

## Mutagenic Effect of Sodium Azide on Seed Germination of *Eruca sativa* (L.)

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**Abstract:** *Eruca sativa* is a very important crop of Mediterranean region, and highly used as salad in European countries. Sodium azide ( $\text{NaN}_3$ ) is a chemical mutagen, and widely used in crops to improve their yield and quality traits. We studied the effect of various concentrations of  $\text{NaN}_3$  ranged (1mM, 2mM, 3 mM, 4 mM and 5 mM) on germination and seedling growth of *Eruca* at various time intervals. The seeds treated at 5 mM of  $\text{NaN}_3$ , the percent germination profoundly affected on days 9 and 12 following its application for 120 min and 180 min of time intervals. The highest and lowest % germination was found for 30 min and 180 min of time intervals, whilst seeds were treated at same concentration of  $\text{NaN}_3$ . The radicle and coleoptile length were decreased as the concentration of  $\text{NaN}_3$  increased, and highly affected at concentrations 3 mM, 4 mM and 5 mM respectively. More variation was found on radicle length than that of coleoptile length at same concentrations and at same time intervals.

**Key words:** *Eruca sativa*, germination, mutagen, sodium azide.

### INTRODUCTION

*Eruca sativa* is a native of southern Europe and central Asia, where it has been cultivated since classical times. It is not a commercial crop in the UK or northern Europe, but is widely grown in kitchen and market gardens in southern France, Italy, Greece and the near East, where it is used for flavoring salads. The plant is naturalized in waste places, road shoulders and fallow fields in northern and Western Europe, well beyond its original range. It is also sometimes referred to as rocket, true rocket, rocket salad, arugula, roquette or white pepper. The young plants are used as a salad, vegetable and as green fodder. Tender leaves are reported to have stimulant, stomachic, diuretic and antiscorbutic activity (Bhandari and Chandel, 1966). The study on chemical mutagenicity on *E. sativa* is limited in literature, and because these mutagens play important role to improve agronomic traits of plant and also produce resistance to them against biotic and abiotic stresses. The seeds are good explants for chemical mutagens to create mutations in a genome of a cell. After treatment of chemical mutagens, seeds show the effects of mutagen as modified morphological traits from disturbed physiological processes. Germination is the process by which a seed initiates growth after a period of quiescence. It requires seed imbibition, and in a strict sense, is defined as the process leading to emergence of the radicle through the testa, a tissue of maternal origin that surrounds the embryo (Bewley, 1967; Koornneef *et al.* 2002). Germination is thus finished once the radicle has emerged. Imbibition, i.e. water uptake by the seeds, is accompanied by cell expansion, cell wall synthesis, and activation of metabolism. Accumulating evidence indicates that, in general, cell division occurs following germination (de Castro *et al.* 2000; Barroco *et al.* 2005). The increase in cell growth that is required for germination is due to cell elongation. In a very short time interval, a limited number of cells elongate and go through differentiation processes based on rapid metabolic changes preceding cell division. The process of germination is under the control of environmental and hormonal factors thus making the system appropriate for the study of plant development and the cellular responses to these factors. In laboratory, the germination depends upon a number of factors such as temperature, pH of the solvent, duration of soaking etc. Chemical mutagens are the one cause of mutation in living organism. These mutagens affect the germination process in seeds. The percent germination in seeds depends on the nature of the mutagen and its treatment dose. Many of these mutagens have clastogenic (chromosome damaging) effects on plants via reactive oxygen-derived radicals (Yuan, 1993). Chemical mutagen generally produce induced mutations, which lead to base pair substitution especially GC→AT

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(guanine : cytosine – adenine : thymine) resulting in amino acid changes, which change the function of proteins, but do not abolish their functions as deletions or frame shift mutations mostly do (Van der Veen, 1966). These chemomutagens also induced a broad variation of morphological and yield structure changes in comparison to normal plants.

Sodium azide ( $\text{NaN}_3$ ) is a common laboratory chemical and is widely used in industry, agriculture, medical practice, and organic synthesis research. It is a common bactericide, pesticide, and industrial nitrogen gas generator, and known to be highly mutagenic in several organisms, including plants and animals (Rines, 1985; Veleminsky and Anglis, 1987; Raicu and Mixich, 1992). It has been reported that  $\text{NaN}_3$  affects plant physiology and decrease cyanide resistant respiration in tobacco callus (Wen and Liang, 1995). The mutagenicity of this chemical is mediated through the production of an organic metabolite of azide compound (Owais and Kleinhofs, 1988). With reference to the mutagenic effect of  $\text{NaN}_3$  at different concentrations in barley, some results are contradictory, probably due to the different treatment conditions (presoaking, pH, temperature, time of exposure, etc.) and to the different varieties used (Ilbas *et al.* 2005). Sodium azide has been used in a number of crops for several biotic and abiotic stresses such as *Zea mays* resistant against pathogen *Striga* (Kiruki *et al.* 2006), *Musa spp.* AAA resistant against *Fusarium oxysporum* f. sp. cubense (Bhagwat and Duncan, 1988), barley resistant against Mildew disease (Molina-Cano *et al.* 2003), *Saccharum officinarum* resistant against red rot disease (Ali *et al.* 2007), *Arachis hypogea* (Mondal *et al.* 2007), *Lactuca sativa* resistant against down mildew disease (Okubara *et al.* 1994), *Glycine max* for enhanced fatty acid content (Hammond and Fehr, 1983b), *Triticum aestivum* (durum wheat) for salt tolerance (Agata *et al.* 2001), *Oryza sativa* for reduced amylase content (Jeng *et al.* 2003), *Oryza sativa* for enhanced yield (Jeng *et al.* 2006), *Halianthus annuus* for enhanced stearic acid content (Skoric *et al.* 2008), *Halianthus annuus* for reduced triacylglycerol content (Venegas-Caleron *et al.* 2008), *Oryza sativa* for silicon deficient (Nakata *et al.* 2008), *Hordeum vulgare* for reduced phytic acid content (Oliver *et al.* 2009), *Oryza sativa* for enhanced amylase content (Suzuki *et al.* 2008) and *Zea mays* for drought tolerance (He *et al.* 2009) respectively. The sodium azide mutagenicity was performed on *E. sativa* in green house experiment, and 3 mM concentration showed reversible inhibitory effect on growth and yield traits after 60 days of sowing (Al-Qurainy *et al.* 2009). In the light of above literature, in the present study, the mutagenic effect of various concentrations of  $\text{NaN}_3$  on seed germination and seedling growth were studied on *E. sativa* after a time interval in petriplate.

## MATERIALS AND METHODS

The seed of *E. sativa* was purchased from a local market of Riyadh, and the experiment was conducted at the Department of Botany and Microbiology, King Saud University, Riyadh, Kingdom of Saudi Arabia. The  $\text{NaN}_3$  was dissolved in water and stock solution made of 1.5 M concentration. Further, it was diluted with 0.1M sodium phosphate buffer of pH 3.2, and dilution was made of various concentrations ranged from 1 to 5 mM. It was also diluted in distilled water, and dilution was made of various concentrations ranged from 1 to 5 mM, and further used for seed treatment for various time intervals.

The seeds were chosen after passing through sieve of size 1.5 x 2 mm. They were soaked into autoclaved distilled water for 12 h with agitation on shaker at room temperature. After soaking into water, they were washed with autoclaved distilled water for five times to remove brown colour appeared from seeds in water. After washing, 50 seeds were kept in various concentrations of  $\text{NaN}_3$  solution for 30 min, 60 min, 120 min and 180 min with agitation on shaker at room temperature. After  $\text{NaN}_3$  treatment, seeds were washed with autoclaved distilled water for five times to remove excess  $\text{NaN}_3$ , and thereafter, each group of seeds (50 each treatment) were transferred to wet Whatmann paper in petri dishes at 21 °C for the investigation of the mutagenic effects of  $\text{NaN}_3$ . The % germination and seedling growth were investigated after 9 days of sowing in all treated and untreated seeds.

### **Statistical Analysis:**

Statistical significance was evaluated with one-way ANOVA analysis followed by Dunnett's multiple comparison test (comparing seedling developed from treated seeds with  $\text{NaN}_3$  to untreated seedling, and also among seedlings of treated seeds).

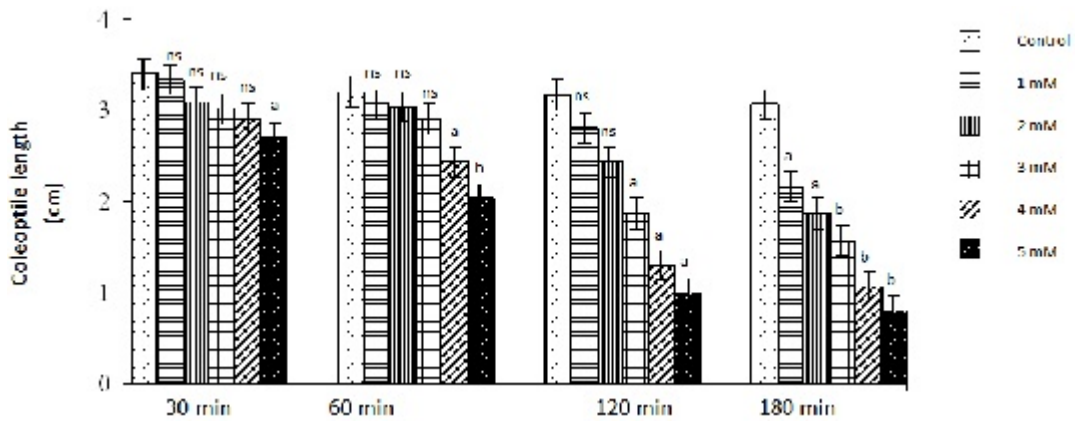
## RESULTS AND DISCUSSION

Sodium azide is highly soluble in water, but fewer number of hydrozoic ions are produced in water, and at low pH the quantities of  $\text{NaN}_3$  dissociated to hydrozoic acid which is theoretically many times greater (at pH 3 there is approximately 19 times more hydrozoic acid than at pH 6, for the same concentration of  $\text{NaN}_3$ ), and that would be the condition for better penetration through the cell membrane and create mutations in the

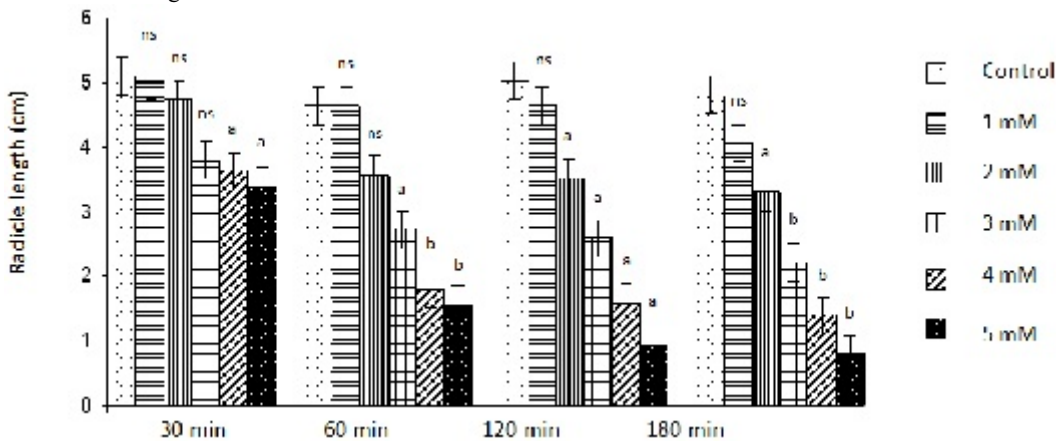
genome of a cell (Nilan et al. 1973; Kleinhofs *et al.* 1974). The pH value of soaking solution affects the efficiency of mutation with  $\text{NaN}_3$ . We performed our experiments in two dilution systems, water as well as phosphate buffer of pH 3.2. The little mutagenic effect of  $\text{NaN}_3$  or negligible effect was observed in water used as dilution system (data not shown). When it was diluted in phosphate buffer of pH 3.2, it had strong mutagenic effect on % germination, radicle and coleoptile lengths respectively.

In our experiment, 180 min treatment duration was very effective and at this treatment duration % germination was found to be 51.11(1 mM), 28.89 (2 mM), 22.32 (3 mM), 6.66 (4 mM) and 4.04 (5 mM) respectively. The seeds treated for 120 min with same concentration as 180 min, % germination was higher at 120 min of time interval than that of 180 min of time intervals, and it was found to be 62.21(1mM), 42.02 (2 mM), 26.56 (3 mM), 17.78 (4 mM) and 11.12 (5 mM) respectively. Similarly, treatment for 60 min of time interval with  $\text{NaN}_3$ , the % germination was found higher than 180 min and 120 min of time interval, and it was found 51.11(1 mM), 45.44 (2 mM), 40 (3 mM), 22.78 (4 mM) and 13.33 (5 mM) respectively. The % germination was found maximum when seeds were treated for 30 min of time interval. Our result was line with the result of Admu and Aliyu (Adamu and Aliyu, 2007) who performed your experiment on tomato, and treatment of  $\text{NaN}_3$  was very effective in inducing mutations and affects the % germination, root length, seedling height, seedling survival, number of branches per plant, and yield per plant. The various concentration of  $\text{NaN}_3$  also affected the seedling survival, and reduction in seedling survival is attributed to the cytogenetic damage and physiological disturbances (Sato and Gaul, 1967; Natrajan and Shivshankar, 1965). The greater sensitivity at higher mutagenic dose has been attributed to various factors such as changes in the metabolic activity of the cells, inhibitory effects of mutagens (Maherchandari, 1975) and to disturbance of balance between promoter and inhibitors of growth regulators (Krishna *et al.* 1984). The % seed germination was decreased at all studied concentrations of  $\text{NaN}_3$  at various time intervals as compared to untreated seeds, which had 85 % of seed germination in all experimental groups. The inverse relation was found among the various concentrations of  $\text{NaN}_3$  for various time intervals and percent seed germination (Table 1). The reduction in seed germination in mutagenic treatments had been explained due to delayed or inhibition of physiological and biological processes necessary for seed germination which include enzyme activity (Chrispeeds and Varner, 1976), hormonal imbalance (39) and inhibition of mitotic process (Ananthaswamy *et al.* 1971). The inhibitory effect of  $\text{NaN}_3$  on germination could be azide anions which are strong inhibitors of cytochrome oxidase, which in turn inhibits oxidative phosphorylation (Kleinhofs *et al.* 1978). In addition, it is a potent inhibitor of the proton pump and 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. Cheng and Gao (Cheng and Gao, 1988) treated barley seeds with  $\text{NaN}_3$  and significant reduction was found in the % germination. Furthermore, the effect of  $\text{NaN}_3$  was measured after 5-7 days from sowing, when the length of the first leaf had not reached its maximum, rendering it impossible to distinguish between delay in germination, and a real length reduction (Gaul, 1970; Konzak *et al.* 1975). As the  $\text{NaN}_3$  concentration increased from 1 mM to 5 mM, there was delay in seed germination, and seeds treated at 5 mM concentration of  $\text{NaN}_3$  for various time intervals (30, 60, 120 and 180 min) showed strong mutagenic effect on germination, and it was found to be 55.54%, 13.30%, 11.12% and 4.04% respectively. The % germination, radicle and coleoptile lengths were profoundly affected, when seed treated with  $\text{NaN}_3$ , diluted into phosphate buffer of pH 3.2. The radicle and coleoptile length were strongly affected by  $\text{NaN}_3$  treatment and as the dose of  $\text{NaN}_3$  increased, the radicle and coleoptile length were decreased, but more effect was observed on radicle length (Fig 1). Among various time intervals, the seeds treated for 180 min showed strong mutagenic affect on radicle and coleoptile lengths. The seeds treated with various concentrations of  $\text{NaN}_3$  at 3 mM, 4 mM and 5 mM for 180 min of time intervals showed high mutagenic effect on radicle length (cm), and it was found to be  $2.22 \pm 0.08$ ,  $1.40 \pm 0.08$  and  $0.80 \pm 0.35$  respectively (Table 3, Fig 2). Similarly, the length of coleoptile (cm) at above concentration was found to be  $1.56 \pm 0.12$ ,  $1.06 \pm 0.09$  and  $0.80 \pm 0.08$  at various concentrations of  $\text{NaN}_3$  including 3mM, 4mM and 5mM respectively (Table 2, Fig 3). The concentrations 1 mM and 2 mM of  $\text{NaN}_3$  for various time intervals showed less mutagenic effect on radicle and coleoptiles length as compared to 3 mM, 4 mM and 5 mM (Fig 1). Our results showed that the high dose of  $\text{NaN}_3$  (5 mM) treatment at various time intervals showed high mutagenic effect on *E. sativa* and it is mentioned in literature that treatments with  $\text{NaN}_3$  at various concentrations, under the same conditions, produce a delay in the initiation of plant growth, as can be observed and mentioned by Pearson *et al.* (1974, 1975). Kleinhofs *et al.* (1978b) suggested that 0.003 M  $\text{NaN}_3$  dose increased mutations in pea. The higher dose of  $\text{NaN}_3$  also caused disturbance in genetical and physiological activities leading to the death of the cells. Prina and Favret (1983) used 0.001 and 0.005 M doses of  $\text{NaN}_3$  on barley, but could not detect any physiological changes on the shoot development. In conclusion,  $\text{NaN}_3$  is a strong mutagen, and affected seed germination, radicle and coleoptile lengths of *E. sativa*, and thus it should be used further on this species to improve its agronomic traits and also produce resistance to them against biotic and abiotic stress by creating mutation.





**Fig. 2:** Mutagenic effect of various concentration of  $\text{NaN}_3$  on coleoptile length at various time intervals, and all treatments were compared to control. Results shows that treatment with  $\text{NaN}_3$  decreased coleoptile length. Data are mean  $\pm$  SD for three replicate done petriplate. Statistical significance was determined by ANOVA (Dunnett's multiple comparison test). Values are mean  $\pm$  S.D for three replicates in each group  
 a  $p < 0.01$ , when compared with control  
 b  $p < 0.001$ , when compared with control  
 ns- Not significant



**Fig. 3:** Mutagenic effect of various concentration of  $\text{NaN}_3$  on radicle length at various time intervals, and all treatments were compared to control. Results shows that treatment with s  $\text{NaN}_3$  decreased radicle length. Data are mean  $\pm$  SD for three replicate done petriplate. Statistical significance was determined by ANOVA (Dunnett's multiple comparison test). Values are mean  $\pm$  S.D for three replicates in each group  
 a  $p < 0.01$ , when compared with control  
 b  $p < 0.001$ , when compared with control  
 ns- Not significant

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