Journal of Climate Change, Vol. 8, No. 2 (2022), pp. 1-6.
DOI 10.3233/JCC220009

Quantitative Analysis of ABA and SA in Rice (*Oryza sativa* L.) Grown Under Drought Stress

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Received April 30, 2022; revised and accepted May 13, 2022

Abstract: Rice cultivation requires plenty of water for its proper growth, development, and productivity. The regular life cycle of rice plants is disrupted by moderate to severe drought. Both abscisic acid (ABA) and salicylic acid (SA) have involvement in rice physiology under drought. With the limited information, we aimed to study the relationship between ABA and SA concentration in leaves of rice plants under drought. The experiment was performed on a drought-sensitive variety of Swarna MTU 7029 rice. The HPLC method was used to analyse the endogenous ABA and SA content. This study provided data on ABA and SA content in 0.5 mM SA treated and untreated 56 days old rice plants at 7, 14 and 28 days of drought. The result showed that the concentration of ABA was enhanced by 74.6%, 82.8%, and 99.4% during 7, 14, and 28 days of drought, respectively, while it was increased by 64.6%, 74.3% and 78.5% in SA treated plants under 7, 14 and 28 days drought, respectively. The concentration of SA enhanced by 132.2%, 19.7%, and 3.0% during 7, 14, and 28 days of drought, while it was increased to 137.5%, 54.8%, and 23.2% in SA treated plants under 7, 14, and 28 days drought, respectively. This explains that on the 7th day of drought stress, SA may suppress the formation of ABA but not much on the 14th and 28th days of drought stress in treated rice plants. This outcome helps study the interaction of ABA and SA at the gene level under drought stress.

Keywords: Salicylic acid; Abscisic acid; Phytohormones; HPLC; Abiotic stress.

Introduction

Agricultural drought causes economic loss in crop productivity (Liu et al., 2014). Rice farming is readily harmed when there is a lack of water (Dien et al., 2019). The temperature of the planet is increasing due to rising population and industrialization, which results in a strain on rice yield. The cultivation of rice crops is mostly dependent on well-irrigated areas; rice consumes nearly 80% of the total supply of freshwater for irrigation (Yang et al., 2019). Drought and salinity disrupt the ion level within and outside the cell, resulting in osmotic stress, which leads to a variety of physiological and biochemical changes as adaptive responses to the detrimental effects of environmental conditions (Llanes et al., 2016). Plant hormones (ABA and SA) regulate many developmental processes under abiotic and biotic stresses (Mur et al., 2006; Chandrasekar et al., 2011). Salicylic acid is a natural phenolic, signaling molecule that minimizes the negative effects produced under abiotic stress and also promotes growth balances between physiological and biochemical traits in plants under non-stress conditions (Shemi et al., 2021; Loutfy et al., 2020). In general, when rice is under stress conditions, ABA and SA-sensitive genes are activated. Upregulation of SA and ABA are under

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the control of many transcription factors (TF families), which are either ABA-dependent or ABA-independent. The amount of ABA in plant tissues is determined by production, catabolism, and the presence of other hormones (Ali et al., 2011). According to one study, endogenous SA boosts ABA levels (Li et al., 2019), while other studies found an antagonistic link between SA and ABA (Cohen and Leach, 2019). According to a study on *B. napus* leaves under drought stress, endogenous SA levels surged in the early days (0-6 days) and then considerably dropped to normal levels in the latter days, while ABA levels grew constantly (Park et al., 2021). Hormonal interactions in plants respond to internal and external signals and these responses are controlled by a complex network of stress-responsive transcriptional genes via signal transduction (Deb et al., 2016). SA ameliorates plant metabolism and plays a defensive role under different stress conditions like low temperature, drought, and salinity (Hussain et al., 2016; Ijaz Ahmad, 2012; El-Taher et al., 2022). Exogenous administration of plant growth regulators has been proposed as a possible technique for reducing the deleterious effects of drought stress (Khan et al., 2015). According to a previous study, exogenous administration of salicylic acid at concentrations of 0.1-0.5 mM enhanced photosynthetic growth and many physiological and biochemical processes, but greater concentrations of more than 1.0 mM inhibited the plant growth (Hayat et al., 2010; Loutfy et al., 2012; Nazar et al., 2015). The responses of SA and ABA under different types of stresses are different in different plants at different stages of the plant's life cycle. Many studies have been done on different plants to study the effect of SA on foliage, like as barley plants (Wang et al., 2018), maize (Shemi et al., 2021), squash plants (Abd El-Mageed et al., 2016), and also at seedling stage in wheat (Kang et al., 2012) whereas studies on seeds, pretreated with SA is limited. In the long-duration, for the drought-sensitive variety (Swarna MTU-7029) of rice at tillering stage (before the flowering stage), research has not been done so far. The present study is focussed on determining the concentration of ABA and SA on the 7th, 14th and 28th days of drought stress.

Materials and Methods

Plant Material

The *Oryza sativa* (variety; Swarna MTU-7029) seeds were acquired from the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, UP, India. The seeds were planted in-vivo in Udai Pratap Botanical Garden, Varanasi, UP, India during the rainy season. The molecular and biochemical study was conducted in the laboratory of Udai Pratap College, Varanasi, UP, India.

Experimentation and Salicylic Acid Treatment

The uniform rice seeds were sorted and sterilised with 5% sodium hypochlorite for 5 minutes followed by thrice washing with distilled water. Rice seeds were separately soaked in 0.5 mM of salicylic acid (treated) and in distilled water (untreated) for 48 hours at room temperature. Seeds were sown in two different pots after presoaking. In a fourth leaf stage (approximately after 21 days from sowing), seedlings were transplanted to four different sets of pots with three replications. The four pots were classified as Control (C), Drought (D), Drought+SA (D+SA), and Salicylic Acid (SA). After 56 days (35 days from transplantation), the rice plants were subjected to drought stress for 7, 14, and 28 days. Biochemical analysis was performed at 7, 14, and 28 days of stress condition.

Sample Extraction

About 50 mg of fresh rice leaves from each study group were treated with liquid nitrogen and crushed in 2 ml of 80% methanol by mortar & pestle. A total of 500 µl of extraction solvent containing 2-propanol: H_2O: HCl (2000: 000: 2, vol/vol/vol) was added and properly mixed by shaking at 100 rpm on a shaker for 30 minutes at 4°C. About 1 ml of dichloromethane was added and the sample was stirred for 30 minutes at 4°C followed by centrifugation at 13,000 g for 5 min at 4°C. After centrifugation, two phages were formed. Approximately 900 µl of solvent from the lower phase was transferred in a screw-cap tube and concentrated by using a nitrogen evaporator. The sample again dissolved in 0.1 ml of methanol. This prepared solvent was stored at -20°C till further use.

Phytochemical Estimation (Endogenous ABA and SA)

The prepared sample was kept in a temperature-controlled autosampler at 8°C. About 50 µL sample was injected into the reverse phase C_{18} Gemini HPLC column for HPLC-ESI-MS/MS analysis. The HPLC (Shimadzu class VP.V6.10) determines the endogenous ABA and SA at 254 nm and 449 nm, respectively. The phytohormones (ABA and SA) were eluted at a 1.5 mL/min flow rate with a gradient of hexane /IPA +0.1% acetic acid. The system was equipped with a column Chiralpak IB (4.6 mm × 250 mm × 5 µM) and a UV detector.

Analysis

The peaks were analysed based on the retention time of the standard solution of ABA and SA. The standard concentration of ABA and SA was dissolved in methanol and determined the retention time at 254 nm and 449 nm, respectively, to prepare a standard calibration curve. The concentrations of ABA and SA were calculated by standard peak area and concentration curve.

Results

Effect of Salicylic Acid on ABA Content in Rice Plant

The typical chromatogram represents the peak of different compounds concerning retention time. The desired ABA compounds from the samples under the proposed conditions were identified by comparing the retention time and UV spectra with that of the authentic standard. The peak area of ABA significantly increased with the increment of the duration of stress (7-28 days). The concentration of ABA (measured in ng/g FW) was determined in study groups based on five gradient concentrations of standard solutions and the regression equation coupled with the calibration curve showed good fitness ($R^2 = 0.9998$) (Figure 1). The concentration of ABA was significantly increased with the duration of stress (7-28 days) (Figure 2). The concentration of ABA was enhanced by 74.6%, 82.8%, and 99.4% during 7, 14, and 28 days of drought condition respectively, while it was increased by 64.6%, 74.3% and 78.5% in SA treated plants under 7, 14 and 28 days drought condition, respectively.

Effect of Salicylic Acid on SA Content in Rice Plant

The typical chromatogram represents the peak of different compounds for retention time. The desired SA compounds from the samples under the proposed conditions were identified by comparing the retention time and UV spectra with that of the authentic standard. The peak area of SA significantly decreased with the increment of the duration of stress (7-28 days). The concentration of SA (measured in µg/g FW) was determined in study groups based on five gradient concentrations of standard solutions and the regression equation coupled with the calibration curve showed good fitness ($R^2 = 0.9999$) (Figure 3). The concentration of SA was significantly decreased with the duration of stress (7-28 days) (Figure 4). The concentration of SA

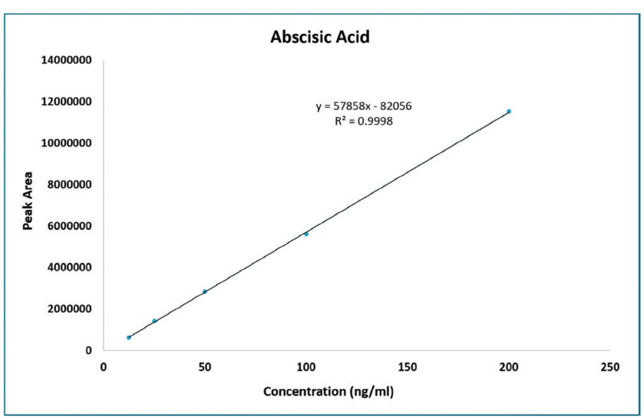

Figure 1: Calibration curve of ABA standard solution.

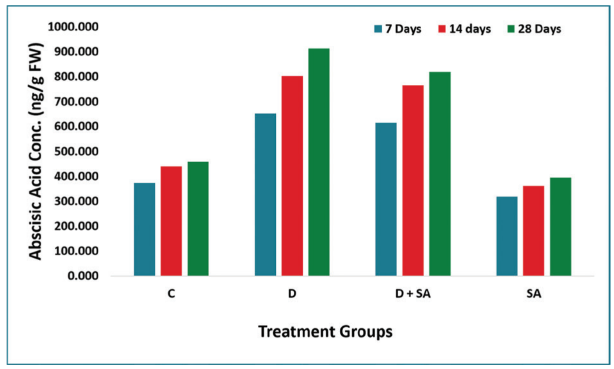

Figure 2: Endogenous ABA content of rice plant leaves in study conditions.

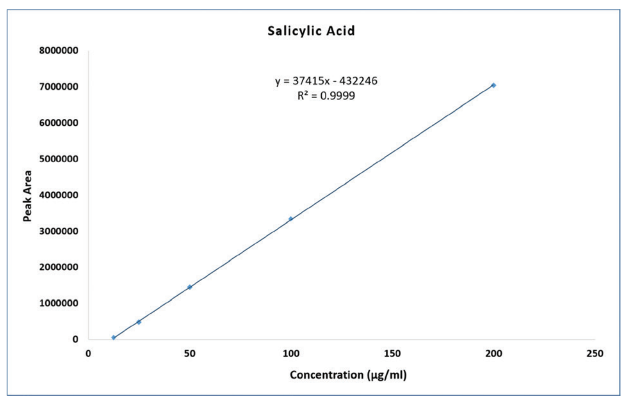

Figure 3: Calibration curve of SA standard solution.

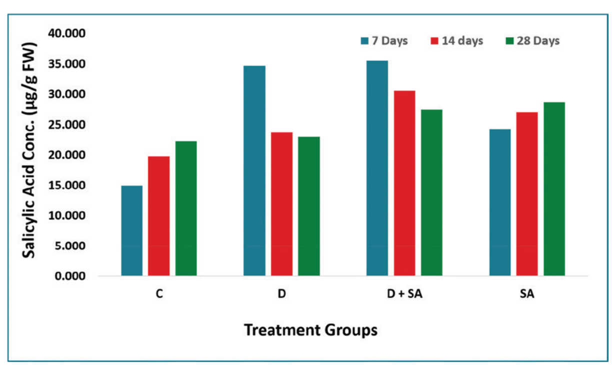

Figure 4: Endogenous SA content of rice plant leaves in study conditions.

enhanced by 132.2%, 19.7%, and 3.0% during 7, 14, and 28 days of drought condition respectively, while it was increased to 137.5%, 54.8%, and 23.2% in SA treated plants under 7, 14, and 28 days of drought condition respectively.

Discussion

Rice, unlike several other crops, is very susceptible to water stress at all stages of development, seedling, vegetative, and flowering (Goswami et al., 2013; Yang et al., 2019). Swarna MTU-7029 is a flood-resistant and drought-sensitive rice genotype (Dash et al., 2020). Presoaking with 0.5 mM SA gave relevant information about ABA and SA concentrations against mild to severe drought conditions. We investigated in our study the relationship between ABA and SA by examining how their concentrations changed over 7, 14, and 28 days of drought stress. ABA-mediated actions were generated in response to water stress (Hu et al., 2016). Due to its major role in plants among all phytohormones during stress conditions, ABA is also known as a stress hormone (Ye et al., 2012). In untreated (sensitive) rice plants, the ABA concentration increased more than treated rice plants under drought stress. Yoshida and coworkers proposed that the increase in the ABA content indicates the involvement of ABA signaling in the closing of stomata to minimise transpiration rate and stops the influx of CO_2 that reduces photosynthetic products then finally halts plant growth and development (Yoshida et al., 2019). ABA supports shoot growth in an *Arabidopsis thaliana* and *Solanum lycopersicum.* Inconsistent with prior research, we found that ABA and SA are secreted in low concentration during the tillering stage of rice in both normal and drought stress conditions to aid plant growth and development, as well as to control physiological activities. Untreated rice plants with significant ABA accumulation are more sensitive to drought and less drought-resistant. On the contrary, drought-tolerant wheat varieties acquired more ABA than sensitive wheat varieties under water stress (Chandrasekar et al., 2011). Plant immunity keeps plants healthy by regulating immune responses in response to environmental challenges. Different stages of plant leaves respond distinctively to immune responses under abiotic and biotic stress conditions. ABA suppressed SA inducing gene and deteriorated the SA receptor gene. In young leaves of *A. thaliana*, SA suppressed the ABA effects and in old leaves, ABA suppressed the effects of SA (Berens et al., 2019). We found that under the influence of salicylic acid, rice plants may have developed tolerance to the negative effects of oxidative stress as a result of drought stress, as evidenced by the reduced concentration of endogenous ABA in SA-treated rice plants compared to untreated rice plants. SA maintains osmotic adjustment by increasing osmolytes, proline, soluble carbohydrate (maize, sunflower) (Saruhan and Saglam, 2012), enhancing antioxidative enzymes (SOD, APX), increasing grain yield (barley, soybean) (Abdelaal et al., 2020; Razmi et al., 2017). In normal conditions, SA concentrations remain low but as the drought conditions created rice plants, the concentration of SA increased. The endogenous levels of SA in *Phillyrea angustifolia* raised five-fold in response to drought stress (Munné-Bosch and Peñuelas, 2003). The concentration of ABA increases proportionally with the saline exposure (Zhang et al., 2006). The ABA level increased until the 28th day of drought, but the SA level rose only until the 7th day of drought, and then declined slightly until the 28th day of drought, in both treated and untreated rice plants under drought circumstances. This manifests that ABA concentration might be increased with a prolonged duration of drought. However, the inactivation of SA synthesising genes under the impact of drought duration may prevent them from functioning. Li and coworkers described the drought-tolerant gene, *OsASDR3*, which suppresses the effect of ROS associated with the ABA-mediated signaling pathway (Li et al., 2021). An experimental work stated that the ABA synthesizing genes were suppressed by the influence of SA pretreated in *B. napus* (La et al., 2019). This finding demonstrates that SA, like ABA, plays a role in drought response control, both work together in times of drought. In terms of ABA and SA, the rice plant responded differently after 7, 14, and 28 days of drought stress. Both were not released at the same rate at different severity levels of drought stress. Some rice genes, such as *OsGRXS17*, play a key role in reducing the effects of water stress (Hu et al., 2017). The function of *OsGRXS17* may be up-regulated by SA. Overexpression of *OsNAC10* and *SAC1* activity improve grain yield and increases tolerance to drought in rice (Liu et al., 2014). The present study suggests that pretreatment of 0.5 mM SA may induce the activity of these genes during periods of drought. Under stress, exogenous SA pretreatment increased endogenous SA levels while reducing endogenous ABA levels. Variations in ABA and SA levels in treated and untreated rice plants in response to drought severity revealed a synergistic and antagonistic connection between them.

Conclusion

The antagonistic link between ABA and SA in the control of numerous aspects of plant development and abiotic signaling cascade is an attractive focus in the field of plant molecular biology. The rice drought avoidance system is indicated by the release of ABA until 28 days of drought; however, under the effect of SA, the rice plant evolved a drought tolerance mechanism to defend itself from drought. Exogenously applied SA to rice seeds increases the level of SA endogenously during drought stress. According to our findings, the endogenous SA did not increase throughout long durations of drought stress, while endogenous ABA rose. Deliberating the level of ABA and SA for over 28 days of drought stress aids in understanding the mechanism of drought stress in rice, and also helps to demonstrate that the ABA cannot be repressed by SA for a long period of drought. Finally, we concluded that rice became drought resistant in later stages of its life cycle after being pretreated with 0.5 mM SA.

Research Significance

The findings of this study could help to improve the efficiency of scientific research. Our research suggests that endogenous changes in SA and ABA in rice could help to improve drought tolerance and crop productivity. It could be beneficial to farmers in preventing hunger and ensuring food security.

Acknowledgement

We thank all staff for their continuous support in the study.

Conflict of Interest

The authors declare that there is no conflict of interest.

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