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Induction of mutations in *Browallia speciosa* using sodium azide and identification of the genetic variation by peroxidase isozyme

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Plants of *Browallia speciosa* were treated with different concentrations of sodium azide (0, 200, 400, 600 and 800 ppm) as a soil drench. The concentration of 800 ppm had simulative effect on the most studied traits. It increased the number of branches and leaves, chlorophyll content, fresh weights of vegetative growth and roots, dry weights of vegetative growth and roots and root length in the M_1 (2011/ 2012) and M_2 (2012/ 2013). All the concentrations of sodium azide produced changes in the flower colour, flower shape and leaf form in both generations. Peroxidase isozyme pattern indicated that seven loci produced of peroxidase are shown in *B. speciosa*. In addition, different peroxidase profiles among mutants were found. The phylogenetic tree indicated that mutant 4 was the most genetically distinct mutant from the control followed by mutant 3.

Key words: *Browallia speciosa*, mutation, sodium azide, isozyme.

INTRODUCTION

Browallia is a genus of flowering plants in the *Solanaceae* family. The plants are perennials in warmer climates, native to the tropical parts of South America, but are usually grown as annuals elsewhere. They grow well in the shade, and are frequently used as a color source in mass plantings or hanging baskets. The primary species grown is *Browallia speciosa*, also known as amethyst flower or bush violet.

Induced mutation using physical and chemical mutagen is a method to create genetic variation resulting in new varieties with better characteristics as reported by Wongpiyasatid et al. (2000) and Arulbalachandran et al. (2009). Sodium azide (NaN_3) is a chemical mutagen which is consider as one of the most powerful mutagens in plants. Its application on plant is easy and inexpensive and creates mutation to improve their traits. The efficiency of mutant production depends on many conditions such as pH, soaking into water, temperature, concentration of azide and treatment duration. It creates point mutation and damages the chromosomes and thus produces tolerance in the plants for numerous adverse conditions (Al-Qurainy and Khan, 2009). Sodium azide was used in many studies to induce mutation as found by El-Nashar (2006) on *Amaranthus caudatus*, Al-Gawwad and Makka (2009) on *Mirabilis jalapa* and Mostafa (2011) on *Helianthus annuus*. Isozymes are widely used as molecular marker to distinguish mutants as reported by Talukdar (2010).

The aim of this work was to study the effect of sodium azide on the growth of *B. speciosa*. Also, it aimed to

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produce genetic variation in the vegetative and flowering growth and use peroxidase isozeme to detect these variation.

MATERIALS AND METHODS

The investigation was carried out at the Nursery of Floricultural and Ornamental Plants, Faculty of Agriculture, Alexandria University, Egypt during two successive generations of 2011/ 2012 and 2012/ 2013. Rooted cuttings of *Browallia* were propagated on 20 cm in clay pots containing the soil mixture of clay and sand (3:1 v/v) on July 19 th 2011. After one week, the plants were treated with the following sodium azide concentrations 0, 200, 400, 600, and 800 ppm) as a soil drench (10 ml for each pot). On April 10th 2012, stem cuttings were taken to establish M_2 generation. Rooted cuttings were transplanted on July 5th. The procedure of transplanting was made likewise the first generation. All plants of the different treatments were examined daily to search for variation in the vegetative and flowering growth. The experimental layout was a randomized complete block design containing three replications (Steel and Torrie, 1982). Each replication contained five treatments and every treatment consisted of seven plants.

All data were recorded after one year from transplanting. The recorded data was plant height (cm), number of branches and leaves per plant, stem diameter (cm), leaf area (cm^2), chlorophyll content (SPAD unit), root length (cm) and fresh and dry weights of vegetative growth and roots. In addition, chlorophyll content was determined using apparatus (SPADA) as described by Yadava (1986). Flowering time as days from transplanting to the opening of the first flower was recorded. All changes in the vegetative and flowering growth were recorded; the valuable mutants were selected and identified using peroxidase isozyme pattern. Peroxidase isozyme activities were studied on the leaf of the mutants and the control of *B*. *speciosa*. Agar-starch-polyvinyl pyrolidin (PVP), gel electrophoresis was carried out according to the procedures described by EL- Metainy et al. (1977) and Rida (2003). Similarity values were calculated to determine the genetic relationships between the mutant plants. Bands on agarose gels were scored as present or absent and a pairwise similarity matrix was constructed using Dice coefficient after Sneath and Sokal (1973), followed by the unweighted pair grouping method of average method (UPGMA) to construct the dendrogram (Figure 5).

RESULTS AND DISCUSSION

The data presented in Tables 1 and 2 indicates that the concentration of 800 ppm sodium azide had stimulative effect on the most studied traits. It increased the number of branches and leaves, chlorophyll content, fresh weights of vegetative growth and roots dry weights of vegetative growth and roots and root length in both generations. The stimulative effect of sodium azide might be attributed to cell division rates as well as to activation of growth hormones, for example, auxin as reported by Joshi et al. (2011). These results are in agreement with the finding of El-Nashar (2006) on *Amaranthus* seedlings and Mostafa (2011) on *H. annuus*

Plants treated with 400 ppm sodium azide increased significantly the plant height in the M_1 compared to control (49.0 and 42.0, respectively), but in the M_2 generation, plants treated with 200 ppm was the tallest compared to control (66.3 and 52.6 cm). Concerning flowering time, plants treated with 400 ppm sodium azide flowered earlier in both generations. In the M1 the treated plant flowered 4 days earlier than the untreated control plants. Also, in the M2 the treated plant flowered about 8 days earlier than the untreated control plants (Table 1). On the other hand, the highest concentration (800 ppm) of sodium azide delayed flowering in both generations. This result agrees with the results of El-Nashar (2006) and Mostafa (2011). No significant effects were recorded for the different sodium azide treatments with respect to stem diameter in the M_1 generation, leaf area and dry weight of vegetative growth in the M_2 generation.

All the concentrations of sodium azide produced changes in the flower colour as shown in Figure 1 in both generations. Flowers with stripped petals with white color and variegated flowers with yellow color were found as a result of the 400 ppm sodium azide treatment. The concentrations of 400, 600 and 800 ppm produced changes in the flowers form, floweres with four petals, with malformed shape or having stamins modified to petals. Flowers with four petals were obtained by 400 and 600ppm compared to the control. Also stamens were modified to petals on some flower as an effect of sodium azide at 800 ppm as shown in Figure 2. All treatments of sodium azide produced malformed leaves forming some plants as shown in Figure 3 in both generations. These changes may be due to chromosomal disturbances; these changes could be referred also to the layer rearrangement as a result of the chemical mutagens effect as reported by Abdel Maksoud (1988) and El Nashar (2006).

It is important to notice that isozyme analysis using electrophoresis offers a very well defined effective tool for the detection of genetic differences among individuals. This makes electrophoresis a useful tool for breeders (Arulsekar and Parfitt, 1986). Four mutants and the control plant were selected for isozyme analysis as shown in Table 3. The electrophoretic banding patterns presented in Figure 4 indicate different profiles among mutants. It can be concluded that a total number of seven loci control the production of peroxidase in *Browallia*. Four bands migrated toward the cathode (-) and the others migrated toward the anode (+) in the electrophoresis field and were designed as prx-1 prx-7. The bands of the loci prx-2 and prx-3 presented in all the evaluated genotypes, but prx-3 was different in the intensity between mutants. Mutant 2 and 5 had very low intensity band for this locus loci prx-5 and prx-6 were found only in the mutant 4. This might be related to the improvement of these mutants' traits comparable to control (Talukdar, 2010). The mutagenesis treatments seemed to activate expression of some genes which resulted in the appearence of some new bands. Mutations have been identified as one of the sources of isozyme variation in higher plants. These results are almost in agreement with those of Bartosova et al. (2005), Malaviya et al. (2006),

Table 1. Effect of different sodium azide concentrations on plant height (cm), number of branches and leaves, stem diameter (cm), chlorophyll content (SPAD unit) and flowering date (days).

Sodium azide conc. (ppm.)	Plant height (cm)		No. of branches		No. of leaves		Stem diameter (cm)		Chlorophyll (SPAD)		Flowering time	
	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
0.0	42.0^{b}	52.6^{b}	6.0^{c}	10.8^{ab}	122.0^{b}	156.6^{bc}	0.67^{a}	0.63^{b}	59.7^{ab}	43.8^{bc}	52.0^{d}	54.4^{a}
200	37.0^{c}	66.3^{a}	6.0^{c}	7.0^{c}	118.0^{b}	123.3^{d}	0.60^{a}	0.63^{b}	66.0^{a}	43.8^{bc}	60.0^{c}	57.1^{a}
400	49.0^{a}	58.5^{ab}	7.0^{b}	14.1^{a}	106.0^{d}	194.3^{ab}	0.56^{a}	0.76^{a}	52.6^{c}	41.8^{c}	48.0^{e}	46.2^{b}
600	37.0^{c}	50.2^{b}	6.0^{c}	7.8^{bc}	112.0^{c}	147.6^{cd}	0.44^{a}	0.58^{b}	58.3^{bc}	47.4^{a}	63.0^{b}	58.1^{a}
800	41.0^{b}	56.7^{b}	8.0^{a}	12.9^{a}	168.0^{a}	203.3^{a}	0.63^{a}	0.55^{b}	65.6^{a}	45.4^{ab}	68.0^{a}	61.0^{a}
$LSD_{0.05}$	1.8**	8.69*	0.4**	3.8*	5.5**	30.4**	NS	0.11*	6.9*	3.2*	3.6**	7.7*

Values in the same column not followed by the same letter are significantly different at the 5% level of probability. NS,*, **= Not significant, significant at p= 0.05 and 0.01 respectively.

Table 2. Effect of different sodium azide concentrations on leaf area (cm^2), fresh and dry weights of vegetative growth and roots (g) and root length.

Sodium azide conc. (ppm.)	Leaf area (cm^2		Fresh weight of vegetative growth(g)		Fresh weight of root (g)		Dry weight of vegetative growth (g)		Dry weight of root (g)		Root length (cm)	
	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
0.0	9.58 bc	13.22 a	66.2 c	88.3 a	17.5 bc	28.3 bc	18.2 a	24.3 a	10.0 b	13.6 b	26.0 a	19.4 d
200	8.84 c	11.17 a	44.0 d	58.3 b	16.2 c	20.0 c	13.0 b	16.8 a	6.2 c	8.2 c	21.6 b	20.5 cd
400	9.96 b	5.39 a	72.0 b	96.6 a	22.9 a	33.3 b	19.5 a	25.6 a	9.0 b	12.3 bc	17.0 c	23.2 bc
600	9.10 c	11.73 a	41.0 d	55.0 b	18.5 b	26.6 bc	13.0 b	18.1 a	9.1 b	12.3 bc	23.6 ab	28.0 a
800	12.01 a	8.47 a	85.3 a	91.6 a	21.3 a	53.3 a	20.0 a	25.0 a	17.0 a	21.3 a	22.3 ab	25.0 ab
$LSD_{0.05}$	0.79**	NS	0.4**	3.8*	5.5**	30.4**	4.2*	NS	2.1**	4.4**	4.3*	3.13**

Values in the same column not followed by the same letter are significantly different at the 5 % level of probability. NS, Not significant; *, **, significant at p= 0.05 and 0.01, respectively.

Table 3. Illustrated the mutants of *B. speciosa* selected for peroxidase analysis.

Mutant no.	Selected variation characteristic
Control (M0)	Normal plant
M1	Changes in the form of the leaf
M2	Flowers with stripped petals with white color
M3	Variegated flowers with yellow color
M4	Stamens modified to petals

Hossain et al. (2006) and Talukdar (2010). On the other hand, the loci prx-4 was absent from the mutants 3, 4 and 5. In addition, the locus



Figure 1. Photograph showing different variegated flowers of *B. speciosa* as a result of the treatment with sodium azide.



Figure 2. Photograph showing changes in the flower form of *B. speciosa* as a result of the treatment with sodium azide.

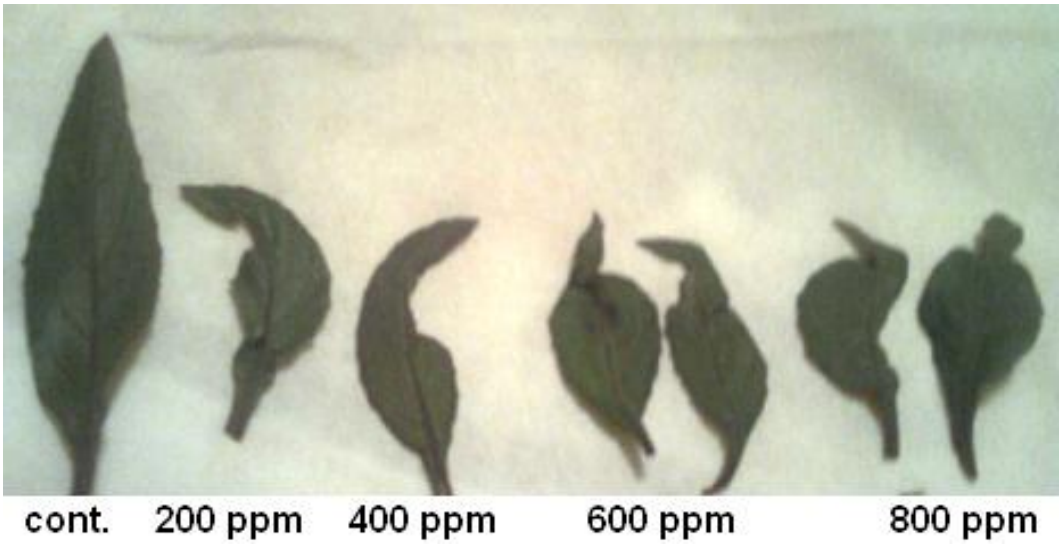


Figure 3. Photograph showing malformed leaves of B. speciosa as a result of the treatment with sodium azide.

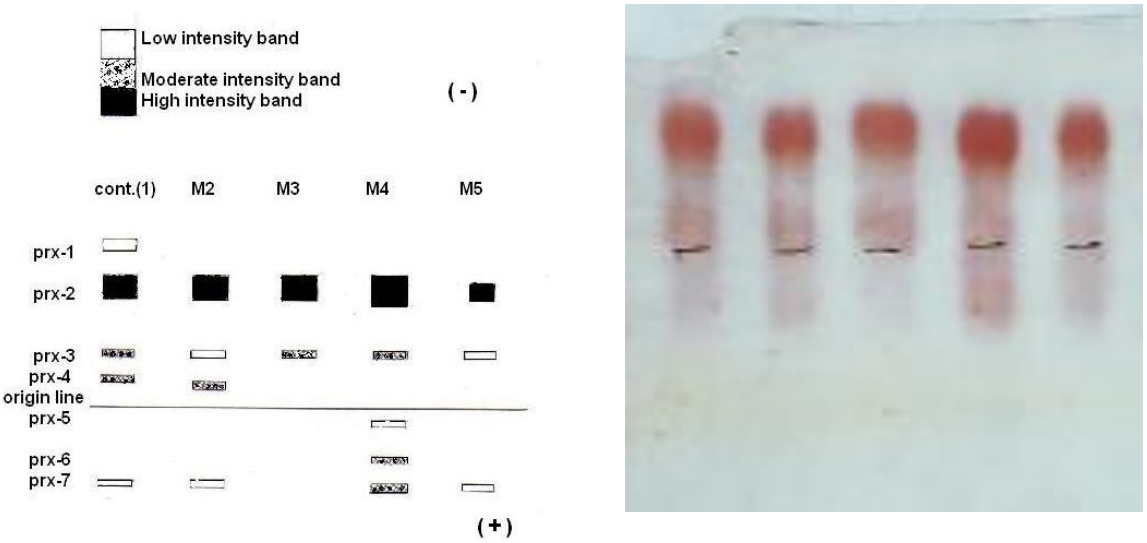


Figure 4. Electrophoretic separation pattern of peroxidase isozyme of *B. speciosa* mutants.

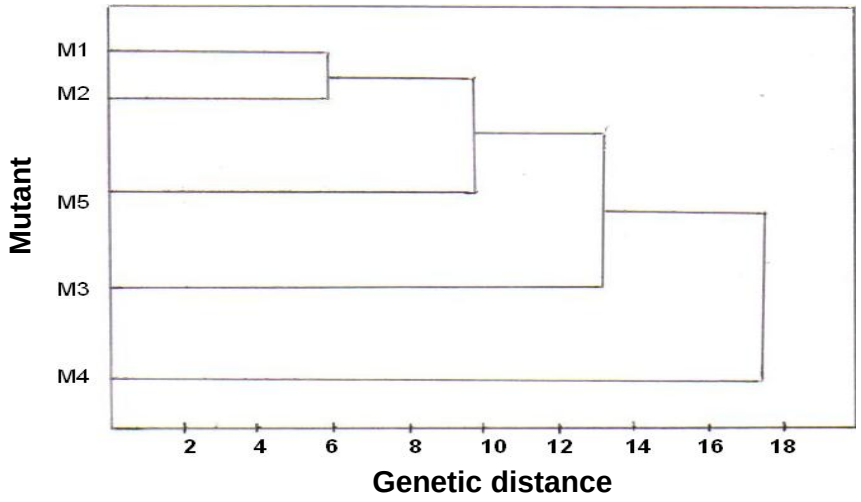


Figure 5. Dendrogram constructed on the basis of peroxidase isozyme profile for wild type (Control) as M0 and four mutants (M1, M2, M3 and M4) of *B. speciosa.*

Table 4. Similarity value among all mutants of *B. speciosa* produced by sodium azide treatments.

	M0	M1	M2	M3	M4
M0 (control)	100				
M1	88.8	100			
M2	74.0	66.6	100		
M3	60.0	66.6	57.1	100	
M5	75.0	85.7	80	75	100

prx-7 disappeared from the mutant 3. This locus showed a low intensity in the mutants 2 and 5 and the control plant, while the loci prx-1 was found only in the control this may be due to the mutagenic effects (Aly and Elsayed, 2006).

Regarding the similarity values, Table 4 show that mutant 2 was more genetically related to control with similarity value of 88.8%. On the other hand, low genetic similarity between control to mutants 3, 4 and 5 (74, 60 and 75% respectively), mutant 2 to mutants 3 and 4 (66.6).

The lowest genetic similarity was found between mutant 3 and 4 (57.1). The phylogenetic tree indicated that mutant 4 was the most distinct mutant from the control followed by mutant 3.

Finally, it can be concluded that sodium azide is a powerful mutagens for the induction mutations in *B. speciosa* and peroxidase isozyme could act as a useful biochemical marker for mutant identification.

REFERENCES

Abd El-Maksoud BA (1988). Effect of different media and mutagenic treatments on *in vitro* obtained Roses. Ph.D. Thesis, Floriculture, Faculty of Agric. Alexandria University.

Arulseker S, Parfitt DE (1986). Isozyme analysis procedure for stone fruit, almonds, grape, walnut and fig. Hortscience. 21:928-933.

Al Gawwad, Makka A (2009). Effect of chemical mutagens on *Mirabilis jalapa*. M.Sc. Thesis, Floriculture, Faculty of Agriculture, Alexandria University.

Al- Qurainy F, Khan S (2009). Mutagenic effects of sodium azide and its application in crop improvement. World Appl. Sci. J. 6:1589-1601.

Aly A, Elsayed HE (2006). Molecular analysis of two bananas genotypes induced *in vitro* under gamma rays stress. Egypt. Soc. Nuclar Sci. Appl. 39:188-194.

Arulbalachandran D, Mullainathan L, Velu S (2009). Screening of mutants in black gram (*Vigna mungo* L. Hepper) with effect of DES and COH in M2 generation. J. Phytol. 1:213- 218.

Bartošova Z, Obert B, Takac T, Kormutak A, Pretova A (2005). Using enzyme polymorphism to identify the gametic origin of flax regenerants. Acta Biologica Cracoviensia series Botanica. 47(1):173-178.

EL-Metainy A, Abou-Yossef YAY and EL-Hadad M (1977). Starch degrading isozymes in *Triticum asetivum,Triticum pyramidal* and their interspecific hybrid .Egypt. J. Gent. Cytol. 6:375-379.

El-Nashar YIA (2006). Effect of chemical mutagens (sodium azide and diethyl sulphate) on growth, flowering and induced variability in *Amaranthus caudatus* L. and A. *hypochondriacus* L. Ph.D. Thesis, Floriculture, Faculty of Agriculture, Alexandria University.

Hossain Z, Mandal AKA, Datta SK, Biswas AK (2006). Development of NaCl-tolerant strain in *Chrysanthemum morifolium* Ramat. through *in vitro* mutagenesis. Plant Biol. 8:1-12.

Joshi N, Ravind ran A, Mahajan V (2011). Investigations on chemical mutagen sensitivity in onion (*Allium cepa* L.). Int. J. Bot. 7(3):243-248.

Malaviya DR, Roy AK, Tiwari A, Kaushal P, Kumar B (2006). *In vitro* callusing and regeneration in *Trifolium resupinatum* -a fodder legume. Cytologia 71:229-235.

Mostafa GG (2011). Effect of sodium azide on the growth and variability induction in *Helianthus annuus* L. International Journal of Plant Breeding and Genetics. 5 (1):76-85.

Rida ME (2003). Cytogenetical studies on the effect of some mutagenic agent on *Gladiolus* (*Gladiolus hybrida* L.) M.Sc. Thesis, Faculity of Agriculture, Saba Basha, Alexandria University.

Sneath PHA, Sokal RR (1973). Numerical Taxonomy: The Principles and Practice of Numerical Classification. Freeman, San Francisco.

Steel RDG, Torrie JH (1982). Principles and procedures of statistics. 3rd Ed., Mc Graw Hill Konga Kusha Ltd. Book Co. Inc., New York.

Taklukdar D (2010). Allozyme variations in leaf esterase and root peroxidase isozymes and linkage with dwarfing genes in induced dwarf mutants of grass pea (*Lathyrus sativus* L.). Int. J. Genet. Mol. Biol. 2(6):112-120.

Wongpiyasatid A, Chotechuen S, Hormchan P, ngampongasai S, Promcham W (2000). Induced mutations in mungbean breeding: Regional yield trial of mungbean mutant lines. Kasetsart J. Nat. Sci. 34:443-449.

Yadava L (1986). Arapid and non-destruction method to determine chlorophyll in intact leaves. Hort Sci. 21:1449-1450.