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Effects of severe drought stress on the physiology and biochemistry characteristics of Welsh onion (*Allium fistulosum* L. var. *giganteum* Makino) seedlings

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Abstract: The physiological regulation of Welsh onion seedlings was explored under short- and long-term drought stress. The Welsh onion cultivar 'Neicong 3' was grown in pots under drought stress (22.5–25% soil water content) or controlled watering conditions (65–67.5% soil water content). Different lengths of drought stress (7, 14, 21, and 28 days) were studied to determine the effects of the drought on the levels of the osmoregulatory substances, including proline (Pro), free amino acids (FAAs), soluble sugar (SS), and soluble protein (SP); the membrane-damage indicator malondialdehyde (MDA); the endogenous hormones indoleacetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), and jasmonic acid (JA); and the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). The soil water content was restored to the control level after the drought stress to analyse the physiological responses of the Welsh onion with regards to rehydration. The results showed that the Welsh onion responded to drought stress primarily by increasing the levels of the osmoregulatory substances and the activities of the protective enzymes. The drought stress resulted in the accumulation of Pro, FAA, SS, MDA, and ABA in the pseudostems, while the IAA and GA levels decreased. Short-term drought stress led to a decrease in the SOD activity and an increase in the CAT activity, whereas the long-term drought stress led to a decrease in the POD activity. The effects of the drought stress on the SP and JA contents were negligible. The rehydration demonstrated that the short-term drought stress (7 or 14 days) did not exceed the tolerance of the Welsh onion; the substances regulating the drought tolerance were restored to normal levels after the stress conditions were relieved. In contrast, after the severe drought stress (28 days), the substances regulating the drought tolerance in the seedlings could not return to normal levels. This study provides a theoretical basis for the cultivation of the Welsh onion under early drought conditions and for breeding of drought-resistant varieties.

Keywords: Welsh onion; severe drought stress; rehydration; physiological characteristics

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The most important component of plant cells is water, on which plant growth and development heavily depend (Stojnic et al. 2018). When the precipitation levels and soil water content are too low, water transport and utilisation are affected in plants. Water loss in plants is mainly reduced through the accumulation of soluble sugars (SS) and proline (Pro); this reduces the protein degradation and protects the cell membrane under drought stress (Lum et al. 2014). By regulating the cellular osmotic potential, plants maintain expansion pressure, allowing cells to normally perform their physiological functions under drought stress (Chen, Jiang 2010). High levels of malondialdehyde (MDA) cause the denaturation of the antioxidant enzymes under drought stress; the MDA content is commonly used as an indicator for the degree of damage sustained by the plant membranes.

Drought stress can have many effects on the antioxidant enzyme systems and endogenous hormones in plants. Gill, Tuteja (2010) and Uzilday et al. (2012) found that plants produce excessive levels of reactive oxygen species (ROS) in response to drought stress, causing damage to cell membranes and resulting in protein denaturation and inactivation. Miller et al. (2010) showed that plants produce the antioxidants superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which can effectively scavenge ROS, especially by acting synergistically.

Endogenous hormones play an important role in a plant's defence against abiotic stresses. The amount of indoleacetic acid (IAA) present directly affects the plant stress tolerance (Park et al. 2007). Gibberellins (GAs) inhibit maturation and senescence (Liu et al. 2013). When a plant senses stress, the accumulation of abscisic acid (ABA) triggers the closure of the leaf stomata, reducing the water dispersal (Sally, William 2010). Increases in the ABA also lead to higher levels of H_2O_2, which induce the expression of antioxidant enzyme genes; this ultimately increases the antioxidant enzyme activity of the plant. Jasmonic acid (JA) is commonly studied as a plant damage signal that produces antagonistic effects in response to abiotic stresses and improves the drought resistance (Ju-Seok et al. 2011).

The Welsh onion (*Allium fistulosum* L. var. *giganteum* Makino) is mostly found in arid areas with little rainfall and serves as a staple seasoning vegetable. Current research on the Welsh onion primarily focuses on germplasm resources (Mi 2020), the nutrient uptake (Zhou 2019), and cultivation techniques (Liu 2019). There have been no published reports on the physiological response of the Welsh onion to drought stress and rehydration. Here, an experiment was designed using controlled conditions to simulate the extreme natural drought to which field-grown Welsh onions are often exposed as a result of water scarcity. The physiological and biochemical responses of the Welsh onion to drought stress and rehydration were uncovered and the associated regulatory mechanisms were studied.

MATERIAL AND METHODS

Plant material. The Welsh onion cultivar 'Neicong 3' was used in this study. 'Neicong 3' is widely grown in China and is a spring-sown variety developed through years of systematic selection and breeding by the Institute of Vegetable and Flower Research at the Inner Mongolia Academy of Agriculture and Animal Husbandry Sciences (Liang et al. 2015).

Experimental design. The plants were grown in pots in a rain-proof shelter at the Inner Mongolia Academy of Agriculture and Animal Husbandry Sciences (40.8°"N, 111.7°"E). This region is a middle temperate semi-arid continental monsoon climate zone. During the test period (28 March to 7 June 2018), the maximum and minimum temperatures were 31.4 °C and –9.2 °C, respectively, and the average temperature was 14.9 °C. The temperature data were provided by the Inner Mongolia Meteorological Bureau Hohhot Vegetable Weather Test Station. A specialised pot (17.3 cm high, 20.0 cm upper width, and 14.2 cm lower width) was filled with filtered sandy loam. Seeds were sown on 28 March, and conventional water management was carried out at the three-leaf stage. A portable soil moisture meter was used to measure the soil water content (SWC).

When the Welsh onion plants reached the three-leaf stage (on 1 May 2018), 96 pots containing similarly sized, healthy-looking Welsh onions were selected for testing. Two moisture levels were used, as described by Hsiao (1973): SWC of 65.0–67.5% (control) or 22.5–25.0% (drought-stressed). Thirty-two pots were randomly selected as the control group. The remaining 64 pots were randomly divided into eight drought treatment groups: 7, 14,

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21, and 28 days of drought stress (T1–T4, respectively), and 7, 14, 21, and 28 days of drought stress followed by three days of rehydration (T*n* + 3, respectively, where *n* = 1–4). The SWC of all the pots was initially at the control level before water was withheld from the eight treatment groups, reducing the SWC to the drought stress level by natural evaporation then maintaining it at that level by re-watering each day at 17:00. The SWC of the rehydrated pots was returned to 65.0–67.5%. The plants in the pots from the control group were sampled at the same time points as the drought-stressed and rehydrated pot plants.

Growth indices and biochemical measurements. For both the control and drought-stressed groups, the plants were sampled at 7 days (T1), 14 days (T2), 21 days (T3), and 28 days (T4) after the water was withheld (Table 1). The water replenishment was started at the end of day 7, 14, 21, and 28 of the drought stress to restore the SWC to 65.0–67.5%, and the plants from the control and treatment groups were sampled 3 days after rehydration (T*n* + 3).

Growth index measurements were taken on day 7, 14, 21, and 28 of the drought stress. Ten seedlings were randomly selected from the treatment and control groups, with the process repeated three times. Plant height: Distance from the base to the highest leaf point measured with a ruler. Pseudostem length: Distance from the base to the leaf exit measured with a ruler. Pseudostem thickness: Maximum diameter measured with callipers. Biomass: Determined by the oven-drying weighing method. Root-shoot ratio: ratio of the fresh weight of the underground to the aboveground parts.

Twenty Welsh onion seedlings of approximately the same size were randomly selected from each treatment group, and 0.5 g of pseudostem was weighed out. Each pot had 30 Welsh onion seedlings. After flash-freezing with liquid nitrogen, the samples were stored at –80 °C. The Pro content was determined by the acidic ninhydrin method. The free amino acid (FAA) content was determined by the ninhydrin colorimetric method. The SS content was determined by the sulfuric acid-anthrone colorimetric method. The SP content was determined by Coomassie brilliant blue staining. The MDA content was determined by the thiobarbituric acid method. The SOD, POD, and CAT activities were determined by the nitro blue tetrazoliume method, guaiacol method and ultraviolet colourimetry method, respectively, and the units of the SOD, POD and CAT activity were presented as units per gram of fresh weight (FW). The osmoregulatory substances (Pro, FAA, SS and SP), MDA, and the activities of the antioxidant enzymes (SOD, POD, and CAT) were tested using fresh samples as described by Gao (2006). The levels of the plant hormones IAA, ABA, GA, and JA were quantitatively analysed by an enzyme-linked immunosorbent assay (ELISA) (Wu 1988). The test kit was provided by Shanghai Research Biological Technology Co., Ltd.

Table 1. Drought-stressed treatment group dehydration and rehydration schemes

Sampling date (2018)	Time point	Treatment group
12/05	T1	drought
15/05	T1 + 3	drought + rehydration
19/05	T2	drought
22/05	T2 + 3	drought + rehydration
26/05	T3	drought
29/05	T3 + 3	drought + rehydration
02/06	T4	drought
05/06	T4 + 3	drought + rehydration

T1 – 7 days; T2 – 14 days; T3 – 21 days; T4 – 28 days

Statistical analysis. The data shown in the tables are the mean of three replicates. The statistical analysis was conducted with SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA). The significance of the differences between the mean values was assessed by a one-way analysis of variance (ANOVA) according to Dennett's test at a $P < 0.05$ level.

RESULTS

The trends of the various growth indices of the Welsh onion seedlings during the seeding stage are shown in Table 2. As shown in the table, the differences between the treatment and the control groups of the Welsh onion seedlings under drought stress for 7 and 14 days are relatively small. The gaps in the plant height, pseudostem length, and diameter between the treatment and control groups become larger as the continuous drought time increases. The plant height, pseudostem length, and diameter of the treatment group were significantly lower than those of the control group after 28 days of continuous drought, decreasing 28.96%, 40.94%, and 31.23%, respectively, indicating that long-

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Table 2. Effects of the drought stress on the plant height, pseudostem length, and diameter of the Welsh onion seedlings during the seeding stage

Time	Group	Plant height (mm)	Pseudostem length (mm)	Pseudostem thickness (mm)
T1	control group	86.64 ± 1.36[d]	6.39 ± 0.36[d]	2.39 ± 0.03[d]
	treatment group	87.39 ± 0.71[d]	6.20 ± 0.07[d]	2.41 ± 0.05[cd]
T2	control group	94.67 ± 0.86[c]	9.18 ± 0.31[bc]	2.77 ± 0.04[b]
	treatment group	92.24 ± 0.55[c]	8.96 ± 0.17[bc]	2.63 ± 0.09[bc]
T3	control group	103.41 ± 1.2[b]	10.12 ± 0.47[b]	2.83 ± 0.10[b]
	treatment group	93.52 ± 0.69[c]	8.55 ± 0.16[c]	2.47 ± 0.05[cd]
T4	control group	121.46 ± 2.31[a]	14.26 ± 0.75[a]	3.25 ± 0.12[a]
	treatment group	86.28 ± 0.88[d]	8.42 ± 0.18[c]	2.24 ± 0.05[e]

[a–d]Shown are the mean ± standard error, and the different lowercase letters in the same column indicate a statistically significant difference at a $P < 0.05$ level; T1 – 7 days; T2 – 14 days; T3 – 21 days; T4 – 28 days

term drought stress is not conducive to the growth of Welsh onion seedlings.

As can be seen from Table 3, continuous drought for 7 and 14 days had little effect on the accumulation of dry matter in the Welsh onion, and there was no significant difference in the various indicators between the treatment groups and the control group. The continuous drought for 21 days mainly affected the aboveground dry weight and root-shoot ratio of the Welsh onion, while, after 28 days of continuous drought, the aboveground dry weight, underground dry weight, and whole plant dry weight of the Welsh onion in the treatment group were significantly lower than those of the control group by 92.93%, 83.70%, and 90.99%, respectively, and the root-shoot ratio was significantly higher than that of the control group by 23.86%. This suggests that drought stress reduced the accumulation of the dry matter in the Welsh onion, with the aboveground part showing a more significant decrease.

Different lengths of drought stress and the subsequent three-day rehydration had varied effects on the osmoregulatory substances in the Welsh onion seedlings (Table 4). The short-term (14 days) drought stress resulted in significant increases in the Pro, FAA, SS, and MDA levels compared to the control group. Until the late stage (28 days) of drought stress, the SP content in the stressed group was significantly lower than in the control group. The rehydration after 7 or 14 days of drought did not significantly change the Pro, FAA, SS, or MDA levels compared to the control group. However, the levels of these substances were significantly higher following rehydration after 21 or 28 days of drought compared to the control group. In summary, the drought stress led to the accumulation of Pro, FAA, SS, and MDA in the pseudostem of the Welsh onion. The rehydration after the short-term drought could effectively relieve the drought stress; however, longer periods of drought stress

Table 3. Effects of the drought stress on the dry matter accumulation of the Welsh onion seedlings during the seeding stage

Time	Group	Shoot dry weight (mg)	Root dry weight (mg)	Whole plant dry weight (mg)	Fresh root-shoot ratio
T1	control group	4.67 ± 0.20[c]	1.40 ± 0.1[b]	6.07 ± 0.11[b]	0.20 ± 0.01[d]
	treatment group	4.62 ± 0.15[c]	1.37 ± 0.09[b]	5.98 ± 0.09[b]	0.21 ± 0.01[d]
T2	control group	5.65 ± 0.21[bc]	1.57 ± 0.06[b]	7.22 ± 0.27[b]	0.19 ± 0.01[d]
	treatment group	5.31 ± 0.17[bc]	1.39 ± 0.05[b]	6.69 ± 0.13[b]	0.20 ± 0.01[d]
T3	control group	8.90 ± 0.42[b]	2.49 ± 0.20 [b]	11.39 ± 0.22[b]	0.27 ± 0.02[cd]
	treatment group	5.31 ± 0.08[bc]	1.24 ± 0.10[b]	6.56 ± 0.13[b]	0.31 ± 0.02[bc]
T4	control group	63.65 ± 3.39[a]	16.99 ± 1.54[a]	80.65 ± 4.67[a]	0.37 ± 0.03[b]
	treatment group	4.50 ± 0.23[c]	2.77 ± 0.20[b]	7.27 ± 0.20[b]	0.46 ± 0.05[a]

[a–d]Shown are the mean ± standard error, and the different lowercase letters in the same column indicate a statistically significant difference at a $P < 0.05$ level; T1 – 7 days; T2 – 14 days; T3 – 21 days; T4 – 28 days

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Table 4. Changes in the osmoregulatory substances in the Welsh onion seedlings after drought stress and rehydration

Time	Group	Pro (ug/g)	FAA (mg/g)	SS (mg/g)	SP (mg/g)	MDA (nmol/g)
T1	control group	9.06 ± 2.50^{f}	0.59 ± 0.02^{j}	7.67 ± 0.60^{gh}	2.21 ± 0.08^{g}	1.34 ± 0.19^{de}
	treatment group	11.57 ± 1.15^{f}	0.63 ± 0.06^{ij}	8.93 ± 0.10^{ef}	2.29 ± 0.15^{g}	1.53 ± 0.05^{d}
T2	control group	18.28 ± 1.01^{e}	0.72 ± 0.02^{ghij}	7.40 ± 0.69^{h}	5.63 ± 0.35^{cedf}	1.38 ± 0.11^{de}
	treatment group	26.95 ± 2.34^{c}	1.35 ± 0.04^{d}	10.81 ± 0.25^{bc}	6.32 ± 0.17^{c}	3.36 ± 0.53^{c}
T3	control group	20.64 ± 0.88^{de}	0.72 ± 0.05^{ghij}	8.12 ± 0.74^{fgh}	5.53 ± 0.24^{def}	0.82 ± 0.08^{def}
	treatment group	27.98 ± 0.97^{c}	3.08 ± 0.010^{c}	11.52 ± 0.39^{ab}	6.18 ± 0.45^{cd}	6.78 ± 0.39^{a}
T4	control group	25.43 ± 1.79^{cd}	0.89 ± 0.06^{fghi}	8.74 ± 0.38^{efg}	7.52 ± 0.14^{ab}	0.47 ± 0.37^{ef}
	treatment group	42.93 ± 2.99^{b}	6.93 ± 0.07^{a}	12.37 ± 0.08^{a}	5.20 ± 0.21^{f}	6.73 ± 0.53^{a}
T1 + 3	control group	17.95 ± 0.96^{e}	0.60 ± 0.05^{j}	8.72 ± 0.44^{efg}	5.53 ± 0.16^{def}	1.30 ± 0.11^{edf}
	treatment group	20.50 ± 2.02^{de}	0.67 ± 0.05^{hij}	9.70 ± 0.28^{ced}	6.01 ± 0.10^{cde}	1.58 ± 0.07^{d}
T2 + 3	control group	19.57 ± 1.01^{e}	0.92 ± 0.03^{fghi}	8.88 ± 0.13^{efg}	5.28 ± 0.34^{ef}	0.89 ± 0.32^{def}
	treatment group	19.71 ± 0.79^{e}	1.21 ± 0.08^{de}	9.30 ± 0.39^{def}	7.22 ± 0.18^{ab}	1.23 ± 0.09^{def}
T3 + 3	control group	25.91 ± 1.93^{cd}	0.75 ± 0.03^{ghij}	9.44 ± 0.14^{de}	7.49 ± 0.16^{ab}	1.08 ± 0.17^{def}
	Treatment Group	28.56 ± 0.91^{c}	0.98 ± 0.24^{efg}	10.43 ± 0.28^{bcd}	7.96 ± 0.03^{a}	5.20 ± 0.41^{b}
T4 + 3	control group	39.99 ± 0.88^{b}	1.06 ± 0.01^{ef}	8.80 ± 0.13^{efg}	7.95 ± 0.05^{a}	0.30 ± 0.17^{f}
	Treatment Group	68.41 ± 3.48^{a}	5.22 ± 0.16^{b}	12.56 ± 0.05^{a}	7.19 ± 0.39^{b}	4.43 ± 0.54^{b}

[a–j]Shown are the mean ± standard error, and the different lowercase letters in the same column indicate a statistically significant difference at a $P < 0.05$ level; T1 — 7 days; T2 — 14 days; T3 — 21 days; T4 — 28 days

Pro – proline; FAA – free amino acid; SS – soluble sugar; SP – soluble protein; MDA – malondialdehydeoxidase

were associated with higher levels of cell membrane esterification, and the drought-induced stress could not be relieved even after the water supply was restored. In addition, the continuous drought stress affected the accumulation of the SP content in the pseudostems, but was not the main causative factor.

The different lengths of drought stress and rehydration also affected the antioxidant enzyme activity in the Welsh onion seedlings (Table 5). Compared to the control group, the SOD activity was significantly lower in the drought-stressed group at 7 days and higher at 14, 21, and 28 days, although these differences were not significant. The CAT activity was significantly higher in the drought-stressed group at 7 and 14 days, but there were no significant differences at 21 or 28 days. The POD activity was not significantly different between the groups at 7 or 14 days, but was significantly lower in the plants that were drought-stressed for 21 and 28 days. These results indicate that the short-term drought led to a decrease in the SOD activity and an increase in the CAT activity, whereas the long-term drought led to a decrease in the POD activity.

The SOD activity was not significantly different in the control group compared to the rehydrated groups after 7, 14, or 21 days of drought, but it was significantly higher in the control group compared to those that were drought-stressed for 28 days prior to rehydration. The CAT activity was significantly higher in the rehydrated group after 7 days of drought than in the control group, but there was no significant difference between the rehydrated and control groups after 14, 21, or 28 days of drought. The POD activity was not significantly different in the control group compared to those rehydrated after 7 or 14 days of drought, but was significantly lower in those rehydrated after 21 and 28 days of drought. This showed that the SOD activity could be effectively recovered after rehydration following the short-term drought, but the increase in the CAT activity was sustained. In addition, the decrease in the SOD activity upon rehydration after the prolonged drought indicated that the protective mechanism activated during the prolonged drought was deactivated upon rehydration, but that POD activity was not effectively restored.

The continuous drought stress resulted in a higher ABA content and a lower IAA, GA, and JA content in the drought-stressed groups than in the control group (Table 6). The IAA content was significantly lower after 14, 21, and 28 days of drought than in the control plants. After 28 days of drought, the ABA levels were significantly higher and the JA

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Table 5. Changes in the peroxidase activity in the Welsh onion seedlings after drought stress and rehydration

Time	Group	SOD (U/g FW)	CAT (U/g FW)	POD (μg/g FW)
T1	control group	396.62 ± 0.12[a]	88.44 ± 0.90[f]	3.92 ± 0.57[bcd]
	treatment group	394.01 ± 0.55[ab]	104.15 ± 0.07[ab]	2.80 ± 0.19[fg]
T2	control group	395.77 ± 0.32[a]	94.44 ± 0.93[def]	3.74 ± 0.26[bcd]
	treatment group	396.55 ± 0.63[a]	100.89 ± 1.11[abcd]	2.88 ± 0.20[efg]
T3	control group	391.83 ± 0.98[abc]	101.78 ± 0.80[abc]	4.56 ± 0.23[b]
	treatment group	396.55 ± 1.43[a]	104.00 ± 2.89[ab]	3.34 ± 0.23[def]
T4	control group	394.93 ± 2.35[a]	101.33 ± 0.38[abc]	4.41 ± 0.31[bc]
	treatment group	397.96 ± 0.83[a]	103.93 ± 0.97[ab]	2.90 ± 0.20[efg]
T1 + 3	control group	378.01 ± 0.95[e]	91.78 ± 3.85[ef]	3.67 ± 0.24[cde]
	treatment group	373.36 ± 3.48[e]	99.04 ± 1.96[abcd]	3.24 ± 0.10[defg]
T2 + 3	control group	385.13 ± 1.17[d]	100.67 ± 0.38[abcd]	3.41 ± 0.25[def]
	treatment group	391.83 ± 2.30[abc]	99.93 ± 0.45[abcd]	2.79 ± 0.14[fg]
T3 + 3	control group	388.37 ± 4.73[bcd]	98.30 ± 0.45[bcd]	3.94 ± 0.11[bcd]
	treatment group	395.70 ± 1.75[a]	96.81 ± 0.45[cde]	1.90 ± 0.13[h]
T4 + 3	control group	395.84 ± 1.68[a]	102.30 ± 4.08[abc]	5.70 ± 0.37[a]
	treatment group	387.88 ± 0.61[cd]	105.85 ± 4.19[a]	2.42 ± 0.17[gh]

[a–h]Shown are the mean ± standard error, and the different lowercase letters in the same column indicate a statistically significant difference at a $P < 0.05$ level; T1 — 7 days; T2 — 14 days; T3 — 21 days; T4 — 28 days
SOD – superoxide dismutase; CAT – catalase; POD – peroxidase

was significantly lower in the drought-stressed group than in the control group. Although the GA content of the drought-stressed group was lower than that of the control group, there was no significant difference. This indicated that the drought stress led to decreases in the IAA and GA, and that longer periods of drought stress were associated with greater decreases in the levels of these hormones; the opposite

Table 6. Changes in the endogenous hormone content in the Welsh onion seedlings after drought stress and rehydration

Time	Group	IAA (μg/g)	ABA (μg/g)	GA (μg/g)	JA (μg/g)
T1	control group	37.37 ± 1.40[abcd]	33.15 ± 1.84[c]	4.38 ± 0.34[ef]	14.07 ± 0.47[cdef]
	treatment group	41.97 ± 3.66[ab]	33.74 ± 1.47[bc]	4.26 ± 0.22[f]	14.60 ± 0.85[cde]
T2	control group	37.91 ± 1.99[abc]	35.56 ± 1.22[bc]	5.26 ± 0.16[abcd]	16.25 ± 0.43[bcd]
	treatment group	30.03 ± 0.75[de]	36.51 ± 3.80[bc]	5.03 ± 0.15[bcde]	16.48 ± 0.32[bc]
T3	control group	41.87 ± 3.27[ab]	34.16 ± 2.32[bc]	5.03 ± 0.23[bcde]	22.69 ± 0.48[a]
	treatment group	31.85 ± 1.02[cde]	39.50 ± 1.81[abc]	4.79 ± 0.08[cdef]	21.46 ± 1.63[a]
T4	control group	42.45 ± 3.39[a]	35.41 ± 1.23[bc]	5.72 ± 0.25[a]	14.12 ± 0.56[cedf]
	treatment group	29.36 ± 1.55[e]	42.73 ± 2.16[a]	4.89 ± 0.09[bcdef]	9.50 ± 0.86[g]
T1 + 3	control group	38.13 ± 1.27[abc]	35.36 ± 0.84[bc]	4.55 ± 0.16[ef]	14.53 ± 0.86[cde]
	treatment group	44.35 ± 1.34[a]	37.56 ± 1.36[abc]	4.72 ± 0.18[def]	14.77 ± 0.85[cde]
T2 + 3	control group	34.36 ± 3.85[bcde]	34.21 ± 2.93[bc]	5.80 ± 0.17[a]	18.33 ± 1.34[b]
	treatment group	37.43 ± 2.64[abcd]	35.84 ± 1.21[bc]	5.95 ± 0.10[a]	13.16 ± 0.68[ef]
T3 + 3	control group	41.94 ± 3.66[ab]	34.83 ± 1.52[bc]	5.57 ± 0.40[ab]	18.00 ± 0.96[b]
	treatment group	41.34 ± 1.99[ab]	39.79 ± 1.60[ab]	5.47 ± 0.15[abc]	13.65 ± 1.05[efg]
T4 + 3	control group	40.05 ± 1.34[ab]	35.09 ± 0.32[bc]	5.49 ± 0.26[ab]	12.30 ± 0.56[ef]
	treatment group	34.17 ± 1.35[bcde]	39.35 ± 1.40[abc]	4.98 ± 0.13[bcde]	11.55 ± 0.48[fg]

[a–h]Shown are the mean ± standard error, and the different lowercase letters in the same column indicate a statistically significant difference at a $P < 0.05$ level; T1 — 7 days; T2 — 14 days; T3 — 21 days; T4 — 28 days
IAA – indoleacetic acid; ABA – abscisic acid; GA – gibberellic acid; JA – jasmonic acid

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effect was seen with the ABA accumulation, where more severe drought stress was associated with higher ABA levels. Drought stress had a much smaller effect on the JA content in the pseudostem. The degree of the IAA content recovery in the Welsh onion after rehydration varied with the duration of the drought stress. After 7 days of drought, the IAA content in the rehydrated group was significantly lower than that of the control group, but there were no significant differences after 14 or 21 days of drought. After 28 days of drought, the IAA levels in the rehydrated group were significantly lower than in the control. The ABA content was significantly higher and the GA content was significantly lower in the rehydrated group compared to the control group after 28 days of drought. The JA content was significantly lower in the rehydrated group than in the control group after 14 or 21 days of drought, but there was no significant difference after rehydration following 28 days of drought. These results indicate that restoring the water supply following the short-term drought effectively relieved the stress, so the IAA and GA levels gradually increased in the pseudostems of the Welsh onion. The rehydration after the prolonged drought affected the IAA and GA accumulation, and the ABA content could not be rapidly reduced, resulting in the observed failure of these hormones to recover to the control group levels.

DISCUSSION

The Welsh onion is a biennial crop with a relatively long growth cycle; therefore, the robustness of its seedlings during the seeding stage directly affects the yield formation after transplanting. Studies have shown that a water deficiency in crops can lead to a reduction in the plant height and biomass, ultimately resulting in decreased yields. The results of this study show that the impact of drought stress on the plant height, pseudostem length, and diameter of Welsh onion seedlings is relatively small at 7 and 14 days. When the drought stress continues to intensify, the plant height, pseudostem length, and diameter are significantly reduced by 28.96%, 40.94%, and 31.23%, indicating that long-term drought stress is not conducive to the growth of Welsh onion seedlings. This experiment also found that the continuous drought stress significantly increased the root-shoot ratio, suggesting that the drought stress reduces the accumulation of dry matter in the Welsh onion, with a more significant decrease in the aboveground part. That is, the drought stress significantly inhibited the growth of the growing parts, and it is speculated that under drought stress, Welsh onion seedlings adopt a drought avoidance mechanism by reducing the synthesis of the aboveground biomass to decrease the water consumption and maintain growth.

Plants undergo many interrelated physiological and biochemical reactions under drought stress. Osmoregulation is the key process used to relieve stress and protect plants from drought damage in the early stages. Accumulation of Pro, a structural component of proteins, can regulate the cellular osmotic pressure (Zhang et al. 2017). An increase in the intracellular Pro content can effectively increase the water potential, aiding plants in reducing water loss. Studies have shown that Pro can be synthesised in large amounts under both short- and long-term stress (Bikash et al. 2004), and Pro levels are positively correlated with drought resistance. In this study, drought stress over 14, 21, and 28 days led to a significant increase in the Pro content. When water was reapplied after 14 days of drought, the Pro content in the seedlings gradually reversed to normal levels, and the osmoregulatory capacity within the Welsh onion pseudostems gradually decreased, indicating a relief of the drought stress. The seedlings were less able to recover to normal Pro levels when watering was resumed after 21 or 28 days of continuous drought, consistent with previous findings in soybeans (Wang et al. 2015). The Pro content correlated with the FAA (0.742**), SS (0.693**), SP (0.502**), MDA (0.464**), CAT (0.474**) and ABA (0.465**) (Table 7). It indicates that, in the present study, the Welsh onion seedlings under drought stress are responding to the drought stress by regulating the content of free proline in the plant body, thus maintaining the normal functioning of the plant. These results suggest that Welsh onion seedlings respond to drought stress by regulating the accumulation of Pro in the pseudostems to maintain growth.

Researchers have found that the FAA content in plants increases with the intensity of the drought stress and with the length of the stress conditions (Ashraf, Iram 2005). The same conclusion was reached in the present study, where the prolonged drought led to an increase in the stress intensity and a sharp increase in the FAA content in the Welsh onion pseudostems. This suggested that when

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Table 7. Correlation analysis of the physiological indices and endogenous hormones

	FAA	SS	SP	MDA	SOD	CAT	POD	IAA	ABA	GA	JA
Pro	0.742**	0.693**	0.502**	0.464**	0.082	0.474**	–0.151	–0.288	0.465**	0.094	–0.385
FAA	1	0.776**	0.085	0.741**	0.215	0.428**	–0.324	–0.533	0.572**	–0.107	–0.395
SS		1	0.269	0.784**	0.067	0.465**	–0.501	–0.424	0.525**	–0.048	–0.219
SP			1	0.079	–0.045	0.250	0.087	0.042	0.172	0.604**	–0.078
MDA				1	0.287*	0.317*	–0.547	–0.479	0.612**	–0.195	–0.108
SOD					1	0.123	0.054	–0.252	0.101	0.065	–0.075
CAT						1	–0.083	–0.099	0.186	0.104	0.024
POD							1	0.205	–0.407	0.151	0.214
IAA								1	–0.215	0.027	0.084
ABA									1	–0.055	–0.195
GA										1	0.066

**Indicates significant correlation at a 0.01 level; * indicates significant correlation at a 0.05 level

Pro – proline; FAA – free amino acid; SS – soluble sugar; SP – soluble protein; MDA – malondialdehyde; SOD – superoxide dismutase; CAT – catalase; POD – peroxidase; IAA – indoleacetic acid; ABA – abscisic acid; GA – gibberellic acid; JA – jasmonic acid

subjected to drought stress, Welsh onions ensure the continuation of normal growth by accumulating large amounts of FAA. In this study, the FAA content was higher in all the drought treatment groups than in the control group, except after 7 days of consecutive drought; at 7 days, moderate drought promotes the synthesis of Pro and other compounds from glutamate (α-pentanedioic acid), resulting in a lower FAA content; FAA changes occur *in vivo* only when stress aggravates a plant beyond its tolerance (Fricke, Pahlich 2010). Because plant responses to drought stress and rehydration are complex and variable, and there are as many as 18 FAA species in plants, only the total FAA content was measured in this study. Therefore, the changes in the FAA content after rehydration require further investigation in future studies to determine the detailed changes in the FAA species. It has previously been shown that the SS content increases in plants under stress conditions (Gideon et al. 2016), and that the SS content corresponds to the level of drought resistance in plants (Liu 2020). In the present experiment, the changes in the SS followed the same pattern as changes in the FAA, consistent with the findings of Liu et al. (2015).

Adverse conditions can regulate the SP content, help plants to reduce the water potential, and prevent cell dehydration (Cui et al. 2015). In the present study, the SP content was higher in the pseudostem of the Welsh onion exposed to drought stress for 7, 14, or 21 days compared to the control. It, thus, appears that the stress did not cause damage to the Welsh onion, but rather induced the production of proteins and enhanced the drought resistance. The SP content was significantly lower at 28 days of drought stress compared to the control, likely because the prolonged drought stress changed the ratio of anabolic to catabolic metabolism and inhibited the protective enzyme activity, thus reducing the protein production. When plants were rehydrated after 14 days of drought, the SP levels increased significantly, suggesting that a relatively limited drought exposure time can improve the drought resistance of the Welsh onion. The changes in the SP content induced by the drought stress and rehydration observed here were consistent with those found in previous studies (Luo et al. 2015). However, there were no significant changes in the SP content, indicating that the contribution of SP to the Welsh onion drought tolerance is low.

MDA is a membrane lipid peroxidation product produced by plants when they are subjected to abiotic stress. The presence of MDA causes a decrease in the cellular water potential, ultimately resulting in plant metabolic disorders (Zhao et al. 2021). In this study, the continuous drought led to an increase in the MDA content in the Welsh onion pseudostems; the MDA content was significantly higher at 14 days of continuous drought compared to the control group, but quickly returned to normal levels after rehydration. This indicated that the

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Welsh onion was sensitive to the water deficit, but that drought stress did not cause irreversible damage over the short term. However, the MDA content did not decrease when the water supply was restored after 21 or 28 days of continuous drought, meaning that the Welsh onion could not recover from the prolonged drought.

When plants experience drought, they reduce the damage caused by free radicals through protective enzyme systems (Zhao et al. 2016; Wang et al. 2019). In this study, it was found that the short-term drought led to a decrease in the SOD activity and an increase in the CAT activity. Plants usually increase the POD activity to resist drought stress (Diao et al. 2014; Zhang et al. 2014). However, it was found that prolonged drought led to a decrease in the POD activity in this study. This is because POD is not only an important enzyme in the plant ROS scavenging system, but is also a key enzyme in catalysing the lignin biosynthesis enzyme. Because the Welsh onion is a vegetable rich in cellulose and lignin, it is presumed that POD functions mainly as a lignin biosynthetic enzyme, diminishing its function as a protective enzyme.

The decrease in the SOD activity upon rehydration following the short-term drought indicates that normal levels could effectively be recovered, whereas the sustained increase in the CAT activity indicates a longer enzymatic activity duration. This increase in the antioxidant enzyme activity exhibited after rehydration indicates that a certain degree of drought is beneficial to exploit the inherent drought resistance of plants. This coincides with the biological water conservation principle of Shan et al. (2006): a water deficit is not always completely harmful to plants, but rather there is a range from beneficial drought adaptation to harm; rehydration after moderate water deficit has the potential to promote plant growth. Here, after the long-term drought, the SOD activity decreased after rehydration, indicating that the protective mechanism activated during the long-term drought was relieved after rehydration, but the POD activity was not effectively restored. The CAT content correlated with the Pro (0.474**), FAA (0.428**), SS (0.465**), and MDA (0.317*) content (Table 7), which indicates that Welsh onion seedlings can adapt to drought adversity and maintain their normal physiological functions by regulating their own antioxidant enzyme activities. Therefore, the Welsh onion has some drought resistance, and a sustained drought promotes the antioxidant enzyme activity, enhancing its ability to scavenge peroxides and reduce the production of toxic substances.

Plant hormones are co-regulated to allow individuals to adapt to their growth needs. The endogenous plant hormone IAA promotes plant growth. GA promotes differentiation, growth, and development of plant leaves. Plants can reduce the damage caused by stress conditions through the accumulation of ABA (known as the stress hormone) to inhibit growth. JA increases drought resistance by regulating the stomatal closure and inducing the expression of specific genes (Sang-Youl et al. 2015). Plants possess the ability to regulate their metabolism and reduce unwanted anabolism under stress conditions (Li et al. 2010). The present study showed that the continuous drought stress led to decreases in the IAA, GA, and JA levels and an increase in the ABA content in the Welsh onion. The ABA content correlated with Pro (0.465**), FAA (0.572**), SS (0.525**) and MDA (0.612**) content and the GA content correlated with the SP (0.604**) content (Table 7), indicating that each endogenous hormone and other osmoregulatory substances in the Welsh onion exercised their functions together under drought stress. This indicates that, under drought stress conditions, Welsh onions can flexibly adjust the endogenous hormone levels to reduce any unnecessary metabolic consumption and cope with the impact of adversity. Rehydration returned the IAA, GA, and ABA to levels that were comparable to the control group after 7–21 days of drought, but not after 28 days; this demonstrated a time limit to the Welsh onion drought tolerance. In addition, the JA content was not significantly different from the control after 14 or 21 days of drought, but was significantly reduced after rehydration. We speculate that the JA content fluctuates very little during the drought stress, due to the generally high JA content. However, this self-defence mechanism was reversed after rehydration, resulting in a significant decrease in the JA content. Thus, a small degree of drought stress may be beneficial in further developing the drought resistance potential of Welsh onions.

In conclusion, drought stress inhibited the increase in the Welsh onion plant height, pseudostem length and transverse diameter, decreased the dry matter accumulation, reduced the SOD and POD activities, and decreased the IAA and GA contents. The accumulation of Pro, FAA, SS, MDA and ABA

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was increased and the CAT activity was increased. The normal level could be restored by rehydrating the seedlings after the short-term drought stress (7 days or 14 days).

CONCLUSION

Severe drought stress (28 days) could not be repaired by water rehydration. This study provides a theoretical basis for an in-depth study of stress resistance in the Welsh onion.

REFERENCES

Ashraf M., Iram A. (2005): Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. Flora, 200: 535–546.

Bikash C.S., Hara M., Uemura M. (2004): Proline synthesis, physiological responses and biomass yield of eggplants during and after repetitive soil moisture stress. Scientia Horticulturae, 103: 387–402.

Chen H., Jiang J. (2010): Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. Environmental Reviews, 18: 309–319.

Cui L.M., Wang X.X., Xuan H.D., Song X.Y., Pei Y.H. (2015): Changes in several physiological and biochemical indices of maize seedling roots caused by drought stress. Plant Diseases and Pests, 6: 35–37, 43.

Diao J.M., Zeng X.L., Chen G.Z. (2014): Effects of drought stress on the growth and physiological characteristics of *Aegiceras corniculatum*. Forest Research, 27: 423–428.

Fricke W., Pahlich E. (2010): The effect of water stress on the vacuole-extravacuole compartmentation of proline in potato cell suspension cultures. Physiologia Plantarum, 78: 374–378.

Gao J.F. (2006): Plant Physiology Experiment Guidance. Beijing, Higher Education Press, 105–108.

Gideon O.O., Richard O.A., Adekunle A.A. (2016): Proline and soluble sugars accumulation in three pepper species (Capsicum spp) in response to water stress imposed at different stages of growth. Sciences in Cold and Arid Regions, 8: 205–211.

Gill S.S., Tuteja N. (2010): Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 48: 909–930.

Hsiao T.C. (1973): Plant response to water stress.Annual Review Plant Physiology, 24: 519–570

Ju-Seok S., Joungsu J., Min-Jeong K., Yeon-Ki K., Baek H.N., Sang I.S., Jong-Joo C., Jong S. L., Ju-Kon K., Yang D.C. (2011): OsbHLH148, a basic helix–loop–helix protein, interacts with OsJAZ proteins in a jasmonate signaling pathway leading to drought tolerance in rice. Plant Journal for Cell & Molecular Biology, 65: 907–921.

Li D.X., Li C.D., Sun C.F., Sun H.C., Liu L.T., Zhang Y.J., Xiao K. (2010): The effects of drought on endogenous hormone contents and balance in main stem leaves of cotton. Cotton Science, 22: 231–235.

Liang Y.R., Zhang Y.L., Cao J.L., An Z.D., Shang H.J., Zhang Y.C. (2015): Inner Mongolia agricultural acience and technology. Inner Mongolia Agricultural Science and Technology 5: 92–93.

Liu Y. (2019): Effect of irrigation and fertilization proportion on yield, quality and water-fertilizer use efficiency of Welsh Onion. [Masters Thesis.] Taian: Shandong Agricultural University.

Liu A.R., Zhang Y.B., Zhang X.P., Wang X.J., Huang S.C., Xie Y.Q. (2015): Physiological responses of *Coleus blumei* to simulated drought stress. Guihaia, 35: 656–662.

Liu K Q. (2020): Effects of water stress on growth and yield components of Oat. [Masters Thesis.] Xining: Qinghai University.

Liu T., Zhu S., Fu L., Yu Y., Tang Q., Tang S. (2013): Morphological and physiological changes of ramie (Boehmeria nivea L. Gaud) in response to drought stress and GA (3) treatment. Russian Journal of Plant Physiology, 60: 749–755.

Lum M.S., Hanafi M.M., Rafii Y.M. (2014): Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. Japs Journal of Animal & Plant Sciences, 24: 1487–1493.

Luo J., Zhou G.L., Hu T.X., Hu H.L., Chen H., Wang Q., Hu Y., Wang B. (2015): Effects of drought stress on growth and physiological parameters of Machilus pingii seedlings. Chinese Journal of Applied & Environmental Biology, 21: 63–570.

Mi Y.H. (2020): Studies on the characteristics and classification of several Allium landraces. [Masters Thesis.] Hangzhou: Zhejiang University.

Miller G., Suzuki N., Ciftci-Yilmaz S., Ciftci-yilmaz, S. Mittler R. (2010): Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell & Environment, 33: 453–467.

Park J.E., Park J.Y., Kim Y.S., Stasvick P.E., Jeon J., Kim S.Y., Kim J., Lee Y.H., Park C.M. (2007): GH3–mediated auxin homeostasis links growth regulation with stress adaptation response in Arabidopsis. The Journal of Biological Chemistry, 282: 10036–10046.

Sally W., William J.D. (2010): Drought, ozone, ABA and ethylene: new insights from cell to plant to community. Plant, Cell & Environment, 33: 510–525.

Sang-Youl P., Francis C.P., Assaf M., Jin Y., Brian F.V., Sean R.C. (2015): Agrochemical control of plant water use using engineered abscisic acid receptors. Nature, 520: 545–548.

https://doi.org/10.17221/58/2022-HORTSCI

Shan L., Deng X.P., Zhang S.Q. (2006): Advances in biological water–saving research: challenge and perspectives. Science Foundation in China, 20: 66–71.

Stojnic S., Suchocka M., Benito-Garzon M., Torres-Ruiz J.M., Cochard H., Bolte A., Cocozza C., Cvjetkovic B., Luis M., Martinez-Vilalta J., Ræbild A., Tognetti R., Delzon S. (2018): Variation in xylem vulnerability to embolism in European beech from geographically marginal populations. Tree Physiology, 38: 173–185.

Sun G.R., Zhang R., Jiang L.F., Yan X.F. (2001): Water metabolism and changes of several osmotica in leaves of birch *Betula platyphylla* seedlings under drought. Bulletion of Botanical Research, 21: 413–415.

Uzilday B., Turkan I., Sekmen A.H., Ozgur R., Karakaya H.C. (2012): Comparison of ROS formation and antioxidant enzymes in cleome gynandra (C4) and cleome spinosa (C3) under drought stress. Plant Science: An International Journal of Experimental Plant Biology, 182: 59–70.

Wang F.X., Xiao K.Z., Jiang S.F., Qu M.Y., Lian L., He W., Chen L.P., Xie H.A., Zhang J.F. (2019): Mechanisms of reactive oxygen species in plants under drought stress. Chinese Science Bulletin, 64: 1765–1779.

Wang L.B., Zu W., Dong S.K., Liu L.J., Xu Y.H., Li X.N. (2015): Effects of drought stresses and times on compensation effect after re-watering in soybean. Transactions of the Chinese Society of Agricultural Engineering, 11: 150–156.

Wu S.R., Chen W.F., Zhou X. (1988): Enzyme Linked immunosorbent assay for endogenous plant hormones. Plant Physiology Conmunication, 5: 53–57.

Zhang M., Wang L.F., Zhang K., Liu F.Z., Wan Y.S. (2017): Drought-induced responses of organic osmolytes and proline metabolism during pre-flowering stage in leaves of peanut (*Arachis hypogaea* L.). Journal of Integrative Agriculture, 16: 2197–2205.

Zhang Y.Y., Liu Y., Bai L. (2014): Effect of drought stress on physiological indexes of 4 Crassulaceae species. Pratacultural Science, 31: 724–731.

Zhao C.Y., Si J.H., Feng Q., Yu T.F., Luo H., Qin J. (2021): Ecophysiological responses to drought stress in *Populus euphratica*. Sciences in Cold and Arid Regions, 13: 326–336.

Zhou L. (2019): Physiological mechanism of nitrogen nutrition on quality formation of Welsh Onion. [Masters Thesis.] Jinan: Qilu University of Technology.

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