INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 4, No. 5, p. 102-109, 2014

RESEARCH PAPER OPEN ACCESS

Physiological impact of sodium azide on *Helianthus annus* seedlings

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Article published on May 26, 2014

Key words: Sodium Azide, Carotenoides, Anti-oxidant Enzymes, Electrophoresis, Lipid Peroxidation.

Abstract

The present study was carried out to demonstrate the effect of sodium azide on *Helianthus annuus* var."Giza 53" at the green house of Botany Department, Faculty of Science, Tanta University, Egypt. Seeds were soaked in different concentrations of sodium azide (0.5, 1.0 and 2.0 mM) for different time intervals (30, 60, 90, 120 and 150 minutes). Control was maintained by soaking the seeds in distilled water only. The increased concentrations of sodium azide and time of soaking had a negative effect on the percentage of germination, shoot height, chla, chlb, lipid peroxidation and catalase activity. Increasing the concentrations and time of soaking of sodium azide stimulated the antioxidant defense of *Helianthus annuus* by increasing carotenoids, peroxidase activity and protein content. Pattern of protein was changed with increasing concentration and time of soaking.

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Introduction

Attempts to produce high yielding locally acceptable resistant cultivars have had limited success (Ogborn, 1987; Parker and Riches, 1993; Ramaiah, 1987). In view of the relative failure of these breeding programs, there is an urgent need for varieties with very high resistance. Potential Mutations are the tools used by the geneticist to study the nature and function of genes which are building blocks and basis of plant growth and development, hence generating raw materials for genetic enhancement of economic crops (*Adamu, et al., 2004*). Mutation methodology has been used to produce many cultivars with improved economic value, and to study genetic development phenomena. The use of mutagens in crop improvement helps to understand the mechanism of mutation induction and to quantify the frequency as well as the pattern of changes in different selected plants by mutagens. Mutation breeding generates a knowledge base that guides future users of mutation technology for crop improvement. Mutation methodology has been used to produce many cultivars with improved economic value (Broertjes and van Harten, 1988). There are several mutagens available for crop improvement and each mutagen has its important role as positive or negative effects on crops. Chemical mutation has been used to create and increase genetic variability in crop species and ultimately improves some plant traits (Ahloowalia and Maluszynski, 2001)**.** The main advantage of chemical mutation is the possibility of improving one or two specific characters without altering other (Salim *et al*., 2009). Sodium azide, a chemical mutagen has become important tool to enhance agronomic traits of crop plants. Adamu and Aliyu (2007) reported that sodium azide is being used to create durability in different susceptible crops to improve their yield and quality characters in opposition to damaging pathogens.

The mutagenic effects of sodium azide have been documented in previous reports. Kleinhofs *et al.* (1978) reported that sodium azide is a very potent mutagen in barley and induced chlorophyll deficiency as well as a wide range of morphological and physiological mutants. The treatment described here for sodium azide can be used for most dry seeds (Diedrick *et al*., 1990). azide creates point mutation in the genome of plants through metabolite and thus produced protein in mutant plants has different function compared to the normal plants. The mutant plants formed by the application of sodium azide are able to withstand a range of unfavorable conditions and have enhanced yields, improved stress tolerance, longer shelf life and reduced agronomic input in comparison to normal plant (Ahloowalia and Maluszynski, 2001). Its application on plant is easy and inexpensive and creates mutation to improve their traits. Sodium azide applied to soil demonstrated broad spectrum activity against weeds, nematodes, and soil borne phytopathogenic fungi (Kelley and Rodriguez-Kabana, 1979; Robertson and Rodriguez-Kabana, 2000).

The present study aimed to follow up response of sunflower seedlings to different concentration and different soaking times in sodium azide by evaluating the changes in the germination, growth, pigmentation, antioxidant enzymes and protein.

Materials and methods

Plant materials

Seeds of *Helianthus annuus* "Giza 53" was obtained from Crop Research Center, Sakha, Kafr Elsheek, Egypt. Seeds were surface sterilized using 0.01% (w/v) mercuric chloride ($HgCl_2$) for 5 minutes, with continuous steering, rinsed thoroughly many times in distilled water.

Sodium azide treatments

The seeds were soaked in three concentrations of sodium azide (0.5, 1.0 and 2.0 mM) for different time intervals (30, 60, 90, 120 and 150 minutes) control samples were maintained by soaking the seeds in distilled water only. Seeds were then washed under running tap water to remove excess chemicals and exudates from the seeds. Thirty seeds from each treatment were sown for in plastic pots containing 3 kg soil. The experiment was carried out in randomized complete block design (RCBD) at the

green house of Botany Department, Faculty of Science, Tanta University, Egypt.

Growth parameters

The pots were observed daily until maximum seed germination was attained and the germination percentage was calculated, seedlings shoot height (cm) for each treatment was measured as growth parameters.

Photosynthetic pigments

Chlorophyll a, b and carotenoids) were estimated following the method of Metzner *et al.* (1965). Pigment content was then expressed as mgg^{-1} fresh weight.

Total soluble protein

Proteins content was estimated quantitatively using phosphate buffer (pH 7) extract according to Bradford (1976). The protein content was expressed as mgg^{-1} fresh weight using calibration curve by Bovine Serum Albumin (BSA).

Antioxidant enzymes

Two antioxidant enzymes, catalase [EC1.11.1.6] and peroxidase [EC1.11.1.7] were assayed. A sample of 0.5 g fresh plant material was homogenized in 50 mM cold phosphate buffer (pH 7.0) (Beauchamp and Fridovich, 1971). The homogenates were centrifuged at 10000 rpm at $4C^{o}$ for 20 minutes. The supernatant was used as a raw extract for enzymatic assay. Catalase and peroxidase activity were assayed according to Kato and Shimizu (1987). Enzyme activity was expressed in units of µM of the substrate converted per min. per gram fresh weight.

Lipid peroxidation product (MDA)

The lipid peroxide concentration was determined by the method measuring the amount of thiobarbituric acid (TBA) reactivity by the amount of malondialdehyde (MDA) formed during acid hydrolysis of the lipid peroxide compound according to Uchiyama and Mihara (1978). The MDA content was calculated according to its extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed as $\mu molg^{-1}$ fresh weight.

Qualitative characterization of protein using SDS-PAGE

A sample of 0.5g frozen plant was homogenized with 1 ml of extraction buffer (25mM Na-acetate, pH 4.5 and 1 mM phenyl methyl sulphonyl fluoride [PMSF]), vortexed and left for 2 hours at $4^{o}C$. The extract was centrifuged at 10,000 rpm at $0^{o}C$ for 15 minutes and the clear supernatant was used as the total protein extract. Characterization and molecular mass determination of proteins were carried out using one dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970).

Results and discussion

The seed germination percentages of *Helianthus annuus* L. under three sodium azide concentrations with different soaking periods were represented in Fig. 1. Germination percentage reached 96.7% under the control treatment. Increasing concentrations of sodium azide and the soaking period for each concentration gradually inhibited seed germination. The lowest percentage reached to 53.3% which was observed with the highest sodium azide concentration (2.0 mM) at 150 minutes soaking period.

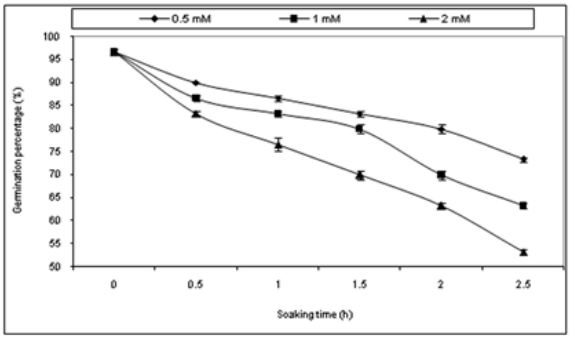

Fig. 1. Effect of different concentrations of sodium azide (control, 0.5, 1.0 and 2,0 mM) at different soaking periods (control, 30, 60, 90, 120 and 150 min) on the percentage of seed germination of *Helianthus annuus* L.

Treatment of the plant with sodium azide under different soaking periods also had an inhibitory effect on the shoot height in comparison with the control. The most pronounced decrease in the shoot height

was recorded at 2.0 mM sodium azide and 150 minutes soaking period (Fig. 2). Sodium azide is a strong mutagen, and growth of plant parts are strongly inhibited with increasing its concentration and treatment duration (Salim *et al*., 2009). The mutational effects of this mutagen has been observed on tomato and it was very effective in inducing mutations with respect to germination percentage, root length, seedling height, seedling survival, number of branches per plant and yield per plant respectively (Adamu and Aliyu, 2007). In this respect, Gehlot (2012) also found that, plants treated with sodium azide exhibited reduced plant growth.

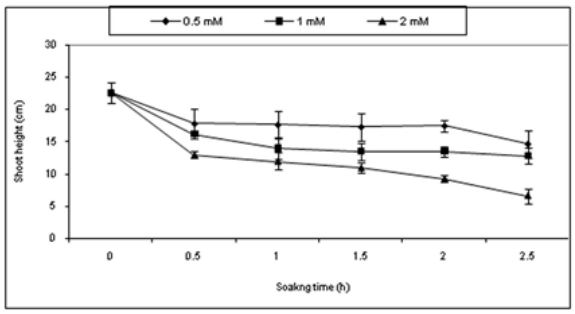

Fig. 2. Effect of different concentrations of sodium azide(control, 0.5, 1.0 and 2.0 mM) at different soaking periods (control, 30, 60, 90, 120 and 150 min) on the shoot height (cm) of *Helianthus annuus* L.

Reduction in seed germination as a result of mutagenic treatments has been explained by delayed or inhibition in physiological and biological processes necessary for seed germination which include enzyme activity as reported by Al-Qurainy and Khan (2009).

The photosynthetic pigments content of *Helianthus annuus* leaves varied in response to the effect of different concentrations of sodium azide at different soaking periods (Fig. 3). Chlorophyll a and chlorophyll b contents significantly decreased with increasing sodium azide concentration and the soaking period. On the contrary, the carotenoids content was increased reaching the highest value at 2mM sodium azide concentration and 150 minutes soaking period. The decrease in chlorophyll content as a result of sodium azide treatment was reported by many authors (Mahmoud and Al-Twaty, 2006; Al-Qurainy and Khan, 2009) and this may be resulted from chloroplast damage or from inhibition of its biosynthesis. Stressed plants increase their crotenoid content to provide protection against the formation of free radicals (Ferrat *et al*., 2003). Also carotenoid may play an important role in photoprotection of chlorophyll and chloroplasts against photooxidative damage (Behera *et al*., 2002) and can reduce lipid peroxidation (Burton and Ingold 1984).

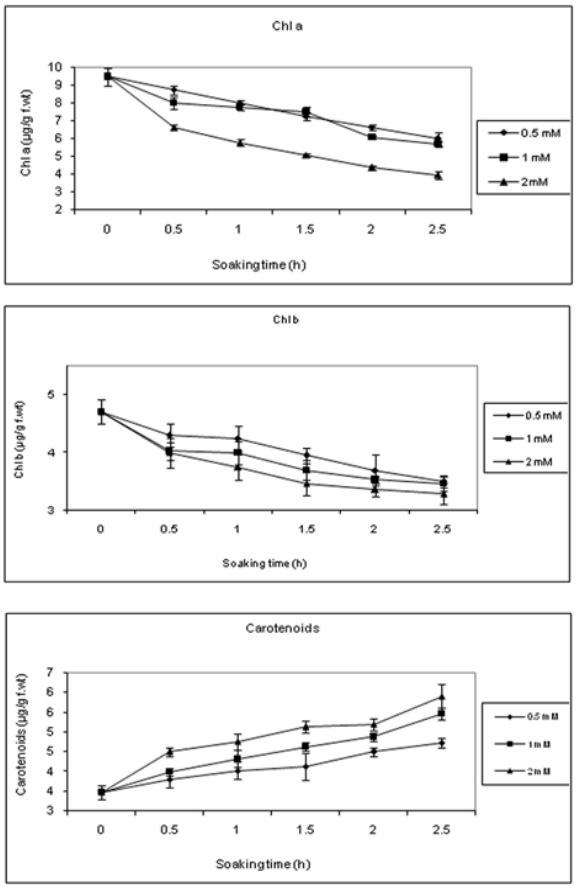

Fig. 3. Effect of different concentrations of sodium azide (control, , 0.5, 1.0 and 2.0 mM) at different soaking periods (30, 60, 90, 120 and 150 min) on the photosynthetic pigments (µgg-1 f.wt) of *Helianthus annuus* L.

The activity of catalase enzyme sharply decreased when increasing soaking periods in different sodium azide concentrations. Catalase activity had the minimum value at 0.5 and 1.0 mM sodium azide concentrations under soaking time 60 minutes and at 2.0 mM sodium azide under soaking time 30 minutes, after that it was gradually increased but it still low compared to control samples (Fig. 4).

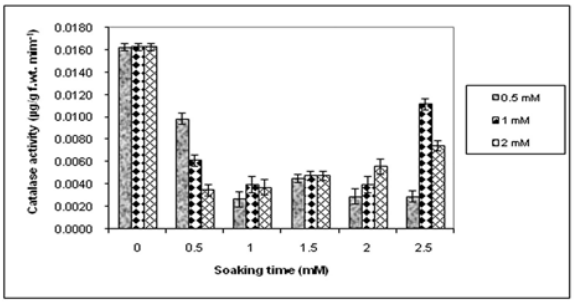

Fig. 4. Effect of different concentrations of sodium azide (control, , 0.5, 1.0 and 2.0 mM) at different soaking periods (30, 60, 90, 120 and 150 min) on the catalase enzyme activity (µgg-1 f.wt.min-1) of *Helianthus annuus* L.

Peroxidase activity gradually increased in response to the different concentrations of sodium azide compared to the control. Maximum peroxidase activity was recorded at 2.0 mM sodium azide and 150 minutes soaking period which increased peroxidase activity by 373.3% compared to the control (Fig. 5). In this respect, Lenoir *et al.* (1986) and Tayefi-Nasrabadi *et al.* (2011) found that sodium azide is a typical catalase and peroxidase inhibitor. It was reported that stresses bring about important alterations in the reactive oxygen metabolism, among these alterations is the disappearance of catalase activity and over production of H_2O_2 (Bailly *et al.,* 2004). The hydrogen peroxide produced is degraded by catalase and peroxidase into H_2O and O_2 in a sequence order. In the present study, there is an increase in peroxidase activity and this increase might compensate the loss in catalase activity.

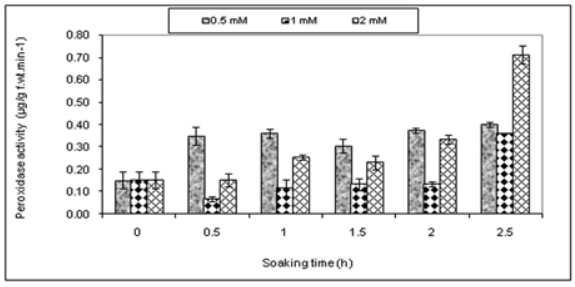

Fig. 5. Effect of different concentrations of sodium azide (control, 0.5, 1.0 and 2.0 mM) at different soaking periods (30, 60, 90, 120 and 150 min) on the peroxidase enzyme activity (µgg-1 f.wt.min-1) of *Helianthus annuus* L.

The data represented in Fig. 6. showed that the applied concentrations of sodium azide increased malondialdehyde (MDA) content in comparison with the control. Increasing the soaking periods resulted in a gradual increase in MDA content for each sodium azide concentration. The maximum MDA content was recorded at 1.0 mM sodium azide and 150 minutes soaking period which increased MDA content by 350.5% compared to the control. The increase in accumulation of malondialdehyde is an indicator of lipid peroxidation. A common aspect of the adverse conditions is the enhanced production of active oxygen species within the cell (Murthy *et al.* 2003) some active oxygen species are extremely reactive and oxidize biological molecules such as DNA, proteins and lipids (Walters, 1998). Free radicals have the potential to change the membrane structure and function (Bailly *et al.,* 1996). As a result, membrane integrity is gradually disrupted causing increased permeability of the plasma membrane and loss of compartmentation of cytoplasmic organelles (Kumar and Knowles, 1993).

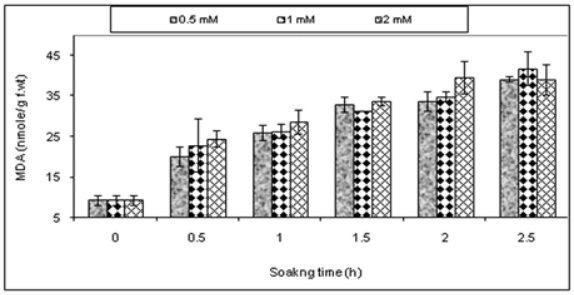

Fig. 6. Effect of different concentrations of sodium azide(control, 0.5, 1.0 and 2.0 mM) at different soaking periods (30, 60, 90, 120 and 150 min) on the malondialdehyde content (n mole-1g.f.wt) of *Helianthus annuus* L.

The protein content significantly increased gradually by increasing the soaking time in 0.5 and 1.0 mM sodium azide concentration. Increasing soaking time in 2.0 mM sodium azide increased the protein content till 60 minutes soaking period then it decreased gradually Fig.7. One possible mechanism for alleviating stress is the increase in soluble protein content. The increase in protein content is possible due to de novo synthesis of stress proteins (Verma and Dubey, 2003). On the other hand, the decrease in

protein content may be caused by enhanced protein degradation process as a result of increased protease activity (Palma *et al*., 2002). It is also likely that sodium azide may have induced fragmentation of proteins due to toxic effects of reactive oxygen species led to reduced protein content.

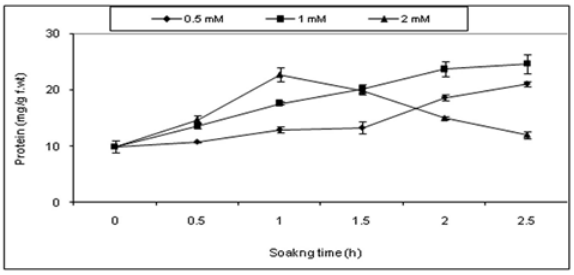

Fig. 7. Effect of different concentrations of sodium azide(control, 0.5, 1.0 and 2.0 mM) at different soaking periods (30, 60, 90, 120 and 150 min) on the total soluble protein content (mgg-1 f.wt) of *Helianthus annuus* L.

From Fig 8, we found that the number, intensity and or density of SDS electrophoretic band for seeds protein varied after sodium azide treatment,. Treatment with 1.0 mM for 90 min leads to appearance of more than one band with molecular weights ranged from 100 to 150 KD, Which been disappeared with increasing soaking time, while treatment of seeds with 0.5 mM sodium azide for 90 min leads to appearance of an intense band with 150 kD, also a new band with a molecular weight of 50 KD. Treatment with 2.0 mM revealed to the appearance of two bands with 50Kd and their intensity increased with increasing soaking times.

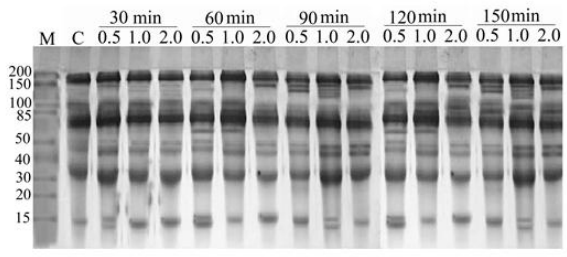

Fig. 8. Effect of different concentrations of sodium azide (control, 0.5, 1.0 and 2.0 mM) at different soaking periods (30, 60, 90, 120 and 150 min) on protein pattern of *Helianthus annuus* L.

The appearance of new bands may be related to the improvement of the studied plant. Treatment seemed to enhance sunflower genome and activate expression of some genes which resulted in the appearance of some new minor bands. These results are almost in agreement with those of Mahmoud and Al-Twaty (2006), Asmahan (1993), and Osama (2002) who found variations in number, intensity and or density of SDS electrophoretic bands of proteins from wheat and maize after sodium azide treatments.

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