

Research Article

# Changes in Antioxidant Enzyme Activities of Two Contrasting Ecotypes of *Arundinella hirta* to Drought Stress

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## ABSTRACT

To understand antioxidant enzyme response of two contrasting *Arundinella hirta* ecotypes to drought stress, drought-tolerant Youngduk and drought-sensitive Jinju-1, were comparatively analyzed changes in the enzymatic activities of peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR). Two ecotypes, drought-tolerant Youngduk and drought-sensitive Jinju-1 were subjected to drought stress by withholding water for 12 days. ROS accumulation level and electrolytic leakage were significantly increased in both *A. hirta* ecotypes by drought stress treatment but less in Youngduk than Jinju-1. The RWC significantly decreased in both the drought stress-treated ecotypes as compared to control, but less in Youngduk than Jinju-1. Soluble sugar and protein content were increased more in drought stress-treated Youngduk as compared to Jinju-1. The activities of antioxidant enzymes such as SOD, CAT, POD, APX, and GR increased significantly in both the drought stress-treated ecotypes Youngduk and Jinju-1 as compared to control. The increase in antioxidant enzyme activity level was more prominent in drought stress-treated Youngduk as compared to Jinju-1. Taken together, these results suggest that Youngduk was more tolerant to drought stress than Jinju-1, and seem to indicate that tolerance of *A. hirta* to drought stress is associated with increased activity of antioxidant enzymes.

**(Key words:** Antioxidant enzymes, *Arundinella hirta*, Drought stress)

## I. INTRODUCTION

Drought is one of the harmful environmental stress features limiting plant growth and development and has been considered a major cause to decrease crop yield and quality (Chakhchar et al., 2016). Reactive oxygen species (ROS) have a key role in changing the acclimation of plants to drought stress (Choudhury et al., 2017). Antioxidant enzymes, such as SOD in coordination with CAT and APX play a critical defensive part in the ROS-detoxifying process (Howarth, 2005). As long as SOD alters superoxide radicals to H<sub>2</sub>O<sub>2</sub>, CAT and POD in the different cellular parts or APX in ascorbate-glutathione cycle reduces them to oxygen (Howarth, 2005). Furthermore, keeping a higher ratio of oxidized to reduced glutathione and ascorbate is vital to inhibit the oxidation of cellular compounds (Gruszka et al., 2017). There is increasing research representing that a well-synchronized antioxidant defense system can recover

the ability to cleanse unnecessary ROS, which donates to shielding the cell against oxidative damage and increasing the drought tolerance of plants (Türkan et al., 2005).

*Arundinella hirta* (*A. hirta*) is a perennial grass species of the Poaceae. It is a rhizomatous grass with tiller nodes that can propagate by both sexual and asexual reproduction. It is found in alpine grasslands of Asian countries, Korea, Japan, India, China and Taiwan (Umberto, 2006). In Korea, it is distributed as a dominant species in natural grasslands and has been used as a forage due to its high palatability to livestock, high feed value and productivity (Yun, 1968). So far, there have been studies on growth characteristic (Li et al., 2015), photosynthetic reaction (Liu et al., 2016), and feed value evaluations (Yun, 1968) in *A. hirta*, but systematic studies on the response of antioxidant enzymes to drought stress have not been reported yet.

Previously we analyzed the physiological and biochemical responses of 5 local ecotypes of *A. hirta* against drought stress

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and concluded that they have different levels of drought stress tolerance (Khan et al., 2019). In the present study, the effects of drought stress on ROS production, possible protection by osmoregulation and the enzymatic antioxidant system were investigated in the drought-tolerant Youngduk and sensitive Jinju-1 ecotypes. The purpose of the study was to understand how ROS production by drought stress is regulated by antioxidant enzymes in *A. hirta*.

In this study, in order to investigate the effects of drought stress on ROS production, possible protection by osmoregulation, and the response of enzymatic antioxidant systems to drought stress in *A. hirta*, two representative contrasting ecotypes, drought tolerant- and sensitive ecotypes which were selected through previous studies, were subjected to drought stress and their responses were compared.

## II. MATERIALS AND METHODS

### 1. Plant materials and growth

Two ecotypes of *A. hirta*, Youngduk (collected from Youngduk region, 36°19'N, 129°16'E) and Jinju-1 (collected from Jinju region, 35°09'N, 128°05'E) were used. The seeds were sown in plastic pots (20 cm × 20 cm × 15 cm) filled with sandy loam soil. Plants were grown in the growth room at 25°C with a relative humidity of 65%, a 16 h photoperiod, and photosynthetically active radiations of about 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and regularly watered with nutrient solution.

### 2. Drought stress treatment

After two months of growth, *A. hirta* plants of growth stage with 5 to 6 leaves were used. In group one, three replicates of the plants were exposed to drought stress for 12 days by withdrawing irrigation. In the second group, three replicates of the plants were watered regularly with an equal amount of water (500 mL / pot / 2 days). On the 12th day, the second and third leaves from the bottom were collected randomly from both the groups and then frozen immediately in liquid nitrogen and were stored at -70°C for future analysis.

### 3. Measurement of $\text{O}_2^-$ and $\text{OH}\cdot$

In collected triplicate samples the rate of superoxide ions ( $\text{O}_2^-$ ) production was measured by the method described by Zhao et al. (2008) and absorbance was measured at 532 nm. The hydroxyl radicals ( $\text{OH}\cdot$ ) production was measured by previous methods (Halliwell et al., 1988).

### 4. Measurement of electrolytic leakage

Electrolyte leakage was measured according to the method described by Chen et al. (2018) with minor modifications. Briefly, fresh leaves from triplicate were washed with deionized water to take away the outward electrolytes and were placed in 10 mL of deionized water for 2 h at room temperature. The initial conductivity ( $C_1$ ) was measured after 2 h by a conductivity meter (Thermo Orion, USA). Samples were then boiled for 20 min and kept for 1 h at 25°C. After 1 h, the final conductivity ( $C_2$ ) was measured by a conductivity meter. The following equation was used for calculating the ion leakage. Relative electrolytic leakage (%) =  $C_1/C_2 \times 100$ .

### 5. Measurement of relative water content (RWC), soluble protein and soluble sugar

Two leaves from each control and drought stress-treated groups were detached and their fresh weight (FW) was measured. The leaves were then put into test tubes and were saturated in deionized water. After 24 h the turgid weight (TW) was measured after blotting dry the leaves. Now to measure the dry weight of the leaves they were kept for 72 h at 70°C in a dry oven. The RWC was determined by using the Smart equation (Smart, 1974).  $\text{RWC (\%)} = (\text{FW}-\text{DW}) / (\text{TW}-\text{DW}) \times 100$ . The determination of soluble protein was performed using bovine serum albumin (BSA) as mentioned by Bradford (1976). The soluble sugar content was determined by the method described by Irigoyen et al. (1992).

### 6. Measurement of antioxidant enzymes activities

For antioxidant enzyme activity analysis leaf samples (0.1 g) were powdered. The activity of SOD was determined at 540 nm using the method of Beauchamp and Fridovich (1971). In brief, 0.1 mM NBT (Sigma-Aldrich), 10  $\mu\text{M}$  riboflavin (Sigma-Aldrich), and 0.1 mM methionine (Sigma-Aldrich) were

added to 10  $\mu\text{L}$  extract of the enzyme. One unit of SOD was the amount of NBT inhibiting 50% of enzyme activity. The activity of POD was determined at 420 nm following the method of Kwak et al. (1995). Briefly, 0.1 M potassium phosphate buffer (pH 6.0), 5% (w/v) pyrogallol (99%, Sigma-Aldrich), 0.147 mM  $\text{H}_2\text{O}_2$  (30%, Daejung), and 20  $\mu\text{L}$  sample were homogenized and the absorbance was recorded for 20 sec. The CAT activity was calculated by the Aebi (Aebi, 1984) method. The 50 mM potassium phosphate buffer (pH 7.0), 150  $\mu\text{L}$   $\text{H}_2\text{O}_2$  (30%, Daejung), in 25 mL 50 mM potassium phosphate buffer, and 100  $\mu\text{L}$  enzyme extract were mixed and the absorbance was determined at 240 nm for 60 sec. The GR activity was determined by Smith et al. (1988) method. Briefly, 50 mM potassium phosphate buffer (pH 7.8), 0.75 mM DTNB (Sigma-Aldrich), 0.1 mM freshly prepared NADPH (Sigma-Aldrich), 1 mM GSSG (Sigma-Aldrich), were mixed in 10  $\mu\text{L}$  enzyme extract and absorbance was calculated at 412 nm for 180 sec. The activity of APX was measured by the method of Nakano and Asada (1981) with minor modification. Simply, the reaction mixture 50 mM HEPES (Sigma-Aldrich), and 0.1 mM EDTA (Sigma-Aldrich), extraction buffer, 0.6 mM ascorbate, 1 mM  $\text{H}_2\text{O}_2$  (30%, Daejung), was put into 20  $\mu\text{L}$  enzyme extract and the absorbance was assayed at 290 nm for 30 sec.

## 7. Statistical analysis

All data were presented as means  $\pm$  SD for three replicates. The data among two *A. hirta* ecotypes were analyzed by two-way (ANOVA). When interactions were found, differences among means were analyzed by a Tukey post-hoc test considered significant at  $p < 0.05$ . All the analysis were conducted using SPSS, version 25.0 (SPSS Inc., Chicago, IL).

## III. RESULTS

### 1. Alterations in ROS and electrolytic leakage

According to our previous physiological and biochemical study (Khan et al., 2019), the ecotype Youngduk was highly tolerant and the others were sensitive to drought stress. In this study, therefore, the Youngduk was chosen for the tolerant ecotype, and the Jinju-1, which shows the most similar growth

characteristics among sensitive ecotypes with the Youngduk, was chosen for the sensitive ecotype. Dehydration usually causes oxidative stress through an increase in excess ROS. Therefore, in this study, we confirmed the changes in the major ROS such as superoxide anionic radicals ( $\text{O}_2^-$ ) and hydroxyl radicals ( $\text{OH}\cdot$ ) in the two contrasting ecotypes of *A. hirta* during drought stress. Drought stress treatment increased  $\text{O}_2^-$  content. The amount of  $\text{O}_2^-$  increased in both of *A. hirta* ecotypes. The less increase in  $\text{O}_2^-$  accumulation occurred in

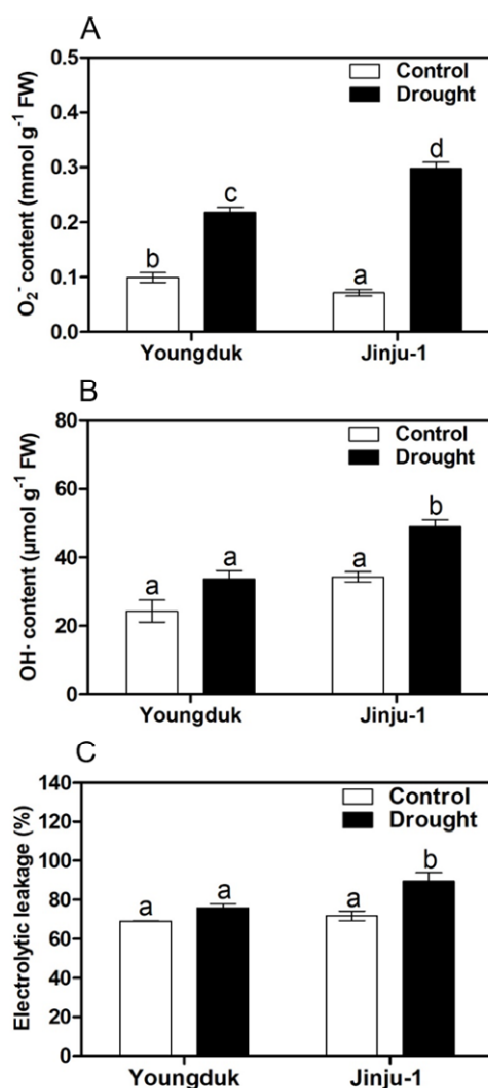


Fig. 1. Changes in superoxide ions ( $\text{O}_2^-$ ) (A), hydroxyl radicals ( $\text{OH}\cdot$ ) (B), and electrolytic leakage (C) of two different ecotypes of *A. hirta* under drought-stressed and unstressed (control) conditions. Different letters above bar graphs indicate a significant difference between control and drought-stressed ecotypes.

Youngduk and the more increase was observed in Jinju-1. The highest increase of 4.17 fold of  $O_2^-$  accumulation by drought stress treatment was observed in Jinju-1 as compared to control, while increase of  $O_2^-$  was observed in Youngduk with 2.2 fold as compared to control (Fig. 1A). The  $OH\cdot$  content was also shown a significant increase in both the drought stress-treated ecotypes. The increase in  $OH\cdot$  level in Jinju-1 was 1.43 fold more in the drought stress-treated as compared to control, while 1.38 fold was increased in Youngduk than control (Fig. 1B). Additionally, the electrolytic leakage was increased in drought stress-treated Youngduk with 1.1 fold and in Jinju-1 with 1.25 fold as compared to control, respectively (Fig. 1B).

## 2. Alterations in RWC, soluble sugar and protein content

During drought stress, plants generally respond through a relative water contents (RWC) and osmoprotectants. Therefore, in this study, we confirmed the levels in the RWC and osmoprotectants in the two ecotypes of *A. hirta* during drought stress. The drought stress decreased the RWC (Fig. 2). The decrease in RWC of Youngduk was 0.75 fold less in drought stress-treated as compared to control, while in Jinju-1 was 0.59 fold less in drought stress-treated as compared to control.

Soluble sugar content was also increased in drought-stress treated *A. hirta*. The higher increase of 1.7 fold was observed in drought stress-treated Youngduk as compared to the control, and the less increase of 1.39 was in Jinju-1 as compared to the

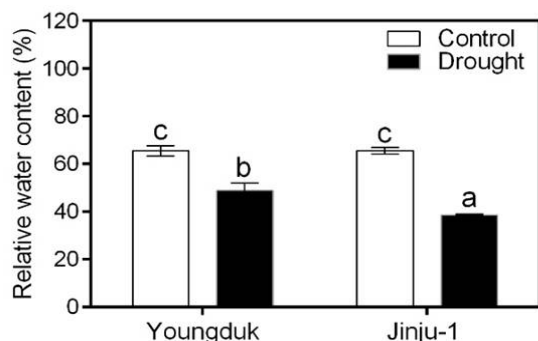


Fig. 2. Changes in relative water content of two different ecotypes of *A. hirta* under drought-stressed and unstressed (control) conditions. Different letters above bar graphs indicate a significant difference between control and drought-stressed ecotypes.

control (Fig. 3A). The protein content was increased higher in the drought stress-treated ecotypes as compared to control. In Youngduk the increase in drought stress-treated was 3.88 fold more as compared to control and in Jinju-1, it was 2.99 fold higher in drought stress-treated as compared to control (Fig. 3B).

## 3. Alterations in antioxidant enzymes activities

Many studies have reported the activities of antioxidant enzymes in plants during drought stress. Therefore, in this study, changes in the activities of SOD, APX, CAT, POD, and GR, which are representative plant antioxidant enzymes, were confirmed in the two ecotypes of *A. hirta* during drought stress. The activities of SOD increased in both the drought stress-treated ecotypes of *A. hirta* compare with control conditions by 1.63 fold in Youngduk and 1.35 fold in Jinju-1, respectively (Fig. 4A). The CAT enzyme activity level, also

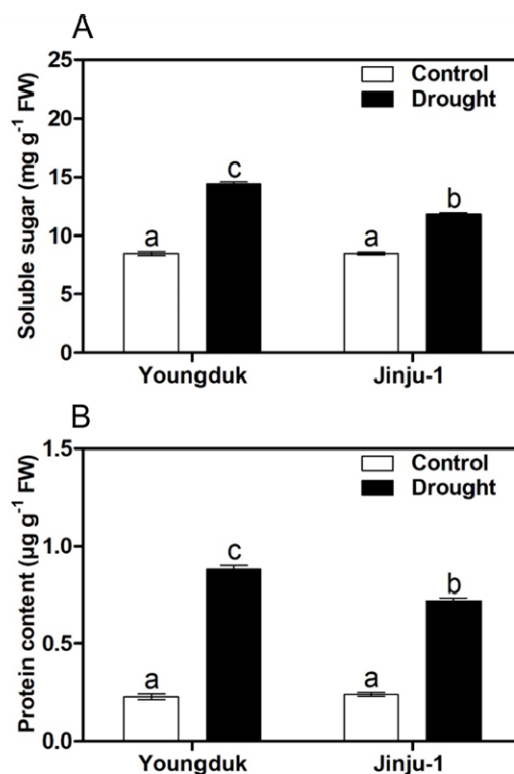


Fig. 3. Changes in soluble sugar content (A), and protein content (B) of two different ecotypes of *A. hirta* under drought-stressed and unstressed (control) conditions. Different letters above bar graphs indicate a significant difference between control and drought-stressed ecotypes.

significantly increased in Youngduk and Jinju-1 of the drought stress-treated ecotypes as compared to the control by 2.76 and 1.7 fold, respectively (Fig. 4B). The POD activity was also shown an increase in the drought stress-treated ecotypes as compared to control by 2.44 and 2.47 fold, respectively (Fig. 4C). The activities of the APX was also increased in Youngduk and Jinju-1 in the drought stress-treated ecotypes as compared to the control by 2.92 fold and 2.3 fold, respectively (Fig. 4D). The GR activity was also shown an increasing trend in the drought stress-treated ecotypes as compared to control by 1.49 fold and 1.25 fold, respectively (Fig. 4E). Activities of the

antioxidant enzymes SOD, APX, CAT, POD, and GR increase was more in Youngduk as compared to Jinju-1.

#### IV. DISCUSSION

In the present study, the content of  $O_2^-$  and  $OH\cdot$  also increased due to drought stress which confirms the previous studies of Marcos et al. (2018) who suggested that drought stress enhances the accumulation of ROS such as  $H_2O_2$ ,  $O_2^-$  and  $OH\cdot$  in the cells. Additionally, the content of electrolytic leakage was also increased

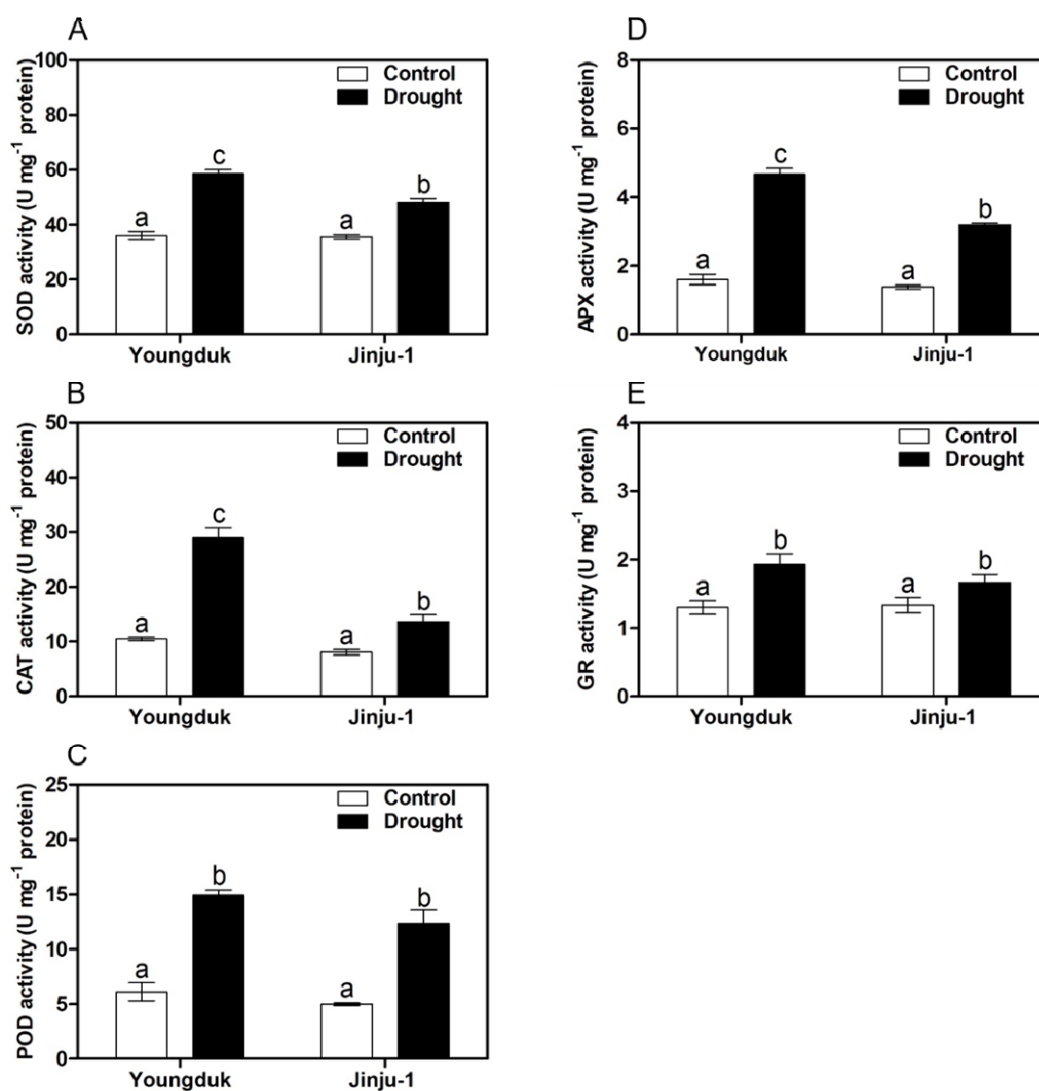


Fig. 4. Changes in SOD (A), CAT (B), POD (C), APX (D), and GR (E) of two different ecotypes of *A. hirta* under drought-stressed and unstressed (control) conditions. Different letters above bar graphs indicate a significant difference between control and drought-stressed ecotypes.

in two ecotypes of drought stress-treated *A. hirta* which are consistent to the previous results of Quan et al. (2004) who found that electrolyte leakage is higher in drought-stressed maize plants than in plants grown under control conditions. As the increase of ROS and electrolytic leakage is more in Jinju-1 drought stress-treated ecotype as compared to drought stress-treated Youngduk, so the cell membrane of Jinju-1 is more injured and hence it has less tolerance to drought stress.

Leaf relative water content (RWC) is the proper measurement of water status in a plant concerning the physiological significance of the deficit of water in the cell (Yamasaki and Dillenburg, 1999). We found that Youngduk exhibited the highest RWC, while Jinju-1 has the lowest RWC. Leaf RWC remained similar under well-watered conditions in both the ecotypes. These results demonstrate that Youngduk leaves lost water less than Jinju-1 during drought stress. Türkan et al. (2005) reported that drought-tolerant ecotypes have higher leaf RWC than drought-sensitive ecotypes.

Soluble sugars are involved in several metabolic events and act as molecules to regulate various genes involved in the synthesis of osmolytes as a response to environmental stresses (Rosa et al., 2009). Our results indicate that the content of soluble sugar was increased during drought stress treatment in the both ecotypes. Similar results were also reported by Ali et al. (2016), who suggested an increase in soluble sugar content in the leaves of drought stress-treated faba bean plants. Soluble sugar functions as ROS scavengers and signaling molecules in plants (Hu et al., 2012; Keunen et al., 2013). It has also been known that ROS-production conditions such as high photosynthetic activity and various stress are associated with the accumulation of soluble sugars in plants (Couée et al., 2006). Accumulation of ROS and soluble sugar, and an increase in antioxidant enzyme activity after drought treatment of *A. hirta* suggests that soluble sugars role as direct ROS scavengers and signaling molecule to enhance tolerance against drought stress.

Antioxidant enzymes such as SOD, CAT, POD, GR, and APX have crucial roles in stressful environments in the plant defense system (Gill and Tuteja, 2010). Drought stress is responsible for an increase in the activity levels of SOD and APX in plants (Fan et al., 2014) which are in agreement with our present results of SOD and APX activities in Youngduk and Jinju-1. Drought stress treatment also increased POD and CAT activities in drought stress-treated Youngduk and Jinju-1.

Similar results were reported by Akitha-Devi and Giridhar (2015) in which SOD, POD, and CAT activities are increased in drought-stressed soybean varieties. The GR activity also increased in drought stress-treated Youngduk and Jinju-1, which is following the results of previous studies (Zhang et al., 2018).

From the results, the Youngduk ecotype could be considered as more drought tolerant as compared to Jinju-1. In further study, this ecotype can be used as a basis for studying the molecular mechanisms underlying *A. hirta* tolerance to drought stress and could be utilized in the development of new forage varieties with drought stress tolerance.

## V. ACKNOWLEDGMENTS

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