

$$\text{Car (}\mu\text{g. mL}^{-1}\text{)} = (1000 \times A_{470} - 1.90 \times \text{Chl } a - 63.14 \times \text{Chl } b)/214$$

where A_{662} , A_{645} , and A_{470} stand for leaf extract absorbance at 662, 645, and 470 nm, respectively.

2.3. Potassium, Calcium, and Magnesium Analyses

After oven-drying at 70 °C until constant weight, the shoot and root samples were ground to fine powder. An amount of about 20 mg dry matter was taken from each sample for nutrient extraction using HNO₃ (0.5%). Three days later, with agitation from time to time, extracts were obtained by filtering the mixtures with ashless filter paper. Potassium (K⁺) concentrations in the extracts were determined using a Corning 480 flame photometer (Corning Medical and Scientific. Ltd., Halstead, Essex, England) while those of calcium (Ca²⁺) and magnesium (Mg²⁺) were determined using a VARIAN 220 FS atomic absorption spectrophotometer (Varian, SpectrAA 220FS, Mulgrave, Australia).

2.4. Proline Assay

Proline content was assayed according to Bates et al. [18]. Dry ground samples from shoots and roots were homogenized in 2 mL ethanol in glass tubes then heated at 85 °C for 1 h using a water bath. By putting the tubes in ice, the reaction was stopped. Thereafter, an aliquot of 1 mL from the upper phase of each tube was added to 1 mL acetic acid and 1 mL of the following mixture: 120 µL distilled water, 300 µL acetic acid, 300 µL orthophosphoric acid, and 25 mg ninhydrin. Following a 30 min boiling period, the mixture was cooled and then added with 5 mL toluene. Proline phase was then collected and quantified by a UV–visible spectrophotometer at 528 nm.

2.5. Total Soluble Sugar Assay

Total soluble sugars were assayed following Shields and Burnet [19]. They were extracted twice from the dried and ground samples of the shoots and roots using hot 80% ethanol. Thereafter, a centrifugation at 3000 rpm for 20 min was performed for the mixtures, and soluble sugar contents were determined in the supernatant by reading the absorbance at 640 nm using the enthrone assay standardized to glucose.

2.6. Statistical Analysis

All replicates (n = 5) were biological replicates. Each replicate consisted of one pot, which was considered the experimental unit. Each pot contained 20 plants per pot, and measurements were taken from representative plants within each pot. All data were subjected to two-way ANOVA using IBM SPSS Statistics 25 and means were compared through a one-way ANOVA according to Duncan's multiple-range test at $p \leq 5\%$. Duncan's multiple-range test was used due to its sensitivity in detecting differences among treatment means in physiological studies where subtle variations are expected. This test is commonly applied in agronomic research to provide detailed pairwise comparisons among treatments.

3. Results and Discussion

Seed germination is a critical step for successful emergence and seedling establishment. However, it is also the most vulnerable stage in the plant life cycle when exposed to environmental stresses [11]. As a result, enhancing seed quality, including both viability and vigor, has become an urgent priority. This improvement is essential not only for the successful establishment of agricultural crops but also to help crops withstand environmental stress. Hydropriming has proven to be an effective agronomic strategy for improving seed vigor and germination performance, particularly under drought stress where osmotic balance is critical [15]. In the present study, we hypothesize that the hydropriming of tall fescue seeds can effectively regulate physiological processes, especially those associated with osmotic adjustment, photosynthesis, and ion homeostasis. Additionally, we suggest that post-priming mechanisms, particularly those involving osmoregulatory systems, may provide tall fescue with enhanced and sustained tolerance to water deficit.

3.1. Effect of Hydropriming on Seedling Growth, Water Status and Photosynthesis Relations

Based on morphological traits, the seedlings from unprimed seeds exposed to drought stress (UPS) showed an obvious reduction in their size as compared to the control ones (UPC). Interestingly, seed hydropriming improved plant vigor under drought conditions (Figure 1).

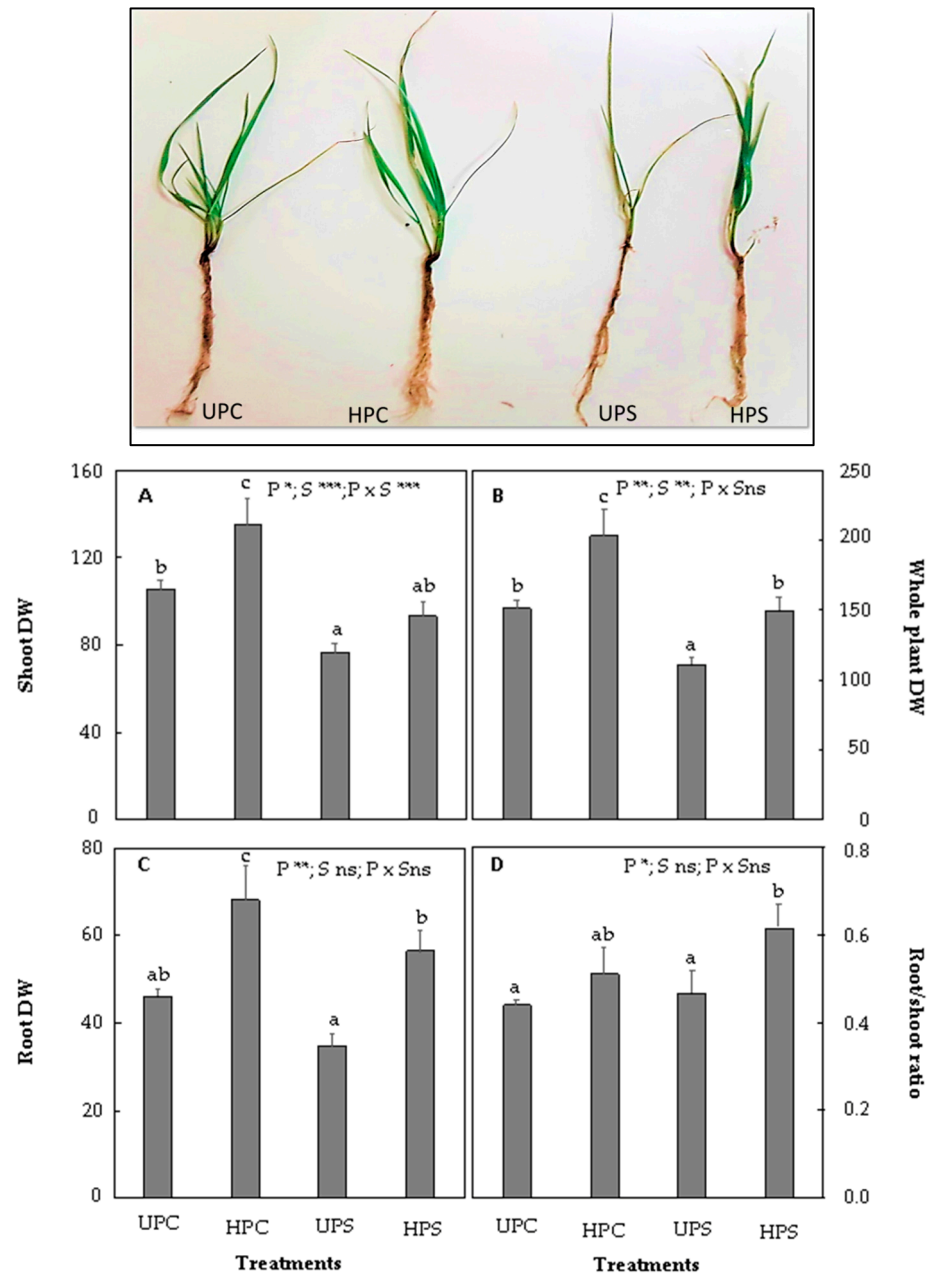


Figure 1. Morphological aspect, shoot (A), whole plant (B), and root DW (C) (mg. plant⁻¹) and root/shoot ratio (D) in *F. arundinacea* plants issued from unprimed (UP) and hydroprimed (HP) seeds and subsequently subjected (UPS and HPS) or not (UPC and HPC) to drought stress for 17 days. Bars are means of 5 replicates + SE. Bars labeled with different letters are significantly different according to Duncan's multiple-range test at 5%. For two-way ANOVA, data about priming (P), stress (S), and their interaction (P × S) effects are presented as asterisks indicating different levels of significance: *: 5%; **: 1%; ***: 0.1%; ns: non-significant.

As shown in Figure 1A–C, the irrigation at 40% of the field capacity statistically ($p < 0.01$) reduced shoot (by 25%) and root (by 24%) biomasses in the seedlings raised

from unprimed seeds. Similarly, a significant decrease in the whole plant dry weight parameter (by 27%) was found in seedlings derived from unprimed seeds sown under drought conditions (Figure 1B). It is important to mention that under the drought state only (without priming), no significant change was recorded in the root/shoot ratio as compared to the non-stressed plants from unprimed seeds (Figure 1D). It is well known that drought stress primarily impacts the root system, altering its morphology and reducing its ability to effectively respond to abiotic stresses [20]. A similar trend was recorded in the present study, where water deficit significantly reduced root and shoot dry weights.

On the contrary, the seedlings from hydroprimed seeds exhibited higher growth performance, under both control and drought conditions. Interestingly, water-deficit treatment significantly ($p < 0.01$) stimulated shoot and root growth by 22 and 35.6%, respectively, when compared to the unprimed stressed seedlings (Figure 1A–C). This was accompanied by a marked increase in whole plant dry weight (by 35%) and root/shoot ratio (by 20%) parameters in comparison with the seedlings raised from unprimed seeds (Figure 1B–D). Our data clearly indicate that seed hydropriming significantly improved the overall growth indices of the tall fescue plants when subjected to water-deficit stress. Additionally, it seems that the improved tolerance of the tall fescue plants under stressful conditions may be associated with strengthened root growth. This may explain in part the increased root/shoot ratio observed in the stressed seedlings derived from hydroprimed seeds. Our findings are supported by Tausz-Posch et al. [21] who assumed that seed priming can boost root biomass to a degree that is beneficial to promoting the root ability to uptake nutrients from the soil. One possible mechanism by which priming enhances stress tolerance is through the rapid activation of a wide range of signaling events triggered in the primed seed itself and then amplified under subsequent stress exposure [22]. This hypothesis was also supported by Forti et al. [23] who reported that hydropriming stimulates the synthesis of enzymes and metabolites involved in the germination process, such as amylases and proteases, which are crucial for breaking down stored nutrients, resulting in the enhanced growth of *Medicago truncatula* species when planted under water-deficit stress.

When considering the seedling hydration, it is possible to observe that hydropriming, drought stress, and their interaction had no significant effect on shoot water content (Figure 2). At the same time, we found that over 17 days of 40% field capacity, water content decreased significantly (by approximately 27%) in the roots of plants arising from unprimed and primed seeds as compared to the control (Figure 2B). Roots, as the primary plant organs to detect soil condition alterations, are a key factor in plant response to water scarcity stress [24,25]. In this context, several studies showed that the particular tolerance of tall fescue species to drought stress is greatly associated with the root system [26,27]. In the present study, we found that tall fescue plants from hydroprimed seeds cope with drought by enhancing both shoot and root growth, while surprisingly limiting root water content. Thus, one may reasonably suggest that during hydropriming, the seeds were sufficiently imbibed to a controlled degree. This allowed optimal hydration and enabled the initiation of metabolic processes that remained latent in the primed seed itself and were subsequently amplified when germination took place under unfavorable conditions [11]. This was supported by Louis et al. [28] who reviewed that during priming, seeds are undoubtedly equipped with plausible and specific molecular changes that govern germination and stress memory, resulting in subsequent stress tolerance. Hence, priming regulates a plethora of pre-germinative molecular events that boost early germination and help in early seedling establishment [29]. However, little is known about these aspects in the context of seed hydropriming, drought and their interaction. Therefore, further in-depth investigations are needed.

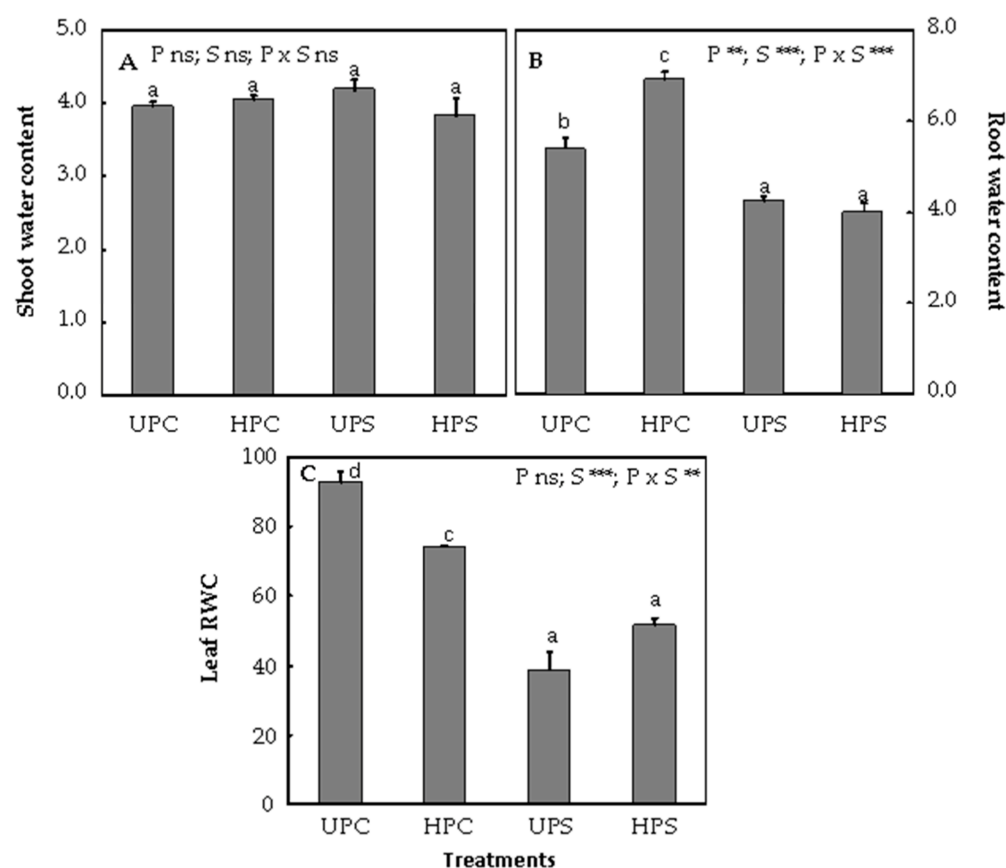


Figure 2. Shoot (A) and root (B) water contents (WC, mL H₂O. g⁻¹ DW) and leaf relative water content (C) (RWC, %) in *F. arundinacea* plants issued from unprimed (UP) and hydroprimed (HP) seeds and subsequently subjected (UPS and HPS) or not (UPC and HPC) to drought stress for 17 days. Means (n = 5) followed by different letters are significantly different according to Duncan's multiple-range test at 5%. For two-way ANOVA, data about priming (P), stress (S), and their interaction (P × S) effects are presented as asterisks indicating different levels of significance: **: 1%; ***: 0.1%; ns: non-significant. Different letters indicate significant differences among treatments.

3.2. Hydropriming–Drought Interaction Modulates Photosynthetic Pigments

Photosynthetic pigment composition in *Festuca arundinacea* exhibited differential sensitivity to drought stress and hydropriming, with responses largely driven by priming × stress interactions rather than single-factor effects (Figure 3). Chl *a* remained statistically unchanged across treatments, indicating that the photosystem II (PSII) reaction centers were largely preserved under the imposed drought conditions. This stability suggests that drought intensity was moderate and did not severely impair primary photochemistry. Such resilience of Chl *a* has been widely reported under mild-to-moderate drought, where plants maintain basal photosynthetic activity while adjusting peripheral components of the photosynthetic apparatus (Yan et al., 2024) [2]. By contrast, Chl *b* was highly responsive to treatments, showing a significant reduction in hydroprimed plants under drought (HPS) and a marked increase in unprimed stressed plants (UPS). The significant effects of priming and the interaction priming × stress confirm that Chl *b* is a key regulatory target in stress acclimation. Given that Chl *b* is predominantly associated with the light-harvesting complex (LHC), its reduction under hydropriming suggests a strategic downscaling of antenna size, limiting excess light absorption and reducing the risk of photoinhibition. This aligns with recent studies showing that drought stress triggers structural and functional adjustments in antenna pigments to optimize energy dissipation and prevent oxidative damage [30]. Conversely, the elevated Chl *b* in UPS may indicate dysregulated pigment

turnover, a phenomenon often associated with stress-sensitive plants lacking pre-adaptive priming mechanisms. Carotenoid (Car) content remained unchanged across treatments, indicating that non-enzymatic antioxidant defenses were maintained at a stable baseline. Carotenoids play a central role in quenching reactive oxygen species (ROS) and dissipating excess excitation energy.

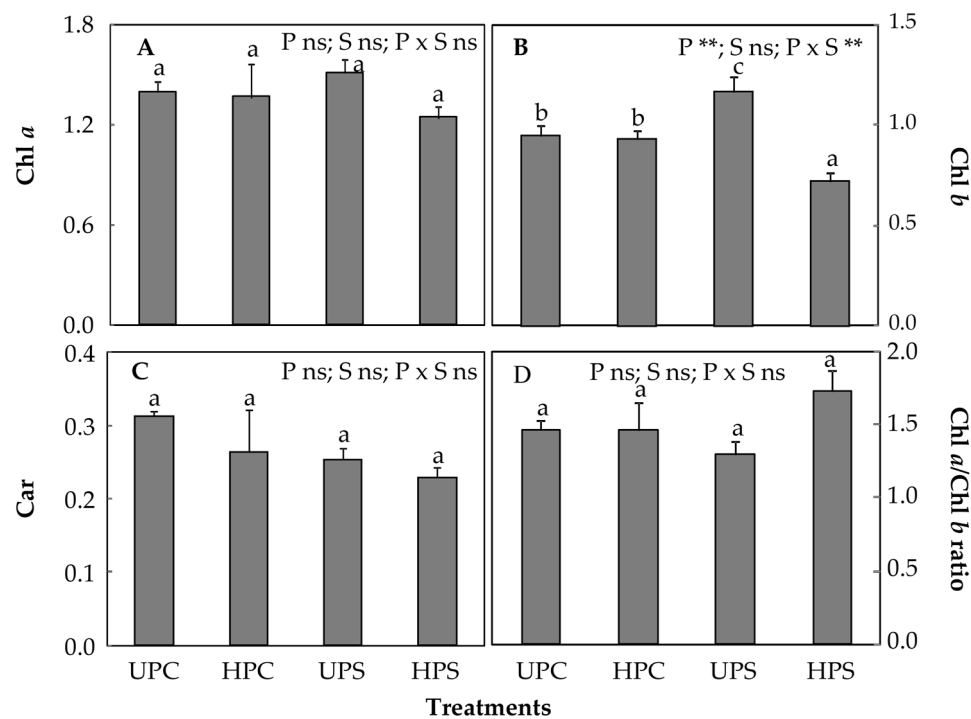


Figure 3. Pigment contents (mg g^{-1} FW) in *Festuca arundinacea*, including chlorophyll a (A), chlorophyll b (B), carotenoids (C), and the chlorophyll a/b ratio (D), in plants derived from unprimed (UP) and hydroprimed (HP) seeds, either subjected (UPS and HPS) or not (UPC and HPC) to drought stress for 17 days. Means ($n = 5$) followed by different letters are significantly different according to Duncan's multiple-range test at 5%. For two-way ANOVA, data about priming (P), stress (S), and their interaction ($P \times S$) effects are presented as asterisks indicating different levels of significance: **: 1%; ns: non-significant. Different letters indicate significant differences among treatments.

The absence of significant variation suggests that drought stress was not severe enough to trigger large-scale oxidative imbalance, or that existing carotenoid pools were sufficient to buffer stress-induced ROS production. Similar stability in carotenoid content under moderate drought has been reported in several crops, where photoprotective systems remain functionally intact despite changes in chlorophyll fractions [2]. Chl a/Chl b ratio showed no significant differences among treatments, indicating that the overall structural organization of the photosynthetic apparatus remained relatively stable. These results suggest that the imposed drought stress did not severely impair photosynthetic processes under the experimental conditions. Therefore, the observed drought tolerance in hydroprimed plants is more likely associated with osmotic adjustment, ion regulation, and root development rather than a direct enhancement of photosynthetic activity.

3.3. Effect of Hydropriming on JIP Test Parameters

Based on our findings, no statistically significant differences were observed between the primed and non-primed states, under control or water-deficit conditions, for the overall JIP test parameters (Table 2). This response, together with the observed maintenance of leaf water status, is a good indicator of tall fescue tolerance to drought stress. In this regard, several studies have further explored the adaptive mechanisms of tall fescue to osmotic

stresses such as drought and salinity. Among these mechanisms, balanced photosynthetic machinery and osmotic adjustment are key factors of tall fescue tolerance [31].

Table 2. Selected JIP test parameters in *F. arundinacea* plants issued from unprimed (UP) and hydroprimed (HP) seeds and subsequently subjected (UPS and HPS) or not (UPC and HPC) to drought stress for 17 days. Means ($n = 5$) followed by the same letter are not significantly different according to Duncan's multiple-range test at 5%. For two-way ANOVA, data about priming (P), stress (S), and their interaction ($P \times S$) effects are presented as asterisks indicating different levels of significance: *: 5%; ns: non-significant.

	UPC	HPC	UPS	HPS	Two-Way ANOVA
Fv/Fm	0.82 a	0.80 a	0.82 a	0.82 a	P ns; S ns; $P \times S$ ns
Fv/Fo	4.41 a	4.12 a	4.43 a	4.50 a	P ns; S ns; $P \times S$ ns
ABS/RC	2.01 a	2.05 a	2.14 a	2.10 a	P ns; S ns; $P \times S$ ns
TRo/RC	1.64 a	1.65 a	1.75 a	1.72 a	P ns; S ns; $P \times S$ ns
Ψ_{Eo}	0.56 a	0.56 a	0.56 a	0.56 a	P ns; S ns; $P \times S$ ns
ETo/RC	0.92 a	0.91 a	0.99 a	0.96 a	P ns; S *; $P \times S$ ns
DTo/RC	0.37 a	0.41 a	0.40 a	0.38 a	P ns; S ns; $P \times S$ ns

3.4. Effect of Hydropriming on Ion Distribution Within Roots and Shoots Under Water Deficit

The decrease in growth and hydration recorded in UPS was accompanied by significant reductions in K^+ , Ca^{2+} , and Mg^{2+} contents in both shoots and roots (Figure 4).

Water deficit significantly ($p < 0.01$) reduced K^+ content by 67% in shoots and 73% roots (Figure 4B) compared to those of the control seedlings from unprimed seeds. At 40% field capacity, the tall fescue seedlings obtained from unprimed seeds exhibited a pronounced decrease in Ca^{2+} accumulation in both shoots (by 67%) and roots (32%), relatively to their homologs of non-stressed plants (Figure 4C,D). Apart from decreasing K^+ and Ca^{2+} nutrition, the water deficit resulted also in a marked decline of Mg^{2+} , by 67% in shoots and 55% in roots (Figure 4E,F), relative to the non-stressed plants from unprimed seeds. Reverse trends were observed in the seedlings issued from primed seeds. In this context, the overall ion contents were significantly restored when the plants were cultivated under both control and water-deficit conditions. At 40% field capacity, shoot K^+ , Ca^{2+} , and Mg^{2+} (Figure 4A,C,E) were significantly improved by 264, 244 and 256% respectively, in comparison with the UPS state. Similar trends were recorded in the roots of plants obtained from hydroprimed seeds; K^+ , Ca^{2+} , and Mg^{2+} contents exhibited spectacular increases when grown under water-deficit stress (Figure 4B,D,F), indicating that seed priming effectively promoted nutrient uptake in both roots and shoots arising from primed seeds. Consequently, seed priming is believed to be intricately linked to improved nutrient mobilization within roots and shoots, even under stress [22,32]. Potassium plays a key role in osmotic regulation and stomatal function, calcium is involved in membrane stability and stress signaling, and magnesium is essential for chlorophyll structure and enzyme activation. These nutrients collectively support cellular homeostasis and stress adaptation under water-deficit conditions. Bibi et al. [33] also documented the beneficial effect of hydropriming on nutrient uptake in canola plants exposed to water-deficit stress. The increased accumulation of K^+ , Ca^{2+} , and Mg^{2+} in hydroprimed plants may contribute to improved drought tolerance.

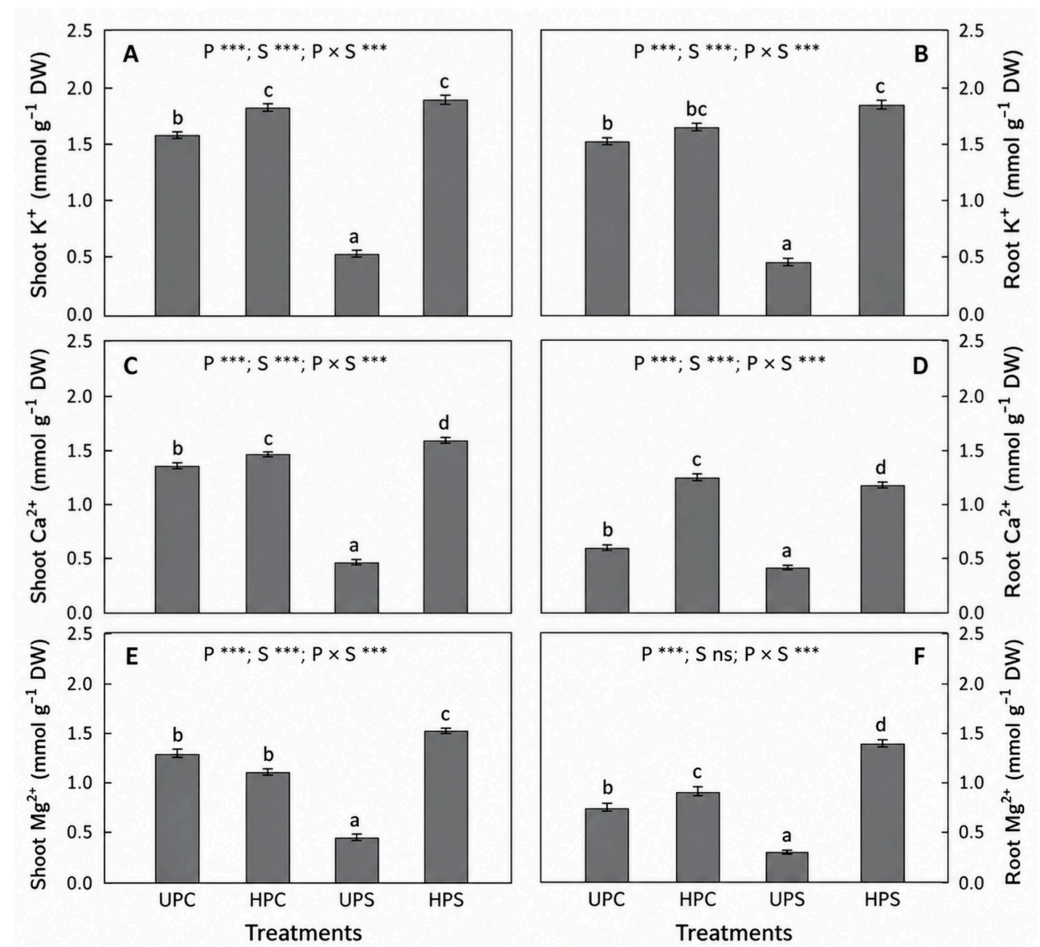


Figure 4. Shoot (A,C,E) and root (B,D,F) contents of K^+ (A,B), Ca^{2+} (C,D), and Mg^{2+} (E,F) ($mmol\ g^{-1}\ DW$) in *Festuca arundinacea* plants derived from unprimed (UP) and hydroprimed (HP) seeds, either subjected (UPS and HPS) or not (UPC and HPC) to drought stress for 17 days. Bars are means of 5 replicates $\pm$ SE. Bars labeled with different letters are significantly different according to Duncan's multiple-range test at 5%. For two-way ANOVA, data about priming (P), stress (S), and their interaction ($P \times S$) effects are presented as asterisks indicating different levels of significance: ***: 0.1%; ns: non-significant. Different letters indicate significant differences among treatments.

3.5. Effect of Hydropriming on Proline and Total Soluble Sugar Contents Under Water Deficit

As shown in Figure 5A–D, proline and total soluble sugar contents were significantly enhanced ($p < 0.01$) in tall fescue seedlings obtained from hydroprimed seeds, when grown under both control and water-deficit conditions.

Interestingly, at 40% field capacity seed hydropriming improved shoot and root proline contents by respectively 83% (Figure 5A) and 96% (Figure 5B) as compared to the control. Similarly, the tall fescue plants arising from hydroprimed seeds and subjected to drought stress had the significantly ($p < 0.01$) higher total sugar contents in both shoots and roots (192% and 123%, respectively) than the 40% field capacity stress-treatment plants from unprimed seeds (Figure 5C,D). One significant physiological response to stress in plants is the active accumulation of solutes within cells, which helps regulate osmotic pressure [34]. The present observations suggest that, without priming, drought stress drastically altered overall seedling osmotic adjustment, as estimated by the decreased proline and sugar contents. Our findings are in agreement with those of Esmailpourmoghdam et al. [31] who found decreased proline and sugar contents in tall fescue plants upon exposure to dual salinity and drought. Since proline, sugar, and potassium accumulate in tall fescue roots and shoots issued from hydroprimed seeds, even under water stress application, they may

act as compatible solutes and osmoregulatory agents by modulating osmoregulation-related channels, including aquaporins and K^+ channels. This hypothesis was also supported by Gong et al. [35]. In the same line of ideas and in the context of priming, many investigations have shown that proline and sugar can serve as signaling molecules and their actions are not limited to osmoregulation; instead, they are greatly implicated as antioxidant systems [1,11].

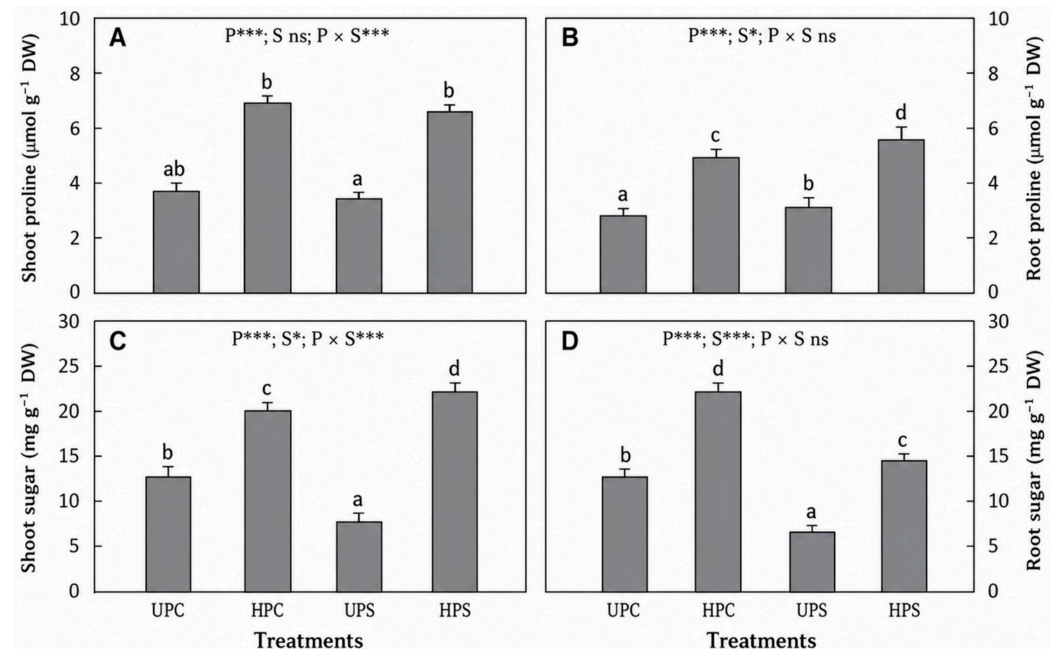


Figure 5. Shoot (A,C) and root (B,D) contents of proline ((A,B); $\mu\text{mol g}^{-1}$ DW) and soluble sugars ((C,D); mg g^{-1} DW) in *Festuca arundinacea* plants derived from unprimed (UP) and hydroprimed (HP) seeds, either subjected (UPS and HPS) or not (UPC and HPC) to drought stress for 17 days. Bars are means of 5 replicates + SE. Bars labeled with different letters are significantly different according to Duncan's multiple-range test at 5%. For two-way ANOVA, data about priming (P), stress (S), and their interaction ($P \times S$) effects are presented as asterisks indicating different levels of significance: *: 5%; ***: 0.1%; ns: non-significant.

A limitation of the present study is the absence of direct measurements of drought severity indicators such as soil water potential, leaf water potential, stomatal conductance, and gas exchange parameters. Consequently, the physiological intensity of drought stress could not be fully quantified. Future studies should integrate these measurements to provide a more comprehensive understanding of plant responses to hydropriming under water-deficit conditions.

4. Conclusions

Based on the results discussed above, we reaffirm the potential role of seed priming in enhancing drought stress tolerance. The present study demonstrated that seed hydropriming stimulated several events that initially occurred in the primed seeds themselves and were later transferred to the entire tall fescue seedlings. The mechanisms by which hydropriming alleviated the effects of water deficit in tall fescue can be summarized as shown in Figure 6. The improved drought tolerance observed in the hydroprimed plants is associated with enhanced ion accumulation (K^+ , Ca^{2+} , Mg^{2+}), osmotic adjustment, and improved root development, while photosynthetic performance remained largely unaffected under the experimental conditions.

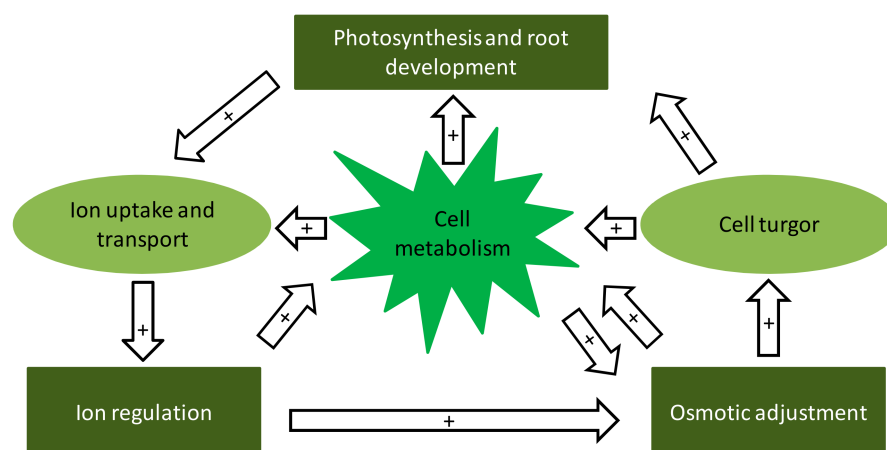


Figure 6. A simplified summary of the studied physiological mechanisms involved in the response to water-deficit stress in *F. arundinacea* plants issued from hydroprimed seeds.

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