ANTIOXIDANT RESPONSES OF *Arachis hypogaea* L. 'Sen' PLANTS TO DROUGHT

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Abstract: *Arachis hypogaea L. 'Sen' is a local peanut cultivar that exhibits desirable agronomic traits to be a promising crop in Nghe An province. The present study aimed to evaluate the antioxidant responses of Sen plants under drought conditions. Drought triggered oxidative stress that was recorded by a burst of superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in Sen peanut leaves. A progressive enhancement in the activities of antioxidative enzymes, including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX), was consistently observed throughout the experiment, effectively facilitating the detoxification of excess endogenous $O_2^{\cdot-}$ and H_2O_2. Furthermore, an increased accumulation of proline appeared to be part of the defense mechanism of Arachis hypogaea L. 'Sen' against oxidative stress caused by drought.*

Keywords: *Sen peanut, drought, oxidative stress, antioxidative enzyme, amino acid.*

1. Introduction

Drought is recognized as one of the most serious abiotic factors affecting plant growth and development. Under water-deficit conditions, plants often face overproduction of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion radical ($O_2^{\cdot-}$), which can trigger oxidative stress leading to cellular damage [2]. To mitigate these negative effects, plants activate antioxidative defense mechanisms including antioxidative enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and peroxidase (POX, EC1.11.1.7), which regulate ROS production and facilitate its detoxification [14]. Additionally, proline, a nitrogen-containing amino acid with antioxidant properties, also is known to play a crucial role in enhancing plant tolerance under drought stress [8].

Sen (also known as "Sen Nghe An"), a local cultivar of peanut (*Arachis hypogaea* L.), exhibits desirable agronomic traits, including high yield, large and uniform seeds, thin seed coat, and adaptability to various soil conditions in Nghe An province. To date, information on its physiological defense mechanisms against abiotic stresses is limited. Therefore, this study aimed to evaluate the antioxidant responses of Sen peanut plants to drought via assessing accumulation of $O_2^{\cdot-}$, H_2O_2, and enhanced activity of SOD, CAT, POX as well as content of proline.

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2. Materials and Methods

2.1. Material and experiment

The antioxidant expression of Sen cultivar was evaluated by using L20, a drought-tolerant peanut cultivar, as the control. Seeds of Sen and L20 peanuts were provided by the Nghe An Agricultural Extension Center.

For each peanut cultivar, uniform seeds were placed on Petri dishes and incubated in the dark at 20ºC for 3 days to ensure germination. Seedlings were cultured in 30 cm pots containing alluvium added macro minerals (each experimental formula included 30 pots; 3 seedlings per pot), and placed under controlled conditions with temperature of 23±2ºC, 65-70% relative humidity, and light intensity of 100-110 µmol photons m^{-2} s^{-1} with a 12h light/12h dark photoperiod.

Experiments included two equal parts:

Drought-treated plants: Seedlings were subjected to drought stress by withholding water at the plantlet stage (40 - 50 days after planting) and the flowering stage (70 - 80 days after planting). Watering of the drought-treated plants were withheld until tensiometer (USA, model 2725 ARL 12, plug in pot about 15 cm deep) reached -80 kPa, then watered again.

Control plants: Peanut seedlings were kept in 70% soil moisture.

Leaves were carefully collected before drought treatment, at the plantlet stage and at the flowering stage for subsequent analyses.

All experiments were conducted in the specialized labs of Vinh University in the years of 2024 - 2025.

2.2. Analytical methods

Relative accumulation of $O_2^{\cdot-}$ in peanut leaves was determined by spectrophotometry based on its ability to reduce nitro blue tetrazolium (NBT, a chemical compound composed of two tetrazole moieties, served as the oxidant) according to the procedure described in [4] and modified in [8]. Generation of H_2O_2 was analyzed following the eFOX (ferrous oxidation-xylenol orange) assay [9].

Activity of SOD (EC 1.15.1.1) was measured spectrophotometrically through its inhibitory effect on the photochemical reduction of NBT [1]. Activity of CAT (EC 1.11.1.6) was determined by monitoring the decomposition of H_2O_2 over in a specified incubation period [5]. Activity of POX (EC1.11.1.7) was assessed using a spectrophotometric method as described previously [10].

Proline content was determined following the colorimetric analysis [11].

2.3. Statistical analysis

All measurements were performed in three independent experiments. Analysis of variance (ANOVA) was conducted to determine whether means from independent analyses differed significantly, with the significant level as P-value < 0.05. Data presented in tables are expressed as means and standard errors (s.e.) of triplicates for each treatment.

3. Results and discussion

3.1. Drought-triggered reactive oxygen species

Drought triggered the strong generation of endogenous ROS in Sen and L20 peanut plants (*Table 1 and Table 2*).

Table 1 shows that a significant accumulation of $O_2^{\cdot-}$ was observed in leaves of Sen peanut under drought conditions. The generation of that free radical increased from the beginning of the experiment (before drought treatment) to the flowering stage, at which the highest level of $O_2^{\cdot-}$ was 1.97 A_{580} g^{-1} FW (fresh weight). That level was 3.79-fold higher than in the beginning of the experiment and 2.32-fold higher than that in control plants at the same stage. Compared with the L20 cultivar, levels of $O_2^{\cdot-}$ in Sen plants were always higher in all investigated time points. It is noted that, $O_2^{\cdot-}$ content in drought-treated plants of both Sen and L20 cultivars differed significantly from that of the respective control plants ($P<0.05$).

***Table 1**. Relative accumulation of superoxide anion radical in the leaves of Sen and L20 peanut plants*

Peanut cultivars	Treatments	Relative accumulation of superoxide anion radical *(A_{580} g^{-1} FW)*		
		Before drought treatment	Drought-treated stage	
			Plantlet	Blossom
Sen	Control	0.56 ± 0.09^{c}	0.67 ± 0.08^{c}	0.85 ± 0.09^{bc}
	Drought		0.92 ± 0.06^{b}	1.97 ± 0.13^{a}
L20	Control	0.53 ± 0.06^{d}	0.59 ± 0.07^{cd}	0.65 ± 0.08^{c}
	Drought		0.73 ± 0.10^{b}	1.41 ± 0.16^{a}

Note: Values are means ± s.e. (n = 3). Different letters within the same peanut cultivar indicate significant differences according to ANOVA at $P < 0.05$.

Similar to $O_2^{\cdot-}$ generation, the content of H_2O_2 in Sen and L20 cultivars showed similar trends. A significant accumulation of H_2O_2 in drought-treated plants was observed to increase throughout experiments. The maximum content obtained in leaves of Sen plants at flowering stage was 18.41 μM g^{-1} FW (*Table 2*). Compared to the L20 cultivar, the content of this ROS in the Sen cultivar was also much higher at all investigated time points. It is noteworthy that the significant differences in H_2O_2 content between drought-treated plants and the controls were recorded in both Sen and L20 cultivars throughout experiment ($P<0.05$).

***Table 2**. Relative accumulation of hydrogen peroxide in the leaves of Sen and L20 peanut plants*

Peanut cultivars	Treatments	Relative accumulation of hydrogen peroxide *(μM g^{-1} FW)*		
		Before drought treatment	Drought-treated stage	
			Plantlet	Blossom
Sen	Control	6.27 ± 0.95^{d}	8.36 ± 0.91^{c}	9.82 ± 0.76^{c}
	Drought		15.27 ± 0.96^{b}	18.41 ± 1.24^{a}
L20	Control	4.99 ± 0.82^{d}	6.45 ± 0.59^{cd}	7.87 ± 0.93^{c}
	Drought		11.86 ± 1.06^{b}	13.12 ± 1.46^{a}

Note: Values are means ± s.e. (n = 3). Different letters within the same peanut cultivar indicate significant differences according to ANOVA at $P < 0.05$.

3.2. Drought-induced activity of antioxidative enzymes

Drought-accumulated activities of antioxidative enzymes such as SOD, CAT, and POX in both peanut cultivars were presented in *Tables 3, 4 and 5*.

Biosynthesis of SOD in the Sen cultivar increased significantly from the beginning of the experiment to the flowering stage. However, the enhanced SOD activity in Sen plants was always lower than that in L20 cultivar (*Table 3*). The highest SOD activity in the leaves of Sen plants at the flowering stage was 24.76 nkat mg^{-1} protein, which was 2.38- and 1.63-fold higher than in the beginning and the control, respectively. Significant differences in SOD activity between drought-treated plants and controls were observed across all experimental stages ($P<0.05$).

Table 3. *Activity of SOD in the leaves of Sen and L20 peanut plants*

Peanut cultivars	**Treatments**	**Activity of SOD** *(nkat mg^{-1} protein)*		
		Before drought treatment	**Drought-treated stage**	
			Plantlet	**Blossom**
Sen	Control	10.41 ± 1.26^{d}	12.39 ± 1.07^{cd}	14.97 ± 1.16^{c}
	Drought		22.91 ± 2.50^{b}	24.76 ± 2.87^{a}
L20	Control	11.07 ± 1.33^{d}	12.70 ± 1.43^{cd}	13.46 ± 1.53^{c}
	Drought		26.46 ± 2.07^{b}	31.62 ± 2.93^{a}

Note: Values are means ± s.e. (n = 3). Different letters within the same peanut cultivar indicate significant differences according to ANOVA at P < 0.05.

Similar to the changes in SOD accumulation, generation of CAT was also induced under drought conditions, and increased during the experiments. However, CAT activity in the Sen cultivar was always lower than that in the L20 cultivar at all investigated times (*Table 4*). The highest CAT activity in Sen plants was 18.08 nkat mg^{-1} protein at the flowering stage, which was 1.23- and 1.92-fold higher than that in the control and at the beginning of the experiment, respectively. Interestingly, the significant differences between enzyme activities in drought-treated and control plants were obtained in both two peanut cultivars during the experimental time ($P<0.05$).

Table 4. *Activity of CAT in the leaves of Sen and L20 peanut plants*

Peanut cultivars	**Treatments**	**Activity of CAT** *(nkat mg^{-1} protein)*		
		Before drought treatment	**Drought-treated stage**	
			Plantlet	**Blossom**
Sen	Control	9.42 ± 0.68^{d}	10.59 ± 0.98^{cd}	14.67 ± 1.86^{c}
	Drought		12.53 ± 1.84^{c}	18.08 ± 1.34^{a}
L20	Control	8.46 ± 0.77^{d}	10.81 ± 0.81^{cd}	15.37 ± 1.35^{c}
	Drought		17.07 ± 1.02^{b}	22.10 ± 2.47^{a}

Note: Values are means ± s.e. (n = 3). Different letters within the same peanut cultivar indicate significant differences according to ANOVA at P < 0.05.

The activity of the enzyme POX in Sen peanut leaves was also enhanced under drought conditions (*Table 5*). The drought-accumulated activity of POX continuously increased throughout the experimental period. The highest activity of POX obtained at the stage of blossom was 9.84 nkat mg^{-1} protein, which was 1.28-fold higher than in the control and 1.98-fold higher than at the beginning of the experiment, respectively. Although POX activity in Sen plants was similar to that observed in L20 cultivar, activities of POX in drought-treated plants in both two peanut cultivars significantly differed from those in controls ($P<0.05$).

***Table 5**. Activity of POX in the leaves of Sen and L20 peanut plants*

Peanut cultivars	Treatments	Activity of POX *(nkat mg^{-1} protein)*		
		Before drought treatment	Drought-treated stage	
			Plantlet	Blossom
Sen	Control	4.98 ± 0.47^{d}	5.81 ± 0.53^{cd}	7.66 ± 0.63^{c}
	Drought		8.07 ± 0.63^{b}	9.84 ± 0.77^{a}
L20	Control	4.76 ± 0.61^{d}	5.72 ± 0.47^{cd}	8.41 ± 0.86^{bc}
	Drought		7.98 ± 0.52^{b}	10.02 ± 0.73^{a}

Note: Values are means ± s.e. (n = 3). Different letters within the same peanut cultivar indicate significant differences according to ANOVA at $P < 0.05$.

Among the antioxidative enzymes, SOD converts the toxic $O_2^{\cdot-}$ radical to H_2O_2, after which CAT catalyzes the decomposition of H_2O_2 to O_2 and water as a by-product of photoreactions [7]. Meanwhile POX utilizes H_2O_2 to drive a variety of oxidation reactions in plant cells [3]. In the present study, increased antioxidant enzyme activities were generally associated with lower ROS accumulation, and vice versa. Compared with the L20 drought-tolerant cultivar, Sen plants exhibited lower activities of SOD and CAT together with higher accumulation of $O_2^{\cdot-}$ and H_2O_2, suggesting a relatively lower efficiency of ROS detoxification in this cultivar. Nevertheless, drought stress still markedly induced the activities of SOD, CAT and POX in Sen plants, indicating the activation of an adaptive antioxidative defense mechanism to maintain cellular redox homeostasis under water deficit conditions.

These findings suggest that drought tolerance in peanut is closely associated with the efficiency of the antioxidant defense system. Although the antioxidative capacity of Sen cultivar appeared lower than that of L20, the substantial induction of antioxidative enzymes demonstrates that Sen peanut possesses a moderate physiological adaptation to drought stress.

3.3. Drought-enhanced content of proline

The content of the amino acid proline in Sen and L20 drought-treated plants increased continuously from the beginning of drought treatment till the flowering stage, whereas that amino acid in control plants changed slightly in lower levels during experiments. However, lack of the statistical difference between values of proline in Sen and L20 cultivars was recorded although the content of that amino acid in the leaves of Sen plants was always higher than that in L20 plants (*Table 6*).

***Table 6**. Content of proline in the leaves of Sen and L20 peanut plants*

Peanut cultivars	Experimental formulae	Content of proline *(µmol g^{-1} FW)*		
		Before drought treatment	Drought-treated stage	
			Plantlet	Blossom
Sen	Control	10.71 ± 0.95^{d}	11.84 ± 1.04^{cd}	13.18 ± 1.12^{c}
	Drought		14.53 ± 1.36^{b}	20.69 ± 2.06^{a}
L20	Control	10.62 ± 0.97^{d}	11.72 ± 1.17^{cd}	12.51 ± 1.27^{c}
	Drought		13.96 ± 1.20^{b}	18.90 ± 1.74^{a}

Note: Values are means ± s.e. (n = 3). Different letters within the same peanut cultivar indicate significant differences according to ANOVA at $P < 0.05$.

Our results are consistent with previous studies showing that proline accumulation is an important component of plant defense mechanisms under drought stress [6, 12, 13]. The remarkable increase in proline content observed in Sen drought-stressed plants suggests an adaptive metabolic adjustment to water deficit conditions. Proline is known to function as an osmoprotectant by regulating cellular osmotic balance and improving water retention capacity in stressed tissues [6]. In addition, proline contributes to membrane stabilization, protection of protein structure and scavenging of reactive oxygen species, thereby helping maintain cellular integrity under oxidative stress conditions [13]. The higher proline accumulation recorded in Sen plants compared with L20 may indicate that proline metabolism plays a compensatory protective role in this cultivar when the activities of enzymatic antioxidants such as SOD and CAT are relatively lower.

4. Conclusion

Drought triggered oxidative stress in Sen peanut leaves through an increased accumulation of $O_2^{\cdot-}$ and H_2O_2. The enhanced activities of antioxidative enzymes, including SOD, CAT and POX, and the increased content of the amino acid proline were essential elements in the antioxidative mechanism to protect Sen cells from damages of the excess of $O_2^{\cdot-}$ and H_2O_2. It is initially recorded in Sen drought-treated plants that the activities of antioxidative enzymes such as SOD and CAT were significantly lower, and the proline content was higher, whereas POX activity was similar to that in L20, a drought-tolerant cultivar. Those physiological and biochemical responses suggest that the Sen peanut cultivar possesses a moderate adaptive capacity to drought stress and may represent a valuable local genetic resource for future peanut breeding programs targeting drought-prone regions under climate change conditions.

References

[1] C. Beauchamp, I. Fridovich (1971), *Superoxide dismutase, improved assays and an assay applicable to acrylamide gels*, Analytical Biochemistry, 44, 276-287.

[2] M.H.C. de Carvalho (2008), *Drought stress and reactive oxygen species*, Plant Signaling Behavior, 3, 156-165.

[3] T. Demiral, I. Turkan (2005), *Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance*, Environmental and Experimental Botany, 53, 247-257.

[4] N. Doke (1983), *Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of Phytophthora infestans and to the hyphal wall components*, Physiological Plant Pathology, 23, 345-357.

[5] M.H. Hadwan, S.S.M. Al-Obaidy, M.J. Hussein, R.M. Mohammed, A.M. Hadwan, H.S. Al-Kawaz, Z.A. Al Talebi (2024), *An improved method for measuring catalase activity in biological samples*, Biology Methods and Protocols, 9(1), bpae015, doi.org/10.1093/biomethods/bpae015.

[6] P.D.R.V. Heerden, G.H.J. Kruger (2002), *Separately and simultaneously induced dark chilling and drought stress effects on photosynthesis, proline accumulation and antioxidant metabolism in peanut*, Journal of Plant Physiology, 159, 1077-1086

[7] C.M. Luna, G.M. Pastori, S. Driscoll, K. Groten, S. Bernard, C.H. Foyer (2005), *Drought controls on H_2O_2 accumulation, catalase (CAT) activity, and CAT gene expression in wheat*, Journal of Experimental Botany, 56, 417-423.

[8] V.C. Mai, W. Bednarski, B. Borowiak-Sobkowiak, B. Wilkaniec, S. Samardakiewicz, I. Morkunas (2013), *Oxidative stress in pea seedling leaves in response to Acyrthosiphon pisum infestation*, Phytochemistry, 93, 49-62.

[9] M. Rahman, T. Asaeda, K. Fukahori, F. Imamura, A. Nohara, M. Matsubayashi (2023), *Hydrogen peroxide measurement can be used to monitor plant oxidative stress rapidly using modified ferrous oxidation xylenol orange and titanium sulfate assay correlation*, International Journal of Plant Biology, 14, 546-557.

[10] P.M. Seevers, J.M. Daly, F.F. Catedral (1971), *The role of peroxidase isozymes in resistance to wheat stem rust disease*, Plant Physiology, 48, 353-360.

[11] M. Senthilkumar, N. Amaresan, A. Sankaranarayanan (2020), *Estimation of proline content in plant tissues. In, Plant-Microbe interactions*, Springer Protocols Handbooks. Humana, New York.

[12] P. Sharma, A. Jha, R. Dubey, M. Pessarakli (2012), *Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions*, Journal of Botany, 26. https://doi.org/10.1155/2012/217037

[13] N. Verbruggen, C. Hermans (2008), *Proline accumulation in plants, a review*, Amino Acids, 35, 753-759.

[14] R. Wu, J. Yang, L. Wang, *et al.* (2019), *Physiological response of flax seedlings with different drought-resistances to drought stress*, Acta Agriculturae Boreali-Sinica, 34, 145-153.