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Annals
Agric. Sci.,
Ain Shams Univ.,
Cairo, 55(2), 179-190, 2010

INVOLVEMENT OF NITRIC OXIDE IN ABA-INDUCED STIMULATION OF ADVENTITIOUS ROOT FORMATION IN DEROOTED MUNG BEAN [*Vigna radiata* (L.) WILCZEK] SEEDLINGS

[14]

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Keywords: Abscisic acid; Adventitious root formation; Antioxidant enzymes; Nitric oxide; *Vigna radiata* cuttings

ABSTRACT

The present study aimed to investigate whether nitric oxide (NO) is involved in the ABA-induced adventitious root formation (ARF) in derooted *Vigna radiata* seedlings due to its nature as a second messenger in stress responses. 7-day-old derooted mung bean seedlings were treated with the plant growth regulator ABA, NO donor sodium nitroprusside (SNP), and ABA in combination with the specific NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO) or the nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-l-arginine (L-NNA). Results showed that application of ABA and SNP significantly stimulated ARF in a concentration dependent manner. However, the NO specific scavenger carboxy-PTIO and the NOS-inhibitor L-NNA suppressed the stimulatory effect of ABA on ARF in derooted *V. radiata* seedlings, indicating that endogenous NO plays a vital role in the ABA induced ARF; and it can be implied that the generation of NO catalyzed by NOS-like enzyme. In addition, data showed that ABA-treated cuttings had higher levels of NO, hydrogen peroxide (H_2O_2), and lower levels of superoxide anion ($O_2^{\cdot-}$) than those of untreated ones. Further, ABA-treated cutting also contained higher activities of the antioxidant enzymes, i.e. [superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX)], than those of the control treatment; however, carboxy-PTIO + ABA-treated cuttings had lower activities of such enzymatic activities at 120 h of ABA treatment. These results suggest that NO is involved in the ABA-induced stimulation of mung bean ARF most probably mediated via creating optimal levels of $O_2^{\cdot-}$, and H_2O_2 and enhancement of antioxidant enzymatic activities. It may be concluded that endogenous NO plays a vital role in ABA-induced *de novo* root formation in derooted *V. radiata* seedlings.

ABBREVIATIONS

ABA, abscisic acid; ARF, adventitious root formation; carboxy-PTIO, 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide;CAT, catalase; H_2O_2, hydrogen peroxide; L-NNA, N^{ω}-nitro-l-arginine; NO, nitric oxide; NOS, nitric oxide synthase; GPX, guaiacol peroxidase; ROS, reactive oxygen species; SNP, sodium nitroprusside; SOD, superoxide dismutase

INTRODUCTION

Adventitious root formation (ARF) process consists of three successive but interdependent physiological phases with different requirements, namely: induction, initiation and expression. The induction phase is comprised of molecular and biochemical events without visible changes. The initiation phase is characterized by cell divisions

(Received August 8, 2010)
(Accepted September 27, 2010)

and the organization of root primordia. Expression phases (intra-stem growth of root primordia and root emergence) have specific requirements (**Fabijan *et al* 1981**).The plant growth regulator abscisic acid (ABA) participates in the control of diverse physiological processes including adventitious root formation (ARF) (**Hartmann *et al* 1997**). ABA promotes ARF in many plant species (**Davis and Sankhla, 1989**). ABA mediates various important plant developmental and physiological processes and responses to stress conditions (**Leung and Giraudat, 1998**). A common feature of a biotic stresses, including wounding, is an imbalance between pro- and antioxidant reactions in the cell, which appears as oxidative stress (**Orozco-Cárdenas and Ryan, 1999; Halliwell, 2006**), which causes rapid changes of different defense genes expression locally and systemically (**León *et al* 2001**). An increasing body of evidence indicates that one mode of ABA action is related to antioxidant defense system. It is documented that ABA can cause an increase generation of reactive oxygen species (ROS) such as $O_2^{\cdot-}$ and H_2O_2 (**Apel and Hirt, 2004; Kwak *et al* 2006**). It can also induce the expression of antioxidant genes encoding superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX) and enhance the activities of these antioxidant enzymes in plant tissues (**Bueno *et al* 1998; Jiang and Zhang, 2003**). Such antioxidant enzymes play a crucial role in the scavenging of ROS during plant metabolism (**Camp *et al* 1997**). Higher antioxidant enzymatic activities trigger cell division and organogenesis (**Tian *et al* 2003**). ROS is an important intermediate component in ABA induced antioxidant defense system against oxidative stress (**Courtois *et al* 2008**).

In addition to ABA, nitric oxide (NO) is also an important inter-and intracellular signaling molecule involved in many plant physiological processes (**Lamattina *et al* 2003; Lamotte *et al* 2005**). It is a reactive nitrogen species, and its concentration-depending effects on different cell types were shown to be either protective or toxic. NO is involved in regulating growth and developmental processes, such as seed germination, de-etiolation, cell senescence and programmed cell death (**Beligni and Lamattina, 2000; Neill *et al* 2003**). Moreover, NO was found to mediate plant responses to abiotic stresses (**Laspina *et al* 2005; Zhao *et al* 2008**). In animal cells, most of the NO is synthesized by nitric oxide synthase (NOS). Recently, NOS was also detected in plants (**Guo *et al* 2003; Chaki *et al* 2009**). Research with different plant explants suggested that NO is an intermediate component of the plant hormone IAA signaling network that controls rooting process (**Pagnussat *et al* 2002, 2003, 2004; Lanteri *et al* 2006**). The latter authors recognized that NO and IAA shared common steps in the signal transduction pathways towards auxin-induced lateral or ARF. To date, there are no reports describing whether NO is involved in ABA-induced stimulation of ARF.

In this investigation, derooted mung bean (*Vigna radiata* (L.) Wilczek) seedlings were used to study the interaction effects of ABA, the NO donor sodium nitroprusside (SNP), the NO specific scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO), or the nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-Larginine (L-NAA) on adventitious rooting process. Any possible relationship between ROS metabolism and ABA-induced activities of antioxidant enzymes after ABA alone or in combination with NO scavenger carboxy-PTIO was also examined.

MATERIALS AND METHODS

1. Plant material and treatments

Seeds of mung bean were germinated as previously described (**Tartoura *et al* 2004**). After germination, the seedlings were incubated in a growth chamber at 25 ± 2°C under continuous white fluorescent illumination (20 W m^{-2} PAR), and 80% of relative humidity. Five uniform cuttings from 7-day-old seedlings, consisting of terminal bud, one pair of primary leaves, epicotyl and 5 cm of hypocotyls, were placed in 10 ml dark glass vials containing a 5 cm solution depth of distilled water (control) or test solutions for 48 h, including ABA and NO individually at 25, 55, 100, and 200 µM; 100 µM ABA plus either 150 µM carboxy-PTIO or 300 µM L-NAA. Then the cuttings were replaced in distilled water for a further 6 days under the same environmental conditions. The number of adventitious root was quantified after 8 days of treatments. Data are the mean values of three independent experiments, each with two replicates of 25 cuttings. At 120 h after ABA and/ or ABA plus carboxy-PTIO application, the concentrations of NO, $O_2^{\cdot-}$, H_2O_2, and activities of the antioxidant enzymes SOD, CAT, GPX, and APX were determined in the basal end of the hypocotyls (10-mm) during the progress of adventitious rooting process.

2. Biochemical parameters determinations

2.1. Quantification of endogenous NO

Nitric oxide was indirectly determined as nitrite using the method described by **Zhou *et al* (2005)**. Briefly, 500 mg sample tissues of 10-mm length from the basal end of hypocotyls were quickly frozen and homogenized in a pre-chilled mortar with 4.0 ml of 50 mM cold acetate buffer pH 3.6, containing 4% (w/v) zinc diacetate. The homogenates were centrifuged at 10,000g for 15 min at 4°C. The supernatant was collected and the pellet was washed by 1.0 ml of extraction buffer and centrifuged as before. The two supernatants were combined and 0.1 g of activated charcoal was added. After vortex and filtration, the filtrate was leached, collected and immediately assayed for NO. Equal amounts of the filtrate and Greiss reagents [1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 5% (w/v) H_3PO_4] were incubated at room temperature for 30 min. Absorbance of the reaction mixture was read at 540 nm and concentration of NO determined from a calibration curve prepared using sodium nitrite as standard.

2.2. Determination of H_2O_2 and $O_2^{\cdot-}$ production

Samples tissues (200 mg FW)) were homogenized with 5 ml of ice-cold 5% (w/v) trichloroacetic acid. The extracted solution (800 µl) was reacted by adding 100 µl of 15% (w/v) titanium sulphate ($TiSO_4$) in 23% (v/v) H_2SO_4. The reaction mixture was then centrifuged at 10,000g for 10 min and absorbance of the orange-yellow color solution measured at 408 nm against hydrogen peroxide as standard (**Nag *et al* 2000**).

Superoxide radical production rate was determined by the modified method according to **Elstner and Heupel (1976)**. Sample tissues of 10-mm length from the basal end of hypocotyls (1.0g) were homogenized in 3 ml of 50 mM potassium phosphate buffer (pH7.8) and centrifuged at12,000g for 20 min. The incubation mixture contained 1ml of supernatant, 1 ml of 50 mM potassium phosphate buffer (pH7.8) and 1 ml of 1mM hydroxylammonium chloride and the mixture was incubated in 25 °C for 20 min. The mixture was subsequently incubated with 2 ml of17 mM sulphanilic acid and 2 ml of 7 mM α-naphthyl amine at 25 °C for 20 min. The final solution was mixed with an equal volume of ethylether, and the absorbance of the pink phase was read at 530 nm. The production rate of superoxide radical was calculated based on a linear standard curve of $NaNO_2$.

2.3. Determination of antioxidant enzymatic activities

Samples tissues (0.5 g) of 10-mm length from the basal end of the hypocotyls were ground in a mortar and pestle in 5 ml of 50 mM cold phosphate buffer (pH 7.8), containing 2% (w/v) polyvinylpolypyrrolidone (PVP). The homogenates were centrifuged at 10,000g for 20 min at 4°C. The resulting supernatant was used for assay of enzymatic activities.

Total superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by the photochemical method described by **Rao and Sresty (2000)**. The 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM nitroblue tetrazolium, 2 mM riboflavin, 10 mM EDTA and 0.1 ml enzyme extract. One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of nitroblue tetrazolium (NBT) reduction measured at 560 nm. SOD activity was expressed as U mg^{-1} protein. Catalase (CAT, EC 1.11.1.6) activity was measured according to the method of **Cakmak and Marschner (1992)** by determination the disappearance of H_2O_2 by measuring the decrease in an absorbance at 240 nm of a reaction mixture containing 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.1 ml enzyme extract. Guaiacol peroxidase (GPX, EC 1.11.1.7) activity was estimated after **Hammerschmidt *et al* (1982)** method. Activity was measured by the increase in absorbance at 470 nm due to guaiacol oxidation. The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol, 1.0 mM H_2O_2 and 0.1 ml enzyme extract. Activity of ascorbate peroxidase (APX, 1.11.1.11) was measured according to **Nakano and Asada (1981)** by monitoring the rate of ascorbate oxidation at 290 nm. The assay mixture contained 0.25 mM ascorbate, 1.0 mM H_2O_2, 0.1 mM EDTA, and 0.1 ml enzyme extract in 25 mM phosphate buffer (pH 7.0). Protein content was estimated according to **Bradford (1976)** using bovine serum albumin as a standard.

3. Statistical analysis

The data were subjected to statistical analysis using COSTAT computer software (CoHort Computer Software, Berkeley, CA, USA). Least significant differences (LSD) test was applied to compare

the treatment means. Graphical presentation of data was carried out using MICROSOFT EXCEL program (Microsoft Corporation, Los Angeles, CA, USA).

RSULTS AND DISCUSSION

Effects of ABA and SNP on adventitious rooting

Figure (1A) shows that the number of adventitious roots increased significantly in response to ABA treatments in a dose-dependent manner, with the maximum effect being at 100 µM. ABA promotes ARF in various plant species, including mung bean cuttings. It is considered to be a rooting cofactor (**Hartmann *et al* 1997**). According to **Davis and Sankhala (1989)**, **Grafi *et al* (1994)**, and **Tartoura (2001)**, ABA stimulated ARF via regulating the levels of both endogenous free and conjugated IAA and gibberellins in favor of inducing a large number of adventitious roots. In addition **Zaghlool and Shehata (2002)** reported that basipetal polar transport of auxin (IAA) through hypocotyls of derooted cucumber seedlings affected ARF. Monitoring and optimization of IAA transport by exogenous application of ABA could be achieved the maximum ARF. Therefore, the role of ABA in adventitious rooting could be mediated by regulation of IAA level.

The NO donor sodium nitroprusside (SNP) treatments also increased adventitious root number in a concentration dependent pattern, with the maximum biological effect attained at 100 µM, as shown in **Fig. (1B)**. NO has recently emerged as a multifunctional bioactive molecule in plant signal transduction pathways participating in a broad spectrum of physiological and developmental processes, including root organogenesis, via direct or indirect processes or through cross talking with the classical plant hormones (**Lamattina *et al* 2003; Pagnussat *et al* 2003; Huang *et al* 2007; Tewari *et al* 2008**).

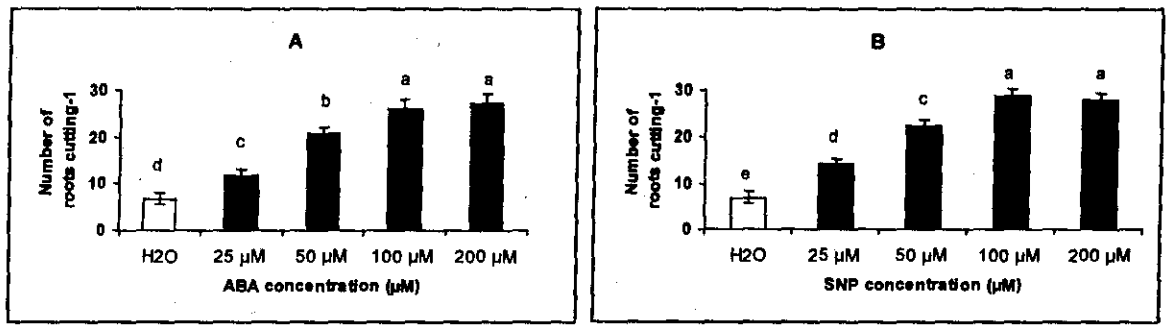

Figure 1. Effect of different concentrations of ABA (A), and SNP (B) on the number of adventitious roots of derooted mung bean seedlings. Vertical bars represent ± SD. Means with different letters are significantly different at 0.05 *P* level.

Interaction effects of ABA, NO scavenger, and NOS inhibitor on adventitious roots

To confirm whether NO is involved in ABA signal transduction pathway leading to ARF, mung bean cuttings were treated with either 150 µM of the specific NO scavenger carboxy-PTIO or 300 µM of NOS-like enzyme (NOS) inhibitor L-NNA in combination with 100 µM ABA. **Figure (2)** shows that carboxy-PTIO and L-NAA suppressed the stimulatory effect of ABA on ARF, indicating the vital role of NO in adventitious rooting since carboxy-PTIO and L-NAA individually blocked the action of ABA. In addition, the inhibitory effect of L-NAA on ABA-induced ARF suggests that the generation of NO catalyzed by a NOS-like enzyme. In this respect, it is well known that two major enzymatic NO-generation pathways were proposed in plants, nitric oxide synthase (**Guo *et al* 2003**) and nitrate reductase (NR) (**Desikan *et al* 2002; Garcia-Mata and Lamattina, 2003**). Because of the NR activity and NO production after ABA treatment in the presence of a NR inhibitor were not measured, the present data cannot rule out the possible involvement of NR in ABA-induced NO formation in *V. radiata* cuttings. Research with different plant explants suggested that NO is an intermediate component of the plant hormone IAA signaling

network that controls rooting process (**Pagnussat *et al* 2002; Lanteri *et al* 2006**). The latter authors recognized that NO and IAA shared common steps in the signal transduction pathways toward auxin-induced lateral or ARF. In agreement with the present results, **Pagnussat *et al* (2002), Correa-Aragunde *et al* (2004) and Huang *et al* (2007)** found that application of NO generating agents stimulated adventitious rooting of several plant species. They also reported that NO-scavengers and inhibitors reduced the stimulatory effect of NO in inducing root initiation suggesting that NO is required for adventitious rooting. Recently, **Correa-Aragunde *et al* (2007)** reported that NO is an intermediate signal in auxin and ABA signal transduction pathways. The data presented here suggested that ABA signal transduction pathway leading to stimulation of mung bean adventitious rooting required the involvement of NO.

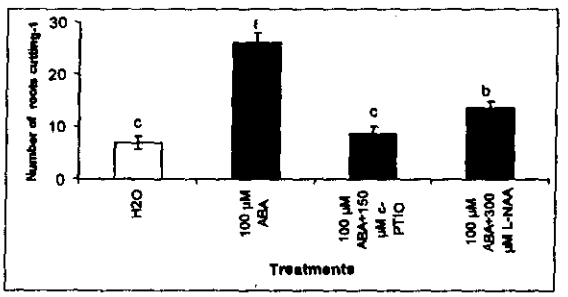

Figure 2. Inhibitory effect of carboxy-PTIO and L-NNA on the stimulatory effect of ABA on adventitious roots of mung bean cuttings. Vertical bars represent ± SD. Means with different letters are significantly different at 0.05 *P* level.

Interaction effects of ABA and NO scavenger on NO and ROS metabolism

This experimental system was used to investigate the possible correlation between the levels of NO, H_2O_2, and $O_2^{\cdot-}$ as well as theactivities of antioxidant enzymes, i.e. SOD, CAT, GPX and APX, and adventitious root formation in response to ABA alone or in combination with carboxy-PTIO 120 h after treatment. **Table (1)** shows that endogenous levels of NO, and H_2O_2 in ABA-treated cuttings were significantly higher than that present in the control cuttings. The opposite trends were recorded after application of carboxy-PTIO + ABA. The level of $O_2^{\cdot-}$ in the latter treatment was about twofold as much as that in the ABA treatment. Low root regeneration capacity was found when endogenous NO was removed by carboxy-PTIO, which confirms the role of NO in ARF in derooted *V. radiata* seedlings **Fig. (1B) and Table (1)**. These results indicated that high H_2O_2 level, and low $O_2^{\cdot-}$ level associated with good root regeneration capacity in ABA-treated cuttings, while the reverse was true in relation to carboxy-PTIO+ ABA-treated ones, as shown in **Fig. (2) and Table (1)**.

Hydrogen peroxide is widely generated in many biological systems. **Li *et al* (2007)** suggested that H_2O_2 may function as a signaling molecule involved in the formation and development of adventitious roots in cucumber. In fact, plant cells contain several sources of H_2O_2, such as cell wall-bound peroxidases and NADPH oxidase in the plasma membrane, which generate H_2O_2 in the apoplast. Moreover, mitochondrial respiration, photosynthetic electron transport in chloroplasts and photorespiratory pathway are powerful suppliers of H_2O_2 (**Apel and Hirt, 2004; Ślesak *et al* 2007**). Results obtained suggest that a certain level of H_2O_2 may function as a signal molecule in the formation of adventitious roots.

In addition to H_2O_2, $O_2^{\cdot-}$ is also recognized as another signaling molecule, regenerated from a one-electron reduction of O_2 by enzyme catalysis or by 'electron leak' from various electron transfer reactions (**Thannickal and Fanburg, 2000**). H_2O_2 and $O_2^{\cdot-}$ can induce different gene expression, in combination or separately, thereby giving more flexibility to the ROS signaling function (**Van Breusegem *et al* 2001**). In this respect, **Tewari *et al* (2008)** reported that NO activate NADPH oxidase activity resulting in the generation of $O_2^{\cdot-}$ and which subsequently induces rootdifferentiation. Moreover, they found that inhibition of NADPH oxidase with the treatment of SNP + diphenyl iodonium chloride (DPI, an inhibitor of NADPH oxidase) or DPI alone retarded growth of adventitious roots, suggesting that a certain level of $O_2^{\cdot-}$ is beneficial for growth of adventitious roots of mountain ginseng. Thus, the present results suggest that NO and ROS might play an important role in ARF in derooted mung bean seedlings.

SOD is a major scavenger of $O_2^{\cdot-}$, catalyzing the dismutation of superoxide radicals to H_2O_2 and O_2. CAT, GPX and APX are important H_2O_2 detoxyfying enzymes. In addition, GPX is implicated in several physiological processes including cell growth and expansion (**Kawano, 2003**), reactive oxygen species generation (**Schopfer *et al* 2002**), and lignification (**Goldberg *et al* 1985**). As shown in **Table (1)**, ABA-treated cuttings exhibited

Table 1. Effect of ABA, and the specific NO scavenger carboxy-PTIO on the levels of NO, $O_2^{\cdot-}$, and H_2O_2, and activities of antioxidant enzymes in the basal end (10-mm) of hypocotyls of mung bean cuttings 120 h after treatment. Means with different letters are significantly different at 0.05 *P* level

Treatment	NO content (nmol g^{-1} DW)	H_2O_2 (µmol g^{-1} FW)	$O_2^{\cdot-}$ (nmol min^{-1} g^{-1} FW)	SOD activity (U mg^{-1} Protein)	CAT activity (µmol min^{-1} mg^{-1} protein)	GPX (U min^{-1} mg^{-1} protein)	APX activity (µmol min^{-1} mg^{-1} protein)
Control	33.68 b	0.481 b	39.30 b	76.90 b	79.57 b	42.64 b	0.404 b
ABA	53.95 a	0.579 a	31.38 c	87.73 a	103.67 a	58.78 a	0.708 a
PTIO - ABA	11.26 c	0.335 c	63.71 a	5.78 c	56.47 c	23.81 c	0.163 c

increases in the activities of antioxidant enzymes, namely SOD, CAT, GPX, and APX. Increases in the activities of previously antioxidant enzymes suggest that ROS induced these increases in different cellular compartments, as also reported by **Logan *et al* (1998)**; whereas a reversed pattern was found when the NO scavenger carboxy-PTIO along with ABA were applied **(Table, 1)**. This is consistent with the postulated role of NO as a signaling molecule involved in inducing increases in the activities of antioxidant enzymes, as reported by **Laspina *et al* (2005), Tewari *et al* (2007) and Zhang *et al* (2009)**. The mode of ABA action in inducing ARF might be explained as follows: ABA might first induce NO synthesis and the ABA induced NO then stimulates the antioxidant enzymes, as also reported by **Zhou *et al* (2005)**. It has been observed in many other plant species that NO stimulates antioxidant enzymes. The inducible effect of the NO donor SNP on the activity of SOD, CAT, GPX, and APX was observed in rice seedlings **(Uchida *et al* 2002)**. The NO donor increased SOD activity in rice under osmotic stress **(Cheng *et al* 2002)**. SOD and CAT are considered key players in the antioxidant response system as they regulate the cellular concentration of $O_2^{\cdot-}$ and H_2O_2 **(Van Breusegem *et al* 2001)**. These two enzymes, together with ascorbate peroxidase, and other antioxidant enzymes, constitute the major defense system against ROS in the plant cell under abiotic stress **(Mittler, 2002)**.

Additionally, recent research suggested that suppressed expression of totipotency in tobacco protoplast was correlated with reduced activity of cellular antioxidant machinery **(Papadakis *et al* 2001)**. Similar results were noted in the present study **(Figs. 1, 2 and Table 1)**. As previously stated, the activities of the antioxidant enzymes in carboxy-PTIO plus ABA were much lower than that in the ABA-treatment, which possibly related to the low regeneration capacity in the former treatment. In fact, antioxidant enzymes play a crucial role in the scavenging of ROS during plant metabolism **(Camp *et al* 1997)**. Higher activities trigger cell division and root organogenesis, as also suggested by **Tian *et al* (2003)**. The present results are also agreement with the observations that ABA induced the expression of SOD, CAT, and APX genes in maize and *Chlamydomonas reinhardtii* **(Zhu and Scandalios, 1994; Yoshida *et al* 2004)**. These data supported the hypothesis that NO may mediate ABA-induced antioxidant enzymes. Thus, endogenous NO, ROS and activities of antioxidant enzymes were required to mediate ABA action on adventitious rooting process in derooted *V. radiata* seedlings. The mode of NO action in stimulating ARF might be related to alleviation of the oxidative damage induced by wounding after removing the primary roots. Its role can be explained as following: because of the existence of an unpaired electron within the molecule, NO can react directly with some ROS, such as $O_2^{\cdot-}$, and H_2O_2 **(Martinez *et al* 2000)**. Reaction of NO with $O_2^{\cdot-}$ produces peroxynitrite ($ONOO^-$), which is considered to be a highly toxic product. However, $ONOO^-$ can be protonated and decomposed to a nitrate anion and a proton or it can react with H_2O_2 to yield a nitrite anion and oxygen **(Martinez *et al* 2000; Wendehenne *et al* 2001)**. Meanwhile, NO can terminate the lipid peroxidation by reacting with lipid alcoxyl (LO·) and peroxyl (LOO·) radicals, which are produced during membrane lipid peroxidation. It has been reported that the reaction between NO and LO·/ LOO· is rapid and in a direct fashion **(Beligni and Lamattina, 1999)**. Root formation is not exception from the targets of NO action **(Correa-**

Aragunde *et al* 2004, 2006; Kolbert *et al* 2008). A high root regeneration capacity in ABA-treated cuttings is owed to significantly increased antioxidant enzymes activities. As a bioactive antioxidant, endogenous NO generation at the potential rooting sites stimulates ARF by reacting with ROS directly or inducing activities of ROS-scavenging enzymes, especially in ABA-treated cuttings, which are better adapted to produce a large number of roots in this research. Thus, it may be concluded that ROS metabolism is important for ARF in derooted mung bean seedlings.

CONCLUSIONS

Treatments with the NO donor SNP, specific NO scavenger carboxy-PTIO and NOS inhibitor L-NNA revealed that NO is involved in ABA induced ARF via enhancement of antioxidant enzymatic activities in derooted *V. radiata* seedlings. ABA-triggered NO production was reversed in the presence of the NOS-inhibitor, indicating that NOS may play an important role in NO-mediated ABA-induced antioxidant enzymes activities and in turn ARF. NO generation has been suggested to be responsible for the regulation of H_2O_2, $O_2^{\cdot-}$ levels and antioxidant enzymatic activities, which may affect *V. radiata* adventitious rooting. It could be postulated that ABA is necessary to control antioxidant enzyme activity via modulating NO and ROS for inducing adventitious rooting.

ACKNOWLEDGMENT

The author wishes to thank Prof. Dr. Mohamed T. Ahmed, Plant Protection Dept, Suez Canal Univ, for critical reviewing of this manuscript.

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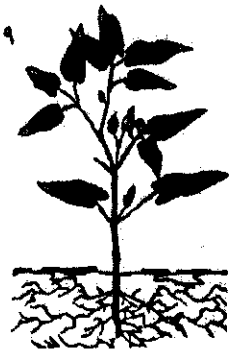

حوليـــات العلـــوم الزراعية
جامعة عين شمس ، القاهرة
مجلد(٥٥)، عدد (٢)، ١٧٩-١٩٠، ٢٠١٠

اشتراك أكسيد النيتريك فى تنشيط حمض الأبسيسيك لتكوين الجذور العرضية فى بادرات اللوبيا مفصولة الجذور

[١٤]

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الموجـــز

ينشط منظم النمو حمض الأبسيسيك تكوين الجـذور العرضية على بادرات اللوبيا مفصولة الجذور، مـن ناحية أخرى يعتبر أكسيد النيتريك أحـد الجزيئـات البيولوجية النشطة فسيولوجيا والتى ثبت حـديثا أنـه يشارك فى معظم العمليات البيوكيماوية والفسيولوجية المرتبطة بنمو وتطور النباتات متضمنة تكوين الجذور العرضية. يهدف البحث الحالى الى دراسـة علاقـة المتكون داخليا كنتيجة nitric oxideأكسيد النيتريك المعاملة الخارجيـة بحمـض الأبسيـسيك وامكانيـة مشاركته فى الأحداث المؤدية الى تنـشيط تكـوين الجذور العرضية على العقل النباتية تحت الدراسـة. عوملت قواعد عقل بادرات اللوبيـا عمـر ٧ أيـام لكل من حمـض 0, 25, 50, 100, 200 µMبتركيزات الأبسيسيك، أكسيد النيتريك كلا على حـدة لمـدة ٤٨ ساعة، قد عوملت العقـل النباتيـة أيـضا بحمـض الأبسيسيك بتركيز ١٠٠ ميكرومولار مضافا اليه إمـا [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, c-PTIO]

أحد المركبات المتخصصة فى ازالة أكسيد النيتريـك the NO specific scavenger بتركيـــز ١٥٠ ميكرومـولارأو (N^{ω}-nitro-Larginine, L-NNA) أحـد المثبطات الكيماوية للانزيم المـسئول عـن تكـوين اكـسيد النيتريـك the nitric oxide synthase (NOS) inhibitor من الحمض الأمينى arginine والأوكسيجين الجزيئى molecular oxygen كمـواد تفاعـل لهـذا الانزيم وذلك بتركيز ٣٠٠ ميكرومولار. أدت معاملة قواعد العقل بكل من حمـض الأبسيـسيك، أكـسيد النيتريك الى تنشيط معنـوى فـى تكـوين الجـذور العرضية وكان هناك تناسبا طرديا بين تركيـز تلـك المواد ذات التأثير المنشط لتكوين الجذور العرضـية، عدد الجذور المتكونة على قواعد تلك العقل. أظهرت نتائج هذا البحث أيضا أن عـدد الجـذور العرضـية المتكونة قد انخفض معنويا عنـدما عوملـت العقـل بحمض الأبسيسيك مضافا اليـه carboxy-PTIO or L-NNA دليلا على أهمية الدور الوظيفى لأكسيد النيتريك فى تكوين الجذور العرضية. بالاضافة الى ما سـبق، تشير النتائج أيضا على أن العقل المعاملـة بحـض الأبسيسيك قد احتوت على مستويات عالية من كل من أكسيد النيتريك، فوق أكسيد الهيدروجين، مـستويات منخفضة من الشق الحـر superoxide anion radical (O_2^-) مقارنة بالعقل غير المعاملة. على النقيض مـن ذلك احتوت العقل المعاملة بـ carboxy-PTIO علـى مستويات منخفضة من اكسيد النيتريك، فوق أكـسيد الهيدروجين، مستويات مرتفعة من superoxide anion radical وكان ذلك مصحوبا بعدد أقل مـن الجـذور العرضية علـى عكـس العقـل المعاملـة بحمـض

تحكيم: أ.د سعيد عواد شحاته
أ.د أحمد حسين حنفى

الأبسيسيك والتى احتوت على عدد أكبر من الجذور العرضية. واكثر من ذلك احتوت العقل المعاملة بحمض الأبسيسيك على مستويات عالية من أنشطة مضادات الأكسدة الإنزيمية [superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX)] مقرنة بالعقل الغير المعاملة، بينما احتوت العقل المعاملة بالمركب المتخصص فى إزالة أكسيد النيتريك على مستويات منخفضة من تلك الأنشطة الإنزيمية وعلى عدد أقل من الجذور العرضية دليلا على أهمية مضادات الأكسدة الإنزيمية تحت الدراسة فى تكوين وتطور تلك الجذور العرضية. يستنتج إجماليا من تلك الدراسة أن تنشيط حمض الأبسيسيك لتكوين الجذور العرضية على بادرات اللوبيا مفصولة الجذور قد تم من خلال اشتراك أكسيد النيتريك الذى لعب دورا هاما فى خلق بيئة مثلى لتكوين الجذور العرضية- مستويات عالية من جزيئات فوق أكسيد الهيدروجين، مستويات منخفضة من الشق الحر O_2^- ، و تنشيط مضادات الأكسدة الإنزيمية المشار اليها سابقا ذات الأهمية الوظيفية لتكوين الجذور العرضية.