Antioxidative Response of *Glycine max* (L.) Merr. cv. Namdan to Drought Stress

Van-Chung Mai, Thi-Kim-Dung Le, Thi-Kim-Chi Nguyen

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ABSTRACT

The endemic variety *Glycine max* (L.) Merr. cv. Namdan is one of the most important crops in the agricultural production in Nghe An province (Vietnam). Experiments were carried out to assess the adaptive capability of that soybean variety in drought condition. The oxidative stress with a burst of $O_2^{\cdot -}$ and H_2O_2 in soybean leaves resulted the obviously cellular membrane damage that was illustrated by a continuous increase in content of products of lipid peroxidation. A high accumulation in activity of enzymatic antioxidants such as SOD, CAT and POX would detoxify the excess of $O_2^{\cdot -}$ and H_2O_2 and decrease oxidative damages. An increase in amino acid proline's content protects the stressed cells by adjusting intercellular osmotic potential. Those results suggested the drought tolerant capability for variety Namdan.

Key words: Antioxidative response, Drought, Namdan, Soybean.

INTRODUCTION

Drought is one of the most harmful abiotic stresses that limits the plant growth and productivity (Çelik *et al.*, 2017). In the cellular level, drought often provokes peroxidation and induced the oxidative burst with strong generation of reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) leading to damages to living cells (de Carvalho, 2008). To protect themselves from drought, plants would mobilize various defense mechanisms, of which antioxidative responses are essential biochemical metabolisms. For example, osmoregulation is an important characteristic of plant drought resistance, which closely controlled degree of the cellular membrane damage via rising in content of products of membrane lipid peroxidation (Li and Nong, 2018; Wang *et al.*, 2019). When plants face drought stress, activities of enzymatic antioxidants such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and peroxidase (POX, EC1.11.1.7) are usually accumulated to control generation and detoxify ROS (Wu *et al.*, 2019). In soybean plants, activity of those enzymes was also observed to induce under the combined effect of drought and other stresses (Ramana *et al.,* 2012). Protein (Boukecha *et al.*, 2018) and several nitrogen-containing organic compounds, *e.g.*, amino acid proline (Luo *et al.*, 2014), polyamines putrescine and spermidine (Zahedi and Abbasi, 2015) were suggested to function as antioxidants as they improve soybean tolerant capability in drought condition.

Glycine max (L.) Merr. cv. Namdan is an endemic variety that plays as important crop in the agricultural production in Nghe An province (Vietnam). There were several documents presented about defense of *G. max* cv. Namdan against aphid (Mai *et al.*, 2016; 2017), however, lack of information has been mentioned about its response to abiotic stresses. This study aims to investigate antioxidative variations of *G. max* cv. Namdan under drought condition, in which, level of $O_2^{\cdot -}$, H_2O_2, content of proline as well as activity of SOD, CAT and POX were assessed. As an additional objective, degree of cellular oxidative damage was also determined. It should be stressed that those aforementioned aspects are novel knowledge about the drought defense mechanism of *G. max* cv. Namdan and will provide the convinced evidences to clarify whether it is a drought-tolerant variety.

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MATERIALS AND METHODS

Materials

Homogenous seeds of two varieties of soybean [*Glycine max* (L.) Merr.], namely Namdan (investigated variety) and DT2008 (drought-tolerant), were obtained from Nghe An Agricultural Extension Centre. Seeds were placed on agar plates and remained at 4°C overnight. Then, they were incubated to 20°C for 3 days in the dark to ensure uniform germination. Seedlings were transferred into 20 cm pots (three seedlings/pot) containing alluvium added macro minerals and placed in the growth chamber with environmental factors controlled. All the pots were normally watered before the implementation of the drought stress.

Seedlings were selected according to their growth uniformity and divided into two parts: the first part was put under drought stress imposition by withholding water (drought treatment formulae) since stage V3 (plants with three trifoliate leaves unrolled) until stage R1 (beginning bloom) and the second one was kept in optimal water condition (control formulae). Leaves were carefully collected before drought treatment and as plants in stages of V3, V5 and R1 for subsequent analyses. Experiments all were carried out in Lab of Plant Physiology (Vinh University, Vietnam).

Analyses

Determination of $O_2^{.-}$ in soybean leaves was based on its ability to reduce nitro blue tetrazolium-NBT (Doke, 1983). Content of H_2O_2 was determined following the spectrophotometric method (Becana *et al.*, 1986). To assess the cellular membrane damage, lipid peroxidation was determined by TBARS assay (Heath and Packer, 1968).

Activity of SOD was spectrophotometrically assayed by measuring its ability to inhibit the photochemical reduction of NBT (Beauchamp and Fridovich, 1971). Activity of CAT was determined by measuring H_2O_2 consumption (Dhindsa *et al.*, 1981). POX activity was calculated following the increase in absorbance at 470 nm followed for 90s (Seevers *et al.*, 1971). The enzyme-prepared protein was determined following method of Bradford (1976). Proline content was determined by the spetrophotometric method of Bates *et al.* (1973).

Statistical analysis

Analysis of variance (ANOVA) was applied to verify whether the statistic differences were recorded in means from experimental variants with the significant level as P-value < 0.05. Data shown in figures are means and standard errors (S.E.) of triplicates for each variant.

RESULTS AND DISCUSSION

Drought-accumulated reactive oxygen species

An accumulated generation of $O_2^{.-}$ was observed in leaves of *G. max* cv. Namdan under drought condition. Level of that ROS increased strongly from stage V3 to stage V5 and then reduced in stage R1. The highest amount of $O_2^{.-}$ recorded in stage V5 was 2.189 $A_{580}.g^{-1}$ FW (fresh weight), having by 3.11- and 2.74-fold higher than that in the beginning and in control, respectively (Fig 1). The $O_2^{.-}$ content in drought-treated plants was always significantly different from that in control (P<0.05). It should be highlighted that, level of $O_2^{.-}$ in Namdan was always higher than that in DT2008 at all investigated time points.

Similar to $O_2^{.-}$ generation, a remarkable accumulation of H_2O_2 in drought-treated plants of Namdan was recorded since stage V3, reached to the highest level in stage V5 (21.161 µM $H_2O_2.g^{-1}$ FW), then reduced in stage R1 (Fig 2). ANOVA showed the significant differences in content of H_2O_2 in drought-treated plants and controls in all estimated time (P<0.05). Content of H_2O_2 in Namdan was also much higher than that in DT2008 although this ROS in both varieties expressed the same tendency to change during experimental time.

Cellular membrane damage

Lipid peroxidation is a well-established mechanism of cellular injury and is measured by amount of TBARS. A continuous increase in content of TBARS was recorded in leaves of Namdan during investigated time and reached to maximum level in stage R1 as 21.259 µM.g^{-1} FW (Fig 3). It was noted

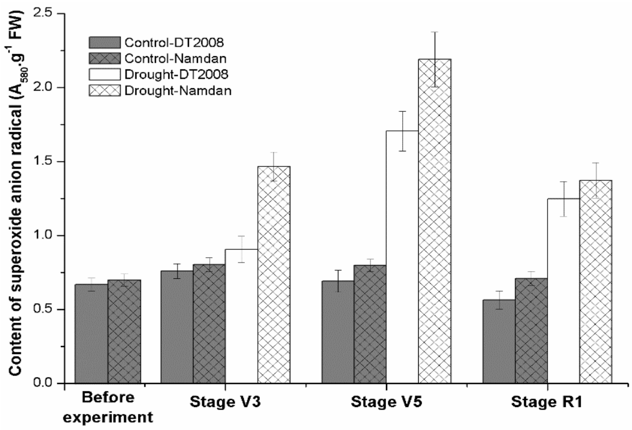

Fig 1: Content of superoxide anion radical in leaves of *G. max* cv. Namdan in comparing with *G. max* cv. DT2008 under drought stress.

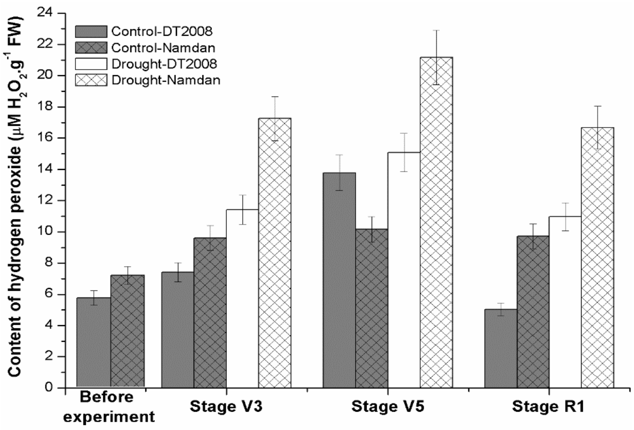

Fig 2: Content of hydrogen peroxide in leaves of *G. max* cv. Namdan in comparing with *G. max* cv. DT2008 under drought stress.

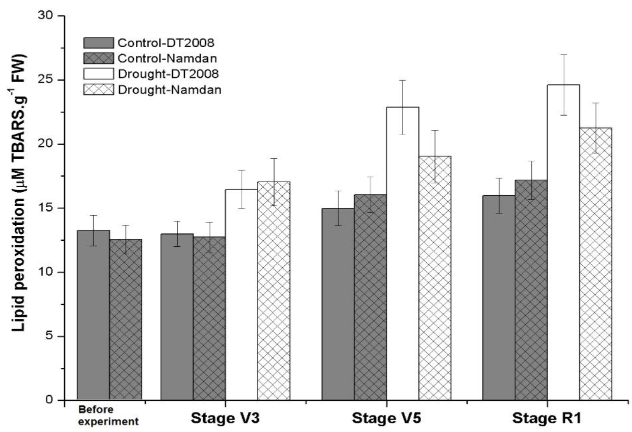

Fig 3: Lipid peroxidation in leaf of *G. max* cv. Namdan in comparing with *G. max* cv. DT2008 under drought stress.

that TBARS contents in drought-treated plants were always significant higher than in control and those substances' levels in Namdan were significantly lower than that in DT2008 within stages of V5 and R1 ($P<0.05$).

Lipid peroxidation has been associated with oxidative damages provoked by environmental stresses. In our study, the accumulation of products of lipid peroxidation such as TBARS in drought-treated plants clearly demonstrated that both soybean varieties as Namdan and DT2008 were exposed to stress conditions leading to membrane lipid peroxidation by means of $O_2^{.-}$ and H_2O_2. This result is in agreement with previous reports on soybean (Iqbal *et al*. 2019) and other crops such as wheat (Simova-Stoilova *et al*., 2009), flax (Wu *et al*., 2019), *etc*., which confirmed that the increase in lipid peroxidation in plants was directly resulted from drought condition.

Drought-accumulated activity of enzymatic antioxidants

In *G. max* cv. Namdan leaves, the drought-accumulated activity of SOD remarkably increased within stages of V3 and V5, then strongly decreased in stage R1 (Fig 4). The highest activity of SOD obtained in stage V5 was 25.307 $nkat.mg^{-1}$ protein, having by 234.77% and 267.83% higher than that in control and at the beginning, respectively. ANOVA recorded the significant difference between the SOD activities in drought-treated plants and controls within stages V3 and V5 ($P<0.05$). It is stressed that, activity of SOD in Namdan was always higher than that in DT2008 at all points of estimated time.

Our results confirmed the high activity of SOD in soybean Namdan resulted a decrease in level of $O_2^{.-}$ in the following time (Fig 1 and 4). Because of counteracting oxidative damage caused by over-generation of $O_2^{.-}$ within the living cells, SOD constitutes as one of the first line of plant defense against that ROS (Grene, 2002).

Similar to SOD, generation of CAT in leaves of Namdan was strongly stimulated under drought condition. This enzyme remarkably induced since stage V3, reached to the highest level in the V5 stage, then decreased in stage R1 (Fig 5). ANOVA revealed a significant difference between activities of CAT in drought-treated and control plants during the experimental time ($P<0.05$).

Activity of enzyme POX in Namdan was remarkably enhanced under drought condition (Fig 6). The drought-accumulated activity of POX progressively increased during the experimental time. The highest value of POX activity obtained in stage R1 was 18.025 $nkat.mg^{-1}$ protein, having by 234.77% and 267.83% higher than that observed in control and at the beginning, respectively. Activity of POX in experimental plants was statistically different from that in control ($P<0.05$). POX activity in Namdan was similar to the changing trend, however, was always higher than that in DT2008.

SOD converts the toxic $O_2^{.-}$ radical to H_2O_2 which must be scavenged by the enzymatic antioxidants such as CAT and POX. CAT is known to catalyze H_2O_2 to O_2 and water as a side product of photoreactions (Luna *et al*., 2005), whereas enzyme POX metabolizes H_2O_2 dependent oxidation reactions in plant cells (Demiral and Turkan, 2005). The accumulated activity of both CAT and POX protected soybean leaf cells from an excess of this ROS product and thus reduced considerable membrane damages.

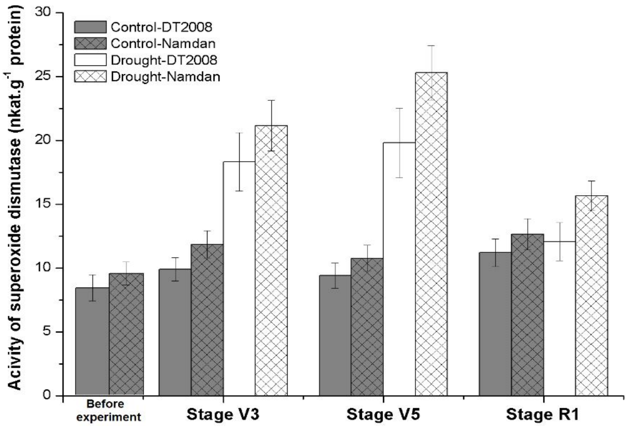

Fig 4: Activity of SOD in leaves of *G. max* cv. Namdan in comparing with *G. max* cv. DT2008 under drought stress.

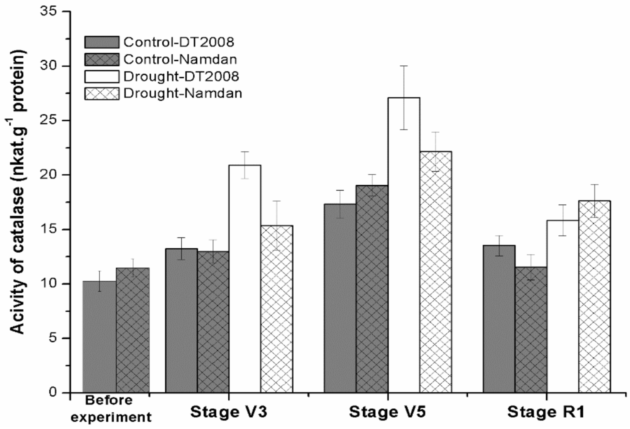

Fig 5: Activity of CAT in leaves of *G. max* cv. Namdan in comparing with *G. max* cv. DT2008 under drought stress.

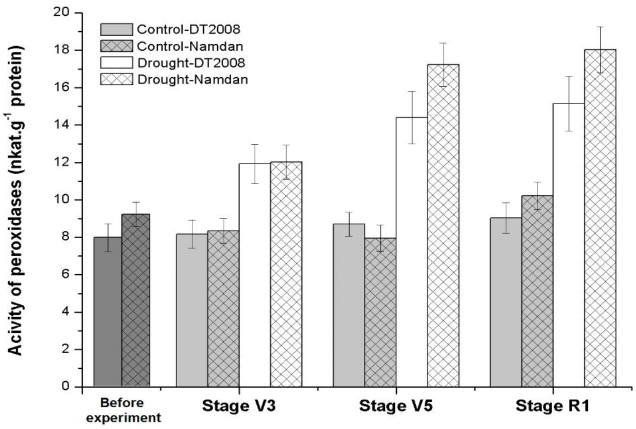

Fig 6: Activity of POX in leaves of *G. max* cv. Namdan in comparing with *G. max* cv. DT2008 under drought stress.

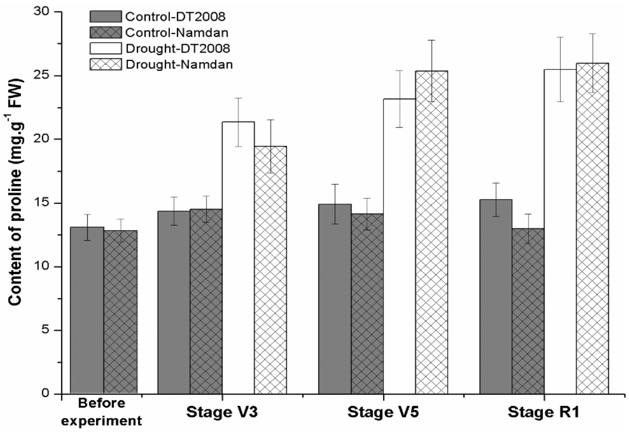

Fig 7: Content of proline in leaves of *G. max* cv. "Nam Dan" in comparing with *G. max* cv. DT2008 under drought stress.

To cope with detrimental effects of oxidative stresses under extremely adverse conditions, legume plants have developed an antioxidant defense system including the enzymatic antioxidants such as SOD, CAT and POX (Hossain and Fujita, 2009; Filippou *et al.*, 2011; Lokhande *et al.*, 2019). Activity of those enzymes in tolerant varieties are always higher than in sensitive ones under various stress conditions (Turkan *et al.*, 2005). In agreement with those reports, the accumulated activity of SOD, CAT and POX in Namdan during drought demonstrated that this soybean variety denoted its drought tolerance.

Drought-accumulated content of amino acid proline

Similar to DT2008, proline content in leaves of Namdan increased continuously in drought condition since stage V3 till stage R1, whereas that amino acid in control plants was in minor change in lower levels following the investigated time (Fig 7). Proline accumulation in leaves of drought-stressed plants has been well-documented. Our results were in agreement with previous researches, which presented a significant increase in content of proline in soybean plants under drought condition (Shen *et al.*, 2010; Sharma *et al.,* 2012). That amino acid protects the stressed cells by adjusting intercellular osmotic potential and attributed to high water retention (Heerden and Kruger, 2002). Proline accumulation also confers plant drought tolerance by protecting proteins' structure and maintaining their stability as well as by directly acting as a free radical scavenger (Verbruggen and Hermans, 2008).

CONCLUSION

Drought resulted the oxidative stress in leaves of *G. max* cv. Namdan with a burst of O_2^- and H_2O_2 generation leading to the cellular membrane damage that was probably confirmed by an increase in TBARS's content in cells. The high accumulation in activity of SOD, CAT and POX is one of the most essential elements in the antioxidative mechanism that detoxifies actively the excess of O_2^- and H_2O_2 and reduces efficiently oxidative damage. Furthermore, the remarkable enhancement of proline would protect living cells from drought stress by adjusting intercellular osmotic potential. From those results, it can suggest that *Glycine max* (L.) Merr. cv. Namdan is a drought-tolerant variety.

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