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Effects of the Application of Different Concentrations of NaN_3 for Different Times on the Morphological and Cytogenetic Characteristics of Barley (*Hordeum vulgare* L.) Seedlings

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Abstract: Sodium azide (NaN_3) has been used in many biological studies as a mutagen. In the present study, the morphological and cytogenetic effects of NaN_3 on barley (*Hordeum vulgare* L.) seedlings were investigated. Seeds of barley were treated with different concentrations of NaN_3 (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mmol/L) and applied for different periods of time (3 and 4 h). Parameters investigated, except for the mitotic index, were determined on Days 7 and 14. Increasing concentrations of NaN_3 affected germination rates on Days 7 and 14 following application for 3 and 4 h. Although the length of the roots and leaves was affected by treatment with NaN_3 on Day 14 of the germination period, coleoptile length was affected by NaN_3 treatment on Day 7. Increasing concentrations of NaN_3 and increased treatment period decreased the mitotic index compared with the untreated control. The inhibitory effects of NaN_3 on the mitotic index indicate that NaN_3 can have genotoxic and mutagenic effects on barley seedlings.

Key words: *Hordeum vulgare*; mitotic index; NaN_3.

The genetic information must be the same in all the cells of a living creature. However, in some cases, variations called mutations may take place in the genetic information, causing a cell or living creature to be different from the others. Chemical mutagens are one cause of mutations in living organisms. It is known that various chemicals have positive or negative effects on living organisms. Many of these chemicals have clastogenic (chromosome-damaging) effects on plants via reactive oxygen-derived radicals (Yuan and Zhang 1993). These effects can occur both spontaneously and artificially following induction by mutagens. Sodium azide (NaN_3), which has been demonstrated to have these effects, is a mutagen. Sodium azide is a common laboratory chemical and is widely used in industry, agriculture, medical practice, and organic synthesis research. In addition, NaN_3 has been used as a nitrogen gas generator in airbags for cars (Kleinhofs *et al*. 1978), as a chemical preservative in hospitals and laboratories, and for pest control in agriculture (farming). NaN_3 is also used in detonators and other explosives. It has been reported that NaN_3 affects plant physiology and decreases cyanide-resistant respiration in tobacco callus (Wen and Liang 1995). The mutagenic potential of NaN_3 has been reported in several screening assays. NaN_3, a common bactericide, pesticide, and industrial nitrogen gas generator, is known to be highly mutagenic in several organisms, including plants and animals (Rines 1985; Veleminsky and Anglis 1987; Owais and Kleinhofs 1988; Raicu and Mixich 1992; Grant and Salamone 1994; González-César and Ramos-Morales 1997). In organisms, NaN_3 is marginally mutagenic (Jones *et al*. 1980; Arenaz *et al.* 1989) and is not mutagenic in several organisms, such as

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Drosophila (Kamra and Gallopudi 1979) and *Arabidopsis* (Gichner and Veleminsky 1977). Previous studies have clearly demonstrated that the mutagenicity of NaN_3 is mediated through the production of an organic metabolite of azide (Owais and Kleinhofs 1988).

NaN_3 has been used recently as both an agent for the study of the repair of radiation-induced genetic damage and as a mutagen in embryonic shoots of barley seeds (Pearson *et al*. 1975). NaN_3 is a very potent mutagen in barley in terms of the induction of chlorophyll deficiency and it appears to induce various morphological, physiological, and cytogenetic mutations. NaN_3 is also a highly efficient mutagen, because it appears to induce chromosome aberrations in barley, vicia, and soybean (Sideris *et al*. 1973; Veleminsky *et al*. 1987; Grant and Salamone 1994; Kanaya *et al*. 1994).

In the present study, NaN_3 was been applied to seeds and embryonic roots of barley. Barley (*Hordeum vulgare* L.) is a monocotyledonous plant belonging to the Gramineae family that is able to grow in unhospitable places because of its ecological tolerance. The chromosome number of barley is 2n=14 (Blattner 2004).

The mitotic index (MI) is a cytogenetic test that is used both *in vivo* and *in vitro* for the examination of the genotoxic effects of a compound over a short period of time and there are considerable variations in the mutagenic effects of agents in different environments. The MI assay is used to characterize proliferating cells and to identify compounds that inhibit or induce mitotic progression. Compounds that inhibit mitotic progression result in a decrease in the MI of a population (Ozkul *et al*. in press).

In the present study, the effect of NaN_3 on the morphological characteristics (germination and length of plants) and MI of barley seedlings was investigated.

1 Materials and Methods

1.1 Treatment with NaN_3

Dry dormant seed samples of barley (*Hordeum vulgare* L.) were treated with 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mmol/L NaN_3 and a phosphate tampon as the control group for 3 and 4 h at 21 ºC.

1.2 Germination

Control (untreated) and treated barley seeds were soaked in distilled water for 1 h at room temperature. Then, each group of seeds was transferred to wet Whatman paper in Petri dishes for 24 h at 21 ºC for the determination of the MI and for 7 and 14 d at room temperature for the investigation of the effects of NaN_3 on the morphological characteristics of the seeds. Each group in which morphological characteristics were investigated consisted of 20 seeds. Germinated seeds were taken from the sand using a sieve after washing them with distilled water. At the end of a definite time period, root tips were cut (1.0–1.5 cm length) for determination of the MI.

1.3 Fixation and preparation

Root tips were pretreated in a saturated aqueous solution of α-monobromonaphtalen for 2 h at 4 ºC and were then fixed in pure glacial acetic acid for 30 min before being rinsed and transferred to 70% alcohol, and then stored in a refrigerator until use. For preparation, root tips were hydrolized in 5 mol/L HCl for 20 min at room temperature and then stained in an acetocarmin solution for 1 h (to prepare the acetocarmin solution, 45% glacial acetic acid is melted and cooled to 50 °C; then, 1 g acetocarmin is added and the mixture is melted for 10 min). Chromosome spreads were made using the squash technique (Savaskan and Toker 1991).

1.4 Examination of morphological and cytogenetic characteristics

The morphological characteristics examined included germination and the length of seedlings. Root, coleoptile, and the leaf length of germinated seeds were measured (in cm). For the MI, the frequency of mitosis was determined by counting the number of dividing cells of 500 cells investigated in the untreated and treated groups.

1.5 Statistical evaluation

The statistical significance of the effects of NaN_3 on MI, germination, and plant length was tested by analysis of variance and differences between groups were determined by the least significant differences (LSD) test, with $P < 0.05$ considered significance.

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2 Results

2.1 Effects of NaN_3 on germination

Generally, treatment with NaN_3 decreased germination rates at Day 7 of the germination period and the germination rates between different NaN_3 treatments and the control group (untreated) were found to be statistically significant ($P < 0.05$; Table 1). The most pronounced decrease in germination rate occurred following treatment with 2.5 mmol/L NaN_3 for 3 h. The lowest germination rate (65%) was obtained following treatment with 2.0 mmol/L NaN_3 for 3 h (Table 1). Based on the germination rate on Day 14, neither different concentrations of NaN_3 nor exposure for different periods of time was found to have any effect. Although the seed germination rate was increased from 90% to 95% on Day 14, differences between NaN_3 treatment groups and the control group (untreated) were not statistically significant ($P > 0.005$). Differences in the effects of NaN_3 on germination rate between Day 7 and Day 14 may be due to the longer germination period from 7 to 14 d. At Day 14, seeds had probably developed tolerance to the inhibitory effect of NaN_3 on germination and had improved their physiological conditions on Day 14 with respect to seed germination.

2.2 Length of plant parts

Generally, the effects of NaN_3 treatments on root length were not statistically significant ($P > 0.005$), except on Day 14 for groups exposed to NaN_3 for 3 h. The lowest root length (9.10 cm) was found on Day 14 for the group exposed to 2.5 mmol/L NaN_3 for 3 h.

Except for groups exposed to NaN_3 for 3 h and measured on Day 7, NaN_3 treatment had a statistically significant effect on leaf length ($P < 0.05$) and this effect appeared to be particularly evident on Day 14 (Table 2).

Measurements made on Day 7 indicated that NaN_3 treatment affected coleoptile length in groups exposed to NaN_3 for both 3 and 4 h. The lowest coleoptile lengths were found in the groups exposed to 3.0 mmol/L NaN_3 for 3 h (2.54 cm) and to 2.0 mmol/L NaN_3 for 4 h (1.59 cm; Table 2). With regard to measurements made on Day 14, the effects of NaN_3 treatments on coleoptile length were not statistically significant ($P > 0.005$).

2.3 Mitotic index

Increasing concentrations of NaN_3 were decreased MI following exposure of seeds for 3 and 4 h. The differences in MI among all groups were statistically significant for all treatment protocols ($P < 0.05$). Following exposure to NaN_3 for 3 h, the highest MI frequency was found to be 5.8% in the control group, whereas the lowest frequency was 0.4% in the group exposed to 3.0 mmol/L NaN_3. In the groups exposed to NaN_3 for 4 h, the highest MI frequency was found to be 5.2% in the control group, whereas the lowest frequency was 0.2% in the group exposed to 3.0 mmol/L NaN_3 (Table 3).

With respect to MI frequency, the differences between groups exposed to NaN_3 for 3 and 4 h were found to be statistically significant ($P < 0.05$), whereas there were no significant interactions found for NaN_3 concentration and period of application ($P > 0.05$). It was clear that the highest concentration of NaN_3 (3.0 mmol/L) resulted in the lowest MI following exposure for both 3 and 4 h. Thus, higher concentrations and longer exposure to NaN_3 causes a greater inhibition of MI.

Table 1 Rate of seed germination for seed treated with NaN_3

NaN_3 (mmol/L)	Germination rate (%)			
	Day 7		Day 14	
	3 h exposure	4 h exposure	3 h exposure	4 h exposure
0 (control)	95a	100a	80a	90a
0.5	95a	85abc	95a	90a
1.0	80ab	80bcd	85a	85a
1.5	80ab	90ab	80a	85a
2.0	90a	65d	80a	95a
2.5	70b	75bcd	85a	95a
3.0	80ab	70cd	95a	95a

Values within a column with different letters are significantly different.

3 Discussion

The present study was undertaken to examine of

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Table 2 Effects of different doses of NaN_3 on the length of plant parts

NaN_3 exposure time	Doses (mmol/L)	Average length (cm)					
		Root		Leaf		Coleoptyle	
		Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
3 h	0 (control)	3.41a	12.94ab	1.88a	9.08abc	3.63ab	3.69a
	0.5	3.21a	14.50a	1.37a	10.47ab	4.05a	3.92a
	1.0	5.24a	11.29ab	1.20a	10.65a	3.30ab	4.44a
	1.5	2.41a	12.33ab	0.50a	8.07bc	3.07ab	3.36a
	2.0	4.91a	12.67ab	0.42a	8.51abc	3.49ab	3.62a
	2.5	2.14a	9.10b	0.23a	7.54c	2.59b	3.77a
	3.0	1.47a	12.24ab	0.23a	8.34abc	2.54b	4.10a
	Average	3.25	12.15	0.83	8.95	3.23	3.84
4 h	0 (control)	3.59a	11.65a	2.93a	8.,23ab	3.88a	4.24a
	0.5	2.55a	10.6a	0.12b	9.14a	3.13ab	3.58a
	1.0	1.75a	10.84a	0.21b	8.82ab	2.04bc	3.54a
	1.5	2.60a	9.27a	0.20b	7.16ab	2.73abc	3.14a
	2.0	1.75a	9.01a	0.13b	7.79ab	1.59c	3.38a
	2.5	2.98a	8.05a	1ab	6.52b	2.95abc	3.02a
	3.0	1.75a	7.82a	0.23ab	6.71ab	2bc	2.96a
	Average	2.42	9.6	0.68	7.76	2.61	3.4

Values within a column with different letters are significantly different.

Table 3 Mitotic index of seeds exposed to different concentrations of NaN_3

Exposure time	NaN_3 (mmol/L)	Total cells counted	Total no. dividing cells	Average MI (%)
3 h	0 (control)	500	29	5.8a
	0.5	500	18	3.6b
	1.0	500	13	2.6bc
	1.5	500	12	2.4bcd
	2.0	500	8	1.6cd
	2.5	500	5	1.0cd
	3.0	500	2	0.4d
4 h	0 (control)	500	26	5.2a
	0.5	500	14	2.8b
	1.0	500	13	2.6bc
	1.5	500	10	2.0bcd
	2.0	500	7	1.4bcd
	2.5	500	4	0.8cd
	3.0	500	1	0.2d

Values within a column with different letters are significantly different.

effect of NaN_3 on morphological and cytogenetic characteristics of barley (*H. vulgare*). The concentrations used and time of exposure to NaN_3 in the present study, as well as the pH and temperature, were determined on the basis of published reports. The pH of the phosphate buffer used in the present study was 3 on the basis of the report of Gichner *et al*. (1994), who determined that the most favorable pH for treatment with NaN_3 is pH 3.

In the present study, generally, NaN_3 treatment decreased germination rate at Day 7. Gichner and Veleminsky (1977) showed that concentrations of 1–5 mmol/L NaN_3 decreased the germination rate of *Arabidopsis thaliana*. Others, such as Hasegawa and Inoue (1984) and Nilan *et al*. (1975), have reported that NaN_3 affects the germination rate of barley. This inhibitory effect of NaN_3 on seed germination was not seen at Day 14 of the germination period. This result can be clarified in the following way: when the germination period extends beyond 7 d to 14 d, seeds probably become tolerant to the inhibitory effect of NaN_3 and improved their own physiological condition. Although it occurred slowly, it may be that meristematic cells continued mitotic division within 14 d; in this way, germination rates again increased or remained constant.

Although no effects of NaN_3 were observed on root length at Day 7, on leaf length at Day 7 following exposure to NaN_3 for 3 h or on coleoptile length at Day 14, generally, NaN_3 treatment decreased the length of leaves and coleoptiles. Pearson *et al*. (1975) observed

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that NaN_3 decreased the length of barley seedlings at pH 3. Muehlbauer and Sanders (1975) determined that 1 mmol/L NaN_3 affected plant length, plant weight, and the form of the leaf following exposure for 4 h. Conversely, Konzak *et al*. (1975) did not find any effect of NaN_3 at concentrations ranging from 2.0 to 2.5 mmol/L following exposure for 2 h with respect to the length of barley seedlings. The results of the present study, as well as results of previous studies, indicate that the effects of NaN_3 on the morphological characteristics of barley differ according to variations in concentrations used, periods of exposure, and the seedling growth period.

The cytogenetic results of the present study showed that NaN_3 treatment decreased MI rates compared with the control (untreated) group. Increasing concentrations of NaN_3 resulted in a decreased number of dividing cells. Pearson *et al*. (1975) determined that NaN_3 treatments decreased the MI of *H. vulgare* seeds treated with concentrations of 10^{-4}, 5×10^{-4}, and 10^{-3} mol/L NaN_3, pH 3, for 2 h. These results are comparable with those of the present study with regard to both the concentrations of NaN_3 used and the pH. As a result, both increased concentrations of NaN_3 and longer periods of exposure decrease the MI. Inhibition of the MI following NaN_3 treatment indicates that NaN_3 has genetoxic and mutagenetic effects on barley. It may be that the mutagenecity of NaN_3 is modified through the production of an organic metabolite of azid (Owais and Kleinhofs 1988). NaN_3 probably induces chromosome aberrations in barley (Sideris *et al.* 1973; Veleminsky *et al.* 1987). The results of the present study are fundamental results and provide direction for future investigations.

References

Arenaz P, Hallberg L, Mancillas F, Gutierrez G, Garcia S (1989). Sodium azide mutagenesis in mammals: Inability of mammalian cells to convert azide to mutagenic intermediate. *Mutat Res* **277**, 63–67.

Blattner FR (2004). Phylogenetic analysis of *Hordeum* (Poaceae) as inferred by nuclear rDNA ITS sequences. *Mol Phylogenet Evol* **33**, 289–299.

Gichner T, Veleminsky J (1977). The very low mutagenic activity of sodium azide in *Arabidopsis thaliana*. *Biol Plantarum* **19**, 153–155.

Gichner T, Badayev SA, Demchenko SI *et al*. (1994). *Arabidopsis* assay for mutagenicity. *Mutat Res* **310**, 249–256.

González-César E, Ramos-Morales P (1997). Sodium azide induces mitotic recombination in *Drosophila melanogaster* larvae. *Mutat Res Gen Tox En* **389**, 157–165.

Grant WF, Salamone MF (1994). Comparative mutagenecity of chemicals selected for test in the International Program on Chemical Safety's collaborative study on plant systems for the detection of environmental mutagens. *Mutat Res Fund Mol M* **310**, 187–209.

Hasegawa H, Inoue M (1984). Influence of temperature during and after sodium azide treatment on M_1 damage and M_2 chlorophyll mutation in barley (*Hordeum vulgare* L.). *Environ Exp Bot* **24**, 3–7.

Jones JA, Starkey JR, Kleinhofs A (1980). Toxicity and mutagenicity of sodium azide in mammalian cultures. *Mutat Res* **77**, 293–299.

Kamra OP, Gallopudi B (1979). Mutagenic effects of sodium azide in *Drosophila melanogaster*. *Mutat Res* **251**, 381–384.

Kanaya N, Gill BS, Grover IS *et al*. (1994). *Vicia faba* chromosomal aberration assay. *Mutat Res Fund Mol M* **310**, 231–247.

Kleinhofs A, Owais WM, Nilan RA (1978). Azide. *Mutat Res* **55**, 165–195.

Konzak CF, Niknejad M, Wickham I, Donaldson E (1975). Mutagenic interaction of sodium azide on mutations induced in barley seeds treated with diethyl sulfate or *N*-methyl-*N*-nitrosourea. *Mutat Res* **30**, 55–62.

Muehlbauer FJ, Sanders C (1975). Mutagenic effects of sodium azide on *Pisum*. *Res Rep* **7**, 32–33.

Nilan RA, Kleinhofs A, Sander C (1975). Azide mutagenesis in barley. *Barley Genet* **111**, 113–122.

Owais WM, Kleinhofs A (1988). Metabolic activation of the mutagen azide in biological systems. *Mutat Res* **197**, 313–323.

Ozkul Y, Silici S, Erogu H (2005). The anticarcinogenic effect of propolis in human lymphocytes culture. *Phytomedicine* (in press).

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Pearson W, Nilan RA, Sander C (1975). The effect of sodium azide on cell processes in the embryonic barley shoot. *Radiat Bot* **15**, 315–322.

Raicu P, Mixich F (1992). Cytogenetic effects of sodium azide encapsulated in liposomes on heteroploid cell cultures. *Mutat Res* **283**, 215–219.

Rines HW (1985). Sodium azide mutagenesis in diploid and hexaploid oats and comparison with ethyl methane-sulfonate treatments. *Environ Exp Bot* **25**, 7–16.

Savaskan C, Toker MC (1991). The effects of various doses of gamma irradiation on the seed germination and root tips chromosomes of rye (*Secale cereale* L.). *Tr J Bot* **15**, 349–359.

Sideris EG, Nilan RA, Bogyo TP (1973). Differential effect of sodium azide on the frequency of radiation-induced chromosome aberrations vs. the frequencies of radiation-induced chlorophyll mutations in *Hordeum vulgare*. *Radiat Bot* **13**, 315–322.

Veleminsky J, Anglis KJ (1987). Effects of sodium azide on replicative and repair DNA synthesis in barley embryos. *Mutat Res* **190**, 125–129.

Veleminsky J, Rosichan JL, Juricek M, Kleinhofs A, Nilan RA, Gichner T (1987). Interaction of the mutagenic metabolite of sodium azide, synthesized *in vitro*, with DNA of barley embryos. *Mutat Res Fund Mol M* **181**, 73–79.

Wen JG, Liang HG (1995). Effects of KCN and NaN_3 pretreatment on the cyanide-resistant respiration in tobacco callus. *Acta Bot Sin* **37**, 711–717.

Yuan HY, Zhang ZI (1993). Effect of free radicals and temperature on sister chromatid exchanges in *Hordeum vulgare* L. *Acta Bot Sin* **35**, 20–26.

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