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Effect of sodium azide on growth criteria, some metabolites, mitotic index and chromosomal abnormalities in *Pisum sativum* and *Vicia faba*

K.M. Saad-Allah, M. Hammouda, W.A. Kasim*

Botany Department, Faculty of Science, Tanta University, Tanta, Egypt

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Key words: Chromosomal abnormalities, metabolites, mitotic index, *Pisum sativum*, Sodium azide, *Vicia faba*.

Abstract

Seeds of *Pisum sativum* and *Vicia faba* were pre-soaked in one of three concentrations (1.0, 2.0 or 4.0 mM) of the mutagenic agent sodium azide (NaN_3) for different periods (30 min, 1 h or 2 hs). The impact of these treatments on seed germination, some growth criteria, photosynthetic pigments, some metabolic activities and cytological behavior in the growing seedlings, the yield parameters as well as the variation in the protein profile of the yielded seeds of the selected mutants grown from the seedlings were studied. The germination percentage, shoot height, root depth, leaflets area, chl. a, ch.b, the initial level of fluorescence (Fo) were decreased with NaN_3 treatments and these decreases were directly proportional to the dosage and duration of treatment. In the two species, the treatments resulted in significant increases in the carotenoid contents, total soluble carbohydrate contents, total soluble protein content, total free amino acids contents and theses increases were directly proportional to the increase in NaN_3 concentration and soaking time. However, all yield criteria were reduced gradually by increasing NaN_3 concentration and soaking duration. The rate of mitotic index (dividing cell frequency) was generally decreased while; the rate of non-dividing cells and the rate of abnormalities were increased with the increase in both the pre-soaking duration and concentration of NaN_3. Marked changes in the protein patterns in the two plant species were recorded where nine polymorphic bands, 16 monomorphic bands and 2 two unique bands were found in the case of pea, while in case of bean, there were 13 polymorphic bands, 15 monomorphic bands and two unique bands.

* **Corresponding Author:** Kasim, W.A. ✉ wedkasim@yahoo.com

Introduction

Pea (*Pisum sativum* L.) is one of the most important legumes crops in Egypt and the world for its versatile use as human food, livestock fodder and as a source of hay (Choudhury *et al.*, 2006). It is widely used because of the high protein content of the seeds, their balanced amino acid composition, good taste and digestibility, as well as high yield. Faba bean, broad bean or field bean (*Vicia faba* L.) is a major food and feed seed legume owing to the high nutritional value of seeds, with 27-34% protein (Link *et al.*, 1995**;** Duc, 1997). In Egypt, faba bean is among the main nutritional source of plant proteins (El-Danasoury *et al.,* 2008; Bakry *et al*., 2011). Mutations are known to enhance the genetic variability of crop plants and result in large number of high yielding varieties of several crops (Pavadai *et al*., 2010 a; Mehta and Nair, 2011). The ability of these mutagens to enter the cell of living organisms to interact with the DNA produces the general toxic effects associated with their mutagenic properties; thus, their effects are mainly due to the direct interaction between the mutagen and the DNA molecules (Kleinhofs *et al*., 1978).

Sodium azide (NaN_3, mol. wt 65.02) is a colorless, odorless and crystalline solid (Nilan *et al.*, 1977); it is relatively safe to handle, inexpensive, non-carcinogenic and is also the least dangerous and the most efficient chemical mutagen. However, it has been reported to be mutagenic in several crop species where its mutagenesis cannot only generate diverse resistance but also provide an efficient method for breeding disease resistant varieties (Adamu and Aliyu, 2007; Mostafa, 2011). Owais and Kleinhofs (1988) stated that the mutagenicity of NaN_3 is arbitrated through the formation of an organic metabolite which enters the nucleus, interacts with DNA and generates point mutations in the genome. Owais *et al.,* (1983) reported that it is metabolized by plant cells to the mutagenic agent azidoalanine. Utilization of NaN_3 in generating genetic variability in plant breeding has been reported in barley (Kleinhofs and Sander, 1975), in groundnut (Mensah and Obadoni, 2007) and other crops (Avila and Murty, 1983; Routaray *et al*, 1995). The main advantage of mutation breeding is the possibility of improving one or two characters without changing the rest of the genotype.

NaN_3 causes cytotoxicity in several animal and plant test systems, by inhibiting the protein synthesis and replicative DNA synthesis at low dosages (Grant and Salamone, 1994), and induces chromosomal aberrations such as break, gap, iso-chromatid break and exchange the cells (Ragunathan and Panneerselvam, 2007). Mutagens induce structural changes in chromosomes and create mutations, which might be responsible for the failure of pairing among homologous chromosomes. Adegoke (1984) reported that NaN_3 induces chromosomal damages leading to bridge formation during mitotic division, and hence increased phenotypic aberration.

Effect of NaN_3 on seed germination and plant survival was reported in *Stevia rebaudiana* seeds by Pande and Khetmalas (2012) and in *Nigella, Plantago and Trigonella* by Prabha *et al.* (2011). The latter three crops showed minimum germination at 4.5 mM concentration of NaN3 for eight hrs of duration of exposure. Similarly, Pearson *et al.* (1974 and 1975) found that various concentrations of NaN_3 delayed the initiation of plant growth in barley. On the other hand, Fay (1975) observed some positive effects of NaN_3 in wild oat seed. In wheat, NaN_3 reduced the root depth and shoot height (Srivastava *et al.* (2011) and in tomato, it decreased each of germination percentage, seedling height, seedling survival, number of leaves per seedling, height at maturity, number of leaves per plant and yield per plant, where these effects are increased with increasing NaN_3 concentration (Adebola, 2013).

Chlorophyll mutations are considered as the most dependable indices for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic markers in basic and applied research. NaN_3 was shown to be a potent mutagen in barley and induced chlorophyll deficiency as well as a wide range of

morphological and physiological mutants (Kleinhofs *et al.*, 1978).

Increase of soluble sugars after exposure to chemical and physical mutagens was reported by many authors (Mohamed and Moussa, 2003; Nassar *et al.*, 2004; Li *et al.*, 2005; El-Shafey *et al.*, 2009 and Naganada *et al.*, 2013). The increase in osmolyte or osmoprotectant contents may come as a defens mechanism via the activation of genes responsible for expression of the enzymes involved in the accumulation of these osmolytes, leading to more protection for the up-regulating enzymes involved in the anabolism of these contents (El-Shafey *et al.*, 2009). Increase of total soluble protein content by NaN_3 was reported in many crop plants as soybean (Pavadai *et al.*, 2010a), sorghum (Dahot *et al.*, 2011) and maize (Gnanamurthy *et al.*, 2013). On the other hand, Sheikh *et al.* (2012) found that it has no significant effect on the mean protein content of wheat grains.

SDS-PAGE analysis of proteins and isozymes aids in the systematic study of polymorphism and in the elucidation of phylogenetic relationships between and within the species (Ghafoor *et al.*, 2002). Analysis of seed storage protein and the induced changes in number and intensity of bands for different proteins may be used to identify and differentiate the species, in variety description and in assessment of the cultivars (Osanyinpeju and Odeigah, 1998; Nayeem *et al.*, 1999; Singh, 2011).

The present study was conducted to investigate the impact of pretreatment of *Pisum sativum* and *Vicia faba* seeds with the mutagenic agent NaN_3 on seed germination, some growth criteria, photosynthetic pigments, some metabolic activities and cytological behavior in the growing seedlings, the yield parameters, as well as the changes in the protein profile of the yielded seeds of the mutants grown from the pre-treated seeds.

Materials and methods

Two legume crops pea (*Pisum sativum* L.) and bean (*Vicia faba* L.) were chosen for the present study. Seeds of these two species were kindly provided by the Agriculture Research Centre (ARC), Giza, Egypt. The two plants were grown for one season (December 2012 to April 2013). The seeds were surface sterilized using 0.01% (w/v) mercuric chloride ($HgCl_2$) for 8 minutes, with continuous stirring, rinsed thoroughly several times in distilled water, then three groups of them were soaked in three concentrations of NaN_3 (1.0, 2.0 or 4.0 mM) for different time intervals (30 min., 1 h. or 2 hs.), and the control was maintained by pre-soaking the seeds in distilled water. The soaked seeds were then washed under running tap water and sown in plastic pots (35 cm diameter x 18 cm depth) each containing 6 kg clay soil. The experiment was carried out in randomized complete block design (RCBD) with split-plot arrangement in the green house under conditions of 25/18°C day/night temperature and natural light(16/8 h day/night). Phosphate fertilizer was applied at 20 lb/acre, as a side band with the seed, at the seedling stage and fruiting stage. The pots were left until maximum seed germination was attained and the germination percentage was calculated.

Growth criteria (shoot height, root depth and leaflet area), photosynthetic pigments (chlorophylls-a and –b, and carotenoids) were determined in the fresh seedling (21days old), following the method of Metzner *et al.* (1965) and expressed as mg/g fresh weight. Chlorophyll fluorescence was measured using portable fluometer (OS-30p). The leaves were dark-adapted for 30 min, then the initial fluorescence (Fo) and the maximum fluorescence (Fm) were assessed. The variable fluorescence (Fv) was calculated as Fv = Fm- Fo and the maximum quantum efficiency of PSII photochemistry (Fv/Fm) was determined. Total soluble carbohydrate contents were determined using phenol sulfuric acid method according to Dubois *et al.* (1956) and expressed as mg/g d.wt. The total soluble protein contents were estimated quantitatively in the phosphate buffer (pH 7) extract using the method described by Bradford (1976). The protein content

was calculated as mg/g f. wt. Amino acids content was determined by ninhydrin assays according to Lee and Takahashi (1966) and calculated as mg/g d. wt.

At the end of the growth season (3 months), yield parameters were determined such as the number of pods per plant, number of seeds per pod, the mass of seeds/legume, the mass of seeds/plant and the mass of 1000 seeds.

To asses the effects of NaN_3 on cell division and chromosomes, seeds were grown in Petri dishes on blotting paper moistened with distilled water until the radicals reached a length of 1-2 cm. At least ten roots of at least ten different seedlings from each treatment were then fixed in freshly prepared fixative composed of absolute ethanol and glacial acetic acid (3:1) for 24 hours and kept in 70% ethanol at 4°C until use for cytological preparations as Feulgen's squash technique to calculate the mitotic index (dividing cell frequency) and % chromosomal abnormalities**.**

The protein patterns of the seedlings and the yielded seeds were studied using Sodium- Dodecyl-Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) as described by Laemmli (1970). The gel was then photographed with digital camera and the presence or absence of bands was scored as 1 or 0, respectively. Molecular weights of protein bands were calculated using Lab Image software version 2.7 produced by Kapelan GmbH, Germany.

Results and discussion

As shown in Fig. 1, the germination percentage was decreased with NaN_3 treatments and it was directly proportional to the dosage and duration, where the treatment with 4mM NaN_3 for 2 h decreased the germination percentage to 30 and 67.6 % for pea and bean, respectively. These reductions in seed germination may be due to the delaying or inhibition of physiological and biological processes necessary for seed germination which include enzyme activities and to the inhibition of mitotic process (Mensah *et al*., 2006). The inhibitory effect of NaN_3 on germination could be also attributed to the azide anions which are considered as strong inhibitors of cytochrome oxidase, which in turn inhibits oxidative phosphorylation (Kleinhofs and Sander, 1975). Azide anion plays an important role in causing mutation by interacting with enzymes and DNA in the cell; in addition, it is a potent inhibitor of the proton pump and it can alters the mitochondrial membrane potential (Zhang, 2000). These effects together may hamper ATP biosynthesis resulting in decreased availability of ATP which may slow the germination rate and reduce the germination percentage. This is consistent with the results of some previous authors who found that the various concentrations of NaN_3 delay the initiation of plant growth and reduce the rate of germination (Mensah and Akomeah, 1992; Mensah *et al*., 2005; Divanli *et al.,* 2006 and Pande and Khetmalas, 2012).

As shown from Fig.2, shoot height, root depth and leaflets area of the two studied species were significantly decreased ($P<0.001$) with the increase in both of the concentration and the soaking time in NaN_3. These results are in accordance with those of Mensah *et al.* (2006) and Adamu and Aliyu (2007) who reported reductions in the growth with the increase in NaN_3 level in cowpea and tomato, respectively. The reduction in plant growth with increasing concentrations of NaN_3 and treatment duration is due to the decrease in the mitotic index of the plant cells as reported by Ilbas *et al.* (2005). It was also reported that NaN_3 decreases the cellular calmodulin level which is a calcium binding protein for signal transduction and cell division, and it is a proton pump inhibitor that blocks secretion and accumulation of cyclic adenosine monophosphate (cAMP) (Kleinhofs *et al*., 1978; Osborn and Weber, 1980; Dinauer *et al*., 1980). Gnanamurthy *et al.* (2013) suggested that such reductions in plant growth might be due to the toxicity of the mutagen NaN_3 on the physiological parameters such as the induction of chlorophyll mutants (Wani and Khan, 2006), the increase in destruction of growth inhibitors, drop in the auxin level or inhibition of auxin synthesis (Al-Qurainy and Khan, 2009), decline of assimilation mechanism and the activation of unusual transposable elements (Snowden *et al*., 2005).

Table 1. Effect of presoaking of pea and bean seeds in different concentrations (1,2 or 4 mM) of NaN_3 for different time intervals (0.5 h, 1h or 2 h) on the photosynthetic pigments (chl a, chl b and carotenoids) of 21- days old seedlings.

Treatment	Photosynthetic pigments (mg/g f.wt)					
	Pea			Bean		
	Chl a	Chl b	Carotenoids	Chl a	Chl b	Carotenoids
Control	9.10±0.87	2.64±0.72	3.28±0.51	9.74±0.34	4.34±0.58	3.28±0.17
1 mM + 0.5 h	8.63±0.35	2.61±0.39	3.30±0.24	8.69±0.14	3.46±0.21	3.35±0.30
2 mM + 0.5 h	8.53±0.52	2.56±0.41	3.41±0.02	8.39±0.33	3.24±0.52	3.45±0.31
4 mM + 0.5 h	8.01±1.85	2.34±0.44	3.57±0.50	8.01±0.74	3.10±0.38	3.33±0.37
1 mM + 1.0 h	8.30±0.82	2.67±0.45	3.38±0.40	8.21±1.10	3.42±0.48	3.51±0.46
2 mM +1.0 h	8.73±0.16	2.57±0.42	3.60±0.11	8.16±0.49	3.20±0.08	3.53±0.42
4 mM + 1.0 h	7.36±0.35	2.39±0.44	3.61±0.09	8.13±0.69	3.06±0.07	3.45±0.29
1 mM + 2.0 h	8.95±0.53	2.50±0.17	3.54±0.19	8.09±0.26	3.36±0.33	4.26±0.37
2 mM + 2.0 h	7.57±0.48	2.38±0.20	3.75±0.02	7.69±0.36	3.09±0.03	3.63±0.45
4 mM + 2.0 h	6.44±0.16	2.20±0.14	3.91±0.38	7.45±0.09	2.70±0.18	3.52±0.20

Table 2. Effect of presoaking of pea and bean seeds in different concentrations (1, 2 or 4 mM) of NaN_3 for different time intervals (0.5 h, 1h or 2 h) on the yield parameters.

Treatments of NaN_3	Pea						Bean					
	Number of legumes /plant	Number of seeds /legume	% of mature seeds	Mass of seeds /legume (g)	Mass of seeds /plant (g)	Mass of 1000 seeds (g)	Number of legumes /plant	Number of seeds /legume	% of mature seeds	Mass of seeds /legume (g)	Mass of seeds /plant (g)	Mass of 1000 seeds (g)
Control	8	7	95.14	1.61	11.49	234	15	7	97.34	2.52	37.91	400
1 mM + 0.5 h	8	7	95.11	1.58	11.09	229	14	7	97.01	2.46	35.65	390
1 mM + 1.0 h	7	7	91.32	1.51	10.38	219	14	6	96.12	2.20	31.15	360
1 mM + 2.0 h	7	7	90.59	1.38	9.49	210	14	6	95.36	1.92	26.10	320
2 mM + 0.5 h	7	7	94.19	1.47	9.99	215	14	6	94.67	2.08	29.66	340
2 mM + 1.0 h	7	6	92.16	1.27	8.26	202	13	6	93.11	1.87	25.04	310
2 mM + 2.0 h	6	6	89.28	1.16	7.30	192	13	6	92.13	1.71	22.28	290
4 mM + 0.5 h	6	6	91.83	1.34	7.92	208	12	5	90.27	1.88	23.46	320
4 mM + 1.0 h	5	6	88.28	1.23	6.86	195	12	5	88.12	1.58	18.93	280
4 mM + 2.0 h	5	6	82.60	1.14	6.22	186	11	5	85.41	1.27	14.26	250

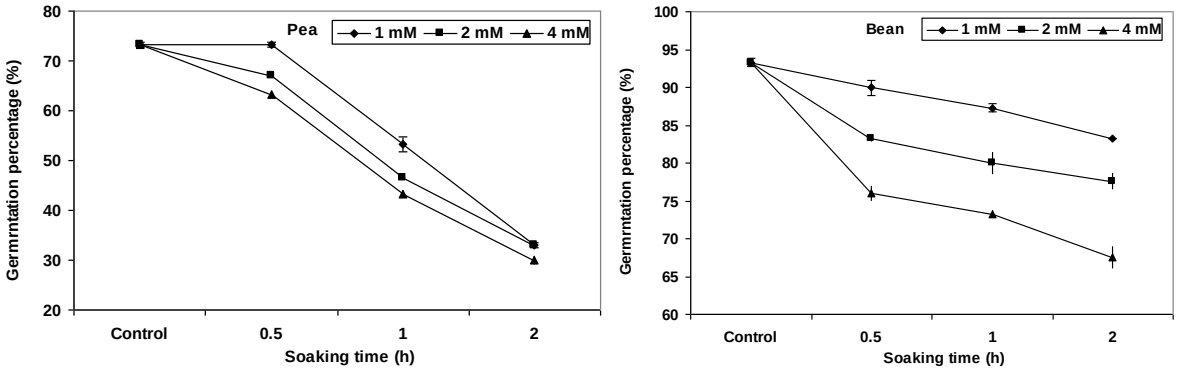

Fig.1. Effect of presoaking of pea and bean seeds in different concentrations (1, 2 or 4 mM) of NaN_3 for different time intervals (0.5 h, 1h or 2 hs) on the percentage of seed germination.

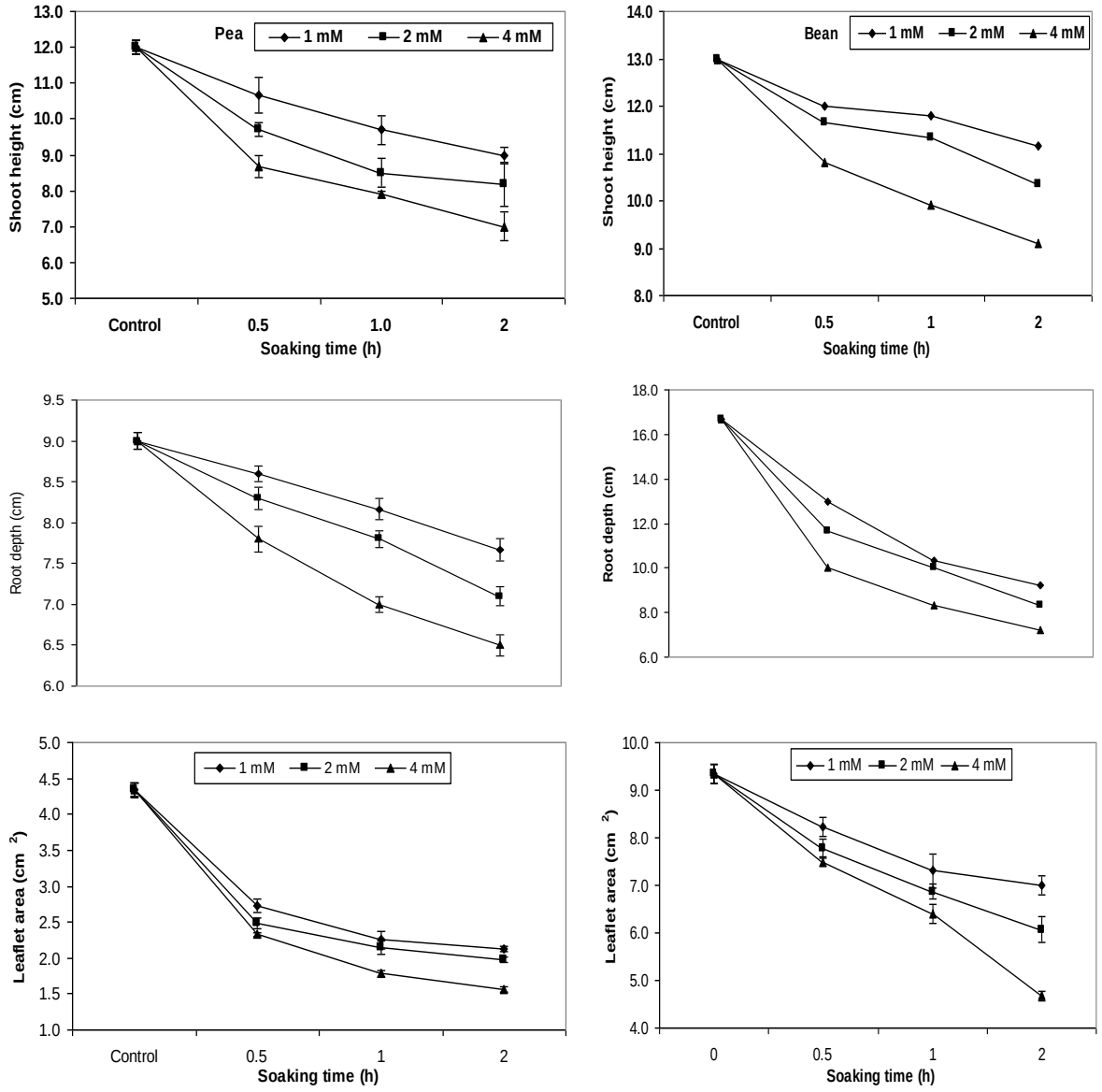

Fig. 2. Effect of presoaking of pea and bean seeds in different concentrations (1,2 or 4 mM) of NaN_3 for different time intervals (0.5 h,1 h or 2 hs) on shoot height, root depth and leaflet area of 21-days old seedlings.

Table 1 shows that presoaking in NaN_3 caused a reduction in chl. a, and ch.b contents in both species compared with the control. The decrease in chlorophyll content was directly proportional to the increase in the concentration and duration of presoaking. This reduction in the chlorophyll content may be due to disturbances at some stage in the chlorophyll apparatus (Mensah *et al.*, 2006) or to chlorophyll mutations (Gaibriyal *et al.*, 2009). Also, the reduction in chlorophyll content may result from inactivation of enzymes responsible for chlorophyll biosynthesis as reported by Rahman *et al.*, (2005). Mshembula *et al.,* (2012) reported that low NaN_3 concentration increased chlorophyll content in cowpea while higher concentrations reduced its levels compared to the control.

In contrast to chlorophyll, NaN_3 presoaking resulted in significant ($P<0.01$) increases in the carotenoid contents of both crops compared to the control (Table 1). The increase in carotenoid contents was directly proportional to the increase in NaN_3 concentration and to the increase in soaking time. The increase in carotenoid contents under stress conditions might be due to the capability of carotenoids to cope with stress conditions since the photosynthetic equipment directly determines the energetic status of plants as a prerequisite for activate defense (Drazkiewicz *et al.*,

2003; Stamp, 2003). In addition, under normal conditions, carotenoid retention in the progress of chlorophyll breakdown directly contributes to photoprotection during leaf senescence (Merzlyak and Gitelson, 1995). The higher carotenoid content under abiotic stress might indicate a relatively better defense strategy against stresses (Middleton and Teramura, 1993).

The effect of NaN_3 treatments on chlorophyll fluorescence was examined in dark adapted leaves of *Pisum sativum* and *Vicia faba* (Fig 3). The initial level of fluorescence (Fo) was decreased with the increase in both concentration and soaking time of NaN_3 for the two species. A similar trend was obtained in the maximum fluorescence (Fm) and the variable fluorescence (Fv) by NaN_3 treatments. The maximum quantum efficiency of PSII photochemistry (Fv/Fm) of *Pisum sativum* was slightly decreased with the increase in NaN_3 concentration and soaking intervals, but that of *Vicia faba* showed some fluctuations with the applied treatments. The maximum capacity of PSII (FV/FM) is a widely used indicator of stress response of plants (Huang *et al.*, 1997). The results were in accordance with those obtained by Bjorkman and Demming (1987) who stated that oxidative stress caused damage to PSII reaction centers leading to a decline in the quantum yield of photosystem II photochemistry (Fv/Fm) ratio. It can be assumed that the higher impact of NaN_3 was given by its effect on the redox state of the electron carriers in photosynthetic machinery. Similar results were found by Evdokimova *et al.* (2013) who reported that NaN_3 caused inhibition of mitochondrial electron transport with increased relative content of non-photoactive proto-chlorophyllide in etiolated leaves, decreased the content of ATP, chlorophylls, and carotenoids and completely suppressed the functional activity of PSII in barley seedlings.

The results illustrated in Fig. (4) Show that NaN_3 treatments significantly increased the total soluble carbohydrate contents in the shoots of *Pisum sativum* and *Vicia faba* seedlings. This increase coincided with the increase in NaN_3 concentration and soaking interval. Similar results were obtained by Khairwal *et al.* (1984) and Naganada *et al.* (2013) in sugar cane and carrot, respectively and in several other reports (e.g. Mohamed and Moussa; 2003, Nassar *et al.*; 2004, Li *et al.*; 2005). The increase in soluble sugars contents may come as another defensive mechanism caused by the pre-exposing to the mutagen through the activation of certain genes responsible for expression of the enzymes involved in the accumulation of these osmolytes (El-Shafey *et al.*, 2009).

NaN_3 treatments significantly increased total soluble protein content of the two species compared to the control (Fig. 5). The increase in protein content was proportional to the increase in NaN_3 concentration and soaking period. The highest protein content was obtained by soaking the seeds for two hours in 4 mM NaN_3 which was 67.6 and 141.2% in *Pisum sativum* and *Vicia faba*, respectively. The results were similar to the findings of Dahot *et al.* (2011) who found that the total protein content was considerably increased in sorghum treated with NaN_3 compared to control. Similarly, in the study of Pavadai *et al.* (2010b) on soybean and of Gnanamurthy *et al.* (2013) on *Zea mays*, they found that NaN_3 and other chemical and physical mutagens significantly increased the total protein and oil contents. Meanwhile, Sheikh *et al.* (2012), reported non-significant increase in the protein content of wheat under NaN_3 treatments.

The total free amino acids contents in *Pisum sativum* and *Vicia faba* were significantly increased by NaN_3 treatments which were proportional to concentration and soaking duration (Fig. 6). Soaking seeds in 4 mM NaN_3 for 4 hours resulted in the highest content of the free amino acids (19.9 and 11.0 mg/g d. wt. in pea and bean, respectively). This result was in agreement with the findings of Satter *et al.* (1990) and Maity *et al.* (2009) who documented increases in essential and nonessential amino acids of *Oryza sativa* and *Cicer arietinum* when irradiated by gamma irradiation. Increase of amino acids could be due to de-polymerization of nucleic acid caused by mutagenic treatments or due to partial dissociation of nucleo-proteins and alterations in their pattern of organization (Kumar *et al.*, 2003).

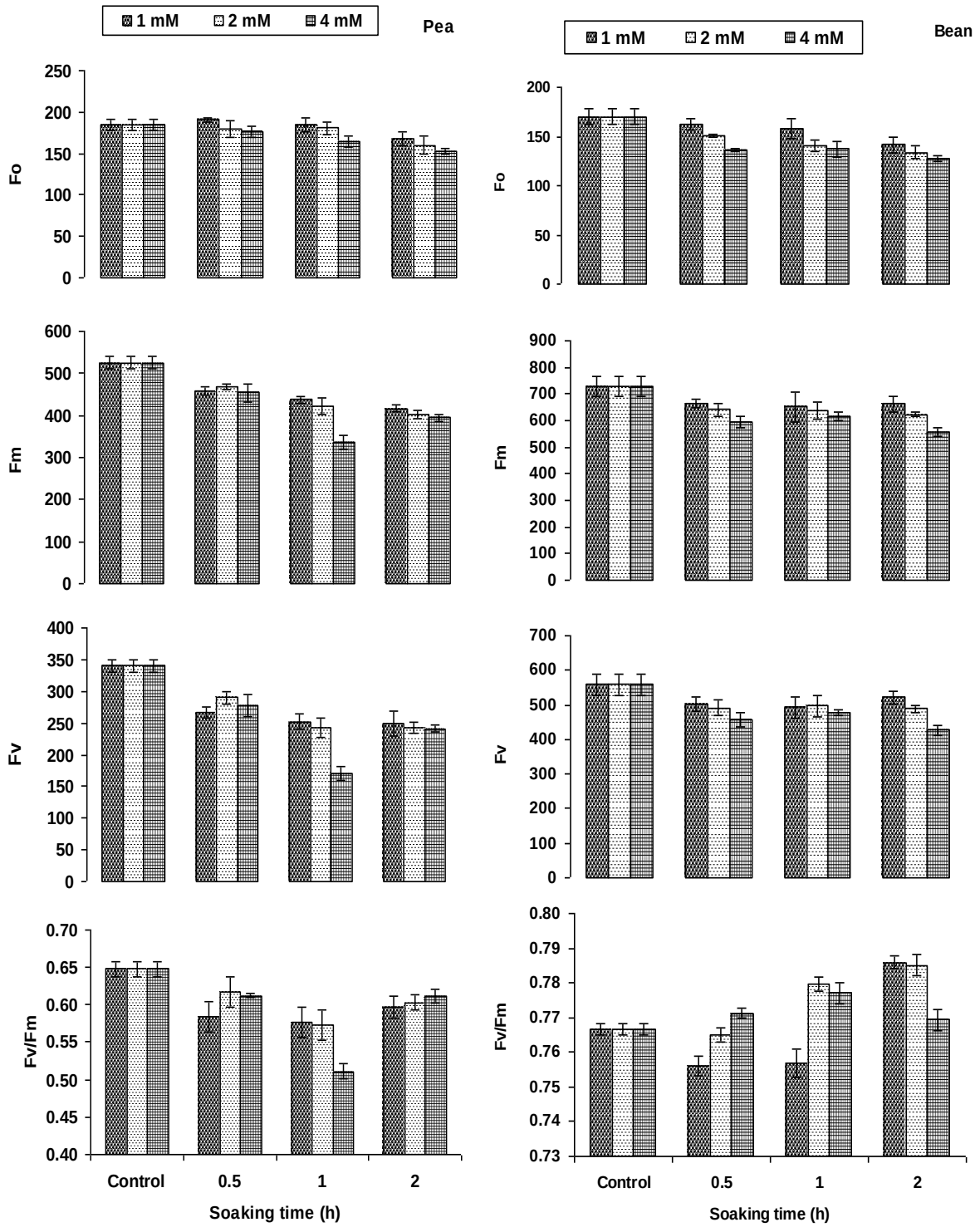

Fig. 3. Effect of presoaking of pea and bean seeds in different concentrations (1, 2 or 4 mM) of NaN_3 for different time intervals (0.5 h, 1 h or 2 hs) on the parameters of chlorophyll fluorescence (Fo, Fm, Fv and Fv/Fm) of 21-days old seedlings.

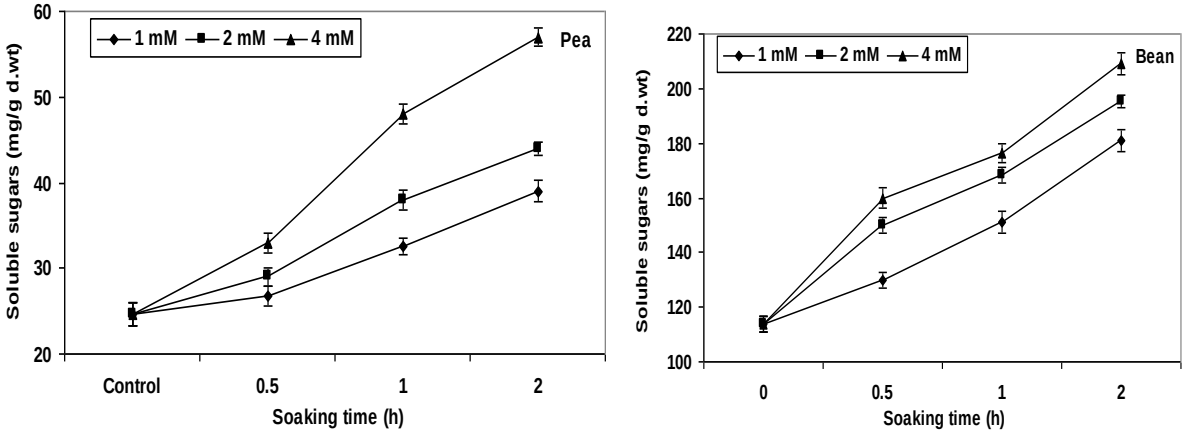

Fig. 4. Effect of presoaking of pea and bean seeds in different concentrations (1,2 or 4 mM) of NaN_3 for different time intervals (0.5 h,1 h or 2 hs) on total soluble sugars content of 21-days old seedlings.

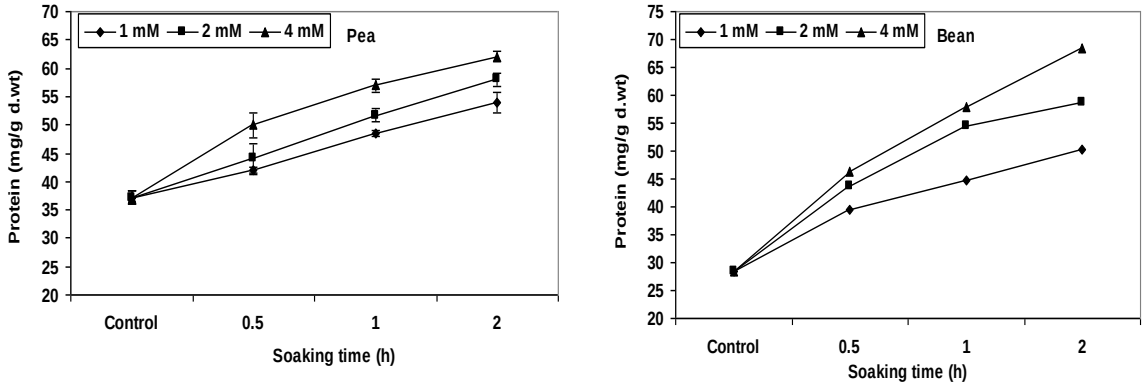

Fig. 5. Effect of presoaking of pea and bean seeds in different concentrations (1, 2 or 4 mM) of NaN_3 for different time intervals (0.5 h, 1 h or 2 hs) on total soluble protein content of 21-days old seedlings.

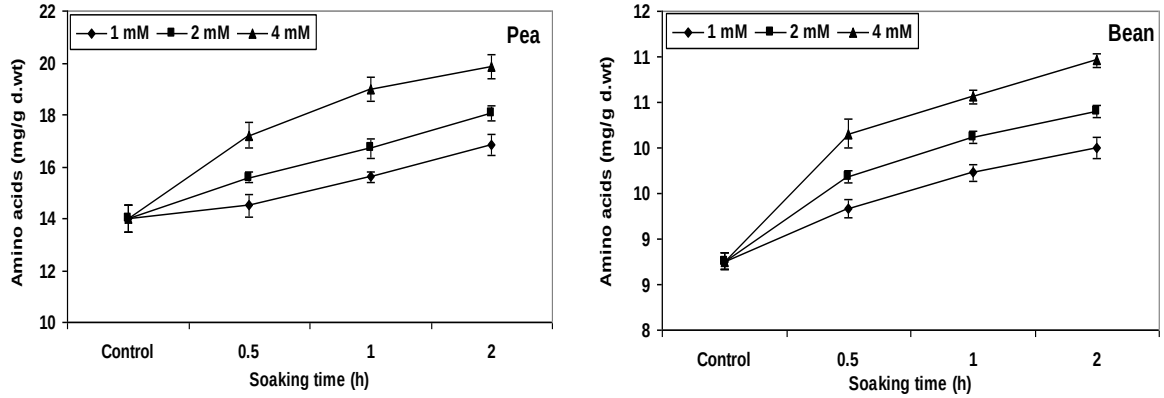

Fig. 6. Effect of presoaking of pea and bean seeds in different concentrations (1, 2 or 4 mM) of NaN_3 for different time intervals (0.5 h, 1h or 2 hs) on total free amino acids contents of 21-days old seedlings.

The data presented in Table (2) showed the response of yield parameters of both *Pisum sativum* and *Vicia faba* to the pre-soaking in NaN_3 treatments. However, all yield criteria were reduced gradually by increasing NaN_3 concentration and soaking duration. Presoaking of the seeds in 4 mM NaN_3 for 2 hours caused 37.5 and 26.7% decrease in the number of legumes/plant and 14.3 and 28.6% decrease in number of seeds/legume in *Pisum sativum* and *Vicia faba*, respectively. Similarly, the maturation percentage was decreased by 13.2 and 12.3% in pea and bean, respectively. The mass of seeds/legume was decreased by 29.2 and 48.4% and the mass of seeds/plant was decrease by 45.8 and 62.4% in pea and bean, respectively. Consequently, the mass of 1000 seeds was decreased by 20.5 and 37.5% in pea and bean, respectively.

Similar results were obtained in wheat (Rachovska and Dimova, 2000), in groundnut (Mensah and Obadoni, 2007), in tomato (Adamu and Aliyu, 2007), in *Capsicum annum* and in *Capsicum frutescens* (Daudu and Falusi, 2011). The decrease in yield parameters induced by NaN_3 may be due to the reduction in the plant growth (Poornananda and Hosakatee, 2009) which in turn may be attributed to the variation in auxin level (Goud and Nayar, 1968), change in the specific activity of a few enzymes (Cherry *et al*., 1962) and physiological injury induced in the seeds and seedlings (Ignacimuthu and Babu, 1988). Evans and Sparrow (1961) suggested that the chromosomal damage and inhibition of cell division are the chief causes of reduced plant growth and productivity.

For cytological study the obtained data cleared out that, relative to the control, the rate of mitotic index (dividing cell frequency) was generally decreased in the two plant species with increasing both concentration and pre-soaking duration of NaN_3 (Fig. 7). This concentration of NaN_3 may be caused errors in the normal behavior of chromosome and metabolic pathway of cell as recorded by Awan *et al*., (1980). These results were constituent with those of Bhat *et al*. (2007) who reported that NaN_3 as mutagen; decrease the rate of mitotic index in *Vicia faba*.

Fig. (8) Also revealed that the abnormality indexes in pea and bean were correlated with NaN_3 concentration; where the rate of abnormalities was increased with the increase of both NaN_3 concentration and soaking duration. These results may be due to the fact that, when NaN_3 is dissolved in water it forms a toxic hydrogen azide gas, with the generation of azide ions being the possible reason for its genotoxicity and cytotoxicity in plant systems; therefore, the occurrence of the disturbance in the examined cell reflected the increasing in aberration cell and decreasing mitotic index as explained by Nilan *et al*., (1977); Ilbas *et al*., (2005); Bhat *et al*., (2007) and Najeeb *et al.,* (2013)

When the seeds of pea and bean were soaked in different concentrations of NaN_3 for long duration, several chromosomal abnormalities were produced (Plate. 1). A broad range of chromosomal aberrations were induced in both species under this investigation such as stickiness of chromosomes which may be due to depolymerization of nucleic acid caused by mutagenic treatment or due to partial dissociation of the nucleoproteins and alterations in their pattern of organization (Kumar *et al*., 2003). Recent reports suggest that chromosome stickiness, may be controlled by a single pair of genes, two pairs of genes or by the interaction of several genes which may be recessive or dominant (Kiihl *et al*., 2011). Micronuclei arise if laggard or non-oriented chromosomes fail to reach the poles in time to be in main telophase nucleus (Utsunomiya *et al*., 2002). Chromosomal bridges were also found which might have arisen through breaks in two chromosomes followed by union of the centric fragments (Shreekrishna, 2006) or due to stickiness of chromosomes at metaphase and their failure to separate at anaphase or due to breakage and reunion of chromosome (Grant, 1978 and Badr, 1988). C-Metaphase was also found and this may result from defective formation of spindle apparatus (Badr, 1986). Lagging chromosome might result from late

chiasma terminalization (Pagliarini, 1990). Lagging chromosome may be explained on the basis of abnormal spindle formation and failure of chromosome movement. Mutagen may have caused chromosomal breakage by binding the DNA at GC rich region and making the DNA unstable and hence formation of fragments and lagging. These findings showed the direct effect of NaN_3 mutagen on spindle apparatus which mostly caused somatic instability (Bhat *et al.*, 2007).

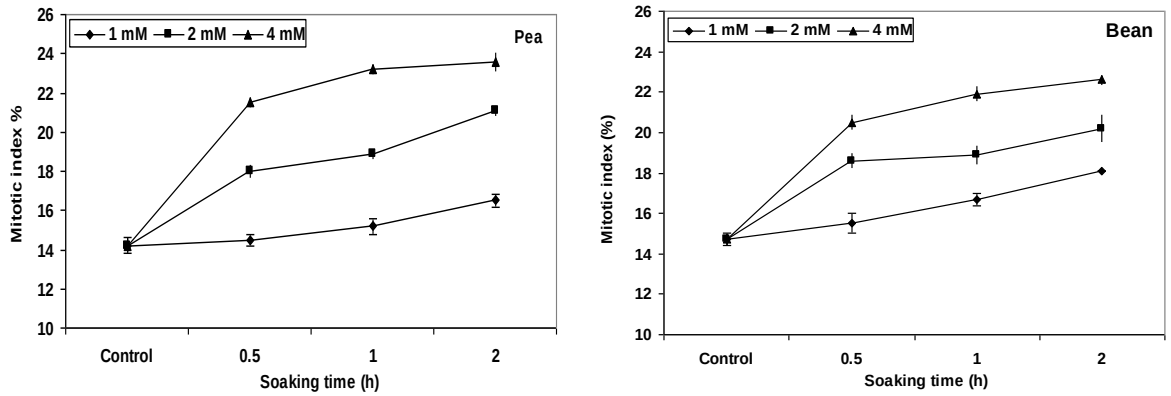

Fig. 7. Effect of presoaking of pea and bean seeds in different concentrations (1,2 or 4 mM) of NaN_3 for different time intervals (0.5 h,1 h or 2 hs) on the mitotic index of 7 days old seedlings.

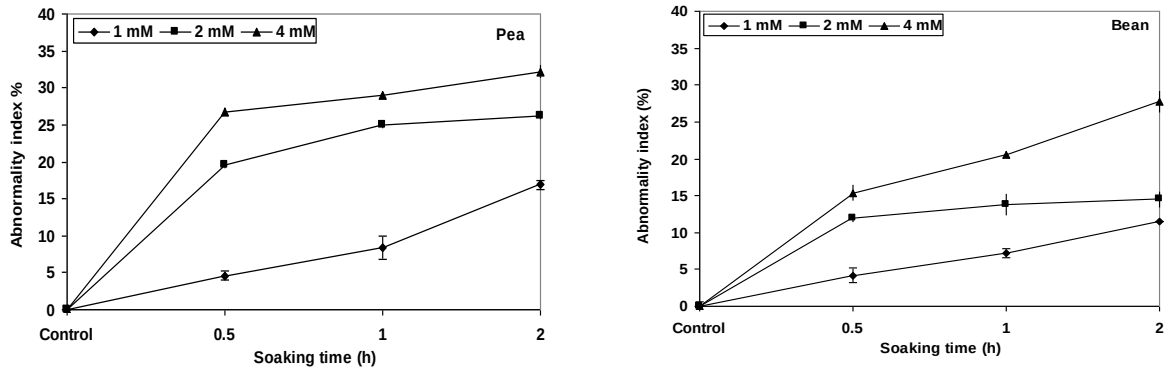

Fig. 8. Effect of presoaking of pea and bean seeds in different concentrations (1, 2 or 4 mM) of NaN_3 for different time intervals (0.5 h, 1h or 2 hs) on the abnormality index of pea and bean root tips of 7-days old seedlings.

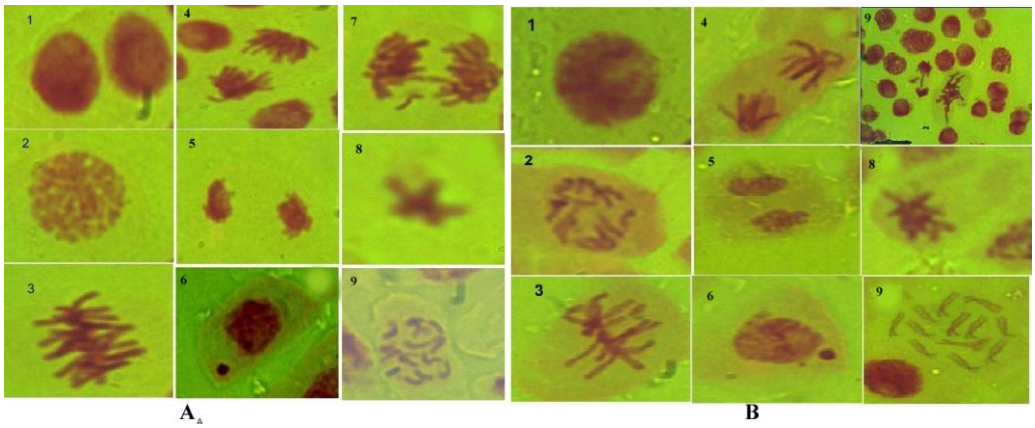

Plate 1. Types of normal cells and chromosomal abnormalities in the root meristems of pea (A) and bean (B) following seed exposure to each of the four concentrations of NaN_3 and for each of the four durations period of presoaking.

Normal cell: **1**- Normal interphase **2-** prophase **3-** Metaphase **4**- Anaphase **5-** Telophase

Abnormal cell: **6-**Micronuclei **7-** Chromosome bridge with lagging **8-** stickiness metaphase **9- C**-Metaphase

The results (Tables 3-6) show marked changes in the protein patterns of pea and bean seeds in response to NaN_3 treatments. Nine, 16 and two polymorphic monomorphic and unique bands were found in case of pea, respectively. In the case of bean, the numbers of polymorphic, monomorphic and unique bands were 13, 15 and two respectively.

The bands which appeared in response to the highest concentration of NaN_3 and the longest duration, have molecular weights 148, 111 and 18 KDa for pea, and 43, 32 and 26 KDa for bean. The appearance of new bands may be due to mutational events at the regulatory system of an unexpressed gene(s) that activate it. These results are in agreement with those of Mahmoud and Al-Twaty (2006), who reported that the use of NaN_3 with different concentrations in tomato through SDS protein electrophoresis enhanced the tomato genome to activate some genes as they are expressed by the appearance of some new minor bands, which is responsible for improvement of the studied tomato traits. Mahmoud (1993) found that NaN_3 was more effective in improvement of yield components in maize hybrids than gamma irradiation and that the NaN_3 treatments changed the number, intensity and/or density of SDS electrophoretic bands for grain proteins than respective control.

Table 3: Effect of presoaking of pea seeds in different concentrations (1, 2 or 4 mM) of NaN_3 for different time intervals (0.5 h, 1h or 2 hs) on the protein patterns of their seedlings as revealed by SDS-PAGE (1= present; 0 = absent, M.Wt. = molecular weight, , M = monomorphic, P = polymorphic, U = unique).

Band number	M.Wt. (KDa)	*Pisum sativum*										Band type
		Different concentration										
		control	1mM			2 mM			4 mM			
		0.0 Min	30 Min	60 Min	120 Min	30 Min	60 Min	120 Min	30 Min	60 Min	120 Min	
01	148	0	0	0	0	0	0	0	0	0	1	U
02	139	0	0	0	0	0	0	1	1	1	1	P
03	128	1	1	1	1	1	1	1	1	1	1	M
04	121	1	1	1	1	1	1	1	1	1	1	M
05	111	0	0	0	1	0	0	1	0	0	1	P
06	105	0	0	0	1	1	1	1	1	1	1	P
07	88	1	1	1	1	1	1	1	1	1	1	M
08	98	1	1	1	1	0	0	0	0	0	0	P
09	68	1	1	1	1	1	1	1	1	1	1	M
10	63	1	1	1	1	1	1	1	1	1	1	M
11	60	0	1	1	1	1	1	1	1	1	1	P
12	48	1	1	1	1	1	1	1	1	1	1	M
13	46	1	1	1	1	1	1	1	1	1	1	M
14	44	1	1	1	1	1	1	1	1	1	1	M
15	39	1	1	1	1	1	1	1	1	1	1	M
16	34	1	1	1	1	1	1	1	1	1	1	M
17	30	1	1	1	1	1	1	1	1	1	1	M
18	24	1	1	1	1	1	1	1	1	1	1	M
19	23	0	0	0	1	1	0	1	0	0	1	P
20	22	1	1	1	1	1	1	1	1	1	1	M
21	20	1	1	1	1	1	1	1	1	1	1	M
22	18	0	0	0	0	0	0	0	1	1	1	P
23	17	0	0	0	0	0	0	1	1	1	1	P
24	15	1	1	1	1	1	1	1	1	1	1	M
25	14	0	0	0	1	1	1	1	1	1	1	P
26	11	1	1	1	1	1	1	1	1	1	1	M
27	9	1	0	0	0	0	0	0	0	0	0	U

Three bands found in the protein profile of the control seeds disappeared with NaN_3 treatment: the 9 KDa band in pea and the 75 and 7 KDa bands in bean. The disappearance of these bands could be explained on the basis of mutational event at the regulatory genes that prevent transcription. Induction of laggards, micronuclei by this mutagenic agent may lead to the loss of their corresponding genes. Similar results were obtained by Prasad and Zha (1992) in *Phaseolus vulgaris* and George and Ghareeb (2001) in *Vicia faba*.

Marked reduction in total protein bands was recorded in the yield stage compared with the seedling stage for the two plant species; whereas, for pea, 27 protein bands were recorded (9 polymorphic, 16 monomorphic and two unique) in the seedling stage while only 23 bands (2 polymorphic and 21 monomorphic) were detected in the yielded seeds. In case of bean, 30 bands (13 polymorphic, two unique and 15 monomorphic) were detected in the seedling stage, while only 24 bands (3 polymorphic and 21 monomorphic) were found in its yielded seeds. The reduction of these bands may be due to the ability of the plant to be recovered from the mutagenic effect. Cell recovery can be related to reversible effects of NaN_3 on protein and/or possibly related to its degradation into less toxic metabolites (Sadiq, 1995). The toxicity of NaN_3 and most of its physiological effects can be traced to its reversible inhibitory effect on enzymes containing a coordinated divalent ion, such as those of cellular respiration (Kleinhofs *et al*., 1978).

Table 4. Effect of presoaking of bean seeds in different concentrations (1, 2 or 4 mM) of sodium azide for different time intervals (0.5 h, 1h or 2 hs) on the protein patterns of their seedlings as revealed by SDS-PAGE (1= present; 0 = absent, M.Wt. = molecular weight, M = monomorphic, P = polymorphic, U = unique).

Band number	M.Wt. (KDa)	*Vicia faba*										Band type
		Different concentration										
		control		1mM		2 mM			4 mM			
		0.0 Min	30 Min	60 Min	120 Min	30 Min	60 Min	120 Min	30 Min	60 Min	120 Min	
01	179	1	1	1	1	1	1	1	1	1	1	M
02	166	0	0	0	0	0	0	0	0	1	1	P
03	162	1	1	1	1	1	1	1	1	1	1	M
04	152	0	0	1	1	1	1	1	1	1	1	P
05	143	1	1	1	1	1	1	1	1	1	1	M
06	137	1	1	1	1	1	1	1	1	1	1	M
07	132	0	1	1	1	1	1	1	1	1	1	P
08	123	1	1	1	1	1	1	1	1	1	1	M
09	113	1	1	1	1	1	1	1	1	1	1	M
10	103	1	1	1	1	1	1	1	1	1	1	M
11	96	1	1	1	1	1	1	1	1	1	1	M
12	92	1	1	1	1	1	1	1	1	1	1	M
13	91	0	0	0	1	1	1	1	1	1	1	P
14	77	0	0	0	0	0	0	1	1	1	1	P
15	75	1	0	0	0	0	0	0	0	0	0	U
16	58	1	1	1	1	1	1	1	1	1	1	M
17	50	0	0	1	1	1	1	1	1	1	1	P
18	48	1	0	0	1	1	1	1	1	1	1	P
19	43	0	0	0	0	0	0	0	1	1	1	P
20	40	1	1	1	1	1	1	1	1	1	1	M
21	36	1	1	1	1	1	1	1	1	1	1	M
22	33	1	1	1	1	1	1	1	1	1	1	M
23	32	0	0	0	0	0	0	0	0	1	1	P
24	27	1	1	1	1	1	1	1	1	1	1	M
25	26	0	0	0	1	0	0	1	0	0	1	P
26	19	0	0	0	0	0	0	0	1	1	1	P
27	17	1	1	1	1	1	1	1	1	1	1	M
28	14	0	0	1	1	1	1	1	1	1	1	P
29	9	0	0	0	1	0	0	0	1	1	1	P
30	7	1	0	0	0	0	0	0	0	0	0	U

Table 5. Effect of presoaking of *Pisum sativum* seeds in different concentrations (1, 2 or 4 mM) of sodium azide for different time intervals (0.5 h, 1h or 2 hs) on the protein patterns of their yielded seeds as revealed by SDS-PAGE (1 = present; 0 = absent, M.Wt. = molecular weight, , M = monomorphic, P = polymorphic).

Band number	M.Wt. (KDa)	*Pisum sativum*										Band type
		Different concentration										
		control	1mM			2 mM			4 mM			
		0.0 Min	30 Min	60 Min	120 Min	30 Min	60 Min	120 Min	30 Min	60 Min	120 Min	
01	148	1	1	1	1	1	1	1	1	1	1	M
02	139	1	1	1	1	1	1	1	1	1	1	M
03	121	1	1	1	1	1	1	1	1	1	1	M
04	111	1	1	1	1	1	1	1	1	1	1	M
05	105	0	0	0	0	0	0	0	1	1	1	P
06	98	1	1	1	1	1	1	1	1	1	1	M
07	88	1	1	1	1	1	1	1	1	1	1	M
08	65	1	1	1	1	1	1	1	1	1	1	M
09	54	1	1	1	1	1	1	1	1	1	1	M
10	48	1	1	1	1	1	1	1	1	1	1	M
11	44	1	1	1	1	1	1	1	1	1	1	M
12	39	1	1	1	1	1	1	1	1	1	1	M
13	34	1	1	1	1	1	1	1	1	1	1	M
14	30	1	1	1	1	1	1	1	1	1	1	M
15	28	1	1	1	1	1	1	1	1	1	1	M
16	26	1	1	1	1	1	1	1	1	1	1	M
17	24	1	1	1	1	1	1	1	1	1	1	M
18	22	1	1	1	1	1	1	1	1	1	1	M
19	20	1	1	1	1	1	1	1	1	1	1	M
20	17	1	1	1	1	1	1	1	1	1	1	M
21	14	1	1	1	1	1	1	1	1	1	1	M
22	9	0	0	0	0	0	0	1	1	1	1	P
23	7	1	1	1	1	1	1	1	1	1	1	M

Table 6. Effect of presoaking of *Vicia faba* seeds in different concentrations (1, 2 or 4 mM) of sodium azide for different time intervals (0.5 h, 1h or 2 hs) on the protein patterns of their yielded seeds as revealed by SDS-PAGE (1 = present; 0 = absent, M.Wt. = molecular weight, con= concentration, dur = duration, M = monomorphic, P = polymorphic).

Band number	M.Wt (KDa)	*Vicia faba*										Band type
		Different concentration										
		control	1mM			2 mM			4 mM			
		0.0 Min	30 Min	60 Min	120 Min	30 Min	60 Min	120 Min	30 Min	60 Min	120 Min	
01	123	1	1	1	1	1	1	1	1	1	1	M
02	113	1	1	1	1	1	1	1	1	1	1	M
03	103	1	1	1	1	1	1	1	1	1	1	M
04	96	1	1	1	1	1	1	1	1	1	1	M
05	91	1	1	1	1	1	1	1	1	1	1	M
06	87	0	0	0	1	0	0	1	0	0	1	P
07	70	1	1	1	1	1	1	1	1	1	1	M
08	58	1	1	1	1	1	1	1	1	1	1	M
09	48	1	1	1	1	1	1	1	1	1	1	M
10	44	1	1	1	1	1	1	1	1	1	1	M
11	43	1	1	1	1	1	1	1	1	1	1	M
12	40	1	1	1	1	1	1	1	1	1	1	M
13	33	1	1	1	1	1	1	1	1	1	1	M
14	32	1	1	1	1	1	1	1	1	1	1	M
15	30	1	1	1	1	1	1	1	1	1	1	M
16	26	0	0	0	0	0	0	0	1	1	1	P
17	27	1	1	1	1	1	1	1	1	1	1	M
18	24	1	1	1	1	1	1	1	1	1	1	M
19	23	1	1	1	1	1	1	1	1	1	1	M
20	19	0	0	0	0	0	0	0	1	1	1	P
21	17	1	1	1	1	1	1	1	1	1	1	M
22	10	1	1	1	1	1	1	1	1	1	1	M
23	7	1	1	1	1	1	1	1	1	1	1	M
24	5	1	1	1	1	1	1	1	1	1	1	M

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