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Research Article
Oxidative Stress and Photosynthesis Reduction of Cultivated (*Glycine max* L.) and Wild Soybean (*G. tomentella* L.) Exposed to Drought and Paraquat

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Abstract

Background and Objective: Drought is an abiotic factor that significantly reduces agriculture production almost every year. Drought stress especially during excessive light results in enhancement of Reactive Oxygen Species (ROS) leading to an occurring oxidative stress. The increase of ROS also occurs in plants applied by herbicide. In this study, oxidative stress of three soybean (*Glycine max* L.) Merr) cultivars and a wild line soybean (*G. tomentella*) were analyzed in response to drought and paraquat treatments. **Methodology:** Drought treatment was performed by withholding water for 12 days (for cultivars) and 22 days (for wild line soybean) in greenhouse experiment during flower initiation. Paraquat treatment was applied using manual sprayer at the same time of drought treatment application. Plant water status and photosynthetic rate were measured during the drought treatment and after rewatering and after paraquat application. During the treatment, malondialdehyde (MDA) and the activity of Glutathione Reductase (GR) and superoxide dismutase (SOD) enzymes were measured. **Results:** Drought treatment decreased plant relative water content up to 33 and 42% in sensitive and tolerant variety respectively. Transpiration and photosynthetic rate decreased almost to zero at the end of drought period, while those of control plant were 4.7 and 12.58 μmol m^{-2} sec^{-1}, respectively. Malondialdehyde content and antioxidative enzymes GR and SOD increased substantially during the drought and paraquat application in all cultivated varieties as well as wild soybean. **Conclusion:** Drought and paraquat application induced oxidative stress in soybean cultivars as well as in wild soybean indicated by dramatic rising of ROS and the increase of malondialdehyde and antioxidative enzyme (GR and SOD) by approximately 2-3 folds but there was no clear pattern of enzyme activities between tolerant and sensitive varieties.

Key words: Drought stress, water stress, oxidative stress, paraquat, photosynthesis, soybean, wild soybean, *Glycine tomentella*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Drought is an abiotic factor that significantly reduces agriculture production almost every year. Drought stress causes the plant photosynthesis rate reduces by decreasing stomatal conductance[1-4] and may also cause the damage of the photosynthesis apparatus including photosystem I (PSI) and photosystem II (PSII) of photosynthesis[5] and loss of ATP synthase[6]. In addition, partial stomatal closure due to drought stress in C3 plants increases photorespiration[7,8] that causes the decrease of carbohydrate accumulation required for growth and seed filling.

The lower CO_2 assimilation rate caused by drought, especially during excessive light exposure, may lead to over-reduction of PSII photosynthesis reaction center[9]. This situation may result in enhancement of Reactive Oxygen Species (ROS) such as superoxide radical ions (O_2^-), hydrogen peroxides (H_2O_2) and oxygen singlets[10-15]. Reactive oxygen species are unstable substances that are harmful to the cells or tissue and a higher degree of ROS accumulation can cause cell and tissue damage[16,17] leading to oxidative stress.

A growing body of evidence indicates that drought stress increases the accumulation of ROS in many species including wheat (*Triticum aestivum* L.)[18], rice[19], maize[20], sunflower (*Helianthus annuus* L.)[16], *Cleome spinosa*[21] and some perennial plants such as *Coffea canephora*[22] and rubber tree, *Hevea brasiliensis*[23]. In soybean (*Glycine max* L.), the high degree of nodule senescence in response to drought stress is also predicted due to high accumulation of ROS during drought stress[24]. Water stress-induced abscisic acid (ABA) accumulation triggers the increased generation of ROS, which in turn, leads to the up-regulation of the antioxidant defense system[25].

The increase of ROS also occurs in plants applied by herbicide such as in pea (*Pisum sativum*)[26], grapevine[27], winter wheat, rye and maize[28], tobacco[29] and crickweed (*Malachium aquaticum*)[30] indicated by the increase of malondialdehyde or other radical compounds such as superoxide[28] anion $O_2^{\bullet -}$. This phenomenon is quite stimulating to observe further the magnitude and pattern of ROS accumulation induced by water stress as compared to that produced by herbicide treatment.

It is still unclear whether the accumulation of ROS and the elimination of this substance by antioxidative enzymes activities are different in tolerant and sensitive plants. Iturbe-Ormaetxe *et al.*[31] have concluded that tolerance to water deficit in terms of oxidative damage largely depends on the cultivar, however, little data exists indicating the differences in antioxidative enzyme activities of tolerant and sensitive plants. Some experiments in soybean have shown that the accumulation of enzymes such as superoxide dismutase (SOD) and ascorbate peroxidase (APX) were lower in drought stressed nodules of mycorrhizal plants than in non-mycorrhizal plants, whereas Glutathione Reductase (GR) activity was higher in nodules from mycorrhizal plants than in non-mycorrhizal plants[24].

In this study the analysis of photosynthetic rate, ROS accumulation and antoxidative enzyme activities of tolerant and sensitive soybean varieties (*G. max* L.) and wild soybean (*G. tomentella* L.) was carried out in response to drought stress and paraquat, a herbicide that is able to induce oxidative stress[31] by accumulation of high ROS in the plants.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse at Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor, Indonesia. The plants used in this experiment were drought-tolerant, moderately tolerant and sensitive soybean varieties (*Glycine max* L.) and a wild soybean (*Glycine tomentella* L.). A drought-tolerant variety (Tidar), a moderately tolerant variety (Burangrang) and a drought-sensitive variety (Panderman) were provided by the Indonesian Beans and Tubers Research Bureau (BALITKABI), Malang, East Java, Indonesia, while a drought-tolerant wild soybean was provided by the Life Science and Biotechnology Research Center, Bogor Agricultural University, Bogor, Indonesia.

Plant growing and treatment experiment: The plants were grown in 8 kg capacity polybags containing a mixture of soil and compost 2:1 (v/v). The plants were fertilized with nitrogen, phosphorus and potassium fertilizer (15:15:15) with the dosage of 2 g per polybag. Four seeds were sown in each polybag and then 1 week old seedlings were thinned and selected to become 2 seedlings per polybag. The experiment carried out using a completely randomized design with two factors. The first factor was plant varieties and species comprised of drought-tolerant [(1) Tidar], moderately tolerant [(2) Burangrang] and drought-sensitive varieties [(3) Panderman] and a drought-tolerant wild soybean [(4) *Glycine tomentella* L.]. The second factor was environmental stress including (1) Drought stress, (2) Paraquat application and (3) Normal watering (of control plants). The stress treatment (drought and paraquat application) was applied to 25 days old plants, when the plants started to flower. Drought stress was administered by withholding water for 12 days until the plants were heavily wilted. After the drought stress period,

the plants were rewatered to recovery (to the condition of the control plants). Paraquat application was performed by spraying paraquat on the leaves in the morning between 07:00-08:00 am with a dosage of 90 g ha^{-1} of active compound. Based on a previous experiment, this dosage did not kill the plants but it reduced by 35% the biomass dry weight after 2 weeks of treatment.

Parameters measurement and photosynthetic analysis: The parameters measured in this experiment were media water content, relative water content of plants, transpiration and photosynthetic rate, antioxidant enzymes activity and lipid peroxidation activity. Gas exchange analysis was carried out to analyze the transpiration and photosynthetic rate of the youngest fully expanded leaves using photosynthetic leaf chamber analyzer type of LCA-4 with the light intensity of approximately 950 µmol m^{-2} sec^{-1}. The photosynthetic rate was measured at 0, 4, 8, 10 and 12 days after drought stress treatment for cultivated soybean and at 0. 4, 10, 18 and 22 days after drought stress treatment for wild soybean. The measurement was again carried out 2 days after rewatering to analyze photosynthetic rate of recovered plants. For the plants treated with paraquat herbicide, the photosynthetic rate measurement was carried out before paraquat application, 4 h after application and 1, 3 and 5 days after application.

Enzymes analysis: For the analysis of antioxidant enzymes, 0.2 g of the youngest fully expanded leaf samples were ground in 4 mL solute containing 50 mM phosphate buffer (pH 7.0), 1% polyvinylpolypyrrolidone and 0.2 mM ascorbic acid, which were placed in an ice bath. The homogenate was centrifuged at 1000 rpm for 30 min at 40°C. The supernatant was used for assay of enzymes activity using method of Jiang and Huang[32] with some modification. The activity of GR was determined by following the decreased of absorbance at 340 nm for 1 min due to the glutathione dependence of NADPH[33]. The reaction mixture contained 1 mM of ethylene diamine tetra acetic acid (EDTA), 0.5 mM of glutathione disulfide (GSSG), 0.15 mM of reduced nicotinamide adenine dinucleotide phosphate (NADPH), 100 mM of phosphate buffer (pH 7.8) and 0.5 mL of extract enzyme. Glutathione reductase activity was determined per weigh of unit protein, while the protein content was determined by Bradford method[34] using bovine serum albumin as a standard. The activity of SOD enzyme was determined using method described by Beauchamp and Fridovich[35] with some modification. The measurement carried out based on the inhibition of blue diformazan with the existence of riboflavin/nitro blue tetrazolium (NBT) and light. Leaf extract (30 µL) was added to the medium containing 50 mM phosphate buffer (pH 7.8), 0.1 mM of EDTA and 0.3 mM of riboflavin. After 5 min incubation at the room temperature, the extract was added by 0.03 mM NBT and placed to an approximately 20 cm under 75 W lamp for 1 min. The initial rate of the reaction was determined by the increase of absorbance for every 30 sec at 560 nm. The solution without sample was used as control. The activity of SOD was determined as U mg^{-1} of protein, 1 U was 50% form inhibition of blue diformazan.

Lipid peroxidation analysis: Lipid peroxidation was estimated as the content of total 2-thiobarbituric acid-(TBA) substances expressed as equivalent to malondialdehyde (MDA) production as described in Ono *et al.*[36] with some modifications. Fresh leaves (0.2 g) were extracted in 0.5 mL of 0.1% (w/v) trichloracetic acid (TCA) at 4°C. The extract then was added to 3 mL of 1% H_3PO_4 and 1 mL of 0.6% TBA dissolved in 20% TCA. The solution was incubated in the oven at a temperature of 100°C for 30 min. After cooling to the room temperature, 4 mL n-butanol was added to the solution and followed by centrifugation at 4200 rpm at 28°C for 20 min. The absorbance of supernatant was then measured using a UV-Vis spectrophotometer at 532 nm and corrected for nonspecific turbidity by subtracting the absorbance at 520 nm. The concentration of MDA was calculated from its extinction coefficient (ε = 155 L $mmol^{-1}$ cm^{-1}).

Statistical data analysis: The collected data were analyzed statistically using SPSS 16 software to identify significant differences among the treatments and t-student test was applied to compare among the means.

RESULTS AND DISCUSSION

Plant water status during the drought and paraquat treatment: To analyze plant water status, Relative Water Content (RWC) of plant was measured periodically during the drought and paraquat treatments. The average of RWC of the control plants was 80.3% with small variation between 78.0-82.8% (Table 1). The drought stress treatment for 12 days significantly decreased RWC of the cultivated plant. At the last day of the drought treatment, the sensitive soybean (Panderman) had lower RWC (33%) than the tolerant (Tidar) and moderate (Burangrang) varieties (42.5 and 42.0% respectively). The most dramatic reduction of RWC occurred after 8 days of drought treatment for cultivated soybean, whereas in wild soybean *G. tomentella* it occurred after 12 days of drought stress (Table 1). *Glycine tomentella*

Table 1: Relative water content (%) of soybean leaves of Tidar, Burangrang, Panderman and *G. tomentella* in response to drought period (days)

	Drought period (days)								
	0	4	8	10	12	14	18	22	24
Soybean	(%)								
Tidar									
Control	80.8	78.0	84.8	78.7	78.0	82.8			
Drought	80.2	72.6	52.0*	49.5*	42.5*	71.9	-	-	-
Burangrang									
Control	78.8	79.0	84.2	79.5	78.7	81.1			
Drought	79.0	70.4*	50.5*	48.1*	42.0*	76.9	-	-	-
Panderman									
Control	79.8	79.4	83.9	78.1	79.0	82.3			
Drought	80.9	79.2	52.0*	40.7*	32.5*	83.7	-	-	-
Glycine tomentella									
Control	80.1	78.5	81.8	79.4	78.6	-	82.5	82.8	83.4
Drought	82.9	83.0	85.5	87.9	60.6*	-	43.0*	39.4*	84.0

Values in the same column significantly different at 5% of t-student

survived 22 days of drought period with the RWC of 39.4%. Two days after rewatering the RWC of all the plants rose again to that of the control plants.

The RWC of plants treated with paraquat was also reduced significantly 1 day after treatment. The RWC started to decline 4 h after paraquat application and the maximum reduction was observed 1 day after treatment. Three days after paraquat application, the RWC rose again for tolerant, moderate and wild soybeans, while it remained low for the sensitive variety (Table 2). Even though the RWC of wild soybean *G. tomentella* dropped dramatically one day after paraquat application, it recovered very well 3 days after application. The RWC of the paraquat-treated sensitive variety recovered 5 days after the treatment (Table 2).

Table 2: Relative water content (%) of soybean leaves of Tidar, Burangrang, Panderman and *G. tomentella* after Paraquat application and control plants

	Days after paraquat application (%)				
Soybean varieties	0	0.16	1	3	5
Control	80.8	80.8	77.9	78.0	77.0
Tidar	80.2	63.6*	63.4*	71.3	74.2
Burangrang	80.9	68.6	37.2*	61.9*	74.4
Panderman	79.0	74.9	54.0*	53.7*	79.9
G. tomentella	82.9	69.2	22.5*	79.2	62.3

Values in the same column significantly different at 5% of t-student

Gas exchange analysis: Transpiration rate (E) varied during the treatment with the average of 4.7 mmol m^{-2} sec^{-1} for control plants. During the drought stress period, the E decreased significantly and dropped almost to zero after 12 days in cultivated soybeans (Tidar, Burangrang and Panderman), whereas in wild soybean (*G. tomentella*) the maximum reduction of E occurred 22 days after the drought stress period. Three days after rewatering, the E increased again to the level of control plants (Table 3). Application of paraquat also reduced E and the reduction became significantly different from that of control plants 3 days after application.

The photosynthesis rate (Pn) measured under green house conditions with average PPFD of 950 mmol m^{-2} fluctuated during the day. The average Pn of control plants and both cultivated and wild soybean, measured between 08:00-10:00 am was 12.6 mmol m^{-2} sec^{-1}. As the drought stress period increased, the Pn reduced gradually with the maximum reduction on 12 and 22 days after drought stress treatment for cultivated and wild soybeans, respectively (Fig. 1). The Pn of the drought-sensitive soybean (Panderman) decreased more than that of the tolerant (Tidar) and moderate (Burangrang) varieties after 8 and 10 days drought stress treatment (Fig. 1). At the period of maximum drought stress, the Pn of all plants dropped to near zero. Meanwhile, rewatering increased the Pn back to the normal (control) condition 2 days after rewatering (Fig. 1).

Paraquat treatment also caused significant reduction of the Pn of all treated plants. Even though Pn reduction was detected 4 h after paraquat application, significant reduction of Pn occurred 1 day after application (Fig. 2). The reduction of Pn continued until it reached the minimum level (approximately 0.14 mmol m^{-2} sec^{-1}) on the 3rd day after application. The Pn increased again 5 days after the plants recovered from stress. In contrast to the Pn recovery after drought stress, the recovery of Pn 5 days after paraquat application did not return back to the level of the control plants (Fig. 2).

Lipid peroxidation: Lipid peroxidation was analyzed by the measurement of MDA accumulation in the leaf tissues as a

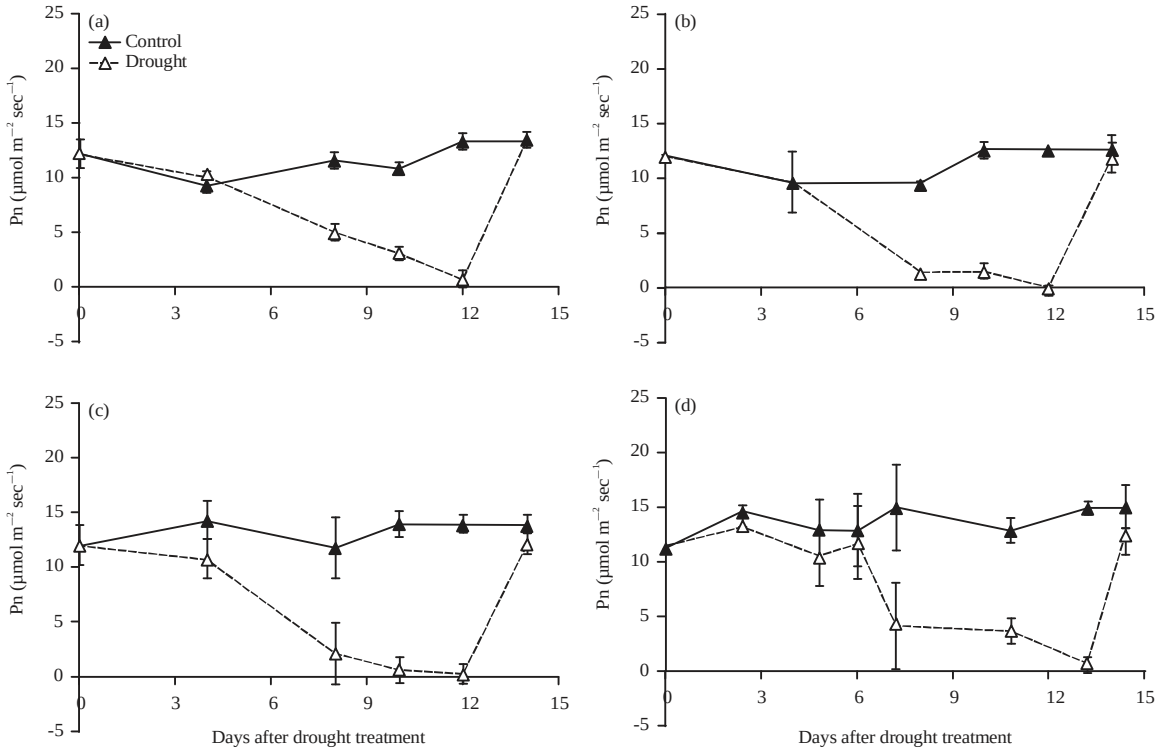

Fig. 1(a-d): Photosynthetic rate (Pn) of cultivated soybean varieties (a) Tidar, (b) Burangrang, (c) Panderman and (d) Wild soybean *Glycine tomentella* during drought period (Mean+SE of t-student test at α of 5%, n = 3)

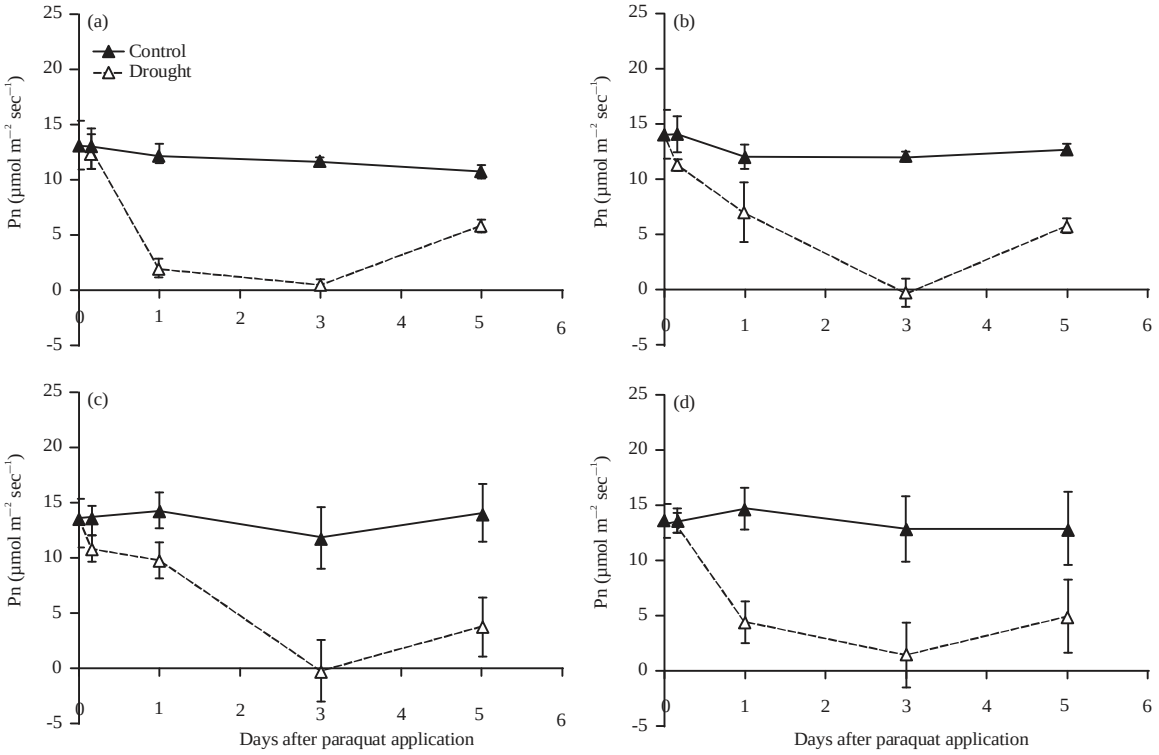

Fig. 2(a-d): Photosynthetic rate (Pn) of cultivated soybean varieties (a) Tidar, (b) Burangrang, (c) Panderman and (d) Wild soybean *Glycine tomentella* during 5 days after paraquat application (Mean+SE of t-student test at α of 5%, n = 3)

result of membrane lipid degradation. At normal conditions indicated by the control plants, the average MDA level in the leaves was 3281 nmol g^{-1} fresh weight. The MDA level increased in drought-stressed plants (Fig. 3). Generally, the MDA level of cultivated soybean significantly increased after 8 days of drought treatment when the plant started wilting. However, in *G. tomentella* MDA levels started to increase when mild drought stress was reached (4 days after drought treatment). The maximum level of MDA (5890 nmol g^{-1} fresh weight) or almost twice that of control plants was reached at 10 days when severe drought stress occurred. After rewatering, the MDA level decreased to that of the control plants or even lower in sensitive variety Panderman (Fig. 3).

Application with paraquat herbicide also increased MDA level in all treated plants 1 day after treatment (Fig. 4). However, the maximum level of MDA was lower in paraquat application compared to that of drought treatment. Five days

Table 3: Transpiration rate (mmol m^{-2} sec^{-1}) of Tidar, Burangrang, Panderman and *G. tomentella* in response to drought period (days)

	Drought period (days)								
	0	4	8	10	12	14	18	22	24
Soybean	(mmol m^{-2} sec^{-1})								
Tidar									
Control	5.3	4.9	4.2	4.5	5.1	5.0			
Drought	4.8	3.7	1.6*	1.1*	0.5*	3.8*	-	-	-
Burangrang									
Control	5.0	3.6	3.7	3.9	5.3	5.1			
Drought	5.1	1.9*	2.0*	0.7*	0.3*	3.8*	-	-	-
Panderman									
Control	4.8	4.7	4.2	3.8	4.6	4.7			
Drought	4.9	3.9	0.5*	0.3*	0.0*	4.2*	-	-	-
Glycine tomentella									
Control	6.5	6.4	4.5	4.4	5.2	-	4.7	5.1	5.1
Drought	6.4	6.2	3.8	2.6	1.4*	-	0.9*	0.4*	3.8*

Values in the same column significantly different at 5% of t-student

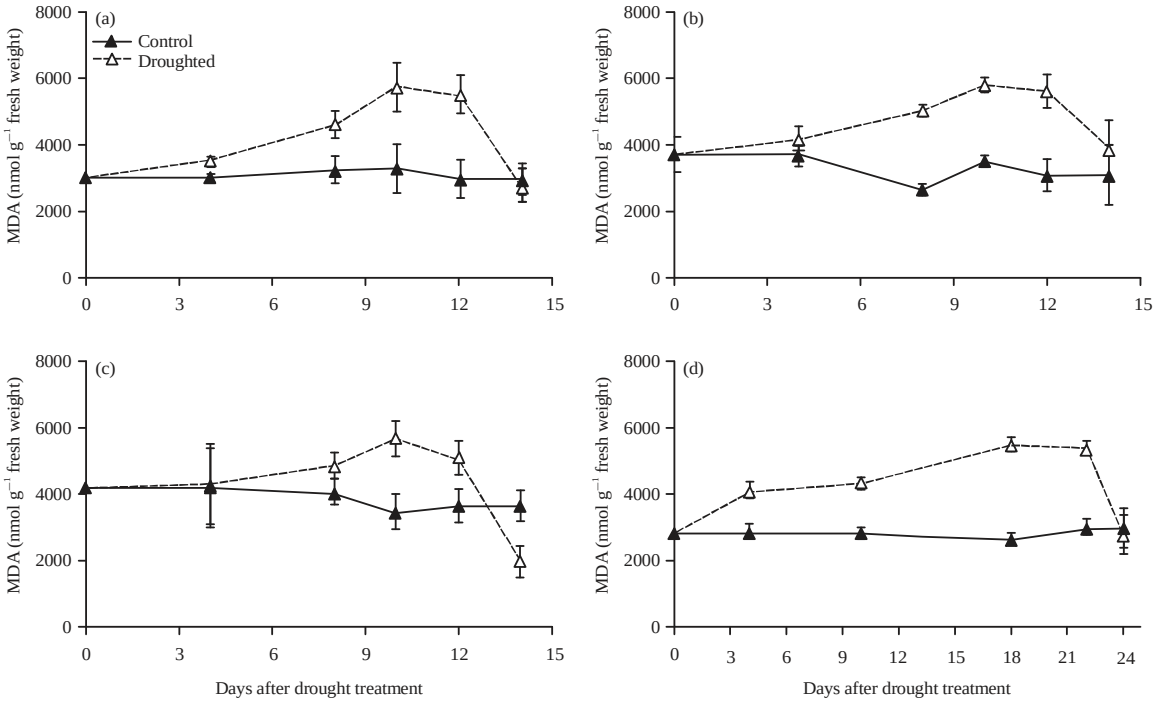

Fig. 3(a-d): Malondialdehyde (MDA) content of soybean leaves of (a) Tidar, (b) Burangrang, (c) Panderman and (d) Wild soybean *Glycine tomentella* during drought period (Mean+SE of t-student test at α of 5%, n = 3)

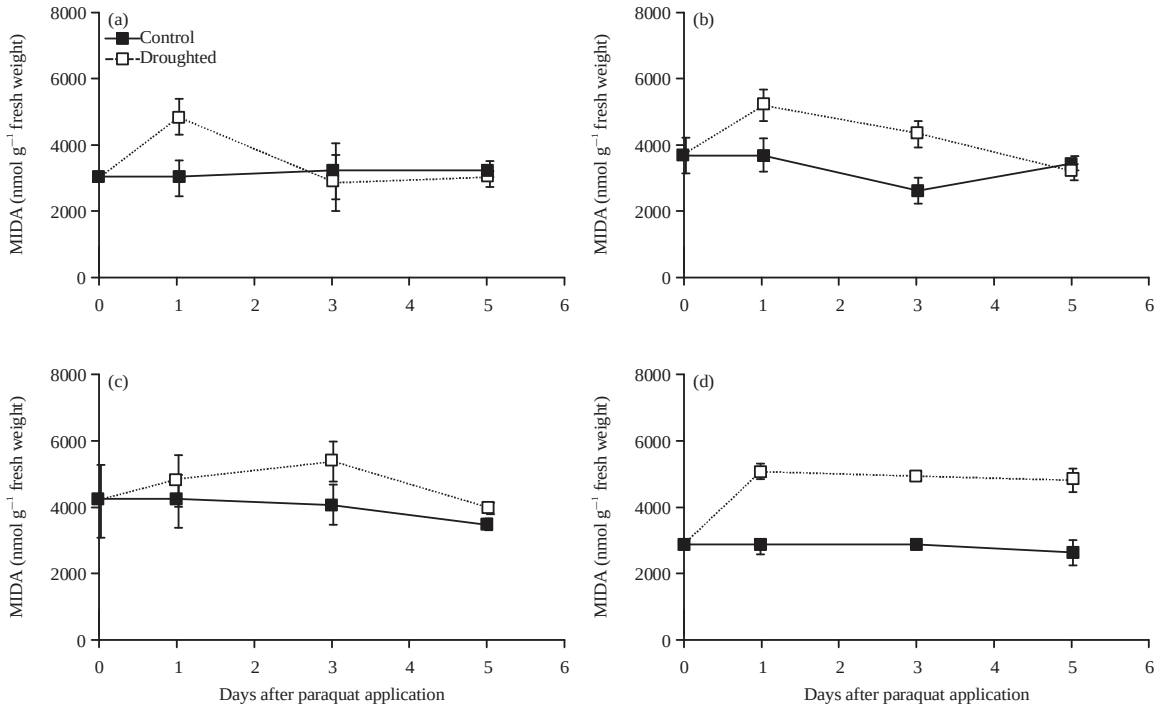

Fig. 4(a-d): Malondialdehyde (MDA) content of soybean leaves of (a) Tidar, (b) Burangrang, (c) Panderman and (d) Wild soybean *Glycine tomentella* 5 days after paraquat application (Mean+SE of t-student test at α of 5%, n = 3)

after paraquat application, the MDA concentration decreased again to the level of that in the control plants, except in *G. tomentella*, which remained unchanged (Fig. 4).

Antioxidative enzyme activity: Drought stress caused an increase of antioxidative enzymes (GR and SOD) activity in all soybean starting from mild drought. The highest activity of the enzymes was recorded after 8 days of the drought for cultivated soybean and the value decreased again at the end of drought period (12 days). There was no different pattern of enzyme activities between tolerant and sensitive soybean varieties (Fig. 5, 6). In *G. tomentella* there was different pattern of GR and SOD enzyme activities, where the activity of GR enzyme increased gradually and reached the maximum level at the end of drought period, while it was not occurred in SOD enzyme (Fig. 5, 6). Only wild soybean that had the highest GR activity during the last period of drought.

The response of antioxidative enzyme activities (GR and SOD) to paraquat treatment was different between GR and SOD. The GR activities tended to increase linearly in response to paraquat treatment starting from the beginning of treatment until 5 days after herbicide application. On the other hand, SOD enzyme was highly active 1 day after herbicide application and then the activity tended to decrease slowly until 5 days after application. There was almost similar pattern of SOD activity in response to paraquat application for all soybean varieties as well as wild soybean (Fig. 7, 8).

Water balance and gas exchange inside the plant during drought stress: Water deficit in plants occurs when water loss due to the transpiration of the leaves exceeds water absorption by the plant roots[37]. When this happens, the plant generally tends to reduce water loss by reducing transpiration rate as indicated by Table 3. Lower transpiration in response to drought stress is associated with the decrease of stomatal conductance which is sensitive to water deficit even before the water potential of the plant decreases[38,39]. The different responses of E and Pn to drought stress treatment between cultivated soybeans and wild soybean (*G. tomentella*), where those parameters dramatically reduced after 12 days in cultivated soybeans varieties, while in *G. tomentella* the reduction occurred after 22 days after drought period may be explained by differences in growth and canopy development. The cultivated soybeans Tidar, Burangrang and Panderman had similar growth and canopy development, while wild soybean *G. tomentella* grew more slowly with smaller leaves than cultivated plants. Therefore, the water was lost from the media through transpiration and consequently caused reduction of RWC of cultivated soybeans faster than wild one (*G. tomentella*).

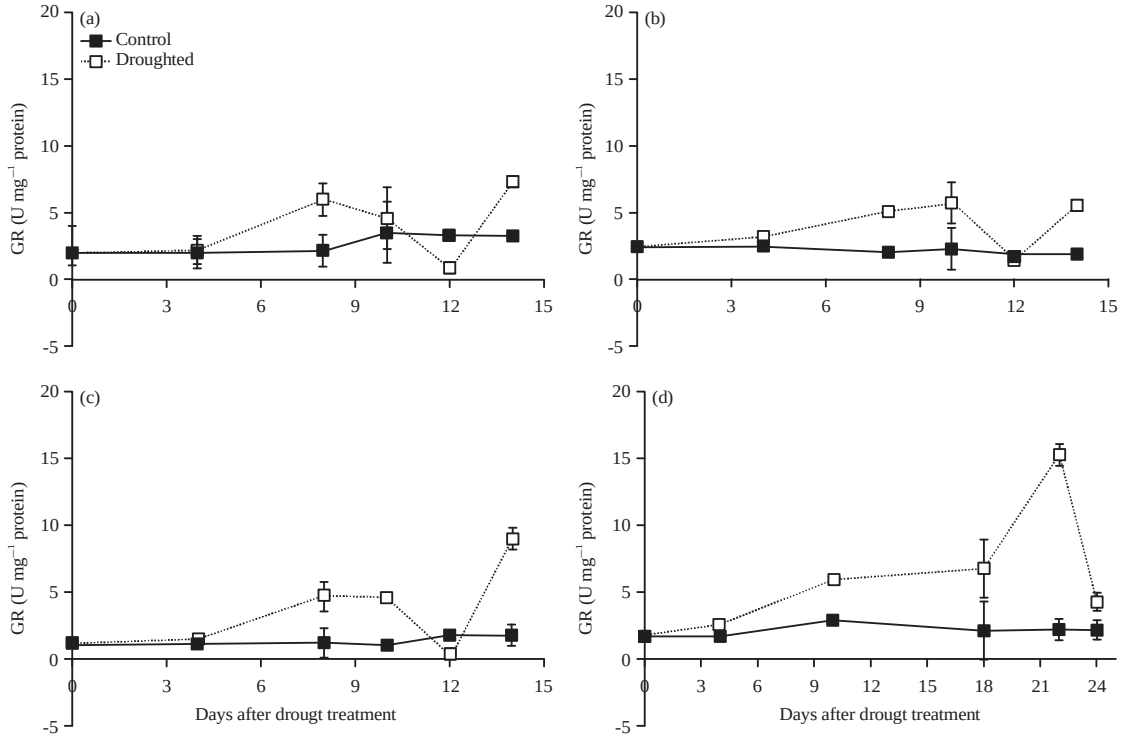

Fig. 5(a-d): Glutathione Reductase Enzyme (GR) of soybean leaves of (a) Tidar, (b) Burangrang, (c) Panderman and (d) Wild soybean *G. tomentella* during drought period (Mean+SE of t-student test at α of 5%, n = 3)

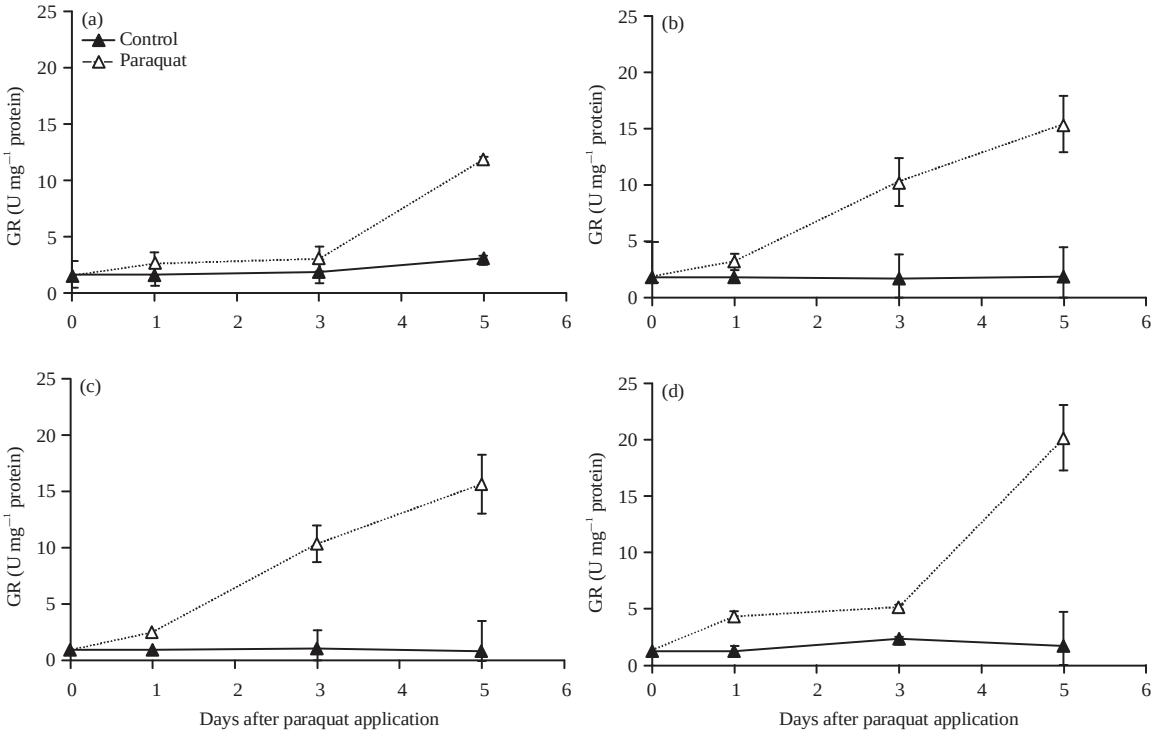

Fig. 6(a-d): Glutathione Reductase Activity (GR) of of soybean leaves of (a) Tidar, (b) Burangrang, (c) Panderman and (d) Wild soybean *G. tomentella* 5 days after paraquat application (Mean+SE of t-student test at α of 5%, n = 3)

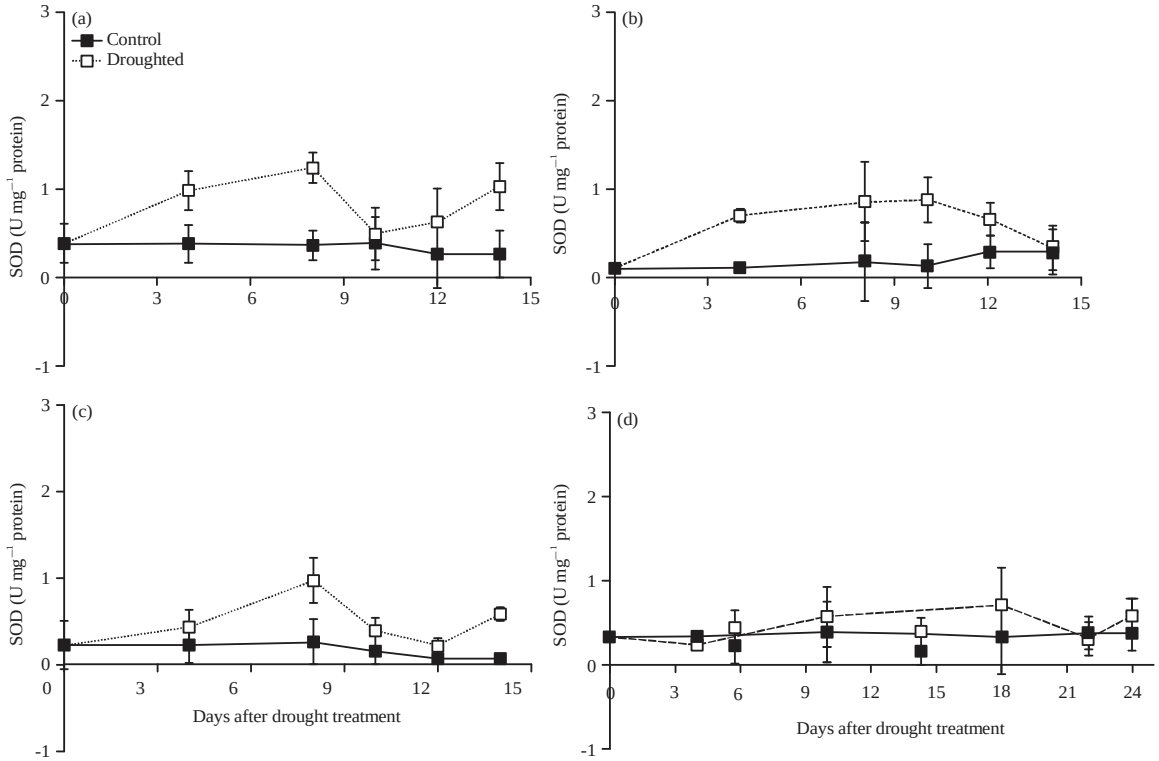

Fig. 7(a-d): Superoxide dismutase (SOD) activity of soybean leaves of (a) Tidar, (b) Burangrang, (c) Panderman and (d) Wild soybean *G. tomentella* during drought period (Mean+SE of t-student test at α of 5%, n = 3)

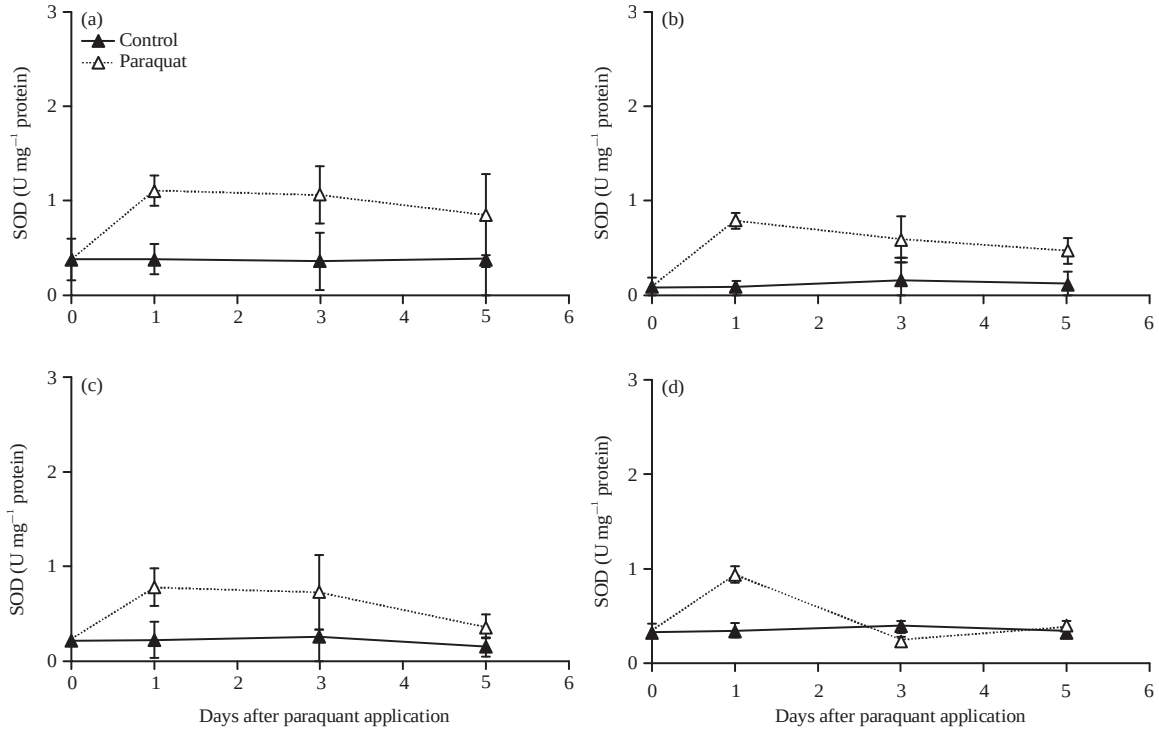

Fig. 8(a-d): Superoxide dismutase (SOD) activity of a soybean leaves of (a) Tidar, (b) Burangrang, (c) Panderman and (d) Wild soybean *G. tomentella* 5 days after paraquat application (Mean+SE of t-student test at α of 5%, n = 3)

The reduction of transpiration rate as a response to drought stress was in accordance with the decrease of photosynthetic rate that dramatically declined after 8 days of drought (Table 3, Fig. 1). The decrease of stomatal conductance reduced CO_2 supply to the chloroplast and then reduced photosynthetic rate[37]. Stomatal conductance is the main factor that influences photosynthesis reduction in plants exposed to drought stress[2].

The application of paraquat herbicide also reduced transpiration as well as photosynthetic rate, however, the reduction of the Pn was faster than the reduction of E (Fig. 2), even though the maximum reduction of Pn occurred as the same time as that of E (3 days after paraquat application). Paraquat is an active compound that accepts electrons from the early acceptors of photosystem I and then reacts with oxygen to form superoxide, a free radical[40]. High accumulation of ROS inside the leaf may cause damage to chloroplast components, especially lipids, which consequently can reduce photosynthetic rate. This herbicide is also known to induce oxidative stress specified by the increase of hydrogen peroxide and malondialdehyde inside the cell and induce ultrastructural changes[41,42]. The evidence of chlorophyll content reduction in *Amaranthus caudatus*, *Celocia argentea* and *Corchorus olitorius* due to paraquat exposure has been obtained by Akinloye *et al.*[43].

The increase of E and Pn back to the level of control plants 2 days after drought-stressed plants were rewatered, indicated that all the plants were capable of recovery after drought (Fig. 2). On the other hand, the E and Pn of paraquat treated plants did not recover well until 5 days after paraquat application (Fig. 2). This treatment may cause damage to photosynthetic and other cellular apparatus, causing malfunction of these components and the need to be rebuilt by the growth of new shoots. Paraquat is a compound that can induce the accumulation of free radicals that cause cellular damage[44,45].

Oxidative stress induced by severe drought stress as well as paraquat: The increase of MDA in all of the soybean plants during severe drought stress indicated that severe drought stress can induce oxidative stress in both cultivated and wild soybean plants. The MDA increased dramatically 8 days after the induction of drought stress and reached the maximum level on the 10th and 12th days of drought when the stress was most severe (Fig. 3). The MDA is a compound resulting from lipid peroxidation at the cellular level and is frequently used as an indicator of lipid peroxidation level due to oxidative stress[31].

The increase of MDA also occurred in the plants treated by paraquat herbicide (Fig. 4). This was also in agreement to the result presented by Akinloye *et al.*[43] that the increase of MDA was associated to the level of paraquat treatments in *A. caudatus*, *C. argentea* and *C. olitorius*. This herbicide is a compound that can be reduced by photosynthetic reaction to become an unstable free radical. This radical compound can be oxidized back by oxygen to form the original ions and hydrogen peroxide (H_2O_2), which significantly inhibits photosynthesis especially photosystem I[46] and also destroys cells and tissues[44]. When this happens, the plant undergoes lipid peroxidation, protein degradation, DNA denaturation and pigment damage[45]. At the cellular level, it causes damage to the cellular membrane and chloroplast[44]. The fact that the increase of MDA levels after 10 days of drought stress was approximately the same as that of the paraquat application, indicated that oxidative stress may be induced by severe drought. The level of MDA decreased to that of the control plant 2 days after the drought-stressed plants were rewatered. The same result has also been demonstrated by Zhang and Kirkham[47] on wheat and Wang and Huang[48] on bluegrass. This seems to indicate that the plants had recovered from oxidative stress after 2 days of rewatering.

The increase of oxidative stress due to drought stress was also confirmed by the dramatic escalation of antioxidative enzymes GR and SOD in all soybean by approximately 2-3 times in response to drought as well as to paraquat application (Fig. 5-8). The SOD is widely known as antioxidative enzyme in many species in response to environmental stress especially drought stress such as in oilseed rape[49], chickpea[50], rice[51] and wheat[52]. Drought stress promotes oxidative stress due to over-generation of ROS and the modulation of the antioxidant defense system is one of the important strategies responsible for drought resistance[52]. Antioxidative enzymes have been confirmed to become an efficient scavenger of ROS such as H_2O_2 produced during drought, which may result in better protection especially for tolerant plant[51].

CONCLUSION

Drought stress caused gradual decrease of relative water content and photosynthesis of three cultivars and one wild line of soybean. Photosynthesis reduction was also occurred due to paraquat treatment with more rapid response, even though there was no correlation with the decrease of water status. The ROS was dramatically accumulated in response to drought as well as paraquat application showed by the

increase of malondialdehyde and antioxidative enzyme GR and SOD by approximately 2-3 folds during severe drought and 1 day after paraquat application suggesting that plants underwent oxidative stress due to severe drought stress. There was no particular distinct level of GR and SOD among tolerant and sensitive cultivars during the period of drought but GR level was almost 3 times higher in wild soybean.

SIGNIFICANT STATEMENT

- Drought stress and paraquat caused Reactive Oxygen Species (ROS) development in soybean cultivar (*Glycine max* L.) and wild soybean (*Glycine tomentella* L.)
- Photosynthesis (Pn) decreased dramatically due to severe drought but recovered completely 2 days after rewatering
- Paraquat application also decreased Pn soon after application but did not recovered completely after several days
- Drought and paraquat induced of antioxidative enzyme activity (GR and SOD) dramatically in cultivar as well as wild soybean

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