

Research Article

Physiological and Biochemical Responses of Local *Arundinella hirta* Collections in Korea against Drought Stress

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ABSTRACT

Drought is one of the key limiting factors that adversely affects the growth and productivity of crop plants. For the enhancement of drought tolerance in crop plants, the identification of basic mechanisms of a plant to drought stress is necessary. In this study, we compared physiological and biochemical responses of five local *Arundinella hirta* ecotypes to drought stress. These ecotypes were previously collected from various parts of Korean peninsula, including Youngduk, Gunsan, Jangsoo, Jinju-1 and Yecheon. *A. hirta* plants were exposed to drought stress for 14 and 17 days respectively, followed by re-watering for 3 days. The results showed that the lipid peroxidation (MDA), hydrogen peroxide (H₂O₂), DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, and proline level were significantly increased while the chlorophyll content was decreased by drought stress in *A. hirta* leaves. The highest proline content and DPPH scavenging activity were shown in Ecotype of Youngduk with least MDA and H₂O₂ levels while the highest MDA and H₂O₂ contents, and least proline and DPPH levels were shown in Gunsan, respectively. These results indicate that the Youngduk is the most tolerant and Gunsan is the most sensitive ecotype among the five different collections. Together, these results provide a new insight of overall physiological responses of *A. hirta* to drought stress.

(Key words: *Arundinella hirta*, Drought stress, Forage, Native grass)

I . INTRODUCTION

As a sessile organism, plants are often exposed to multiple environmental stresses such as heat, drought, flood, cold, salinity, and heavy metals which greatly influence on plant growth and productivity (Gaspar et al., 2002). Abiotic stress tolerance is very complex phenomenon due to different factors and various biochemical, physiochemical and molecular actions which affect plants developments and growth (Razmjoo et al., 2008). Drought is one of the key limiting factors that alter a series of physiological processes in plants (Chakhchar et al., 2016). Drought stress is produced when water is reduced gradually from the soil, in a consequence plants continuously lost water from the leaves due to the process of transpiration. Changing of climate, temperature and shortage of precipitation are greatly influenced to the drought stress in plants globally (IPCC 2013). In addition, the desertification is the another factor which is also responsible for affecting approximately 30% of the world arable land (Ali et al., 2014). At physio-biochemical, morphological and molecular levels, plants have developed different strategies for drought tolerance (Hasanuzzaman et al., 2018). There are four major

drought tolerance mechanisms in plants: drought avoidance, drought escape, drought recovery and drought tolerance (Hasanuzzaman et al., 2018). Among these, tolerance of drought by reducing water loss and enhancing water uptake and drought tolerance by osmo-protection, osmotic adjustment and also antioxidant system of defense in drought tolerance of plants are widely studied (Fang and Xiong, 2015). The role of reactive oxygen species (ROS) is also very important in modifying plants acclimation to drought stress (Choudhury et al., 2017). At normal condition ROS can be generated as by-products at low levels in various cell compartments during the metabolism in plants (Silva et al., 2016). Therefore, it is imperative to study drought tolerance and/or resistance of plants for a better understanding of physiological, morphological, molecular responses as well as yield performance (Martinez et al., 2007).

Arundinella hirta is monocotyledonous perennial plant belonged to family Poaceae, and propagated by both seed and rhizome. It is broadly distributed in Korea, Japan and China, and has been used as a native forage grass in Korea. In this study, we have explored the physiological and biochemical responses of five local *A. hirta* ecotypes to prolonged drought stress.

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II. MATERIALS AND METHODS

1. Collection of *Arundinella hirta* ecotypes, growing and drought treatment

Arundinella hirta (Thunb.) ecotypes were previously collected from Youngduk, Gunsan, Jangsoo, Jinju-1 and Yecheon in Korean peninsula. Mature seeds were planted in pots with garden soil and grown in a growth chamber (25°C, 16 h-light/8 h-dark, 150 $\mu\text{mol m}^{-2}\text{s}^{-1}$). Four weeks old *A. hirta* plants were transferred to growth chamber at 25°C, and subjected to drought stress treatment by withdrawing water supply. At day 14 and 17, leaf samples were collected from both control and drought stress- treated plants. Then drought stress-treated plants were watered for three days and again leaf samples were collected. All the samples were frozen in liquid nitrogen and kept at -80°C for future analysis.

2. Estimation of chlorophyll content

Chlorophyll content of five *A. hirta* ecotypes was estimated by the method of Zhang et al. (2019) with some modifications. Briefly, 100 mg of leaf powder was suspended in 1 ml of 100% dimethyl formamide (DMF). The homogenate was centrifuge at 10000 \times g for 10 min, at room temperature. The supernatant was collected and the absorbance was measured at 664 nm, and 647 nm, respectively. Total chlorophyll content was calculated by using the formula $17.90 \times A_{647} + 8.08 \times A_{664}$.

3. Estimation of malondialdehyde (MDA) content

The content of MDA was determined by the method of Rahman et al. (2015). Briefly, 100 mg of leaf samples was mixed with 1 ml trichloroacetic acid (TCA) 20% and centrifuge at 13000 rpm for 15 min. In 0.7 ml supernatant was added 0.7 ml TCA 20% containing TBA 0.5% (w/v). After incubation at 95°C for 30 min, samples were kept on ice for 5 min for cooling, and centrifuge again at 13000 rpm for 15 min. Then absorbance of supernatant was read at 532 nm and 600 nm.

4. Determination of H₂O₂ content

The hydrogen peroxide content was determined by the method of Lee et al. (2018) with little changes. Briefly, 100 mg of leaf sample of five different ecotypes was dissolved in 1 ml potassium phosphate buffer (50 mmol L⁻¹, pH 6.8) and centrifuge at 12000

rpm for 20 min at 24°C. Subsequently, 0.7 ml of supernatant was mixed with 0.7 ml reagent (20% H₂SO₄ + 100 μl TiCl) and centrifuge at 12000 rpm for 15 min at 24°C. The absorbance was read at 410 nm, and H₂O₂ level was calculated by extinction co-efficient 0.28 $\mu\text{mol}^{-1}\text{cm}^{-1}$.

5. Free proline content determination

The estimation of free proline content was done by Rahman et al. (2015) method with minor changes. Leaf sample (80 mg) was dissolved in 1 ml 3% aqueous sulfosalicylic acid and centrifuged for 12 min at 12000 rpm. Then 700 μl of supernatant was mixed with 700 μl acid ninhydrine and 700 μl glacial acetic acid. The mixture was kept at 95°C for 1 hour. After cooling for 5 min, 1.4 ml toluene was added and centrifuged again for 10 min at 3500 rpm. The absorbance was checked at 520 nm using toluene as blank. The proline content was finally calculated by using L-proline as standard.

6. DPPH-radical scavenging activity determination

Kang and Salveit (2001) method was used for DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity. For this, 200 mg of leaf sample was taken and added 2 ml of absolute ethanol. The homogenate was centrifuged at 3500 rpm for 15 min at 4°C. The 500 μl supernatant was mixed with 250 μl DPPH (0.5 mmol L⁻¹) and 500 μl acetate buffer (100 mmol L⁻¹, pH 5.5). After keeping the supernatant for 30 min at room temperature the absorbance was read at 517 nm.

7. Statistical Analysis

All the results are presented as mean values \pm standard error (S.E.) of at least three independent experiments. Data were statistically analyzed using two way ANOVA between control and drought-treated samples, and also analyzed among drought-treated samples by one way ANOVA. The value of $P < 0.05$ was considered as statistically significant.

III. RESULTS AND DISCUSSION

1. Effect of drought on chlorophyll content

Drought stress adversely affects the chlorophyll content in

plants. Our present results also showed that at day 14, the chlorophyll content was decreased little in the five ecotypes of *A. hirta* in which the most decrease occur on Gunsan followed by Youngduk and Jangsoo compared to the ecotypes of Jinju-1 and Yecheon, respectively (Fig. 1). At day 17 of drought stress, the chlorophyll content has decreased more, which is due to breakdown of chloroplast from drought stress. On re-watering the results have been reverted and the chlorophyll level has increased. Decrease in chlorophyll content by drought stress treatment was also reported from a number of other plant species such as cotton (Massacci et al., 2008) and sunflower (Kiani et al., 2008).

2. Changes in DPPH-scavenging activity and free proline accumulation

DPPH was used to evaluate the antioxidant activity in leaves of plants. The present results showed that the DPPH radical scavenging activity was increased during the drought stress treatment (Fig. 2A, B, and C). The most increase occurred in Youngduk, Yecheon and Jinju-1, while in Gunsan and Jangsoo the increase was less at day 14. Similarly, at day 17 the DPPH level increased more in which the highest was in Youngduk followed by Yecheon, Jinju-1 and Jangsoo, while in Gunsan the DPPH level increased less than all the others. When plants were re-watered the DPPH level was decreased in all the ecotypes. Previously, significant increase of DPPH activity was also observed in rice plants had been exposed to heat shock and chilling stresses (Kang and Saltveit 2001). Similarly, the proline content has also increased with the drought stress

treatment at day 14 in which the highest increase occurred in Youngduk and the least in Gunsan (Fig. 2D, E, and F). The increase in proline level of all the other three cultivars Jangsoo, Jinju-1 and Yecheon are nearly same. At day 17 the level of proline increased more and reverted by re-watering. From the level of increase in DPPH and proline it is evident that Youngduk is the most drought tolerant because it has showed high increase in DPPH and proline than the other *A. hirta* ecotypes. The least drought tolerant is Gunsan as increase in DPPH and proline is less in Gunsan as compared to the other cultivars. It has been known that proline contributes as a source of nitrogen and carbon during post stress recovery and growth (Kishor et al., 2005). In this study the proline level was increased in all *A. hirta* ecotypes during drought stress. This finding is in accordance with the results of previous studies in which the proline level has increased during drought stress (Liu et al., 2010; Wang et al., 2008).

3. Changes of malondialdehyde (MDA) and H₂O₂ content

Malondialdehyde (MDA) indicates the damage of plasma membrane and is a cytotoxic product of lipid peroxidation. The results of MDA content showed that it has increased in the leaves of *A. hirta* plants during the drought stress at day 14 (Fig. 3A, B, and C). The highest increase occurred in Gunsan while the least in Youngduk and it increased more on day 17 as compared to 14. In the same way the H₂O₂ content has also increased in the leaves of *A. hirta* as compared to control during the drought stress (Fig. 3D, E, and F). Gunsan showed the highest increase in H₂O₂ level while Youngduk showed the

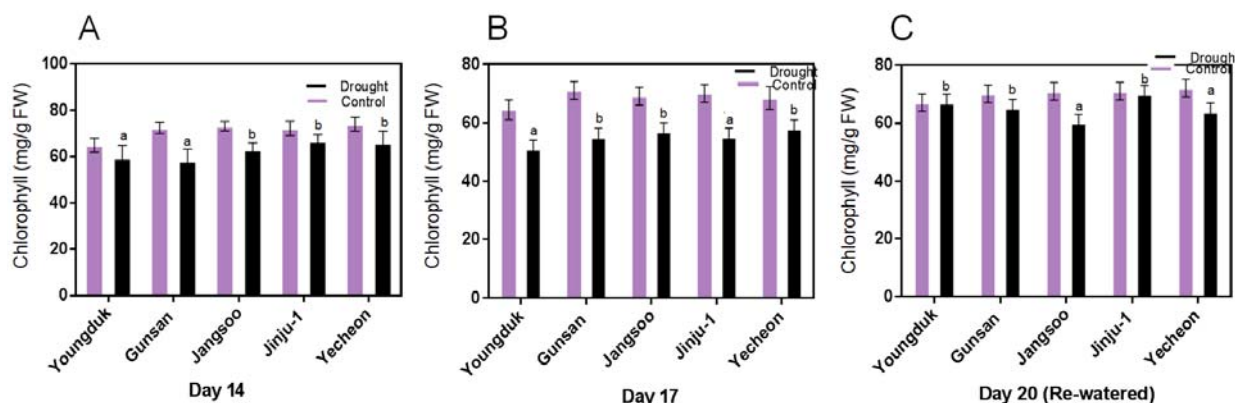


Fig. 1. Effect of drought stress on chlorophyll content in *A. hirta* plants. Total chlorophyll content in leaves of 14-day (A) and 17-day drought stress treatment (B), and re-watered for 3 days (C). Data represent the mean values and SE of three independent experiments.

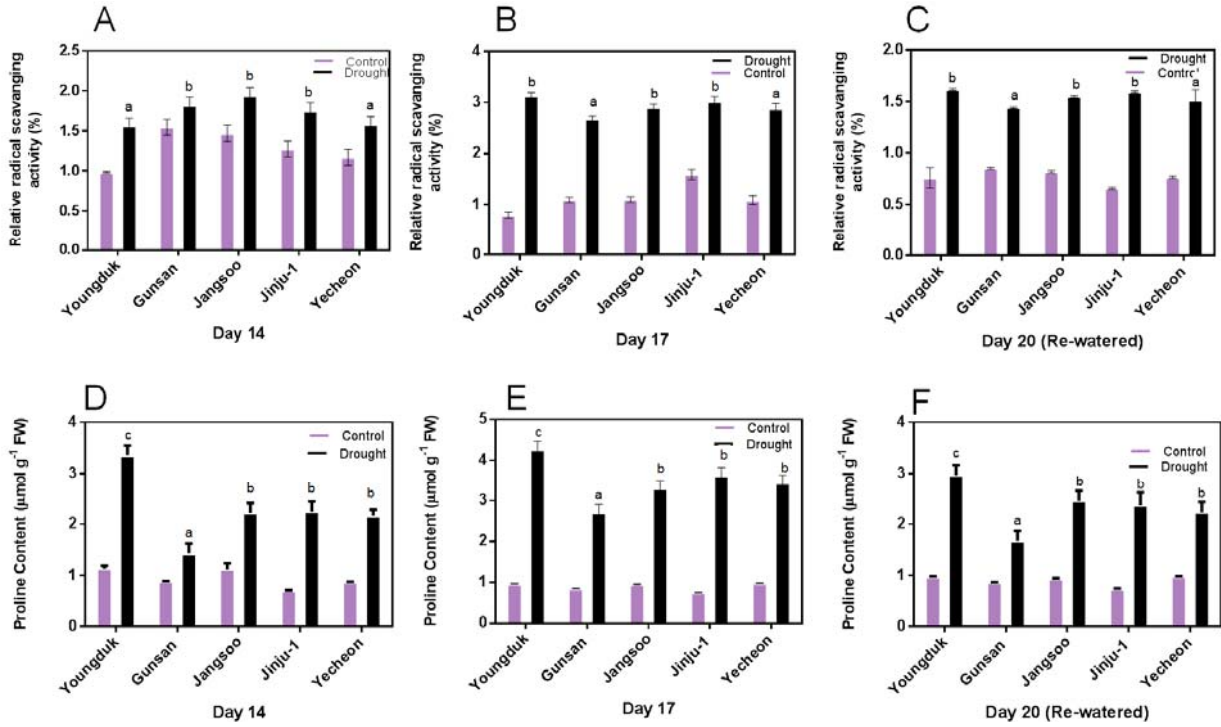


Fig. 2. Effect of drought stress on DPPH radical scavenging activity and proline content in *A. hirta* plants. (A) 14-day treatment, (B) 17-day treatment and (C) re-watered for DPPH. (D) 14-day treatment, (E) 17-day treatment and (F) re-watered for proline. Data represent the mean values and SE of three independent experiments.

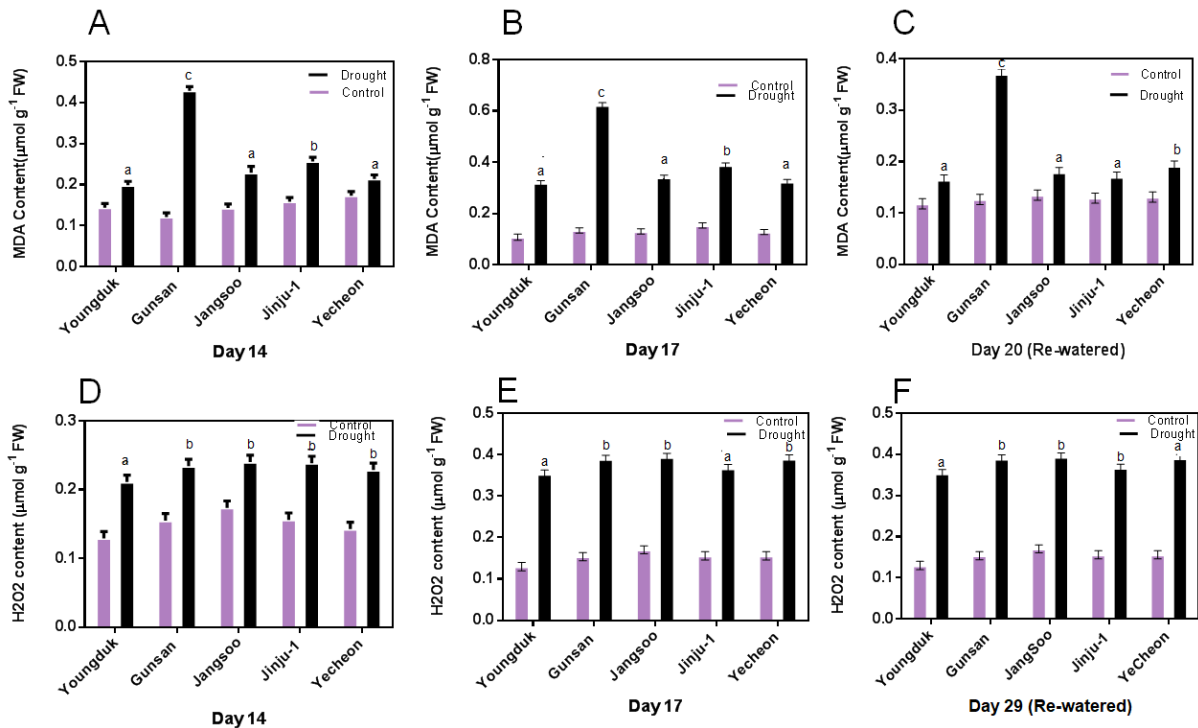


Fig. 3. Effect of drought stress on MDA and H₂O₂ content in *A. hirta* plants. (A) 14-day treatment, (B) 17-day treatment and (C) re-watered for MDA (D) 14-day treatment (E) 17-day treatment and (F) re-watered for H₂O₂. Data represent the mean values and SE of three independent experiments.

little increase. From the results of MDA and H₂O₂ analysis it is evident that Gunsan has more cell membrane damage while Youngduk has the least, which means that Gunsan is the drought sensitive while Youngduk is the drought tolerant. In cotton leaves, MDA and H₂O₂ content was also increased in response to drought stress (Deeba et al., 2012). In summary it is evident that the five ecotypes of *A. hirta* have different level of drought stress tolerance in which the Youngduk is the most drought tolerant species while the Gunsan is the least tolerant. Further investigation is also needed for the crossbreeding and drought tolerance genes identification so that to improve its drought stress tolerance more and can be used as a forage and turf crop in future.

IV. CONCLUSION

In the present study the drought stress response of five ecotypes of *A. hirta* leaves were analyzed. The results showed an increase in proline, antioxidant scavenging activity, H₂O₂ and MDA level while decrease in chlorophyll content. The increase in H₂O₂ and MDA indicate the cell membrane injury while the increase in proline and antioxidant scavenging activity prove that the plant has coped with drought stress. As this was a comparative study in which Youngduk indicated the highest drought stress tolerance and Gunsan is the least drought stress tolerance. Together, the results provided that *A. hirta* has the capability to withstand water drought tolerance. This study will provide bases for further investigation of drought stress tolerance mechanism on *A. hirta*.

V. ACKNOWLEDGEMENT

This work was supported by the Gyeongsang National University Fund for Professors on Sabbatical Leave, 2016.

VI. REFERENCES

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(Received : March 12, 2019 | Revised : March 14, 2019 | Accepted : March 18, 2019)