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Sodium Azide Induced Complementary Effect of Chromosomal Stickiness in *Brassica campestris* L.

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Abstract

Present investigation reports the mutagenic efficacy of sodium azide with a view to study its effects on meiotic behavior and phenotypic quantitative traits of *Brassica campestris* L. Seeds were treated with three concentration of sodium azide i.e. 0.3%, 0.5% and 0.7%, along with control. Morphological/phenotypic parameters studied were plant height, number of primary branches, number of secondary branches, seeds/siliqua, husk yield, seed yield and 1000-seed weight. All these traits showed the significant positive shifts in mean at 0.3% and 0.7% doses of azide as compared to control and 0.5% dose. However, cytogenetic assessment of the treated plants clearly revealed the preponderance of stickiness distributed in all the phases of meiotic division that persisted up to the tetrad stage. The phenomenon displayed mild to severe case of stickiness on the basis of number of genome involved during meiosis. Manifestation of stickiness has also been resulted in several other meiotic abnormalities such as bridge (1.88%), unorientation (0.64%), micronuclei (1.38%), asynchronous division (0.77%), and precocious chromosomes (3.89%) along with some other anomalies (laggards, univalents etc.). Frequency of these anomalies along with severe stickiness was higher at 0.5% dose of azide that must have compromised with pollen fertility resulting declined fertility rate. Though, pollen fertility at 0.3% and 0.7% doses had not been much affected and had fallen near control. Thus, from present investigation inference can be drawn that the sodium azide induced stickiness might have resulted in some meiotic mutant that has imposed some novel genetic combinations thereby positively affecting morphological traits of the crop.

Key Words: *Brassica campestris* L.; Mutation Breeding; Pollen Fertility; Sodium Azide; Stickiness

1. Introduction

Economies all over the world understand that rapid urbanization and industrialization are the surest ways of achieving accelerated economic growth (Oliveria, 2011). However, these cause many other problems such as population explosion, lack of agriculture field and environmental pollution that ultimately results in an increasing food scarcity. There is a growing food shortage problem in many areas around the world and is becoming a matter of worldwide concern. Thus, to overcome the drastic situation of food shortage and increasing world food security, mutation breeding is an effective, inexpensive and dependable approach. Since food crop varieties embedded with various induced mutations have contributed to the significant increase of crop production (Kharkwal and Shu, 2009). It paves a path to induce genetic variability in some economic important self pollinated crops where crossing/hybridization is quite difficult viz. wheat **(**Srivastava *et al.*, 2011**)** and fenugreek **(**Chaudhary and Singh, 2001; Basu *et al.*, 2008). Hence, utilization of mutagenesis, undoubtedly, capable of increasing genetic variability in number of crops as reported by (Mahandjiev *et al.*, 2001; Tai *et al.*, 2007; Khan and Goyal, 2009; Kozgar *et al.*, 2011; Srivastava *et al.*, 2011). Mutations are the tools that being used to study the nature and function of genes which are the building blocks and basis of plant growth and development thereby, producing new raw materials for genetic improvement of economic important crops (Adamu and Aliyu, 2007). Till to date, several mutagens have been known to us that are used in mutation breeding and proved to be valuable in the achievement of crops with beneficial and desired traits such as high yield and resistant mutant (Mahmoud *et al.*, 2006; Tomer *et al.*, 2007; Basu *et al.*, 2008; Srivastava *et al.*, 2011).

Chemical mutagenesis is considered as an effective means in improving the yield and quality traits of crop plants (Srivastava *et al.*, 2011). Among numerous alkylating chemical mutagens such as ethyl methane sulphonate (EMS), sodium azide, hydrazine hydrate etc., sodium azide had been and is still used as potent mutagens in variety of crop improvement programs. Its mutagenic potential has been reported in several

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screening assays (Raicu and Mixich, 1992; Grant and Salamone, 1994; Srivastava and Kapoor, 2008; Srivastava *et al.*, 2011). Notwithstanding the potency, sodium azide is marginally mutagenic in humans and animals (Sadiq and Owais, 2000) thus safe to use.

Brassica is the important oilseed crops throughout the world which rank third among the oilseed crops after soybean and oil palm in production of vegetable oils (Kauser *et al.*, 2006). It is the most economically important genus in the Brassicaceae family (syn. Cruciferae) which contains little fat, and is sources of vitamins, minerals, and fiber (Cardoza and Stewart, 2004). The plant is also used to produce a high quality protein and after extraction of this oil, the residual high protein meal can be soaked in water and fed to cattle (Downey, 2003).

Thus, considering the above points in mind, the present study was performed to appraise the mutagenic effectiveness and efficacy of sodium azide on the chromosomal behavior and morphological characters of *Brassica campestris* L. Also, it was aimed to obtain some desirable mutant lines.

2. Materials and Methods

2.1. Seeds Procurement and Treatment

Inbred seeds of cultivar *Brassica campestris* L. accession number-IC363713 were obtained from National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Seeds were pre-soaked for 6 h in distilled water and later blotted, dried. Thereafter dried seeds were treated with freshly prepared sodium azide (NaN_3) solution for three doses (0.3% 0.5% and 0.7%, w/v) for 5 h at room temperature (26±2ºC) with intermitted stirring (Srivastava *et al.*, 2011). Meanwhile, some seeds were kept in distilled water to maintain control sets. Seeds were washed thoroughly with running water and blotted with filter paper in separate petri plates each and sown in nursery pots in triplicates.

2.2. Meiotic Preparation

Unopened flower buds of *Brassica campestris* L. were collected in vials from adult plants at an early winter's morning. Buds were fixed in glacial acetic acid – alcohol (1:3, v/v) for 24 h at room temperature and transferred to 70% alcohol, refrigerated at 4ºC until use. The anthers were squashed in 2% standard aceto-carmine stain (Fürste, 1962) and the slides were covered with cover slips following gentle tapping. After preparation slides were observed under optical microscope (Olympus CH20i) and photomicrographs of pollen mother cells (PMCs) were made using pinnacle PCTV capture device. Habib *et al.* (2004) reported that chromosomes of *Brassica* species are very small and their identification through the ordinary cytogenetic techniques is extremely difficult. Thus, meiotic analysis of *Brassica campestris* L. has become really complicated yet it was done by the preparation of large number of slides and their thorough selection for each stage of meiosis. Ten slides per plant were prepared and 10fields/slide was cytologically tested. Morphological observations were made in three replicates per dose whereas five plants per replicate were randomly sampled along with control. Frequency of the meiotic abnormalities was documented and pollen fertility was estimated using a glyceroacetocarmine (1:1) mixture as well.

2.3. Statistical Analysis

Variations of the different studied morphological parameters were subjected to one-way variance analysis (ANOVA) and Duncan's test *($p<0.05$)* using Statiatica-8 software (Stat Soft).

3. Results

3.1. Chromosomal Study

Cytological study revealed the normal course of meiotic chromosomes comprised 10 bivalents at diakinesis (2n=20) and its segregation into 10:10 at anaphase (Figures A, B). While treated sets shared an array of abnormalities distributed in all the phases of reduction division namely stickiness (Figures C-L), Unorientation-0.64%, (Figures F, G, I, L), micronuclei-1.38%, precocious chromosomes-3.89% (Figure J), incomplete bridge-1.88% (Figure L) and asynchronous division-0.77%. Some other anomalies such as laggards (Figure G), univalents and picnotic nuclei have also been registered in low frequency. However, among the all anomaly observed, the most prominent anomaly documented was stickiness, found to be distributed in various phases of meiosis i.e. metaphase-I/II to anaphase-I/II. It ranged from mild to severe on basis of number of genome involved. Mild stickiness has been documented at 0.3% and 0.7% doses in which few chromosomes were involved that might be resulted in to aneuploids bearing desirable traits. While intense or severe case of stickiness was found at the medium dose of azide i.e., 0.5% (Table 1). The latter has resulted in clumping or completely intermingling of genome due to which chromosome has lost its distinctiveness completely and resulting genome damage. Whereas occurrence of 0.03% stickiness at control might be due to environmental factor (Table 1).

Despite stickiness, many other abnormalities have been recorded which ranged from 0.03% to 12.88%, where the highest dose has lesser values of aberrations as compared to 0.5%. Further, pollen fertility recorded in 0.3% and 0.7% doses was found to be 96.68% and 94.63%, respectively as compared to control which possessed 99.81% pollen fertility rate. However, the intermediate dose registered a sharp fall in its mean i.e. 73.58% (Table 1).

3.2. Morphological Variability

In present investigation various doses of sodim azide have considerably affected some phenotypic traits of the present crop. Phenotypic characters used to assess the effects of mutagen were plant height, number of primary branches, number of secondary branches, number of seeds/siliqua, husk yield, seed yield and 1000-seed weight. Table 2 clearly depicted that the 0.3% and 0.7% doses of sodium azide were significant and found to be in positive correlation with higher value of number of branches, seeds/siliqua, husk yield, seed yield and 1000-

seed weight as against controls and 0.5% dose of azide. More surprisingly, it was recorded that the lowest as well as the highest doses were promising in inducing desirable traits in the present crop. On the other hand, 0.5% dose was negatively correlated with yield related traits with the greatest share of meiotic aberration (Table 1). As far as plant height is concerned, treated plants were significantly shorter as compared to control ($p<0.05$), which is in fact a desirable trait. Since it prevents plants from lodging as lodging damage might result in reducing the yield capacity of crop.

4. Discussion

The prime strategy in mutation breeding has been to upgrade the well-adapted plant varieties by altering one or two major traits which limit their productivity or enhance their quality (Srivastava *et al.*, 2011). Thus, in view of the above statement, it has found that sodium azide has well suited for mutation breeding since the mutagenic effects of azide appear soon after sowing the seeds and can be observed by naked eyes (Srivastava *et al.*, 2011). Moreover, due to its ease in availability and in being reasonably priced, could be effortlessly utilized in mutation breeding program. Many researchers have been exploited the mutagenic effectiveness of sodium azide in different agronomic crops such as *Triticum aestivum*, *Trigonella foenum-graecum*, *Nigella sativa* and *Plantago ovate* (Srivastava and Kapoor, 2008; Prabha *et al.*, 2010 a, b; Srivastava *et al.*, 2011).

Cytological analysis provides a genetic basis for chromosomal behavior at different stages of cell cycle and hence provides an authentic mean to determine the efficiency of mutagens. Mutagens may cause error in the normal behavior of chromosome. Hence, any disturbance in normal cytological behavior of chromosomes (either positive or negative) reflects in phenotypic traits of plants. As in the present case, positive effects of mutagens have been displayed by the obtainment of meiotic sticky mutant having enviable trait(s) at 0.3% and 0.7% doses of chemical mutagen. Meiotic observations presented in Table 1 clearly showed the dominance of chromosomal stickiness at all the doses of azide. However it had also taken together with meiotic manifestation mainly at 0.5% dose resulting marked reduction in pollen fertility.

Sticky chromosomes were first reported in maize by Beadle (1932), and he attributed such irregularity to a mutation caused by a recessive gene called sticky (*st*). Bione *et al.* (2000) reported that the phenotypic manifestation of stickiness may vary from mild, when only a few chromosomes of the genome are involved, to intense that may involve the entire genome. In the present case, prevalence of stickiness at medium dose (0.5%) could be arisen from improper folding of the entire chromosomes into single chromatids and chromosomes, as a result of which chromatin fibers intermingled (Klasterskii *et al.*, 1976) hence causing genome damage. Such genome damage could be the reason for decreased pollen fertility at this particular dose. However, mild stickiness registered at two optimal doses (0.3% and 0.7%) might be resulted in aneuploids bearing peculiar and enviable genomic constitution resulted in higher mean value of some quantitative parameters over control.

Occurrence of mild stickiness at utmost dose (0.7%) could be due to the effect of azide that might have induced meiotic mutant having beneficial traits over 0.5% dose. Plenty of reports, however, are available which showed the stimulatory effects of lower dose of treatment (Luckey, 1980; Kim *et al.*, 2004) and similar finding has been reported in present case as well.

Table 1. Effect of sodium azide on meiotic courses of *Brassica campestris* L.

Conc.%	TCO	% stickiness at metaphase (M)		% stickiness at anaphase (A)		Oth.Ab.%	Pollen fertility %
		I	II	I	II		
Control	1021	-	-	-	-	0.03	99.81 ± 0.07*
0.3	1005	1.19±0.11*	1.21 ± 0.10	1.04 ± 0.09*	0.85 ± 0.18	3.57	96.68 ± 0.97
0.5	918	10.45±0.29	10.20 ± 0.35	9.04 ± 0.19	7.31 ± 0.20	12.88	73.58 ± 2.45
0.7	922	4.17 ± 0.28	5.04 ± 0.33	4.17 ± 0.10	3.53 ± 0.32	7.28	94.63 ± 1.20

TCO- total cell observed, Oth. Ab.- other meiotic anomaly (laggards, precocious chromosomes, bridge etc.)
*Mean±SE

Table 2. Effect of sodium azide on some yield attributing traits of *Brassica campestris* L.

Conc. %	Plant height (m)*	No. of primary branches*	No. of secondary branches*	Seeds/siliqua*	Husk yield(g)*	Seed yield(g)*	1000-seed weight(g)*
Control	1.57±0.38^{b}	4.0±0.77^{a}	7.4±1.43^{a}	19.7±1.52^{b}	14.3±0.63^{b}	15.57±0.46^{c}	2.50±0.04^{b}
0.3	1.30±0.67^{a}	5.8±0.66^{b}	13.0±2.48^{c}	21.8±1.29^{c}	17.7±2.72^{c}	16.43±2.24^{c}	3.28±0.21^{d}
0.5	1.22±0.38^{a}	3.75±0.37^{a}	8.0±0.63^{ab}	19.5±1.71^{a}	11.9±1.70^{a}	11.44±2.87^{a}	2.24±0.10^{a}
0.7	1.32±0.11^{a}	5.75±0.74^{b}	8.75±1.93^{b}	20.0±0.64^{a}	12.3±2.52^{a}	13.82±1.87^{b}	2.83±0.05^{c}

*Mean±SE, For a given means within each column of each section followed by the different lowercase letter are statistically significant at $p < 0.05$.

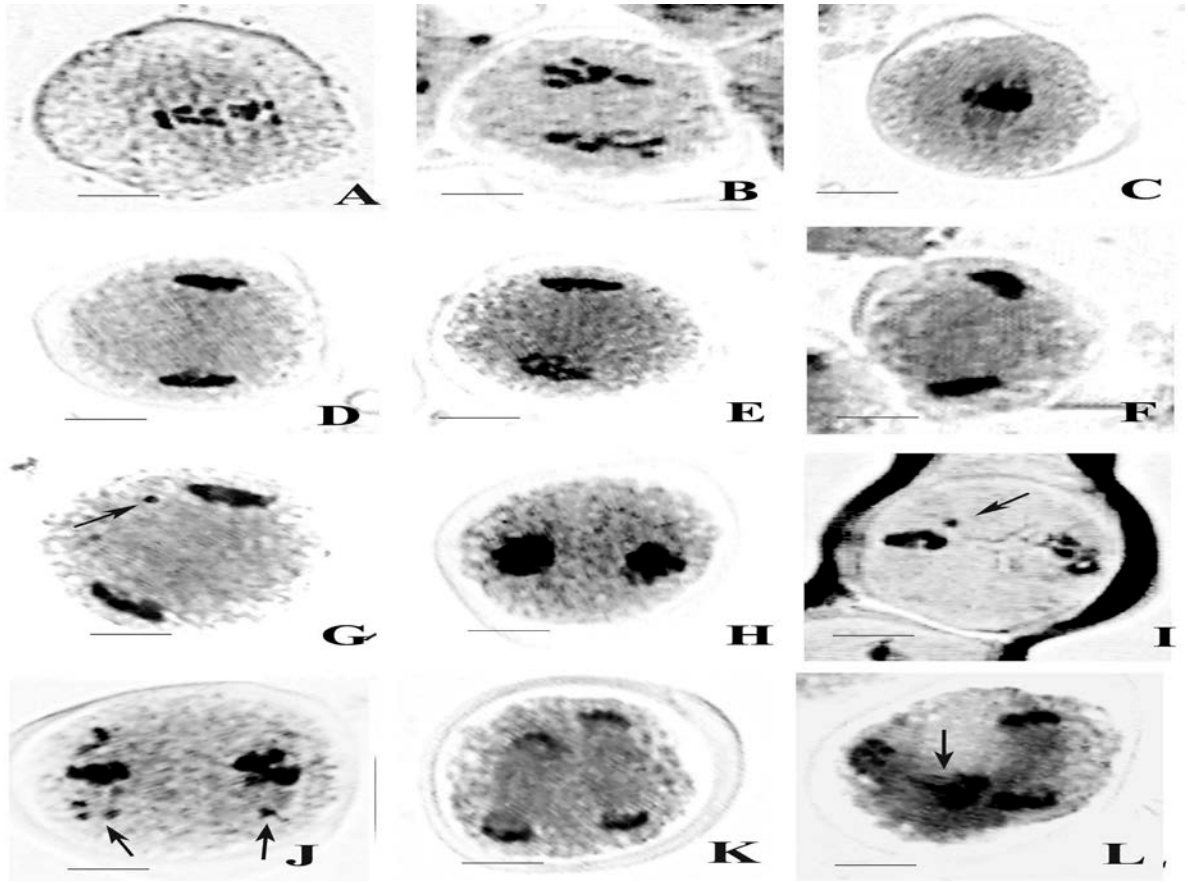

Figure 1. A. Normal metaphase (n=10); **B**. normal anaphase (10:10); **C-** stickiness at metaphase-I; **D-** stickiness at anaphase-I; **E**. stickiness at only one pole of anaphase-I; **F**. intense unoriented sticky mass of chromosomes observed at both pole of anaphase-I; **G**. unoriented sticky mass of chromosomes with laggard; **H**. clumping at metaphase-II; **I**. genome elimination with sticky metaphase-II; **J.** multi precocious condition at metaphase-II; **K.** stickiness at anaphase-II; **L.** unoriented sticky anaphase-II with incomplete bridge.(40×, scale bar=4.2µm).

Several agents have been reported to cause chromosome stickiness, including physical mutagens (Rao and Rao, 1977; Al Achkar *et al.*, 1989), temperature (Eriksson, 1968), and chemicals (Kumar and Singh, 2002; Srivastava and Kapoor, 2008; Kumar *et al.*, 2010). Proper interpretation for the occurrence of stickiness is still yet to be known, however, according to Pagliarini (2000), it may be of either hereditary, caused by mutation in the structural genes coding for them or induced by the direct action of mutagens. Recent reports suggest that chromosome stickiness may be under genetic control, or rather, it 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) resulting into meiotic mutants. Present study also contradict the finding of Kumar and Singh (2002), who noticed the detrimental impact of stickiness on meiotic course and pollen fertility provoked by another chemical mutagen EMS.

During morphological studies, positive impact of some specific doses of chemical mutagens has been verified by assaying some phenotypic traits. Result shows the considerable decrease in plant height in treated sets over control that is in fact desirable as it prevents crop from lodging damage. Since due to lodging, falling of the crops occurs at the time of harvesting, which results in the significant reduction in the yield due to stem breakage at the ground level (Islam and Evans, 1994).

In most of the cases, relationship of different quantitative traits is not positive therefore, difficult to manipulate through mutation breeding yet a trait, number of seeds per siliqua is inherited monogenically and therefore easy to manipulate (Sinhamahapatra *et al.*, 2010). However, in present case this particular trait has been efficiently manipulated by mutation breeding experiment along with some other important parameters as presented in Table 1.

Conclusively, on the basis of our observations, it is worth suggesting that the azide has induced some sticky mutant that had definitely provoked some advantageous variations at gene level which affected the yield and yield attributed traits through mutagenesis. Thus, these meiotic mutants induced via sodium azide, signifying a complementary and regulatory effect of stickiness during microsporogenesis of *Brassica campestris* L. beyond the some instances of impaired male meiosis.

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