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## Role of H<sub>2</sub>O<sub>2</sub> on photosynthetic characteristics of soybean genotypes under low water input

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### Abstract

Soybean is subjected to abiotic stresses that immensely affect its productivity during its lifespan and threaten food security globally. Recent research recommends that chemical substances could be applied to plants as an alternative to traditional agriculture to better abide abiotic stresses. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is a potential agent that can serve for this purpose. Up to today, responses of exogenous H<sub>2</sub>O<sub>2</sub> on photosynthetic machinery in plants exposed to drought is poorly investigated. Therefore, the effects of exogenous low dose H<sub>2</sub>O<sub>2</sub> on plant chlorophyll fluorescence in two soybean genotypes (*Glycine max* L. Merrill), 537 (tolerant) and 520 (susceptible), under drought were evaluated. Drought which we had found significantly reduced in two genotypes, did not cause change in Fv/Fm and ΦPSII of tolerant genotype, contrarily subsided qP and ETR values. However, Fv/Fm, ΦPSII, qP and ETR failed in susceptible genotypes under drought. Increases in NPQ were determined under stress in both genotypes. Exogenous H<sub>2</sub>O<sub>2</sub> mitigated the drought-induced impairment in photosystem II efficiency in both genotypes. This data indicates that low dose H<sub>2</sub>O<sub>2</sub> further enhanced the tolerance to drought via regulation of the photochemical process in both genotypes.

**Keywords:** soybean, hydrogen peroxide, water scarcity, chlorophyll fluorescence

### 1. INTRODUCTION

Changing climate and drought have become the vital limiting factors to crop yield and the food security. Fluctuating rainfall profiles are causing the repeated onset of droughts on earth [1]. Severe drought causes substantial decline in productivity through injurious effects on plant physiology [2]. Under this alarming situation, a sustainable food supply must be guaranteed to feed the population. In this scenario, a convenient way could be to bring in drought resistant crops in the food production system. Photosynthesis is one of the main mechanisms which drought affects. Low level of CO<sub>2</sub> limits diffusion through the stomata

or the alterations of photosynthetic machinery or secondary effects might occur, as oxidative stress [3].

On the other hand, several researchers have been attempting to discover methods to alleviate drought stress or overcome drought injury in plants. Among them, the exogenous application of signaling molecules such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), melatonin (Mel), hydrogen sulfide (H<sub>2</sub>S), nitric oxide (NO) and polyamines (PAs) is attracting considerable attention in recent years. H<sub>2</sub>O<sub>2</sub> is a major radical generated as a result of oxidative stress in plants. Yet, it is also a signaling molecule [4] and acts as a second messenger in response to both abiotic and biotic stresses [5]. Some authors have suggested that H<sub>2</sub>O<sub>2</sub> shows a

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dual dynamic in plants: At high concentration, it initiates apoptosis and reduces the photosynthesis rate [6] whereas at low concentration, it acts as a signal molecule triggering tolerance against stress [5,7]. H<sub>2</sub>O<sub>2</sub> is a key regulator in a variety of steps in growth and development, especially photosynthesis [8] and have been shown to contribute to the amelioration of negative impacts caused by drought [9,10]. As a non-destructive and rapid method chlorophyll fluorescence technique has long been used to monitor and screen stress tolerance of plants and evaluate the damage to the photosynthetic machinery under environmental stresses [11,12].

Soybean is one of the major legume crops in the world serves as an abundant source of protein-rich food for several organisms, yet its growth and yield are affected by abiotic stresses. which particularly is challenged by drought, decreasing up to 40% of the yield annually [13]. Drought stress occurring at the early stages of growth significantly increases the pod failure, subsequently decreases total soy seed yield. The changing climate further intensify the situation. For this reason, more drought resistant soy bean varieties should be selected for future food continuity [14]. The regulatory role of exogenous applications of H<sub>2</sub>O<sub>2</sub> on drought tolerance in plants has been continuously demonstrated. For example, it was put forth in our previous study that low-dose H<sub>2</sub>O<sub>2</sub> might alleviate the hazardous effect of drought stress by regulating endogenous H<sub>2</sub>O<sub>2</sub> level and inducing antioxidant system in the seedlings [15]. However, investigations concerning on the responses of H<sub>2</sub>O<sub>2</sub> on photosynthetic machinery in plants exposed to drought stress, especially soybean, is rather rare. It is very important to know parameters affecting photosynthetic yield for developing high-yield genotypes under drought. Therefore, in this study, we measured responses of certain parameters associated with photosynthesis in two soy bean genotypes differing in tolerance, in order to better understand the mechanisms of action of H<sub>2</sub>O<sub>2</sub> application to increase drought tolerance.

## 2. MATERIAL AND METHODS

### 2.1. Plant growth and stress applications

Two *Glycine max* L. genotypes show sensitivity to water scarcity in different levels (*Glycine max* L. Merrill 537 (drought tolerant) and *Glycine max* L. Merrill 520 (drought susceptible)) were used in the experiments. Seeds were sterilized in 0.1% HgCl<sub>2</sub> for 3 min. and repeatedly washed with ddH<sub>2</sub>O then were sown in 14x16x11 cm pots containing planting mix (5:1 soil and peat). Seedlings were grown in a chamber (23 ± 2°C, 60% ± 5 humidity and 400 μmol m<sup>-2</sup> s<sup>-1</sup> light) during 30 days. Four weeks after germination (radicle emergence), either 1 mM H<sub>2</sub>O<sub>2</sub> or dH<sub>2</sub>O was sprayed as foliar once in a day for 3 days, then irrigation was stopped. Applications for H<sub>2</sub>O<sub>2</sub> or dH<sub>2</sub>O were followed by drought period each time. Plants with no spray and well-watered throughout the experimental period were used as control.

### 2.2. Chlorophyll fluorescence measurements

Measurements were performed using OS5p chlorophyll fluorometer (OptiScience Corporation, USA). For each application, a cm<sup>2</sup> of circular area of the top leaves (fully mature) from different group of plants were used for the analysis. Leaves were exposed to an initial darkness period of 20 min by leaf clips right before the measurements. Min. and max. fluorescence in dark (F<sub>o</sub> and F<sub>m</sub>), changing levels of fluorescence (F<sub>v</sub>), the excitation efficiency captured by Photosystem II (PSII) reaction centers (F<sub>v</sub>/F<sub>m</sub>) were documented by saturation pulse method. The PSII photochemistry's quantum yield (ΦPSII) was detected with a steady state light adapted yield protocol. The electron transport rate (ETR), non-photochemical quenching (NPQ) and photochemical fluorescence quenching (qP) were recorded by PAR (Photosynthetic Active Radiation) clip and a specific quenching protocol.

### 2.3. Statistical analysis

Analyses was performed with biological and technical triplicates. Duncan multiple comparison test using SPSS were used for variance analysis of data and significance level was set to 5% (P<0.05).

### 3. RESULTS

#### 3.1. Chlorophyll fluorescence

Drought stress and H<sub>2</sub>O<sub>2</sub> applications did not cause a significant change in PSII photochemistry's maximum quantum yield (Fv/Fm) of the tolerant genotype 537. There was a similar change in PSII photochemistry's effective quantum yield (ΦPSII) in the tolerant genotype. Conversely, drought stress decreased Fv/Fm and ΦPSII of susceptible genotype 520. However, H<sub>2</sub>O<sub>2</sub> alleviated the inhibitory impact of water scarcity on the plants and enhanced the efficiency of PSII up to 4% in susceptible genotype when compared to the drought treatment only. ΦPSII in the H<sub>2</sub>O<sub>2</sub> applied plants after drought stress was 3% higher than that of drought treatment alone. Non-photochemical quenching (NPQ) which represents heat dissipation in PSII was induced by drought period in two genotypes. The increase in NPQ of susceptible genotype 520 was greater than tolerant genotype 537. Conversely, H<sub>2</sub>O<sub>2</sub> exerted a decrease in NPQ for drought stress treatment (Figure 1). On the other hand, photochemical quenching (qP) of both genotypes decreased under drought stress. Exogenous H<sub>2</sub>O<sub>2</sub> alleviated the decrease in the qP. qP values of tolerant and susceptible lines in drought applied groups after H<sub>2</sub>O<sub>2</sub> application were 20% and 16% higher than those of drought treatment only, respectively. The exposure of soybean genotypes to drought stress decreased the electron transport rate (ETR). However, this effect is reversed by application of exogenous H<sub>2</sub>O<sub>2</sub> in both genotypes (Figure 1).

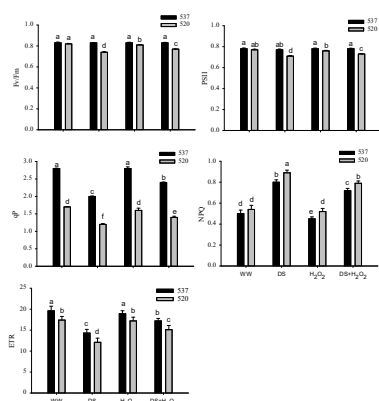


Figure 1. Chlorophyll fluorescence parameters (Fv/Fm, ΦPSII, NPQ, qP and ETR) of two soybean genotypes, 537 (black square) and 520

(grey square), influenced by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) application under drought stress. The seedlings were subjected to four different treatments: (i) the control seedlings were only exposed to sufficient water supply (WW); (ii) pretreated with H<sub>2</sub>O<sub>2</sub> and not drought stressed (H<sub>2</sub>O<sub>2</sub>); (iii) only drought-stressed (DS); (iv) pretreated with H<sub>2</sub>O<sub>2</sub> and drought stressed (DS+H<sub>2</sub>O<sub>2</sub>). Vertical bars represent standard deviation. Different letters indicate significant differences (P ≤ 0.05) between the two genotypes along with different treatments.

### 4. DISCUSSION

Chlorophyll fluorescence is a tool that indicates the photosynthetic activity of genotypes [22] and allow estimation of excessive energy removal [23]. Preservation of photochemical efficiency is a major drought tolerance indicator [24]. In this study, significant differences between the two genotypes were observed under drought conditions. For example, Fv/Fm and ΦPSII values of tolerant genotype 537 did not change significantly with drought stress and H<sub>2</sub>O<sub>2</sub> application. These results pointed out that the photochemical activity and electron transport chain of tolerant genotype was capable of well-maintained under drought stress and thus genotype 537 could be resistant to water deficiency. On the other hand, the values of Fv/Fm and ΦPSII of susceptible genotype 520 were remarkably less under drought stress, suggesting that drought caused the inhibition of electron transport and spreaded the over-abundant excitation in the form of heat. Conversely, exogenously applied H<sub>2</sub>O<sub>2</sub> in susceptible genotype 520 advanced the photochemical efficiency under drought thanks to higher ΦPSII activity.

Photochemical quenching is a marker on whether PSII reaction centers are open or the energy is enough for driving photosynthesis [25,26]. qP here was found to be less in drought exposed both genotypes, and the qP in stressed plants with H<sub>2</sub>O<sub>2</sub> gave higher values showing H<sub>2</sub>O<sub>2</sub> eased the damage possibly affect PSII reaction center and provided a higher ETR between electron acceptors and sources, resulting in weakening possible photo-inhibition [27].

Non-photochemical quenching (qN) is the other marker for excessive energy damage in the photosynthetic apparatus. The plants' capability for heat dissipation is shown by the NPQ data here. Drought could possibly lead increase in the NPQ [25]. The NPQ value undoubtedly increased in both genotypes under drought compared to the well-watered conditions. In this case, both soybean genotypes might protect themselves from detrimental effects of drought stress through increased NPQ, which has dissipated light energy and decreased the efficiency of photochemical reactions of photosynthesis. On the other hand, photo-inhibition and reduced photosynthetic efficiency was reversed by exogenous H<sub>2</sub>O<sub>2</sub>. This result showed that H<sub>2</sub>O<sub>2</sub> protected photosynthetic activation centers against drought-induced photo inhibition by enhancing energy dissipation process.

Sustaining optimal water levels especially under harsh environmental conditions is critical in plants. In this context, our previous report had also indicated that drought significantly reduced the leaf water potential, yet H<sub>2</sub>O<sub>2</sub> applied soybean genotypes showed better water potential than controls under drought period, which had demonstrated that H<sub>2</sub>O<sub>2</sub> maintained higher water status and enhanced the tolerance of soybean against water scarcity [15]. Such an ameliorative effect on water potential might be due to the role of H<sub>2</sub>O<sub>2</sub> in ensuring the accumulation of compatible solutes such as soluble sugar, proline, and polyamine amounts under drought conditions [10, 15, 18]. On the other hand, researchers found that the photosynthetic pigments of the target genotypes decreased remarkably [16,17] upon drought exposure which can be attributed to radical biogenesis [19] or might be due to the inhibition of chlorophyll biosynthesis or acceleration of chlorophyll degradation by the enzyme chlorophyllase [19, 20]. This reduction in pigment contents could also be a pathway in order to reduce the light harvest of chloroplasts. We further had found that drought induced endogenous H<sub>2</sub>O<sub>2</sub> level itself yet this decrease was reduced by exogenously applied H<sub>2</sub>O<sub>2</sub> [15] and foliar H<sub>2</sub>O<sub>2</sub> spray improved the intactness of photosynthetic pigments in both genotypes. [15]. Previous studies reported that the exogenous H<sub>2</sub>O<sub>2</sub> promotes chlorophyll intactness under well-

watered or stressful environments [21, 9]. This means that H<sub>2</sub>O<sub>2</sub> certainly has the capability of protection against the degradation of chlorophyll which distributes, conveys and transforms of light energy in photosynthesis and is a potential inducer to tolerate drought leading to better growth and development under water shortage conditions.

## 5. CONCLUSIONS

In conclusion, these results proved that exogenous H<sub>2</sub>O<sub>2</sub> mitigated the negative effects of drought on the growth and photochemical efficiency of two different soybean genotypes. Plants especially tolerant genotype received supplemental H<sub>2</sub>O<sub>2</sub> maintained an enhanced photochemical efficiency, so were able to maintain photosynthesis. H<sub>2</sub>O<sub>2</sub> may be considered as a potential signaling molecule for augmenting photosynthetic potential of soybean plants under drought stress conditions.

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