# ANTIOXIDANT EXPRESSION IN PEANUT (*Arachis hypogaea* L. cv. CNC1) UNDER DROUGHT CONDITION

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**Abstract**: The current study was carried out to assess the antioxidant expression of the seed's black-testa peanut cultivar namely CNC1 in the artifical drought condition. The oxidative stress with a burst of $O_2^{\cdot-}$ and $H_2O_2$ in peanut leaves resulted the obviously cellular membrane damage that was illustrated by an increase of lipid peroxidation. A high induced activity of enzymatic antioxidants such as SOD, CAT and POX would detoxify the excess of $O_2^{\cdot-}$ and $H_2O_2$. An increase in content of amino acid proline protects the stressed cells by adjusting intercellular osmotic potential. The antioxidative expression of CNC1 plants was similar to that in L20 cutivar - a drought tolerant cutivar of peanut.

**Keywords:** Peanut; CNC1; drought; antioxidative stress.

## 1. Introduction

Drought is one of the most harmful abiotic stresses that limit plant' growth and productivity [5]. Drought often induces the oxidative burst with strong generation of reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$), etc..., which provoke peroxidation and lead to damages to living cells [6]. When plants face drought stress, activity 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) is usually accumulated to control the generation and detoxification of ROS [19]. Furthermore, amino acid proline, a nitrogen-containing organic compound functioned as an antioxidant, also is known to improve the tolerant capability of plants under drought condition [15].

*Arachis hypogaea* L. cv. CNC1, a seeds' black-testa peanut cutivar, poccesses many bioactive subtances such as anthocyanins, flavonoids, vitamins and essential mineral elements. That cultivar has been assessed as a promising crop in agricultural production in Viet Nam. To date, lack of information has been mentioned about its response to abiotic stresses. This study aims to investigate antioxidative changes of *A. hypogaea* cv. CNC1 under drought condition, in which, level of $O_2^{\cdot-}$, $H_2O_2$, activity of SOD, CAT and POX as well as content of proline were assessed. As an additional objective, degree of cellular oxidative damage was also determined. It should be stressed that those aforementioned aspects will provide the convinced evidences to clarify whether *A. hypogaea* cv. CNC1 is a drought-tolerant cultivar.

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## 2. Materials and methods

### *2.1. Materials*

Homogenous seeds of two peanut (*Arachis hypogaea* L.) cultivars namely CNC1 (investigated cultivar) and L20 (drought-tolerant cultivar, used as control plant) were provided by Viet Nam Agricultural Genetics Institute. Seeds were placed on agar plates and remained at 4$^o$C overnight. They were incubated to 20$^o$C for 3 days in the dark to ensure uniform their germination. Seedlings were transferred into 20 cm pots (three seedlings/pot) containing alluvium added macro minerals, and placed in the growth chamber with temperature of 23±2$^o$C, the relative humidity of 65-70%, light intensity of 100-110 $\mu M.m^{-2}.s^{-1}$ and light rhyme of 12 light/12 dark hours. All pots were normally watered before the implementation of drought.

Seedlings were divided into two parts: the first part was put under drought stress imposition by withholding water (drought treatment formulae) in the plantlet stage and the blossom stage, and the second one was kept in the soil moisture of 70% (control formulae). The drought treatment formulae were stopped watering until tensiometer (the measuring instrument of osmotic pressure, SKU-2725ARL06, USA) plug in a pot, about 15 cm deep) reaches to -80 KPa, then watering again. Leaves were carefully collected in the plantlet stage (40-50 days after planting) and the blossom stage (70 - 80 days after planting), when the soil moisture in drought treatment formulae was -80 Kpa, for subsequent analyses. Experiments all were carried out in the Lab of Plant Physiology (Vinh University, Vietnam).

### *2.2. Analytical methods*

Determination of $O_2^{\cdot -}$ in peanut leaves was based on its ability to reduce nitro blue tetrazolium-NBT [9]. Content of $H_2O_2$ was determined following the spectrophotometric method [3]. To assess the cellular membrane damage, lipid peroxidation was determined by the thiobarbituric acid reactive substances (TBARS) assay [10].

Activity of SOD was spectrophotometrically assayed by measuring its ability to inhibit the photochemical reduction of NBT [2]. Activity of CAT was determined by measuring $H_2O_2$ consumption [8]. POX activity was calculated following the spectrophotometrical assay [15]. The enzyme-prepared protein was determined following method of Bradford (1976) [4]. Proline content was determined following the spetrophotometric method of Bates *et al.* (1973) with minor modification [1].

### *2.3. Statistical analysis*

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

## 3. Results and discussion

### *3.1. Drought-accumulated reactive oxygen species*

An accumulated generation of $O_2^{\cdot -}$ was observed in leaves of *A. hypogaea* cv. CNC1 under drought condition. The highest level of $O_2^{\cdot -}$ recorded in leaves in stage of

blossom was 2.19 $A_{580}.g^{-1}$ FW, having by 3.12- and 2.78-fold higher than that in the beginning (before drought treatment) and in control, respectively (*Table 1*). The $O_2^{\cdot-}$ level in drought-treated plants of both CNC1 and L20 cultivars was always significantly different from that in control plants ($P<0.05$).

**Table 1**: *Content of superoxide anion radical in leaves of A. hypogaea cv. CNC1 and A. hypogaea cv. L20*

| Peanut cultivars | Experimental formulae | Superoxide content *(A580.g$^{-1}$ FW)* | | |
|---|---|---|---|---|
| | | Before drought treatment | Drought-treated stage | |
| | | | Plantlet | Blossom |
| L20 | Control | 0.67 ± 0.08 | 0.76 ± 0.08 | 0.69 ± 0.08 |
| | Drought | | 0.91 ± 0.08 | 1.71 ± 0.13 |
| CNC1 | Control | 0.70 ± 0.06 | 0.80 ± 0.07 | 0.79 ± 0.09 |
| | Drought | | 0.90 ± 0.10 | 2.19 ± 0.18 |

**Table 2**: *Content of hydrogen peroxide in leaves of A. hypogaea cv. CNC1 and A. hypogaea cv. L20*

| Peanut cultivars | Experimental formulae | Hydrogen peroxide content *(μM.g$^{-1}$ FW)* | | |
|---|---|---|---|---|
| | | Before drought treatment | Drought-treated stage | |
| | | | Plantlet | Blossom |
| L20 | Control | 5.74 ± 0.59 | 7.42 ± 0.69 | 13.78 ± 1.13 |
| | Drought | | 11.41 ± 0.94 | 15.08 ± 1.24 |
| CNC1 | Control | 7.18 ± 0.87 | 9.61 ± 0.79 | 10.15 ± 0.83 |
| | Drought | | 17.25 ± 1.46 | 21.16 ± 1.74 |

Similar to $O_2^{\cdot-}$ generation, a remarkable accumulation of $H_2O_2$ in CNC1 drought-treated plants was recorded since stage of plantlet, obtained the highest level in stage of blossom (21.16 μM $H_2O_2.g^{-1}$ FW) (*Table 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 leaves of CNC1 plants was also much higher than that in L20 cultivar although this ROS in both two cultivars expressed the same tendency to change during experimental time.

### *3.2. Cellular membrane damage*

Lipid peroxidation is a well-established mechanism of cellular injury and is measured by amount of TBARS. An increase in content of TBARS was recorded in leaves of CNC1 plants during investigated time and reached to maximum level in stage of plantlet as 22.03 μM.g$^{-1}$ FW (*Table 3*). It was noted that TBARS contents in

drought-treated plants were always significantly higher than in control, levels of TBARS in CNC1 leaves were similar to those substances in L20 cultivar within experimental stages ($P<0.05$).

**Table 3**: *Lipid peroxidation in leaves of A. hypogaea cv. CNC1 and A. hypogaea cv. L20*

| Peanut cultivars | Experimental formulae | Lipid peroxidation ($\mu M\ TBARS.g^{-1}\ FW$) | | |
|---|---|---|---|---|
| | | Before drought treatment | Drought-treated stage | |
| | | | Plantlet | Blossom |
| L20 | Control | 12.43 ± 1.14 | 15.99 ± 1.48 | 14.99 ± 1.62 |
| | Drought | | 22.87 ± 2.09 | 16.45 ± 1.60 |
| CNC1 | Control | 12.52 ± 1.50 | 17.06 ± 1.55 | 13.71 ± 1.23 |
| | Drought | | 22.03 ± 2.41 | 17.04 ± 1.84 |

In our study, the accumulation of products of lipid peroxidation such as TBARS in drought-treated plants clearly demonstrated that both peanut cultivars as CNC1 and L20 were exposed to stress condition leading to peroxidize the membrane lipid by means of ROS. That result is in agreement with previous reports on peanut plants which confirmed that the increase in lipid peroxidation in plants was directly resulted from drought condition [12].

### *3.3. Drought-accumulated activity of enzymatic antioxidants*

In *A. hypogaea* cv. CNC1 leaves, the drought-accumulated activity of SOD increased continuously and remarkably within stages of plantley and blossom (*Table 4*). The highest activity of SOD obtained in blossom stage was 29.56 $nkat.mg^{-1}$ protein, having by 267.03% and 234.79% higher than that in control and at the beginning, respectively. ANOVA recorded the difference between the SOD activities in drought-treated plants and controls within experimental stages ($P<0.05$). It is stressed that, the induced activity of SOD in leaves of CNC1 plants was always higher than that in L20 cultivar at all points of estimated time.

**Table 4**: *Activity of SOD in leaves of A. hypogaea cv. CNC1 and A. hypogaea cv. L20*

| Peanut cultivars | Experimental formulae | Activity of SOD ($nkat.g^{-1}\ protein$) | | |
|---|---|---|---|---|
| | | Before drought treatment | Drought-treated stage | |
| | | | Plantlet | Blossom |
| L20 | Control | 9.73 ± 1.19 | 11.58 ± 1.06 | 13.99 ± 1.16 |
| | Drought | | 21.41 ± 2.57 | 23.15 ± 3.19 |
| CNC1 | Control | 11.07 ± 1.05 | 11.85 ± 1.26 | 12.59 ± 1.18 |
| | Drought | | 24.73 ± 2.31 | 29.56 ± 2.50 |

Similar to SOD, generation of CAT in leaves of CNC1 was strongly stimulated under drought condition. This enzyme remarkably induced and reached to the highest level in the blossom stage (*Table 5*). ANOVA revealed a significant difference between activities of CAT in drought-treated and control plants during the experimental time ($P<0.05$). However, the induced activity of CAT in leaves of CNC1 planst was always lower than that in leaves L20 at all points of drought time.

**Table 5:** *Activity of CAT in leaves of A. hypogaea cv. CNC1 and A. hypogaea cv. L20*

| **Peanut cultivars** | **Experimental formulae** | **Activity of CAT** *(nkat.g$^{-1}$ protein)* | | |
|---|---|---|---|---|
| | | **Before drought treatment** | **Drought-treated stage** | |
| | | | **Plantlet** | **Blossom** |
| L20 | Control | 8.46 ± 0.77 | 10.81 ± 0.81 | 15.37 ± 1.35 |
| | Drought | | 17.07 ± 1.02 | 22.10 ± 2.47 |
| CNC1 | Control | 9.42 ± 0.68 | 10.59 ± 0.98 | 14.71 ± 1.86 |
| | Drought | | 12.53 ± 1.84 | 18.08 ± 1.34 |

Activity of enzyme POX in CNC1 plants was remarkably enhanced under drought condition (*Table 6*). The drought-accumulated activity of POX progressively increased during the experimental time. The highest value of POX activity obtained in stage of blossom was 10.82 nkat.mg$^{-1}$ protein, having by 187.83% and 128.66% 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 leaves of CNC1 plants was always higher than that in L20 cultivar.

**Table 6**: *Activity of POX in leaves of A. hypogaea cv. CNC1 and A. hypogaea cv. L20*

| **Peanut cultivars** | **Experimental formulae** | **Activity of POX** *(nkat.g$^{-1}$ protein)* | | |
|---|---|---|---|---|
| | | **Before drought treatment** | **Drought-treated stage** | |
| | | | **Plantlet** | **Blossom** |
| L20 | Control | 4.99 ± 0.47 | 5.16 ± 0.48 | 7.66 ± 0.68 |
| | Drought | | 7.08 ± 0.66 | 9.04 ± 0.97 |
| CNC1 | Control | 5.76 ± 0.61 | 5.87 ± 0.42 | 8.41 ± 0.82 |
| | Drought | | 8.18 ± 0.57 | 10.82 ± 0.73 |

SOD converts the toxic $O_2^{\cdot-}$ radical to $H_2O_2$, then CAT catalyzes $H_2O_2$ to $O_2$ and water as a side product of photoreactions [14], whereas POX metabolizes $H_2O_2$ dependent oxidation reactions in plant cells [7]. The accumulated activity of both CAT and POX protected peanut leaf cells from an excess of this ROS product, and thus reduced considerable membrane damages.

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 [13]. Activity of those enzymes in

tolerant cultivars are always higher than in sensitive ones under various stress conditions [17]. In agreement with those reports, the accumulated activity of SOD, CAT and POX in CNC1 leaves during drought-treament introduced that this peanut cultivar denoted its drought tolerance.

### *3.4. Drought-accumulated content of amino acid proline*

Similar to L20 cultivar, proline content in leaves of CNC1 peanut increased continuously in drought condition since the plantlet stage till the blossom stage, whereas that amino acid in control plants was in minor change in lower levels following the investigated time (*Table 7*). Proline accumulation in leaves of drought-stressed plants has been well-documented. Our results agreed with previous research, which presented a significant increase in content of proline in plants under drought condition [16]. That amino acid protects the stressed cells by adjusting intercellular osmotic potential and attributed to high water retention [11]. 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 [18].

**Table 7:** *Content of proline in leaves of A. hypogaea cv. CNC1 and A. hypogaea cv. L20*

| **Peanut cultivars** | **Experimental formulae** | **Content of proline** *(nkat.g$^{-1}$ protein)* | | |
|---|---|---|---|---|
| | | **Before drought treatment** | **Drought-treated stage** | |
| | | | **Plantlet** | **Blossom** |
| L20 | Control | 10.62 ± 0.97 | 11.72 ± 1.07 | 13.81 ± 1.27 |
| | Drought | | 13.06 ± 1.20 | 18.90 ± 1.74 |
| CNC1 | Control | 10.41 ± 0.95 | 11.84 ± 1.09 | 12.18 ± 1.12 |
| | Drought | | 15.86 ± 1.46 | 20.69 ± 1.90 |

## 4. Conclusion

Drought resulted the oxidative stress in leaves of *A. hypogaea* cv. CNC1 with a burst of $O_2^{\cdot -}$ and $H_2O_2$ generation leading to the cellular membrane damage that was probably confirmed by an increase in TBARS' content in leaves' 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 $O_2^{\cdot -}$ 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.

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## TÓM TẮT

## MỘT SỐ BIỂU HIỆN CHỐNG STRESS ÔXY HÓA CỦA GIỐNG LẠC ĐEN CNC1 TRONG ĐIỀU KIỆN HẠN

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Nghiên cứu được thực hiện nhằm đánh giá một số biểu hiện chống "stress ôxy hóa" của giống lạc đen (*Arachis hypogaea* L. cv. CNC1) trong điều kiện chịu hạn nhân tạo. Hạn đã gây hiện tượng "stress ôxy hóa" thông qua gia tăng các dạng ôxy hoạt hóa như gốc tự do superoxide ($O_2^{\cdot-}$) và hydrogen peroxide ($H_2O_2$), làm tổn thương cho tế bào lá lạc. Để giảm ảnh hưởng của "stress ôxy hóa", cây lạc đen CNC1 đã hình thành cơ chế tự bảo vệ thông qua sự cảm ứng gia tăng hoạt động của các enzym chống ôxy hóa superoxide dismutase (SOD), catalase (CAT), peroxidases (POX) và tổng hợp mạnh mẽ axit amin proline. Biểu hiện chống "stress ôxy hóa" của cây lạc đen CNC1 tương đồng với giống lạc chịu hạn L20.

**Từ khóa:** Lạc; CNC1; hạn; stress ôxy hóa.