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Cytology of induced morphological mutants in *Vigna mungo* (L.) Hepper

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Abstract

Seeds of one blackgram variety were subjected to mutagenic treatments with ethyl methane sulphonate and sodium azide, which induced three morphological mutants in the M_2 generation: *dwarf*, *bushy* and *narrow-leaf*. Meiotic analysis revealed the presence of chromosomal abnormalities, such as univalents and stickiness at metaphase-I, and bridges and laggards at anaphase-I. Cytomixis was noticed at telophase-II. The induction of meiotic aberrations was higher in ethyl methane sulphonate than sodium azide treatments. A reduction in pollen fertility was also noticed. These mutants may be used as valuable breeding stocks for blackgram breeding.

Keywords: urdbean, chemical mutagens

Introduction

Vigna mungo (L.) Hepper (Fabaceae), commonly known as urdbean or blackgram, is an important pulse crop of India. Its beans are regarded as a major source of protein and minerals. Broading the genetic base to augment and recreate wide genetic variability for crop improvement can be achieved through induced mutagenesis. Induced mutants in plants are often associated with cytological abnormalities. Although meiotic mutants have been extensively reported (Perison *et al*. 1996, Pagliarini *et al*. 2000, Kumar & Gupta 2007) in higher plants, there are few studies dealing with the effects of chemical mutagens on the meiotic behaviour of *Vigna mungo*. This paper reports the cytological behaviour of morphological mutants of blackgram induced by ethyl methane sulphonate and sodium azide.

Materials & Methods

A field experiment was conducted during kharif (summer) seasons of 2006 and 2007 at the Agricultural Farm, Aligarh Muslim University, Aligarh. Seeds of urdbean (*Vigna mungo* (L.) Hepper) var Pant U-30, presoaked in distilled water for 9 hours were treated with chemical mutagens: 0.3% and 0.4% ethyl methane sulphonate (EMS) and 0.02% sodium azide (SA) for 6 hours. Mutagenic solutions were prepared in phosphate buffer pH 7 and pH 3 for EMS and SA respectively. Seed soaked in distilled water were used as controls. Seeds harvested from individual M_1 plants were sown as M_2 families in three replicates in the field. Three morphological mutants were isolated from the M_2 generation: bushy (0.3% EMS), dwarf (0.4% EMS) and narrow leaves (0.02% SA) . The mean and standard error (SE) were estimated for plant height, nodes/plant, pods/plant, seeds/pod and weight of 100 seeds. Significant differences were identified using the Least Significant Difference estimated from the error mean square and tabulated 't' values at the 1% level of significance.

For meiotic studies, young unopened flower buds of the mutants were fixed in freshly prepared Carnoy's fixative (absolute alcohol, chloroform and glacial acetic acid in 6:3:1 ratio) for 24 hours, washed thoroughly and stored in 70% alcohol. Squashes were made in 1% acetocarmine.

Results

Meiosis was found to be normal in control (untreated) plants, which formed 11 bivalents at metaphase I and normal separation (11:11) in the anaphase I cells. Three types of

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morphological mutants were isolated in the M_2 generation (Table 1, Figs 1a-d). These mutants bred true in the M_3 generation.

Morphological parameters	Control (untreated) mean ± SE	Mutants mean ± SE			CD (p=0.01)
		Dwarf	Bushy	Narrow leaf	
Plant height (cm)	26.64 ± 0.06	9.6 ± 0.1	21.1 ± 0.1	20.6 ± 0.1	3.27
No. nodes/plant	8.0 ± 0.6	10.0 ± 0.9	7.0 ± 0.6	8.0 ± 0.4	1.10
No. of pods/plant	31.0 ± 0.6	18.0 ± 0.8	24.0 ± 0.6	22.0 ± 0.9	2.90
No. of seeds/pod	5.0 ± 0.6	4.0 ± 0.3	4.0 ± 0.6	3.0 ± 0.2	0.65
100-seed weight	3.7 ± 0.02	3.03 ± 0.02	3.18 ± 0.01	2.85 ± 0.06	0.20

Table 1: Morphological parameters of the M_2 mutants.

The *Dwarf* mutant was isolated at the higher concentration (0.4%) of EMS, with short internodes, reducing the height to 9.6 cm as compared to 26.6 cm in the control. The yield and yield-related characters were markedly reduced. The *bushy* mutant was isolated from the 0.3% EMS treatment, and had reduced plant height, but the number of branches increased, manifesting a bushy appearance. The number of pods and seeds per pod were reduced relative to control plants, and flowering and maturity were delayed. The *narrow-leaf* mutant was isolated from the 0.02% SA treatment; it had a stout stem and narrow leaves which were smaller and deep green. Its pods were smaller, with small-sized seeds; pollen fertility was similar to that of control.

Figure 1a: Control plant

Figure 1b: Dwarf mutant

Figure 1c: Bushy mutant

Figure 1d: Narrow leaves mutant

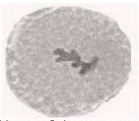

Figure 2a: Stickiness of chromosomes at MetaphaseI

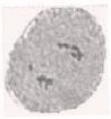

Figure 2b: Lagging chromosome at Anaphase I

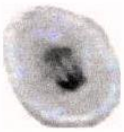

Figure 2c: Bridge formation at Anaphase I

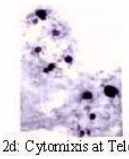

Figure 2d: Cytomixis at Telophase II

Besides the presence of univalents at metaphase I, the *bushy* and *dwarf* mutants showed other abnormalities, such as stickiness, bridge formation and laggards (Table 2, Figs 2a-d). Due to chromosome abnormalities, the process of gamete formation must be disturbed. This was evident from the reduction in pollen fertility of the bushy (70%) and dwarf (48.3%) mutants relative to the control (97%). The *narrow-leaf* mutant showed a low level of pollen mother cells with cytomixis at telophase II in the 0.02% SA treatment. This mutant did not show any other chromosomal aberrations.

Treatment/ Mutant	Total no. of M_2 plants	No. of mutant plants	Frequency (%)	Pollen Mother Cells: Total number observed	Total number abnormal	Metaphase I (%): Univalents	Metaphase I (%): Stickiness	Anaphase I (%): Bridges	Anaphase I (%): Laggards	telo-phase I (%): Cytomixis	Total Abnormal (%)	% pollen fertility
Control	896	0	0	248	0	0	0	0	0	0	0	97
0.3% EMS Bushy	750	3	0.4	238	9	0.8 (2)	--	1.3 (3)	1.7 (4)	0	3.8	70
0.4% EMS Dwarf	872	5	0.6	249	14	2.8 (7)	0.8 (2)	0	2.0 (5)	0	5.6	48
0.02% SA Narrow leaf	832	9	1.1	225	3	0	0	0	0	1.3 (3)	1.3	96

Table 2: Meiotic abnormalities induced by chemical mutagens in various morphological mutants.

Discussion

The present study proved fruitful in inducing a number of morphological mutants isolated on screening the M_2 generation, including changes in growth habit (bushy), plant height (dwarf) and leaf morphology (narrow leaves). The isolated mutants exhibited negative selection value; these might be useful to plant breeders, as a source of many beneficial genes in hybridization programmes.

The induced mutants were often associated with cytological abnormalities. In higher plants, meiotic mutants have been extensively reported (Chaudhary *et al.* 1992; Peirson *et al.* 1996). In the present study, the percentage of meiotic abnormalities was found to be maximum in the dwarf mutant, followed by bushy and narrow-leaf mutants.

During desynapsis, the chromosomes pair normally at zygotene, but they fall a part at diplotene due to failure of chiasma formation as due to the relaxation of pairing at the points of chiasma. At metaphase-I, all such chromosomes appear as univalents scattered around the cell space. This desynaptic behaviour of chromosomes was found from the reduced frequency of chiasma and subsequent univalent formation in the bushy and dwarf mutants isolated from the EMS-treated population, more frequent in the dwarf than the bushy mutants. The prevalence of univalents at metaphase-I gives an indication of pairing disturbances, but normal pairing ensures bivalent formation at early prophase-I.

It is possible that some defects in post-pairing events might be responsible for desynapsis. Processes other than synapsis that mediate exchange have been ascribed for the original desynaptic mutant (Baker *et al*. 1976). Prakken (1943) used the number of univalents at metaphase I as a measure of the degree of desynapsis, and accordingly categorized desynapsis into weak, medium and strong. The meiotic behaviour of desynaptic mutants of *Vigna mungo* described here can be grouped under the weak category, because a high frequency of bivalents per cell was found in the mutants in comparison to the univalents.

The bushy and dwarf mutants with desynaptic behaviour of chromosomes showed reduced seed yield and high pollen sterility. Desynapsis has been found to reduce pollen fertility, affecting seed yield (Consolaro *et al*. 1996, Khazanderi & Jones 1997). As more and more abnormalities accumulate, the process of gamete formation is affected leading to non-viable gametes that considerably reduce plant fertility (Kumar & Gupta 2007). Chromosome

abnormalities have been reported to decrease pollen fertility in pearl millet (Rao *et al*. 1990), canola (Souza & Pagliarini 1996) and black cumin (Kumar & Gupta, 2007). Gottschalk & Kaul (1980) reviewed desynapsis in a number of plant species, and showed that the phenomenon is usually not associated with morphological change, but here the EMS-treated plants showed bushy and dwarf phenotypes together with desynaptic behaviour of chromosomes.

Chromosome stickiness may be caused by genetic or environmental factors (Aclkar *et al.* 1989, Caetano-Pereira *et al*. 1995). Gaulden (1987) postulated that stickiness may result from defective functioning of one or two types of specific non-histone proteins involved in chromosome organization neccessary for chromatid separation and segregation. The altered functioning of these proteins leading to stickiness is caused by mutation in structural genes coding for them (hereditary stickiness) or by the action of mutagens on the proteins (induced stickiness).

The mutant 'bushy' and 'dwarf' also showed bridges and laggards at anaphase-I. A possible explanation for the formation of bridges could be either that the broken ends containing the centromere from two different chromosomes unite, forming a dicentric chromosome, or the chromosomes are often unable to separate completely at anaphase due to stickiness, and hence form an anaphase bridge. Bridges at anaphase-I have been reported in irradiated material by several workers in various crops (Kumar & Singh 2003, Kumar & Gupta 2007). The laggards observed in the dwarf mutant might be due to delayed terminalization, stickiness of chromosomal ends or because of failure of chromosome movement (Jayabalan & Rao 1987, Soheir *et al*. 1989).

Cytomixis, the meiotic abnormality observed in the narrow-leaf mutant, was first described by Gates (1908), who observed delicate threads of cytoplasm connecting adjacent pollen mother cells in *Oenothera rubrinervis.* The transmigration of chromatin material with cytomictic connections might have resulted in an altered number of chromosomes. In a few pollen mother cells of the narrow-leaf mutant, the deviation of chromosome numbers from normal was attributed to cytomixis.

According to Heslop-Harrison (1966), the role of cytoplasmic channels is related to the transport of nutrients between microsporocytes. Caetano-Pereira & Pagliarini (1997) proposed that various factors such as the influence of genes, pathological conditions, herbicides and temperature are responsible for cytomixis. Cytomixis may have serious genetic consequences by causing deviations in chromosome number, and may represent an additional mechanism for the origin of aneuploidy and polyploidy (Sarvella 1958).

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الملخص العربي

دراسات خلوية على الطفرات المورفولوجية المستحثة فى نبات *Vigno mungo*

سونو جوال و ساميللاه خان

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تم تعريض مجموعة من بذور نبات *Vigna mungo* لمعالجات تسبب طفرات من سلفونات الميثان الإيثيلي وأزيد الصوديوم والتي تسببت في حدوث ثلاث طفرات للجيل الثاني وهى: تقزم النبات - التفاف النبات - وضيق الأوراق بالنبات. كشفت دراسة تحليل الانقسام الميوزي عن وجود تشوهات صبغية مثل ظهور الكروموسومات الأحادية والتصاق بعض الكروموسومات خلال الطور الاستوائي الأول من الانقسام ، كما ظهر انحراف وتباطؤ في الحركة في الطور الانفصالي الأول. وفي الطور النهائي الثاني لوحظ بعض الاختلاط الخلوى. وكان للمعالجة بسلفونات الميثان الإيثيلي الأثر الأكبر في تشوه الانقسام الميوزي عن أزيد الصوديوم .كما لوحظ انخفاض في معدلات خصوبة حبوب اللقاح .ويمكن استخدام تلك الطفرات كمخزون تكاثرى قيم لزراعة نبات *Vigna mungo* .