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Morpho-physiological responses and recovery of evening primrose (*Oenothera biennis* L.) to water deficit and re-irrigation

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Abstract

Objective: Evening primrose (*Oenothera biennis* L.) is an important oilseed plant in temperate regions, facing challenges from drought and water scarcity. This study examined the effect of drought stress, sampling time, and accession type on vegetative, physiologic, and biochemical characteristics of evening primrose, and its recovery through re-irrigation.

Methods: Experimental plants underwent four irrigation intervals (5 days as control, 10 days, 14 days, and 18 days) in Gorgan, Iran, using a factorial arrangement based on a randomized complete block design, with three replications. The pot experiment was conducted in a polyethylene-covered outdoor setting and continued until capsule formation. Pre- and post-irrigation sampling allowed for the comparison of plant morphological, physiological, and biochemical characteristics in both Iranian and German accessions.

Results: Results showed no evening primrose recovery at the 18-day irrigation interval. Extended watering cycles led to decreased leaf dry weight and membrane stability, and increased proline, total phenols, total flavonoids, and the activity of antioxidant enzymes catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), and phenylalanine ammonia-lyase (PAL). Levels of proline, CAT, POD, SOD, APX, and PAL varied significantly across different sampling times. Accession differences were evident in antioxidant enzyme activities, reflecting distinct stress and recovery responses. Recovered samples exhibited 1.3 times lower antioxidant activity compared to stressed samples. PAL activity decreased after recovery, indicating improved photosynthetic efficiency after recovery.

Conclusion: Drought stress decreased the vegetative growth and membrane stability, and increased the activity of various antioxidant enzymes, proline, phenols, proteins, and flavonoids. The increase in proline and antioxidant enzymes during drought stress suggests evening primrose's stress response and recovery capabilities. In Gorgan's conditions, optimal evening primrose recovery intervals appear to be around 10 days, with potential recovery even at 14-day intervals. While understanding evening primrose recovery is critical, further investigations are necessary for making informed decisions in the field.

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Introduction

Drought and water scarcity present growing challenges to the production and resilience of medicinal plants (Karimi *et al.* 2021). Understanding how plants tolerate water deficit and recover following rehydration is essential for optimizing water resource management and improving crop performance (Cooper and Farrant 2002). Among such species, *Oenothera biennis* L. (Evening Primrose) holds notable value as a biennial medicinal and oilseed plant, with its seed oil widely used in pharmaceutical, cosmetic, and personal care industries (Ghasemnezhad and Honermeier 2008). However, like many high-value crops, *O. biennis* is sensitive to abiotic stresses, particularly drought, which can substantially reduce yield and quality (Nemeskéri and Helyes 2019).

Physiological and agronomic traits associated with drought tolerance are essential criteria for identifying genotypes with improved resilience (Li *et al.* 2006). Additionally, biochemical mechanisms such as the accumulation of stress-responsive metabolites and the activation of antioxidant defense systems play central roles in mitigating oxidative damage caused by reactive oxygen species (ROS) during drought (Foyer and Noctor 2005; Gill and Tuteja 2010). Enzymatic compounds like catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX), together with non-enzymatic antioxidants such as carotenoids, α-tocopherol, and flavonoids, contribute to ROS homeostasis and overall stress adaptation (Choudhary and Padda 2015).

Although several studies have examined plant responses to drought and subsequent re-irrigation (e.g., Xu *et al.* 2009), most recent work has focused either on short-term physiological recovery or on general drought-tolerance screening, without exploring how re-hydration influences the duration and extent of endurance in medicinal oilseed crops. Moreover, *O. biennis*, despite its commercial importance, has received limited attention in studies that couple drought dynamics with post-stress recovery. This study addresses these gaps by simultaneously evaluating drought tolerance and re-irrigation recovery in Evening Primrose, with an emphasis on endurance duration, and physiological and biochemical response mechanisms that govern recovery after water is restored. By integrating

agronomic, physiological, and antioxidant-related metrics, this research provides a more comprehensive understanding of how *O. biennis* responds to both stress and subsequent recovery, offering insights that are distinct from, and more detailed than, existing drought re-irrigation studies.

Materials and Methods

Plant materials

Two seed accessions of *Oenothera biennis* were utilized in this study. One seed population was purchased from Rühlemann's Kräuter & Duftpflanzen Company in Horstedt, Germany, while another seed population was obtained from the Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Seeds were sown in December 2019 in 6-kg pots containing a specialized soil mixture, with each seed sample having three replicates. Following germination and upon reaching the 6-leaf stage, a single plant per pot was retained. Pots were uniformly and consistently irrigated until stem development occurred.

Experimental design

The experimental design encompassed a factorial arrangement, employing a completely randomized design with three replications. Upon entering the stem production phase, treatments were applied to the two accessions. Drought stress was induced by subjecting the plants to four irrigation intervals: 5 days (control), 10 days, 14 days, and 18 days; however, all samples were destroyed after an 18-day irrigation cycle. Therefore, in the data analysis, only three irrigation cycles were used. The irrigation intervals were maintained until the flowering. Sampling was conducted both before and 24 hours after irrigation, serving as the measurement time for all traits.

Measurement of physiological and biochemical traits

Relative leaf water content was assessed according to Gonzalez and Gonzalez-Vilar (2003). Ion leakage was determined according to Bajji *et al.* (2000). Proline content of the samples was measured using the method described by Irigoyen *et al.* (1992). The Bradford method was employed to analyze protein content in plant tissues (Bradford 1976). The total phenol content was determined following the procedure outlined by McDonald *et al.* (2001), while flavonoid content was assessed based on Chang *et al.* (2002). MDA accumulation was measured using the method described by Heath and Packer (1968).

Measurement of antioxidant enzyme activity

To measure the antioxidant enzyme activity, a standardized extraction method was used. Fresh leaf tissue (5.0 g) was ground using liquid nitrogen, and the resulting sample was mixed with a 50 mM phosphate buffer containing 5.0 mM EDTA and 2% PVPP. After centrifugation, the resulting solution was used to determine the activities of peroxidase (POD), SOD, CAT, and APX.

The CAT activity was assessed using the method described by Luck (1965). The SOD activity was measured following the approach outlined by Giannopolitis and Ries (1977). The APX activity was determined based on the method proposed by Nakano and Asada (1981). The POD activity was evaluated using the method established by Weston (1989). The activity of phenylalanine ammonia-lyase (PAL) was measured by homogenizing the fresh leaf tissue in Tris-HCl buffer and assessing the conversion of phenylalanine to trans-cinnamic acid (Wang *et al.* 2019).

Data analysis

Mean comparisons were conducted using the LSD test at the significance level of 5%. Statistical analysis was performed using SPSS version 16 software. Graphs were generated using Microsoft Excel software.

Results

Effect of the experimental factors on leaf dry weight, relative water content, and ion leakage

The effect of sampling time was significant on the leaf relative water content. Irrigation interval exerted an effect on leaf dry weight. There were significant differences between the two accessions for ion leakage and the leaf dry weight. Also, the irrigation interval × sampling time interaction was significant for the ion leakage (Table 1).

After conducting a comparison of means, it became evident that RWC after recovery exhibited a significant increase (Figure 1). However, no significant difference emerged between the two accessions (Table 1). The results about the effect of irrigation duration on the leaf dry weight are shown in Figure 2. Notably, plants subjected to an irrigation duration of 14 days exhibited a substantial reduction in leaf dry weight in comparison to both the 5-day (control) and 10-day intervals.

Based on Figure 3, in the first year, ion leakage displayed distinct trends. Notably, ion leakage was markedly higher when subjected to a 14-day irrigation interval as opposed to the 10-day interval and the control. Conversely, no significant difference was observed between the 10-day interval and the control. Following the recovery phase, ion leakage significantly decreased in the14-day interval

Table 1. Analysis of variance of the effect of irrigation interval, sampling time, and accessions of the evening primrose on leaf dry weight, relative water content, and ion leakage.

Source of variation	df	Mean squares		
		Leaf DW	Relative water content	Ion leakage
ST	1	0.002	0.31**	0.02
IT	2	0.03**	0.04	0.05
PA	1	0.04**	0.006	0.17*
IT × ST	2	0.004	0.017	0.90**
ST × PA	1	0.000002	0.004	0.06
PA × IT	2	0.004	0.0006	0.08
PA × IT × ST	2	0.0001	0.009	0.05
Error	24	0.001	0.013	0.04
CV (%)		14.65	18.5	30.8

*,**: Significant at 0.05 and 0.01 probability levels, respevtively; ST: Sampling time, IT: Irrigation interval, PA: Plant accession, DW: Dry weight.

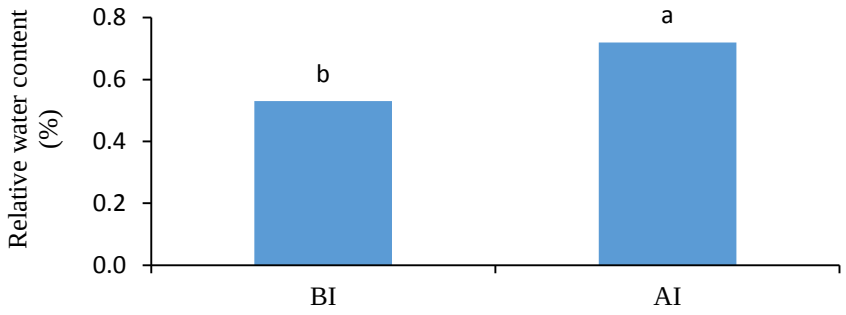

Figure 1. The effect of sampling time on the relative water content of evening primrose; BI: Before irrigation, AI: After irrigation; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

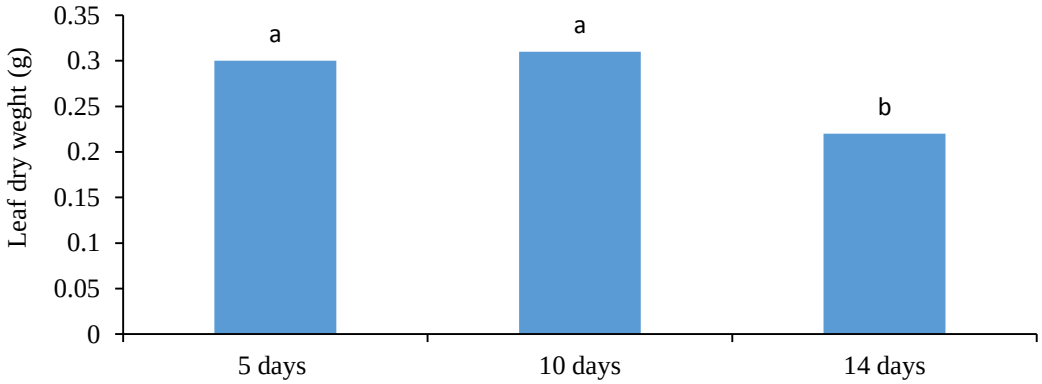

Figure 2. Effect of irrigation intervals on leaf dry weight of evening primrose; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

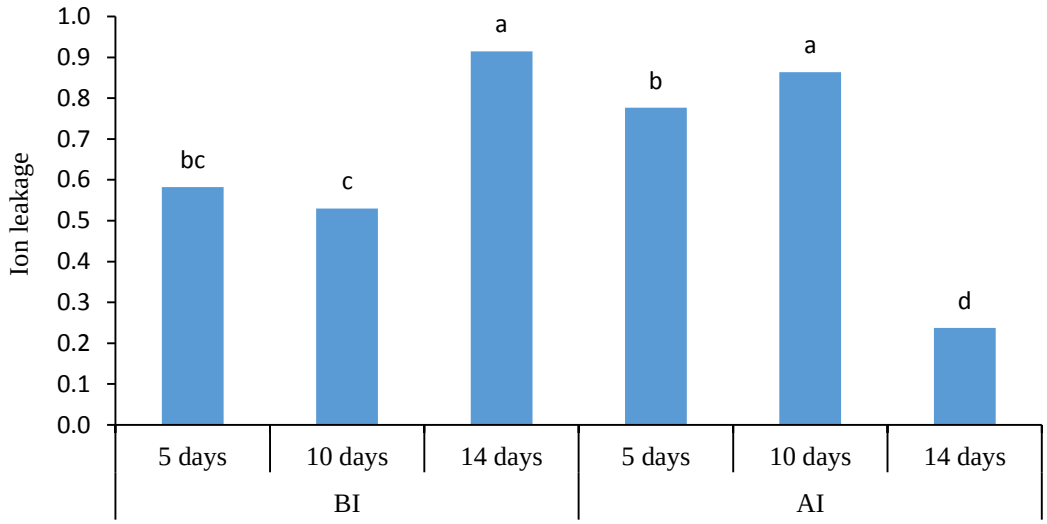

Figure 3. Effect of sampling time and irrigation interval on the ion leakage rate of evening primrose; BI: Before irrigation, AI: After irrigation; Means with different letters within each year are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

compared to the 10-day interval and the control. Ion leakage exhibited a significant increase in the 10-day stress after recovery when compared with the control. Furthermore, ion leakage was significantly higher when plants were subjected to a 14-day irrigation interval compared to the post-recovery period. In contrast, ion leakage witnessed a significant reduction in the 10-day interval compared to the 10-day post-recovery interval.

Effect of the experimental factors on the biochemical characteristics

Based on the outcomes outlined in Table 2, following the analysis of variance, it is apparent that the studied factors elicited some impacts on the biochemical traits. The sampling time showed a significant influence on most biochemical traits, including total phenols, total flavonoids, antioxidant activity, protein, CAT, POD, SOD, PAL, and APX. Furthermore, the irrigation interval exhibited discernible effects across the majority of biochemical traits, including total phenols, antioxidant activity, MDA, CAT, POD, and APX. Also, the two accessions significantly differed for all biochemical characteristics.

Additionally, significant interactions were observed between the experimental factors for the biochemical characteristics of the evening primrose (Table 2). A significant interaction between irrigation interval and sampling time was noted for the traits such as total phenols, total flavonoids, protein, CAT, POD, SOD, and APX. Also, the interaction of irrigation interval with accession was significant for most of the biochemical traits, including total phenols, proline, protein, MDA, POD, SOD, PAL, and APX. However, the sampling time × accession interaction was only significant for

the POD and APX enzymes. Furthermore, no significant sampling time × accession × irrigation interval was observed for the biochemical traits, except for APX.

Table 2. Analysis of variance of the effect of irrigation interval and re-irrigation on the accumulation of proline, protein, MDA and the activity of some antioxidant enzymes in the evening primrose accessions.

Source of variation	df	TPH	TFLA	ANTO	PR	PRO	MDA	CAT	POD	SOD	PAL	APX
ST	1	299.9**	84.2**	1567**	3.67	11.33**	7.4	0.10**	342.3**	0.03**	50.3**	0.95**
IT	1	172.9**	0.5	345.3**	0.23	0.02	92.5**	0.09**	473.8**	0.0009	3.5	2.74**
PA	2	2708.0**	480.3**	3604**	47.49**	69.25**	3595**	1.04**	4816**	0.87**	2654**	63.2**
ST × PA	1	17.2	1.1	0.6	0.01	0.19	0.78	0.0001	17.9*	0.004	7.0	0.95**
IT × ST	2	48.2*	19.1*	4.9	2.09	0.61*	8.0	0.01**	101.2**	0.009**	4.6	0.34**
PA × IT	2	42.1*	8.7	22.5	5.64*	1.66**	23.3*	0.0008	39.9**	0.01**	27.6**	0.46**
PA × IT × ST	2	8.0	2.0	3.06	0.04	0.17	11.5	0.002	2.4	0.0018	5.4	0.29**
Error		10.11	5.01	11.7	1.4	0.15	5.5	0.0009	2.8	0.001	2.2	0.03
CV (%)		11.5	14.0	7.3	6.1	5.4	6.7	5.6	4.8	6.1	6.7	4.7

*,**: Significant at 0.05 and 0.01 probability levels, respectively; Exp: Experiment, ST: Sampling time, IT: Irrigation interval, PA: Plant accession, TPH: Total phenols, TFLA: Total flavonoids, ANTO: Antioxidant activity, PR: Proline, PRO: Protein, MDA:, CAT: Catalase, POD: Peroxidase, SOD: Superoxide dismutase, PAL: Phenylalanine ammonia-lyase, ASCO: Ascorbate peroxidase.

As the irrigation interval increased from the normal 5 days to 10 and 14 days, there was a significant enhancement in antioxidant activity when compared with the control (5-day irrigation interval). Also the Iranian population displayed a higher antioxidant activity compared to the German population (Figure 4). Moreover, the levels of antioxidant activity were higher before re-irrigation, as compared to the conditions after re-irrigation.

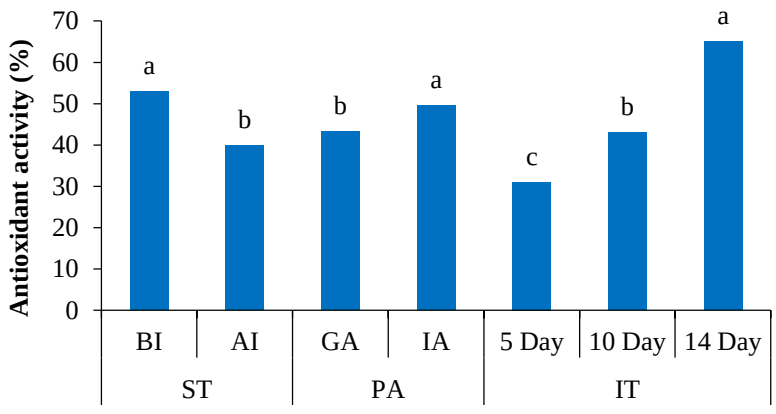

Figure 4. Separate effects of sampling time, plant accession, and irrigation interval on antioxidant activity of the leaf extract in the evening primrose; ST: Sampling time, IT: Irrigation interval, PA: Plant accession, BI: Before irrigation, AI: After irrigation, GA: German accession, IA: Iranian accession; Means with different letters within each factor are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

The CAT enzyme demonstrated an accession-dependent variation. Notably, the activity of this enzyme in the Iranian accession significantly surpassed that of the German accession (Figure 5 -left). Also, the sampling time × irrigation interval indicated that by increasing the drought stress level, the CAT activity increased both before and after irrigation; however, the CAT activity was higher before irrigation compared to the after-irrigation sampling time.

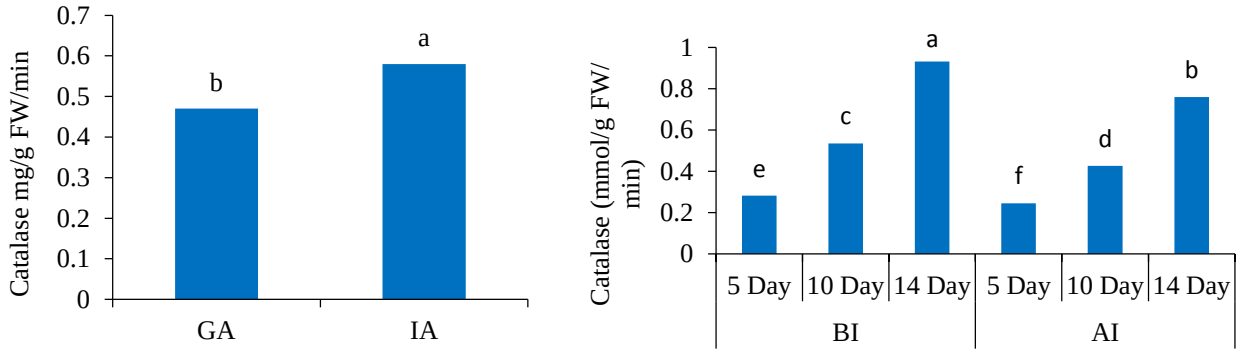

Figure 5. Effect of plant accession (left) and sampling time × irrigation interval (right) on the catalase activity in the evening primrose; GA: German accession, IA: Iranian accession, BI: Before irrigation, AI: After irrigation; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

The interaction of sampling time × irrigation interval for the total flavonoid content is shown in Figure 6. By increasing drought stress, the flavonoid content increased. At the 14-day irrigation interval, the flavonoid content increased drastically as compared to the 10-day interval and the control. The 14-day irrigation interval also had a significantly higher flavonoid content than the other two conditions; however, it was significantly lower than the 14-day interval at pre-irrigation conditions.

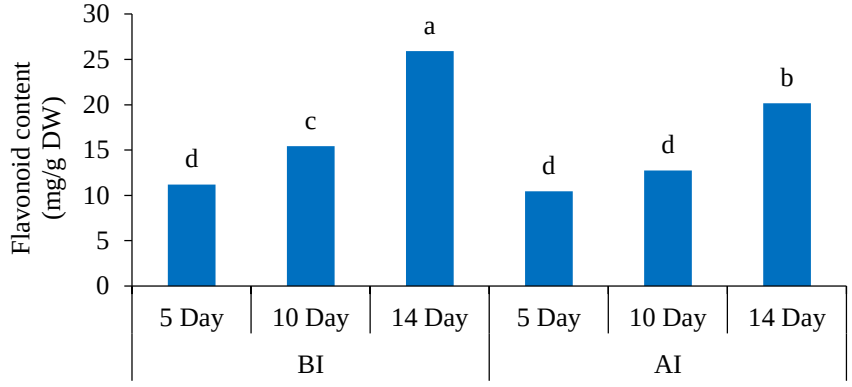

Figure 6. Effect of sampling time and irrigation interval on the total flavonoid content of the evening primrose; BI: Before irrigation, AI: After irrigation; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

The interaction of sampling time with irrigation interval for the total phenol content of evening primrose is shown in Figure 7 (left). Total phenol content exhibited a distinct trend across various irrigation intervals. Notably, before irrigation and subsequent to irrigation, a higher accumulation of total phenols was observed in the treatments involving a 14-day interval compared to those with 10-day and 5-day (control) intervals. In the case of plants subjected to a 14-day irrigation interval, the total phenol content surged to more than 45 mg/g, a considerable rise from the control level of 15 mg/g (Figure 7-left). A progressive increase in phenol accumulation was also observed with extended irrigation intervals after re-irrigation.

Based on Figure 7 (right), it becomes evident that the total phenol content experienced an augmentation across both plant accessions (Iranian and German) as the irrigation interval expanded. Notably, when compared to the control condition, the increase was particularly remarkable under the 14-day interval regime. This augmentation was approximately three and five times higher in the Iranian and German accessions, respectively, compared to the control conditions. The phenol accumulation, reaching approximately 50 mg/g, was notably observed in the German accession subjected to the 14-day interval regime. This value was nearly five times higher than the total phenol content of the control samples.

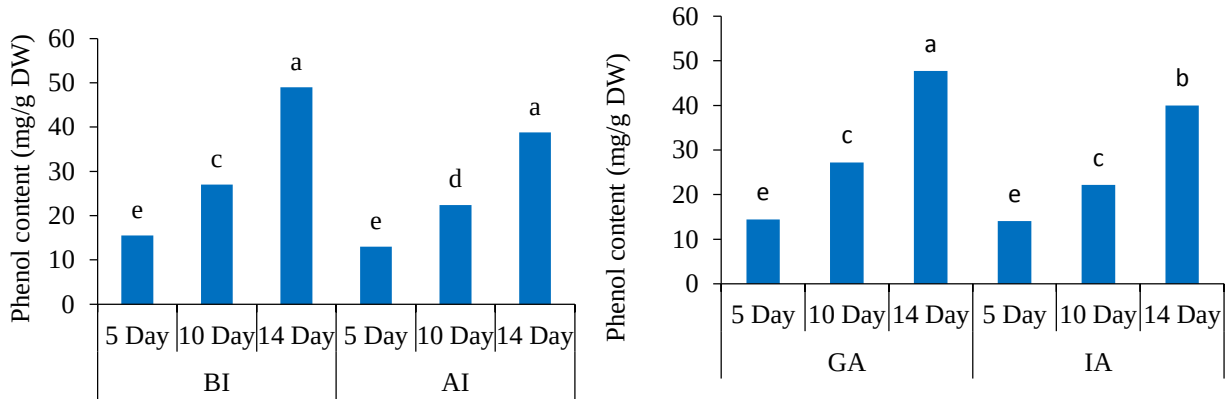

Figure 7. Effect of sampling time and irrigation interval (left), and also accession and irrigation interval (right) interaction on the total phenol content of evening primrose; BI: Before irrigation, AI: After irrigation, GA: German accession; IA: Iranian accession; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

The results demonstrate that in both evening primrose accessions, a significant increase in proline concentration occurred under the 14-day irrigation conditions as compared to the control (Figure 8). In contrast, the control treatment, characterized by a 5-day irrigation cycle, resulted in the lowest proline content.

The interaction of sampling time with irrigation interval for SOD is presented in Figure 8. Before irrigation, the SOD activity was elevated in both the 10-day and 14-day interval irrigations compared

to the control condition. Although, following irrigation, there was an unexpected increase in SOD activity in both the 10-day and 14-day interval treatments, but the values were significantly lower than those in the pre-irrigation conditions. The control conditions showed no significant difference in SOD activity before and after irrigation.

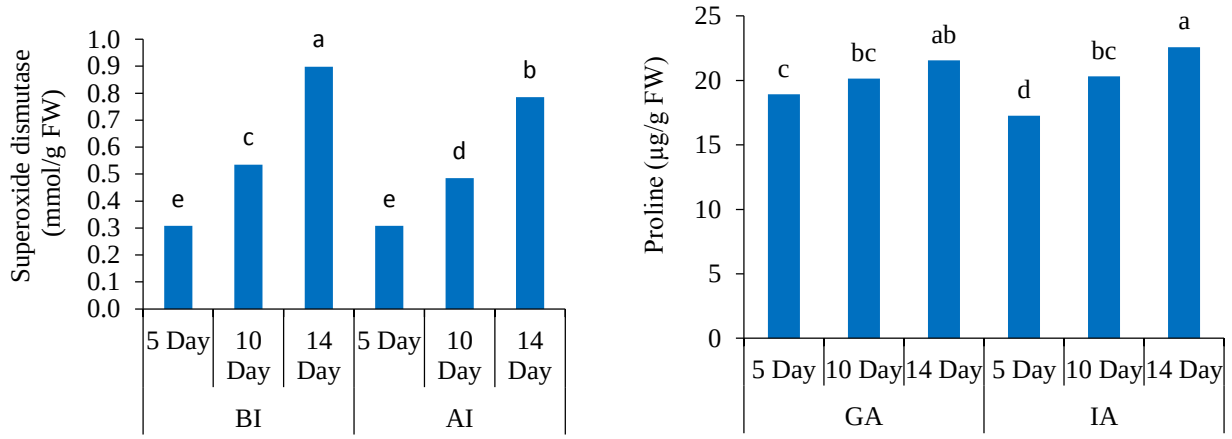

Figure 8. Effect of of sampling time and irrigation interval on superoxide dismutase (left), and also accession and irrigation interval on the proline content (right) of evenening primrose; BI: Before irrigation, AI: After irrigation, GA: German accession, IA: Iranian accession; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

As illustrated in Figure 9, a clear pattern regarding protein content across varying irrigation intervals is apparent in both accessions. Notably, for the 14-day irrigation treatment, protein content increased significantly in both accessions compared to the 10-day irrigation interval and the control. Similarly, the 10-day treatment exhibited elevated protein levels in both accessions relative to the control (5-day interval). Furthermore, the 14-day irrigation interval exhibited higher protein concentration in the German accession compared to the Iranian accession. However, in the control conditions, the protein content in the Iranian accession was significantly higher than the German accession.

The interaction between sampling time and irrigation had a significant impact on the peroxidase enzyme activity (Figure 10, right). Peroxidase activity increased in the treatments with 10-day and 14-day intervals compared to the control (5-day interval). After re-irrigation, a similar increase in peroxidase activity was observed in the 10-day and 14-day interval treatments relative to the control. However, both the 10-day and 14-day interval irrigations exhibited a decrease in peroxidase activity after re-irrigation. In contrast, the control samples showed no significant difference in peroxidase activity before and after irrigation, as depicted in Figure 10 (right).

Figure 10 (left) illustrates that the interaction between plant accession and sampling time significantly affected peroxidase enzyme activity. The German population consistently exhibited higher peroxidase activity than the Iranian population at both sampling times. However, the peroxidase activity after re-irrigation was significantly reduced in both accessions as compared to the conditions before re-irrigation. Also, the Iranian accession had a higher decrease in peroxidase enzyme activity compared to the German accession, with a reduction of 11.1 units for the Iranian and 4.1 units for the German accessions, respectively.

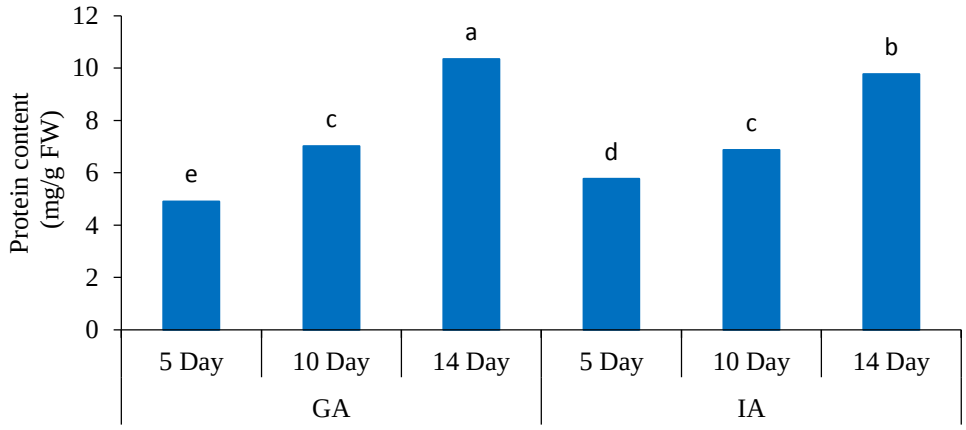

Figure 9. Effect of irrigation interval and accession on protein content of evening primrose; GA: German accession, IA: Iranian accession; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

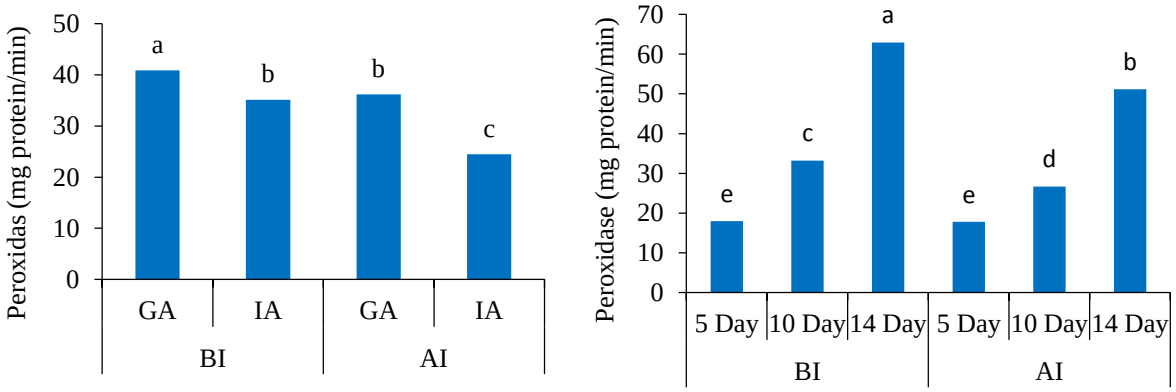

Figure 10. Effect of sampling time and accession, and also, sampling time and irrigation interval on peroxidase in evening primrose; BI: Before irrigation, AI: After irrigation, GA: German accession, IA: Iranian accession; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

Before irrigation, the PAL enzyme activity was 23 nmol/g FW/min. However, immediately following irrigation, the PAL activity experienced a reduction, dropping to 21 nmol/g FW/min, indicating a 9% decrease compared to the pre-recovery value (Figure 11-left).

The interaction between plant accession and irrigation interval on PAL activity is shown in Figure 11. A discernible trend becomes apparent in both accessions: as the irrigation interval extends, a notable increase in PAL activity was observed. When comparing the German and Iranian accessions, the Iranian accession showed a higher increase in PAL activity as the irrigation distance extended to 14 days. Conversely, when subjected to a 10-day interval, the PAL activity of the German accession exceeds that of the Iranian accession. No significant difference in PAL activity was observed between the two accessions under control conditions.

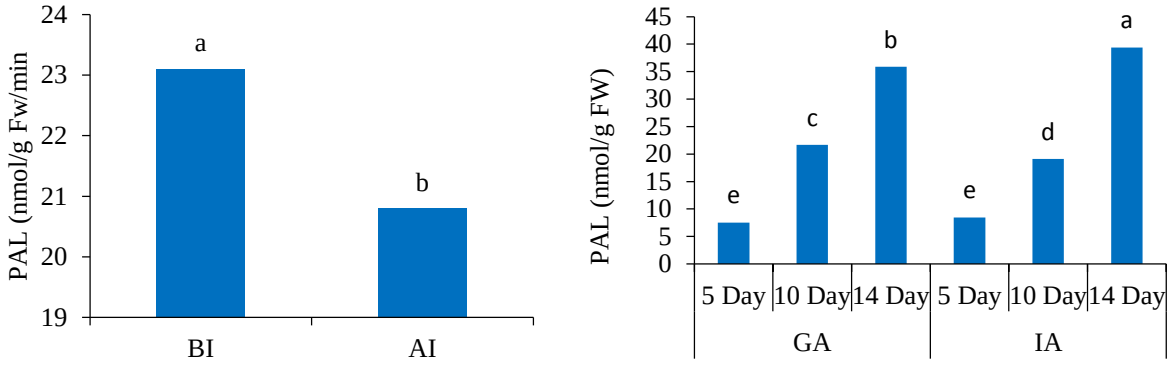

Figure 11. Effect of sampling time (left) and the interaction of accession by irrigation interval (right) on phenylalanine ammonia-lyase activity in evening primrose; PAL: Phenylalanine ammonia-lyase, GA: German accession, IA: Iranian accession, BI: Before irrigation, AI: After irrigation,; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

The outcomes illustrated in Figure 12 underscore the considerable impact of the interaction between plant accession and irrigation interval on the MDA content. A discernible trend was apparent in both accessions: as the irrigation interval extended, a notable increase in MDA was observed. The highest MDA content was observed under 14-day interval conditions in both accessions, followed by the 10-day interval. Both stress conditions showed significantly higher MDA content than the control in both accessions.

Figure 13 presents the sampling time × plant accession × irrigation interval interaction for the ascorbate peroxidase. In both accessions, a clear pattern emerged: as the irrigation interval increased, ascorbate peroxidase activity also rose. There was no significant difference in peroxidase activity between the two accessions before re-irrigation under the 14-day interval. However, the Iranian accession showed higher ascorbate peroxidase activity than the German accession before irrigation at the 10-day interval conditions, while this difference disappeared under control conditions with a 5-day interval. Moreover, after re-irrigation, an elevation in ascorbate peroxidase enzyme activity, compared to the control, was observed in the Iranian population subjected to the 14-day irrigation

interval, surpassing the German population. Similarly, in the context of the 10-day interval, the increase in ascorbate peroxidase activity of the Iranian accession, as compared to the control, was higher than the German accession.

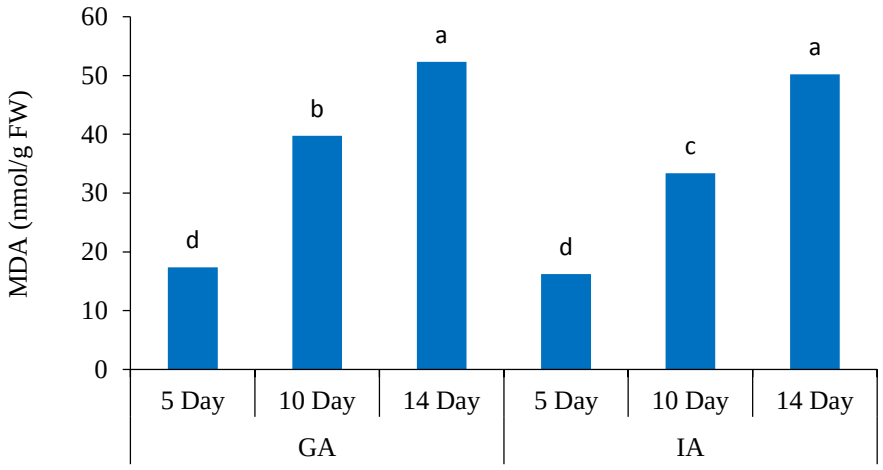

Figure 12. Effect of accession and irrigation interval on malondialdehyde content in evening primrose; MDA: Malondialdehyde, GA: German accession, IA: Iranian accession; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

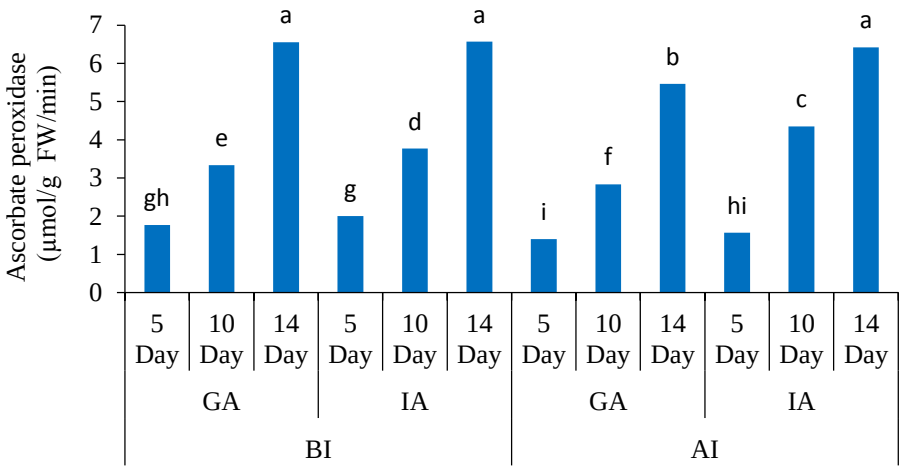

Figure 13. Effect of sampling time, accession, and irrigation interval on ascorbate peroxidase activity of evening primrose; BI: Before irrigation, AI: After irrigation, GA: German accession, IA: Iranian accession; Means with different letters are significantly different at $p \leq 0.05$, based on Duncan's multiple range test.

Discussion

In regions with decreasing rainfall and increased water deficit stress, adopting effective agronomic characteristics such as "recovery" becomes crucial. Recovery refers to a plant's ability to bounce back after a period of drought stress through proper irrigation. The success of recovery plays a pivotal role in determining the overall cultivation value of the plant. The findings of the present study indicate

that an irrigation interval of 18 days was excessively long for the evening primrose cultivation in Gorgan weather conditions. This extended interval doesn't provide the plant with sufficient time to recover post-irrigation due to the prevalent water stress. Re-irrigation (rewatering) acts as a mechanism to restore physiological functions that have been reduced by water stress (Ramírez et al. 2020). In other words, recovery is an important component for adaptation of plants to drought (Vanková *et al.* 2012). In this study, re-irrigation partially mitigated the effect of water-deficit stress on physiological and biochemical characteristics of evening primrose. The observed 1.3-fold reduction in antioxidant activity in recovered samples compared to pre-irrigation conditions indicates a reduction in stress effects and the plant's ability to restore and normalize its physiological conditions upon water absorption. However, the response mechanism of plants to drought and re-irrigation is still not fully understood (Xu *et al.* 2009). Bian and Jiang (2009) reported that the recovery process after re-irrigation cannot necessarily limit ROS production. In other words, plant recovery after re-watering can be affected by stress intensity, stress occurrence time, and genotype and environmental conditions (Palliotti *et al.* 2014). ROS level during the period of drought stress and recovery may be the potential for oxidative stress or signaling in the plant (Foyer and Noctor 2005).

In this research, prolonging the irrigation period decreased the leaf dry weight and increased the ion leakage and MDA in the leaves of the evening primrose. Drought increases the production of ROS and disrupts the physiological system (Bagci *et al*. 2007), including photosynthesis (Navarro *et al.* 2006; Liu *et al.* 2018), causing a reduction in growth characteristics such as leaf weight. Also, when plants are exposed to drought stress, increasing the concentration of ROS causes oxidative damage to cell metabolism (Bartels and Sunkar 2005), resulting in an increase in ion leakage. Considering that the membrane system is the first cell organ to be damaged by oxidative stress (Tina *et al.* 2017), the response of plants to stress has a direct relationship with the bio-membrane resistance. Furthermore, the superoxide radicals produced during the drought period cause lipid peroxidation (Tina *et al*, 2009). MDA is a by-product of lipid peroxidation, which is known as an indicator of cell membrane damage (Hussain *et al.* 2019). Therefore, the ability of plants to protect membrane integrity under drought stress determines plant tolerance to drought stress (Zahedi *et al*. 2020). The overall increase in the peroxidation of membrane lipids is proportional to the severity of drought stress and may result from the spontaneous reactions of ROS with organic molecules in the membrane (Gill and Tuteja 2010). However, it is possible that a decrease in membrane fluidity (Xu *et al.* 2009) or an increase in membrane leakage does not coincide with an increase in lipid peroxidation (Beck et al. 2007). Investigations showed that the amount of MDA production during drought stress is very different between different cultivars (Hosseini Boldaji *et al.* 2012). Heydari *et al.* (2019) reported that

the MDA level of two species of *Echinacea* was different under water deficit conditions. As an increase in MDA was observed in one species, in other species, the amount of MDA did not significantly differ. In another study Hosseini Boldaji *et al.* (2012) reported that the content of MDA increases in drought-sensitive cultivars more than tolerant cultivars.

The extension of the irrigation interval from 5 days to 10 and 14 days resulted in an increase in water-deficit stress in the evening primrose plants. In response to this heightened water-deficit stress, plants initiated various mechanisms to counteract the stress. In our study, total phenol content increased as the water-deficit stress increased in both German and Iranian accessions. Phenolic compounds assume a pivotal role as a vital component of the plant's defense system against environmental stresses. Under the influence of drought, the expression of many phenylpropanoid metabolism genes increases to accumulate the phenolic compounds in plants (Sharma *et al.* 2020). In other words, phenoxy radicals are less reactive than oxygen radicals (Abdallah *et al.* 2017).

In our research, the proline content increased as the water-deficit stress increased by prolonged irrigation in both German and Iranian accessions. Proline accumulation in many plant species is one of the adaptive metabolic responses to non-living stresses (Bandurska *et al.* 2017). The significant accumulation of proline after abiotic stresses can be attributed to its increased synthesis or decreased degradation (Kavi Kishor *et al.* 2005). Free proline acts as an osmotic protector, protein stabilizer, metal chelator, and $OH^{-\bullet}$ and 1O2 trap (Ashraf and Foolad 2007). Although high concentrations of proline are beneficial for plants under drought stress due to the role of this compound in regulating the osmotic potential (Wang *et al.* 2019), proline accumulation does not occur during stress in some species (Selmar and Kleinwächter 2013). In other words, the pattern of proline change depends on the plant genotypes and species (Dien *et al.* 2019). In potato, it was shown that genotypes sensitive to drought have more proline than resistant genotypes (Schafleitner *et al.* 2007). Meanwhile, in resistant genotypes of long fescue, proline was more than in the sensitive types (Man *et al.* 2011).

Protein content increased in both accessions of the evening primrose as water-deficit stress increased. Some researchers have also reported the protein accumulation under stress conditions (Ghorbanpour *et al.* 2020). The increase in protein can be caused by the increase in protein biosynthesis to adapt to new conditions and withstand stress. Such proteins, which are known as main proteins or stress-induced proteins, are synthesized to regulate the osmotic potential of the cells to maintain and regulate the physiological processes of the cell (Li *et al.* 2010). On the other hand, a decrease in protein synthesis and accumulation during drought stress has been reported (Li *et al*. 2010; Wu *et a*l. 2016). The reduction of protein content during stress conditions can also be caused by the

reduction of protein synthesis, the reduction of access to amino acids, and the change in the structure of enzymes during protein synthesis (Rollins *et al.* 2013).

In response to environmental stresses such as drought, plants perform special defense mechanisms that are associated with a series of morphological, physiological, biochemical, and molecular changes (Ghorbanpour *et al.* 2020). The antioxidant system of plants is one of the most important mechanisms of plants to deal with water-deficit conditions. In our research, POD, SOD, CAT, APX, PAL, and flavonoids increased significantly due water-defict stress. Studies have shown that the activity of antioxidant enzymes is related to the tolerance of plants to drought (Xu *et al.* 2010). Enzymatic compounds like CAT, SOD, and APX, together with non-enzymatic antioxidants such as flavonoids, contribute to ROS homeostasis and result in adaptation to stress conditions (Choudhary and Padda 2015). SOD is the first defense barrier of plants to eliminate free radicals (Gill and Tuteja 2010). Under stress conditions, in the cell, SOD decomposes the superoxide radical into hydrogen peroxide and oxygen (Abid *et al.* 2018). However, it should be mentioned that the activity of antioxidant enzymes depends on the plant species and intensity of drought stress (Gale *et al.* 2010), and it may increase (Abid *et al.* 2018), remain constant (Gunes *et al.* 2008), or even decrease (Tan *et al.* 2006).

Since the phenolic compounds can destroy free radicals due to their antioxidant properties, they protect the cell membrane from peroxidation and thereby protect plant cells from oxidative damage. PAL, one of the most important enzymes in the phenylpropanoid pathway, plays a role in the defense response of plant cells and is known as an indicator of environmental stress in different plant species (Khademi Astaneh *et al.* 2018). Therefore, this enzyme plays an important role in the growth and survival of plants (Yusuf *et al.* 2018).

Conclusion

Regardless of plant accessions, extending the irrigation interval (inducing drought stress) led to a decrease in the vegetative growth, and an increase in protein content, proline content, ion leakage, MDA, total phenols, total flavonoids, and CAT, POD, SOD, PAL, and APX enzymes activity in evening primrose. Physiological changes in response to drought and recovery conditions, particularly indicators of plant stress response like proline content, CAT, POD, SOD, PAL, and APX, exhibited significant differences influenced by sampling time. Furthermore, the activity of antioxidant enzymes showed significant differences under the influence of plant accession type under stress and recovery conditions. Although there was a decreasing trend in antioxidant capacity among recovered plants, the increase in antioxidant activity after recovery in the 14-day interval treatment highlights the strong

relationship between stress duration and recovery efficiency. The activity of PAL, a key enzyme in primary and secondary plant metabolism, demonstrated the plant's ability to recover within a 14-day irrigation period under Gorgan's climatic conditions. Nonetheless, considering the studied recovery indicators of evening primrose, it is suggested that the 10-day irrigation interval offers better recovery potential than the 14-day interval. This recommendation takes into account the plant's physiological responses and growth parameters, aiming to optimize recovery and minimize stress-induced effects on plant health and productivity.

Conflict of Interest

The authors have no relevant financial or non-financial interests with any individual or organization.

Ethics declaration

Not applicable.

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