

# Mutagenicity of sodium azide in *Phaseolus vulgaris* L.\*

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## ABSTRACT

Dormant seeds of *Phaseolus vulgaris* L., cv. Milionário 1732, were treated with sodium azide (SA) solutions at concentrations 0 (control), 0.04 mM, 0.12 mM, 0.36 mM, and 1.08 mM, prepared in 0.1 M phosphate buffer, pH 3. Under these conditions, SA was only weakly mutagenic, in contrast to what has been previously reported in barley. Profound metabolic disturbances were caused by the high acidity of the buffer solution, resulting in heritable phenotypic changes as found in the control treatment. The pH of the buffer solution and pregermination of seeds should be the major variables used in future experiments aiming at optimizing SA treatments of bean seeds.

## INTRODUCTION

The utilization of new mutagenic agents in several plant species has played an important role in mutation breeding. Like gamma rays and EMS, sodium azide (SA) is one of the most commonly applied mutagens in plants. SA was used for the first time as a mutagen in barley by Nilan *et al.* (1973). They reported a high frequency (17.3%) of chlorophyll mutations at pH 3, a low frequency at pH 7, and no effect at pH 11. Efficiency was even higher when water-presoaked seeds were used.

SA has been reported to induce high frequency of point mutations (base substitutions) and no detectable chromosomal aberrations (Nilan *et al.*, 1973). As compared to other mutagens, SA is relatively safe to handle, inexpensive, and non-carcinogenic (Nilan *et al.*, 1977).

The mutagenic efficiency of SA has been reported in several plant species, such as barley, peas, *Petunia hybrida*, maize, sorghum, and diploid oats, but

not in *Arabidopsis*, African violets, and *Streptocarpus hybridus* (references in Silva, 1993).

Data on mutagenicity of SA in the common bean (*Phaseolus vulgaris* L.) are lacking. Preliminary data reported by Ando and Tulmann Neto (1975, 1976) were limited to the M<sub>1</sub> generation. Brunner (1977) reported that SA enhanced the mutation rates in grain legumes, but did not present data on kinds and frequencies of the induced mutants. This paper presents preliminary data on the mutagenic effects of SA in *P. vulgaris*.

## MATERIAL AND METHODS

Seeds (ca. 12% moisture) of cultivar Milionário 1732 (M 1732) were treated with SA solutions at concentrations 0 (control), 0.04 mM, 0.12 mM, 0.36 mM and 1.08 mM. The solutions were prepared in 0.1 M potassium phosphate buffer, pH 3. In each treatment, 300 seeds were soaked in 250 ml of the mutagen solution in 500 ml Erlenmeyer flask and shaken in a "Burrel Wrist-Action" agitator for 3 h at room temperature (ca. 23°C). Afterwards they were washed in running tap water for 30 min, dried in paper towel, and immediately taken to the field for planting. A randomized complete block design with six replications was used. Each plot

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had two rows 60 cm apart and 5 m long, with seeds planted 20 cm apart in the row.

Each  $M_1$  plant was harvested and thrashed separately. A sample of 10 to 60 seeds was taken from each  $M_1$  plant, depending on seed availability, and planted in a single row. Spacing was the same as used in the previous generation.

As soon as emergence started, seedlings were examined for the presence of chlorophyll-deficient and hypocotyl color mutants. During plant development, data were collected on the frequency and kinds of morphological deviates. After harvesting individual  $M_2$  progeny rows, each plant was thrashed to score for possible seedcoat mutants. The complete absence of pods or seeds was used as a measure of total sterility.

## RESULTS AND DISCUSSION

A total of 25,071  $M_2$  plants was examined, 4,220 of them deriving from the control treatment. The mutants or deviant types detected were grouped as follows: chlorophyll-deficient mutants, morphological variants, completely sterile plants, and mutants affecting seedcoat color or shine.

### Chlorophyll-deficient mutants

Four categories of chlorophyll mutants were found: *albina*, *xantha*, *viridis*, and *chlorotica*. The first three types were also induced after treating the same variety with EMS (Barbosa *et al.*, 1988b) or gamma rays (Sena and Barbosa, 1992; Paula, 1992).

The highest mutant frequency was induced by 1.08 mM azide treatment (Table I). However, increase in SA concentration up to 0.36 mM did not seem to be associated with increase in mutant frequency. A rather

high mutant frequency was unexpectedly found in the control group. This may be associated with the physiological damages induced by all treatment, including control, in the  $M_1$  generation, as reported elsewhere (Silva *et al.*, 1994). It is believed that the high acidity of the buffer solution caused profound alterations in cellular metabolism, resulting in genetic changes as found in the control. According to Sander *et al.* (1978), buffer solution at pH 3 may cause chromosomal aberrations.

Contrary to the results in Table I, increase in mutant frequency usually accompanies an increase in SA concentration (Rines, 1985). On the other hand, Prina and Favret (1983) reported highest mutant frequency at intermediate concentrations, even in the absence of physiological damages at the highest concentration. However, they used pH 6, much higher than the pH 3 commonly used in SA treatments. At pH 3, increase in azide concentration is usually associated with physiological injuries (Rines, 1985). Using high SA concentrations, mutants may not be recovered due to increased lethality. This was confirmed by the almost total lethality of concentrations higher than 1.08 mM (Silva *et al.*, 1994).

Most of the segregating  $M_2$  progenies yielded deficits of mutants (Table II). This may be attributed to the chimeric nature of the respective  $M_1$  plants resulting from the presence of a mutated cell in a multicellular embryo. Another cause of mutant deficit might be diplontic (intrasomatic) and/or haplontic (gametic) selection (Gaul, 1961). However, Paula (1992) did not find evidence of either diplontic or haplontic selection among cells carrying chlorophyll mutations induced by gamma rays in M 1732. Evidence against diplontic selection was also presented by Motto *et al.* (1975) after treating bean seeds with EMS.

Segregations of normal to mutant fitting the 3:1 ratio (Table II) were found in progenies 43 ( $\chi^2 = 1.92$ ;

Table I - Frequencies of  $M_2$  progenies segregating for induced changes.

Azide concentration (mM)	No. of $M_1$ plants	$M_2$ progenies segregating for					
		Chlorophyll mutants		Morphological variants		Sterile plants	
		% of treated seeds	% of $M_1$ plants	% of treated seeds	% of $M_1$ plants	% of treated seeds	% of $M_1$ plants
0 (control)	96	—*	1.04	—*	16.67	—*	6.25
0.04	180	0.66	1.11	9.33	15.56	2.33	3.88
0.12	185	0.33	0.54	8.67	14.05	2.33	3.78
0.36	151	0.66	1.32	8.67	17.22	2.33	4.64
1.08	94	1.33	4.26	9.00	28.72	2.00	6.38

\* Irrelevant since the number of  $M_1$  plants used for the control treatment (96) represents a sample.

**Table II** - Segregation for chlorophyll deficient mutants in M<sub>2</sub> progenies.

Azide concentration (mM)	Identification number of M <sub>2</sub> progeny	No. of M <sub>2</sub> plants		Chlorophyll mutant
		Normal	Mutant	
0 (control)	735	37	3	Xantha
0.04	43	22	12	Chlorotica
	79	30	10	Xantha
0.12	236	53	1	Viridis
0.36	416	44	3	Viridis
	443	39	16	Xantha
1.08	563	57	2	Viridis
	583	45	5	Albina
			4	Viridis
	597	54	5	Viridis
	603	55	2	Viridis

0.10 < P < 0.20), 79 ( $\chi^2 = 0.0$ ; P > 0.99), and 443 ( $\chi^2 = 0.49$ ; 0.30 < P < 0.50). Such cases indicate that the corresponding M<sub>1</sub> plants were non-chimeric, i.e., derived from a single cell heterozygous for the mutation. Segregation for two different chlorophyll mutations (*albina* and *viridis*) was found in progeny 583. Similar findings have been reported by Barbosa and Sena (1992) and Paula (1992) in M 1732 treated with gamma rays.

### Morphological variants

Many morphological deviates were found in M<sub>2</sub>, including reduction in plant height, reduced leaf size, and thicker leaves. Anomalies prevailing in all treatments were three cotyledonary leaves, small and elongated leaflets usually with lighter color, leaflets with folded margins and darker color, and plants with shorter internodes and excess of branches.

The high frequency of morphological changes found in the control treatment (Table I) was similar to the frequencies for the SA treatments with concentrations of up to 0.36 mM when estimated on M<sub>1</sub> plant basis. This may be ascribed to the high acidity of the buffer solution, as was found for the chlorophyll mutants. The highest frequency of deviate types, recovered with 1.08 mM SA treatment, parallels the data on chlorophyll mutants.

Most of the anomalies described above were similar to those reported by several authors (see Sena and Barbosa, 1992, and references therein). Sena and Barbosa (1992) proposed that most of these morphological anomalies may not have a genetic origin.

### Completely sterile plants

Great variation was found among the sterile M<sub>2</sub> plants. Some did not produce flowers, others had flowers but did not develop pods, while others had pods but did not set seed. As seen in Table I, increase in SA concentration was not associated with increase in sterility. The highest azide concentration induced as much sterility as the control treatment. No report on the mutagenicity of the buffer solution at pH 3 was found in the literature, with the possible exception of that by Sander *et al.* (1978).

Chromosome aberrations are probably the major cause of mutagen-induced sterility (Gaul, 1977). Other causes are gene mutations, cytoplasmic changes, and physiological injuries. Almost all cases of sterility detected in the present work were associated with morphological anomalies, mainly the presence of small and elongated leaflets. If SA is not effective in causing chromosome breaks, as stated by Nilan *et al.* (1973) and others, it is possible that most of the sterility reported here is due to the damaging effects of the buffer solution.

### Seedcoat mutants

Only two plants in one M<sub>2</sub> progeny of 1.08 mM azide treatment developed dark brown mutant seedcoat color. This corresponds to 0.33% of treated seeds and to 1.06% of the number of M<sub>1</sub> plants. No hypocotyl or flower color change was detected in these or any other M<sub>2</sub> plant.

Shiny seedcoat mutants were induced by 0.12 mM azide treatment and recovered from two plants from different M<sub>2</sub> progenies (one mutant per progeny). Such mutants have been induced quite easily in beans (Sena *et al.*, 1991). The dominant nature of this trait has been reported (Moh and Alan, 1964).

Contrary to the results reported in barley (Nilan *et al.*, 1973), the data presented here indicate that treatment of dormant seeds of *Phaseolus vulgaris*, cv. M 1732, with SA at pH 3 is not efficient in inducing mutations. Higher mutation rates have been reported with EMS (Barbosa *et al.*, 1988a,b) or gamma rays (Sena *et al.*, 1991; Sena and Barbosa, 1992) applied to the same variety. However, data on the mutagenicity of SA in *P. vulgaris* are lacking. The pH of mutagen solutions and pregermination should be the main factors to be considered in future experiments with SA mutagenesis in beans. Ando and Tulmann Neto (1975, 1976) have emphasized the importance of pH in SA treatments of bean seeds. They concluded that pH 3 causes more M<sub>1</sub> plant damage than pH 6 when seeds are presoaked in

water, but not in the absence of presoaking. Besides, there is difference in sensitivity to SA among varieties associated with metabolic differences (Kleinhofs *et al.*, 1978). Owais and Kleinhofs (1988) have shown that SA must be metabolized to be mutagenic. Pregermination of seeds may stimulate metabolism and thus azide mutagenicity. So, before making a fair comparison between the mutagenic effects of SA in *P. vulgaris* and those of other mutagens, further experiments should be conducted aiming at optimizing SA applications in this species.

## RESUMO

Sementes de *Phaseolus vulgaris* L., cv. Milionário 1732, foram submetidas às seguintes concentrações de azida sódica (AS): 0 (controle), 0,04 mM; 0,12 mM; 0,36 mM e 1,08 mM. As soluções foram preparadas em tampão fosfato 0,1 M, pH 3 e aplicadas em sementes sem pré-germinação. Nestas condições a AS apresentou baixa atividade mutagênica, ao contrário do que foi relatado originalmente em cevada por outros autores. Concluiu-se que a alta acidez da solução tampão tenha causado profundas alterações metabólicas, resultando em mudanças fenotípicas herdáveis como as encontradas no controle. O pH da solução mutagênica e a pré-germinação das sementes devem ser os principais fatores a serem considerados em futuros experimentos visando otimizar a aplicação de AS em sementes de feijão.

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