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EFFECT OF SODIUM NITROPRUSSIDE AND GAMMA RADIATION ON GROWTH AND SOME PHYSIOLOGICAL PROCESS OF *(Zea mays* L.) PLANTS

Noha E. Eliwa* and Iman A. Farag

Nat. Prod. Dept., Nat. Cent. Rad. Res. and Technol., Atomic Ener. Authority, Cairo, Egypt

ABSTRACT

Sodium nitroprusside (SNP) is an important signaling molecule with diverse physiological functions in plants. In the present study, grains of maize (strain G_4) which were irradiated with gamma irradiation doses (15 and 30 Gy) or sprayed with sodium nitroprusside (150 and 300µM) induced significant enhancement on growth, photosynthetic pigments, and the content of Fe, Zn , Mg , Ca and Cu. The most enhancement effect was observed with spraying treatment (300µM SNP) or (15 Gy) gamma radiation dose. Antioxidant enzymes peroxidase, malate dehydrogenase, and Polyphenyl oxidase (PPO) isoenzymes electrophoresis showed appearance and disappearance of some bands with variation in density.

Key words: Maize (*Zea mays* L.), nitric oxide (NO), sodium nitroprusside (SNP), foliar spray, total pigments, mineral concentrations, isoenzymes electrophoresis.

INTRODUCTION

Nitric oxide (NO) is an important signaling molecule with diverse physiological functions in plants. In the last years, many advances have been obtained regarding NO synthesis and its physiological effects in plants. It was found to play a crucial role in plant growth and development, starting from germination to flowering, ripening of fruit and senescence of organs, respiratory metabolism, as well as plant response to abiotic and biotic stress. However, the molecular mechanisms underlying its effects remain poorly understood (Popova and Tuan, 2010). Plant metabolism is highly influenced by NO, although some complications arise by the mode of its application. NO is a gas, but in most experiments it is applied in the form of donor compounds that release NO into solution, such as sodium nitroprusside (SNP), S-Nitrosoglutathione, and S-nitroso-N-acetyl penicillinamin. The concentration of NO inside the plant tissue depends on some factors, temperature, kinetics of release, the level of concentration, variety of plant systems, and alltogether, these factors led to great variety in plant responses. In a rapidly growing pea foliage application, NO had a dual behavior. Low micro-molar concentrations produced an increase in the rate of leaf expansion, whereas no promotive effect occurred at higher concentrations. A similar dual behavior of NO donor SNP was also noted in wheat (Tian and Lei, 2006). Maize (*Zea mays* L.) is one of the most important grown plants in the world. Maize is the third most important cereal crop after wheat and rice. It is necessary for global food security (Cassman, 1999). Superior position of maize is due to its very wide and variety utilization. During the centuries maize plant was known for it's multifariously use. Maize is used like a human food, livestock feed, for producing alcohol and non- alcohol drinks, built material, like a fuel, and like medical and ornamental plant (Alahdadi *et al.,* 2011; Khodarahmpour, 2011). Gamma rays represent one of the important physical mutagens for improving vegetative growth and consequently the yield of many plants (Abdel-Tawab *et al.,* 2001). Gamma irradiation considered as a valuable tool in many purposes, from which, developing varieties that economically and agriculturally important and

* **Corresponding author: Tel. :** +201006267908
E-mail address: nohaeliwa@hotmail.com

have high productivity potential (Hussein *et al.*, 2012).

MATERIALS AND METHODS

Grains of Maize (strain G_4) were obtained from the Agricultural Research Center, Ministry of Agricultural. Giza. Egypt. Sodium nitroprusside was obtained from Sigma Company, Egypt. Grains were divided into 3 groups. The first group of dry grains was irradiated with 15 and 30 Gy (cobalt- 60 gamma rays) with dose rate 7.5 rad/ sec at National Center for Research and Technology, Nasr City, Cairo, Egypt. The second group of dry grains were sprayed with sodium nitroprusside with concentrations of 150 and 300µM after germination. The third group untreated grains were used as control. Treated and untreated (control) grains were sown in pots in randomized complete block experiment with five replications. The experiment was carried out using regular irrigation every 10 days. Data were recorded after 50 days from sowing. Results were statistically analyzed using Multiplie Range Test (Duncan, 1955). Different letters indicates significant variation.

Total Pigments

Fresh leaves were extracted with N.N. dimethyl formamide solution [$HCON(CH_3)_2$] and placed over night at cool temperature (5°C). Chlorophyll a, chlorophyll b and carotenoids were measured by Shimadzu UV-120-02 spectrophotometer at wave length 663,647 and 470 nm, respectively. Chlorophyll and carotenoids were calculated according to the equation described by Nornai (1982).

Chlorophyll a $=12.7A_{663}-2.79A_{647}$

Cholophyll b $=20.76\ A_{647}-4.62\ A_{663}$

Total carotenoids $=[1000A_{470}- (3.72\ chl._{(a)} - 104\ Chl_{(b)})]/ 299$

Measurement of Mineral Concentrations

Dried plants parts were ground into fine powder and used after digestion of samples according to AOCS (1984) for measurement of mineral concentrations. The minerals Ca, Mg, Mn, Fe, and Zn were estimated on Atomic Absorption Spectrophotometer (SOLAR- UNICAM 989) in NCRRT laboratory after complete digestion according to an appropriate dilution.

Isoenzymes electrophoresis

Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted to identify isozyme variations among studied treatments using three isozymes system according to Stegemann *et al.* (1985).

Fresh and young leaf samples were used separately for isozymes extraction. The utilized isozymes are Peroxidase (Px), Polyphenyl Oxidase (PPO) and Malatedehydrogenase (Mdh).

Gel preparation

The following stock solutions were prepared:

Acrylamide stock solution (30%)

The solution was prepared by dissolving 30 g acrylamide and 0.8 g N, N, methylene bis-acrylamide in about 70 ml distilled water, then the volume was completed to 100 ml by distilled water. The stock solution was kept at 4°C.

M Tris-HCl, pH 8.8

The buffer was prepared by dissolving 18.15 g Tris in 50 ml distilled water and shacked well with magnetic stirrer, and then pH was adjusted to 8.8 by conc. HCl solution. Then the volume was completed to 100 ml with distilled water and kept at 4°C.

Ammonium persulfate solution (APS 10% W/V)

The solution was prepared by dissolving 1.0 g ammonium persulfate in 10 ml distilled water. The solution is unstable and must be immediately prepared before use.

Monomer gel preparation

Acrylamide	8.3 ml
1.5 MTris	6.3 ml
D.W	9.9 ml
APS	250µl
TEMED	10 µl

Running buffer (5X)

This buffer was prepared by adding 15.0 g Tris and 72.0 g glycine to 1 liter distilled water and shacked well with magnetic stirrer. Then the volume was completed to 5 liters with distilled water and kept at 4°C.

Extraction of isozymes

Isozymes extracted from homogenizing 0.5 g fresh leaves samples in 1 ml extraction buffer (10% glycrol) using a mortar and pestle. The extract was then transferred into clean eppendorf tubes and centrifuged at 10000 rpm for 5 minutes. The supernatant was transferred to new clean eppendorf tubes and kept at –20°C until use for electrophoretic analysis.

Application of samples

A volume of 40 µl extract of each sample was mixed with 20 µl sucarose and 10 µ bromophenol blue, then a volume of 50 µl from this mixture was applied to each well.

Electrophoresis conditions

The run was performed at 150 volt until the bromophenol blue dye has reached the separating gel and then the voltage was increased to 200 volt. Electrophoresis apparatus was placed inside a refrigerator during running duration.

Isozyme staining and detection

After electrophoresis, the gels were stained according to their enzyme systems with the appropriate substrate and chemical solutions then incubated at room temperature in dark for complete staining. In most cases incubation for about 1 to 2 hours is enough.

Peroxidase (Px)

Benzidine di Hcl	0.125 gm
Glacial acetic acid	2 ml
D.W up to	50 ml

Gel was placed into this solution and 5 drops of hydrogen peroxide was added. The gel was incubated at room temperature until bands appear (Brown, 1978).

Malate dehydrogenase (Mdh)

0.1M Tris-pH (7.5)	100 ml
NAD	30 mg
MTT	20 mg
PMS	5 mg
Mleic Acid	1.2gm

Gel was placed into this solution and Incubate at 30°C for 30 min until bands appeared.

Polyphenyl oxidase (PPO)

After electrophoresis, the gel was soaked in 0.1 M Sodium Mono Di Phosphate buffer (pH 6.8) solved in 100mg Sulfanilic acid, then mixed with 30mg Cathecol solved in 1ml acetone. The gel incubated at 37°C until bands appear.

RESULTS AND DISCUSSION

Growth Parameters

The data presented in Table 1 revealed that spraying maize plants by SNP (300µM), significantly increased plant length, leaf area, ear leaf area, stem diameter, dry weight of leaves, and specific leaf area as compared with control plants, whereas No. of leaves and Specific leaf weigh had no significant increase.

SNP (150µM) was less effective on all growth parameters studied compared with (300 µM).

On the other hand gamma radiation (15 Gy) appeared to improve growth over that of control plant. Such response was reversed with increasing gamma radiation dose to (30 Gy).

Nitric oxide (NO) is a small, highly diffusible gaseous bioactive molecule. Its chemical properties make NO a versatile signal molecule that functions through interactions with cellular targets via either redox or additive chemistry (Lamattina *et al.*, 2003). Plant metabolism is highly influenced by NO, although some complications arise by the mode of its application. It has been found that the treatment of spinach plants with a low concentration of NO gas (ambient atmosphere with 200 nl l^{-1} NO gas) significantly increased the shoot biomass of the soil cultivated plants as compared with the control treatment (ambient atmosphere) (Jin *et al.*, 2009).

Exogenously applied NO (SNP) alleviates browning of tuber explants by reducing H_2O_2 accumulation, thereby promoting a higher *in vitro* proliferation frequency of Discoreaopposita (Xu *et al.*, 2009).

The effect of spraying SNP, on growth characters of cotton plants under drought stress, was studied by (Shallan *et al.*, 2012). They showed that all growth characters (plant height, number of nodes, inter-node length, *etc.*) of cotton plants were decreased under drought stress conditions in comparison with control plants. Spraying of cotton plants with SNP (0.05,

Table 1. Effect of gamma rays and SNP on the growth criteria of *Zea mays* plants

	Plant length (cm)	Leaf area (cm^2/plant)	No. of leaves	Stem diameter (cm)	Dry weight of leaves (mg) plant	Specific leaf area (SLA)	Specific leaf weigh (SLW)
Control	175.78^{d}	380.61^{e}	5.9	3.6^{d}	11.32^{e}	33.63^{d}	0.0297
150µ MSNP	193.17^{c}	488.18^{d}	6.9	4.0^{c}	12.41^{d}	39.33^{b}	0.0254
300µ MSNP	227.63^{a}	721.11^{a}	7.8	4.9^{a}	16.22^{a}	44.46^{a}	0.0225
15Gy	209.61^{b}	622.81^{b}	7.1	4.8^{a}	15.39^{b}	40.47^{b}	0.0247
30 Gy	187.48^{c}	517.50^{c}	6.4	4.3^{b}	14.19^{c}	36.48^{c}	0.0274
f	**	**	N.S	**	**	**	N.S

Specific leaf area (SLA) = leaf area (cm^2/plant) / dry weight of leaves (mg) plant
Specific leaf weigh (SLW) = dry weight of leaves (mg) plant / leaf area (cm^2/plant)

0.1 and 1 mM) under drought stress conditions increased the growth characters of cotton plants to be near of untreated plants (control). Pre-treatment of cotton plants under drought stress with SNP decreased adverse effects of drought stress, supporting that NO is actively involved in the regulation of plant growth. Previous studies have demonstrated that the exogenous NO, mitigated decrease in plant growth caused by drought is through increasing antioxidant system, alleviating oxidative damage and accelerate proline accumulation, augmented the synthesis of compatible solutes, enhance photosynthesis (Farooq *et al.*, 2008; Anjum *et al.*, 2011). Aftab *et al.* (2012) reported that treatment of NO donor favoured growth and improved the photosynthetic efficiency in stressed as well as non-stressed plants and exogenous application of SNP promoted root elongation in both stressed and non-stressed plants.

Pinkard *et al.* (2007) observed increases in stem diameter of *Eucalyptus globulus* due to N fertilization of defoliated trees suggested increases in leaf area development, and there were changes in the leaf area: leaf dry mass ratio that may have increased light absorption by the crown. Nitrogen fertilization also increased partitioning of dry mass to branches at the expense of main stems, suggesting that N supply was important in rebuilding crowns following a defoliation event.

Chang-li *et al.* (2011) indicated that exogenous NO could significantly improved stem height and diameter, number of leaves, fresh and dry mass and healthy index of seedlings. Moreover, exogenous NO could markedly increase chlorophyll and soluble protein content, free proline content, (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) activies, while significantly restrain the production of MDA. These results suggested that NO could regulate the antioxidative enzymes activity, mass-eliminate ROS, which improved salt-tolerance ability of Brassica

Guo *et al.* (2009) reported that the dry weights of both roots and shoots of *K. virginica* seedlings were significantly increased by exogenous NO treatment.

Girija and Dhanavel (2009) tested the effect of gamma irradiation on cowpea (*Vigna unguiculata* L. Walp) at 15, 20, 25, 30 and 35 KR. They found that the germination percentage of cowpea decreased with the increase in the dose concentration and it was estimated that using 50% reduction in seed germination was observed at 25KR dose.

Hamideldine (2010) irradiated seeds of two cultivars of tomato (*Lycopersicon esculentum* Mill) Marm and Nima with 0, 20 and 40 Gy. There were no significant variations in germination percentage in cultivar Marm, but irradiation by 40 Gy decreased it significantly in cultivar Nima. The dose 20 Gy gave the highest increase with significant variation in shoot length, number of leaves and fresh weight in cultivar Marm. In cultivar Nima, significant changes were found in shoot length, number of leaves, leaf area and fresh weight.

Hegazi and Hamideldin (2010) studied the effect of different doses of gamma irradiation (300,400 and 500 Gray) on growth of two okra varieties, results showed that 400 Gy gave the highest number of branches per plant, leaf area and fresh and dry weight per plant followed by 300, then 500 Gy.

Hussein (2010) studied the effect of gamma rays (0-250Gy) on mungbean (*Vigna radiate* L). The results showed that the highest dose of radiation (250 Gy) caused stunted growth on mungbean seedlings while the lower doses (50 and 100 Gy) stimulated the growth of these seedlings significantly. Helaly and El- Hosieny (2011) studied the effectiveness of gamma irradiated protoplasts at 0, 5, 10 and 20 Kr. It was found that highest fresh weight was recorded at 10 and 20 Kr.

El-Sherif *et al.* (2011) revealed that gamma irradiation significantly increased plant height, number of branches, number of roots and root length of roselle plant as compared with control.

Ramesh *et al.* (2012) reported that leaf contributing parameters such as leaf area and length of internodes which are desirable morpho-economic traits in mulberry were also affected due to irradiation. Mutants with enlarged leaf area were observed specially at 4kR gamma irradiation.

Total Pigments

The increase in growth parameters was accompanied with an increase in photosynthetic pigments, *i.e.* (300μM SNP) gave higher increment in photosynthetic pigments compared to control plants or 150μM SNP treated plants. Concerning radiation effect, it is cleared that 15 Gy significantly increased Chl. a, Chl. b, carotenoids and total pigments studied compared with 30 Gy treated plants.

Effect of gamma rays and SNP on the photosynthetic pigments (mg/g) fresh weight of corn plant.

Jasid *et al.* (2006) suggested that at least two pathways for NO production are operative in chloroplasts of soybean, one dependent on NOS-like enzyme activity and another on nitrite. Under high NO concentration (*i.e.* high nitrite content in chloroplasts), the generation of reactive nitrogen species may lead to impairment of the photosynthetic machinery.

Treatment with SNP improved the rate of photosynthesis, chlorophyll content, transpiration rate and stomatal conductance in cucumber seedlings (Fan *et al.*, 2007).

Treatment with SNP delayed yellowing and retarded the chlorophyll degradation in broccoli (Hyang *et al.*, 2009). Therefore, NO from SNP increased the Fe content might play important role in alleviation of cucumber leaf chlorosis (Lixu *et al.*, 2013). There were some other investigations indicated that NO was a key component in the regulation of iron uptake and homeostasis in plants (Chen *et al.*, 2010b), In accordance with the chlorosis alleviation by application of SNP, photosynthesis of cucumber was significantly promoted and plant growth was increased, similar function of SNP has been observed in Cd-treated barley (Chen *et al.*, 2010a).

Moussa and Abdul Jaleel (2011) studied the effect of gamma radiation doses (0.0, 25, 50, 100 and 150 Gy) on *Trigonella foenumgraecum* L.). It was found that gamma irradiation used, increased total chlorophyll values for plants treated with 150 Gy.

Table 2. Effect of gamma rays and SNP on the photosynthetic pigments (mg/g) fresh weight of corn plant

Treatments	Chl.a	Chl.b	Carotenoids	Total pigments
control	2.220^{ab}	0.656^{b}	1.029^{a}	4.234^{b}
150μM SNP	1.381^{b}	0.812^{b}	1.402^{a}	3.595^{b}
300μ M SNP	2.201^{ab}	1.230^{ab}	1.140^{a}	4.571^{ab}
15Gy	3.052^{a}	1.844^{a}	2.168^{a}	7.064^{a}
30 G	2.369^{ab}	1.237^{b}	1.166^{a}	4.772^{ab}

Different letters indicate significant variation.

Minerals

Table 3 illustrate that the SNP (300µM) induced a significant increase in the contents of Fe, Zn, Mg, Ca and Cu. The same results were found in plants irradiated with 15 Gy compared to control plants or other treatments.

Application of NO reduced the accumulation of Na+ and enhanced that of K+ (Zhang *et al.*, 2004).Consequently, exogenous application of NO proved to be helpful for crop establishment (Zheng *et al.*, 2009).

Hussein *et al.* (2012) treated (*Ambrosia maritime* L.) seeds before sowing by gamma radiation and noticed that there was generally increase in Na percentage in damsisa plant during vegetative stage of growth as compared by its corresponding control or un-irradiated and unstressed control. Concerning the effect of γ-rays, it was observed that, the first stage of growth, all radiation dose decreased Mg (%) comparing with normal control (unstressed and un-irradiated). Hamideldin and Hussein (2014) revealed that Gamma irradiation increased Cl, K, Na and P contents and decreased Ca, Fe and Mg contents in variety Silana. In variety Daimont, gamma irradiation increased the contents of Ca, Cl, Fe, K and Na but decreased thos of Mg and P.

Isoenzymes

Peroxidase isoenzymes

The electrophoretic patterns of peroxidase isoenzyme was photographed and illustrated in Fig. 1 and Table 4. It is observed that band (1) was appeared in all treatments used. It is also cleared that band (1) was very dark under all treatments which means high concentration of isoenzyme except for treatment (2) (150µM SNP) which is less darkness. Band (2) appeared also in all treatments but showed variation in density from faint to very faint. 150 µM No treatment showed inhibition of Band (3), while other treatments showed appearance of Band (3) with low density.

Malate dehydrogenase (Mdh)

It is cleared from electrophoretic pattern of malate dehydrogenase isoenzyme (Fig. 2 and Table 5) that band (1) disappeared in control and 15 Gy treated plants, while it was faint in all treatments which means low concentration in all other treatments.

Band (2) and Band (3) were present in all treatments used but varied in concentration from dark to very dark. Figure (2)

Polyphenyl oxidase enzyme

Electrophoretic pattern of polyphenyl oxidase eisoenzyme (Fig. 3 and Table 6) showed differences in density and number of bands among control and treated plants. The band (1) was very dark in all-treatments which means high concentration of isoenzyme. In band (2) the concentration of the band separated from plants treated with 150 µM SNP was very low as compared with control and other treatments. Band (3) was absent in plants treated with 150µM SNP, 30 Gy treated plants increased the concentration of isoenzymes than control treatments which means high concentration of isoenzyme. In band (2) the concentration of the band separated from plants treated with 150 µM.

SNP and 15 Gy was very low as compared with control and other treatments Aftab *et al.* (2012) supporting that application of SNP, increases the activity of antioxidant enzymes such as CAT, POX and SOD and total antioxidant capacity.

Hayat *et al.* (2014) found that the seed soaking treatment of SNP (10-5 M) enhanced the activities of antioxidant enzymes (CAT, POX, SOD) in the tomato seedlings. Similar results have been reported in canola leaves (Kazemi *et al.*, 2010) and wheat roots (Wang *et al.*, 2010) by the application of NO. The enhanced activity of these enzymes by NO may be due to NO-mediated increased availability of iron in plants and improved biosynthesis of these enzymes (Kazemi, 2012). Moreover, it has been reported that NO acts as an antioxidant and quenches ROS that is generated during oxidative stress (Xiong *et al.*, 2010).

Hamideldin and Eliwa (2015) found that treated plant with gamma radiation increased density of 0.3 band of peroxidase isoenzymeunder normal condition. Concerning malate dehydrogenase isoenzyme it is observed that irradiated plant with gamma radiation increases the density of 0.2 band as compared with the control.

Table 3. Effect of gamma rays and SNP on Mineral content of corn plants

Treatments	Fe ($mg\ Kg^{-1}$)	Zn ($mg\ Kg^{-1}$)	Mg ($X10^3$) ($mg\ Kg^{-1}$)	Ca ($mg\ Kg^{-1}$)	Cu ($mg\ Kg^{-1}$)
Control	30.8^{d}	41.9^{d}	13.7^{bc}	6956^{c}	9.7^{c}
150µM SNP	94.0^{b}	42.5^{d}	19.1^{a}	1060^{a}	8.7^{d}
300µ M SNP	267.6^{a}	55.3^{b}	20.2^{a}	6666^{d}	16.0^{a}
15Gy	111.2^{ab}	70.3^{a}	15.0^{ab}	6666^{d}	10.5^{b}
30 Gy	44.5^{c}	50.8^{c}	8.61^{c}	10416^{b}	6.5^{e}

Different letters indicate significant variation.

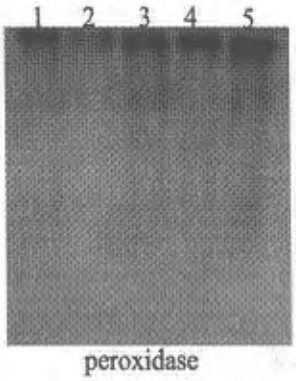
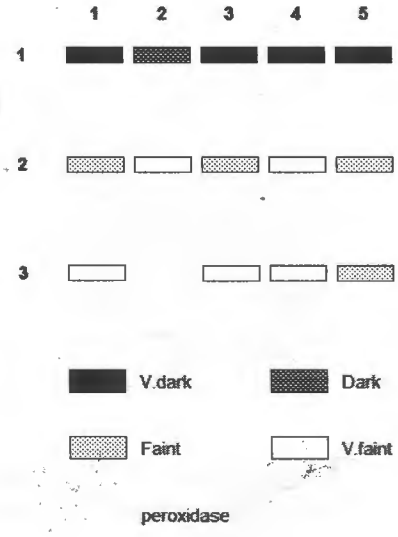


Fig. 1. Ideogram analysis for peroxidase isozyme banding patterns of 50-days-old maize plants. 1. Control 2. 150µM SNP 3. 300µ M SNP 4. 15Gy 5-30 Gy

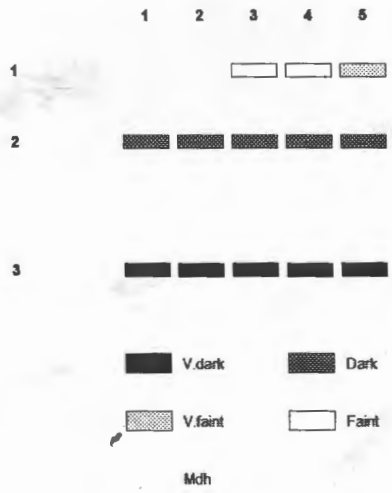
Table 4. Densitometry analysis of band density and present or absent of band for peroxidase isozyme of 50-days-old maize plants. 1. Control 2. 150µM SNP 3. 300µ M SNP 4. 15Gy 5-30 Gy

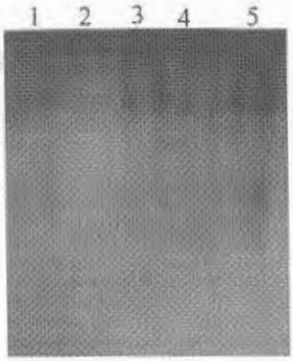


Malate dehydrogenase

Fig. 2. Ideogram analysis for malate dehydrogenase isozyme banding patterns of 50- days-old maize plants. 1. Control 2. 150µM SNP 3. 300µ M SNP 4. 15Gy 5-30 Gy

Table 5. Densitometry analysis of band density and present or absent of band for malate dehydrogenase isozyme of 50-days-old maize plants. 1. Control 2. 150µM SNP 3. 300µ M SNP 4-15Gy 5-30 Gy

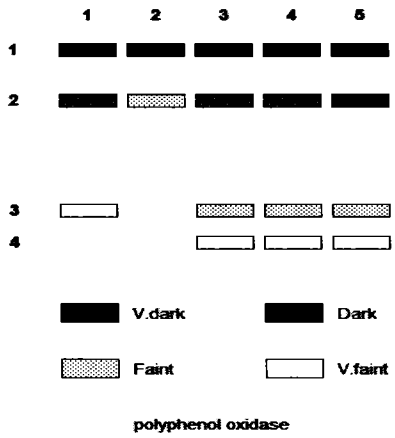




Polyphenol oxidase

Fig. 3. Ideogram analysis for poly phenoloxidase isozyme banding patterns of 50- days-old maize plants. 1. Control 2. 150µM SNP 3. 300µ M SNP 4. 15Gy 5-30 Gy

Table 6. Densitometry analysis of band density and present or absent of band for polyphenol oxidase isozyme of 50-days-old maize plants. 1. Control 2. 150µM SNP 3. 300µ M SNP 4. 15Gy 5-30 Gy



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تأثير نتروبروسيد الصوديوم وأشعة جاما على النمو وبعض العمليات الفسيولوجية لنبات الذرة

نهي عيد عليوة - إيمان عبدالله فرج

قسم المنتجات الطبيعية - المركز القومي لبحوث وتكنولوجيا الإشعاع- هيئة الطاقة الذرية - القاهرة - مصر

الذرة الشامية هي واحدة من النباتات النجيلية الأكثر أهمية في العالم، الذرة هي ثالث أهم محاصيل الحبوب بعد القمح والأرز، يستخدم الذرة كغذاء للإنسان وعلف للماشية، ولإنتاج الكحول والمشروبات الكحولية غير المواد المبنية، مثل الوقود، وتمثل أشعة جاما واحدة من المطفرات الهامة لتحسين النمو الخضري وبالتالي العائد على العديد من النباتات، كما يعد أكسيد النيتريك (NO) جزء مهم ويرتبط بكثير من الوظائف الفسيولوجية المختلفة في النباتات، في الدراسة الحالية أدي تشعيع حبوب الذرة من سلالة نقية (G4) بأشعة جاما بجرعات (١٥ و٣٠ جراي) أو رشها بنتروبروسيد الصوديوم بتركيز (١٥٠-٣٠٠Mμ) إلى زيادة ملحوظة في النمو والصبغات النباتية ومحتوى الحديد والزنك والمغنسيوم والكالسيوم والنحاس، وظهرت أفضل نتيجة باستخدام NO (٣٠٠Mμ) أو أشعة جاما ١٥ جراي، أظهر الفصل الكهربائي للإنزيمات المضادة للأكسدة بيراوكسيديز-ماليت ديهدروجينيز وبوليفينيل أوكسيديز ظهور واختفاء بعض الطرز مع الاختلاف في الكثافة.

المحكمون :

١- **أ.د. نـــادية حســـين كـــامل** أستاذ النبات الزراعي المتفرغ – كلية الزراعة – جامعة الزقازيق.

٢- **أ.د. السيد محمود محمد مقبيل** أستاذ النبات الزراعي المتفرغ – كلية الزراعة – جامعة الزقازيق.