Turkish Journal of Biology

Volume 33 | Number 2 Article 5

1-1-2009

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TÜBİTAK

Turk J Biol
33 (2009) 123-128
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doi:10.3906/biy-0807-29

Induced Karyomorphological Variations in Three Phenodeviants of *Capsicum annuum* L.

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Received: 22.07.2008

Abstract: EMS was used as a mutagen in *Capsicum annuum* L. to isolate karyomorphological mutants. Seeds of *Capsicum annuum* L. var Azad were first pre-soaked in distilled water and then treated with 0.5% solution of EMS for 3 durations, i.e. 3, 5, and 7 h, and genetic segregation was closely observed. Many chromosomal anomalies like stickiness, bridges, and multivalent, secondary associations, laggards, and precocious movement were observed after all 3 durations of treatment. These anomalies showed a dose-dependent increase in frequency. The morphological parameters showed a decreasing trend along with the increasing dose. However, with the 7-h dose 3 morphologically distinct plants were isolated, which varied not only from other sib plants but also from control plants. Their cytological analysis revealed a predominance of bridges and increased frequency of bridges in all 3 plants. Therefore, it can be presumed that these 2 factors together may be responsible for creating deviations in these 3 plants.

Key Words: EMS, 7 h, bridge, chiasma frequency, chromosomal anomalies

Capsicum annuum L. Bitkisinin üç Farklı Fenotipinde İndüklenmiş Karyomorfolojik Varyasyonlar

Özet: EMS *Capsicum annuum* L.'de bir mutajen olarak karyomorfolojik mutantları izole etmek için kullanılmıştır. *Capsicum annuum* L. var Azad'ın tohumları ilk olarak distile suda ıslatıldı ve sonra 3, 5 ve 7 saat sure ile % 0,5 EMS çözeltisi ile muamele edilmiştir ve genetik ayrım yakından gözlemlenmiştir. Yapışık, köprü ve multivalen, ikincil bağlantılı, geri kalmış, erken gelişmiş hareketli gibi birçok kromozomal anomali üç muamelede de gözlenmiştir. Bu anomaliler doza bağımlı olarak sıklıkta artış göstermiştir. Morfolojik parametreler doz artışı ile azalan bir eğilim göstermiştir. Fakat 7 saatlik dozda morfolojik olarak 3 farklı bitki sadece diğer yakın bitkilerden değil aynı zamanda kontrol bitkilerinden de çeşitlilik gösteren bitki izole edilmiştir. Bunların sitolojik analizleri üç bitkinin hepsinde kromozomlar arası köprülerin baskınlığını ve artmış sıklıkta köprüler olduğunu göstermiştir. Bu yüzden bu iki faktörün birarada bu üç bitkinin farklılık göstermesinden sorumlu olduğu düşünülebilir.

Anahtar Sözcükler: EMS, 7 saat, köprü, kiazma sıklığı, kromozomal anomaliler

Introduction

Capsicum annuum L. is an important spice-yielding plant. Its fruits are used to stimulate gastric activities and increase blood circulation. It is also a stimulant and carminative used for neuralgia and rheumatism.

Despite the fact that many chemicals with mutagenic properties have been discovered, relatively few of them nowadays are applied frequently for practical plant breeding purposes. It has been mentioned for many years that by far the most important mutagens belonged to the group of alkylating agents with ethylmethane sulphonate (1,2) and nitroso compounds (3,4). Studies suggest that EMS is an effective mutagen and can be used to induce genetic variability in a number of crop plants (5,6). Therefore, the present study aimed to document and report some valuable information on the cytological behaviour of *C. annuum* and isolation of mutants induced by EMS.

Materials and Methods

The seeds of *Capsicum annum* L. var Azad, obtained from Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, were treated with 0.5% EMS for 3 different durations: 3, 5, and 7 h. The seeds were washed thoroughly in running tap water and excess moisture was blotted off. These seeds were then sown in 3 replicates under natural conditions to raise the M1 generation. The controls were maintained separately.

For meiotic analysis, young floral buds were fixed in 3:1 solution of absolute alcohol and glacial acetic acid and later preserved in 70% alcohol. Slides were prepared via the standard acetocarmine squash technique using 2% acetocarmine. Approximately 750-850 PMCs were analysed in control and treated sets at different stages of meiosis (Table 1).

Observations

During the cytomorphological analysis of the treated sets, a number of significant variations were observed. There was a dose-dependent decrease in the different morphological parameters like plant height, number of nodes, leaf area, and 100-seed weight, and an increase in the days to maturity as compared to the control (Table 2). The cytology of the control plants was found to be normal, with diakinesis (Figure 1A), normal metaphase (Figure 1B), and normal anaphase (Figure 1C). The cytological abnormalities in the treated sets were found to increase along with the increasing doses (Table 1). Different abnormalities observed in the treated sets were multivalent formation (Figure 1D), bridges (Figure 1E), laggards (Figure 1F), stickiness (Figure 1G, H), precocious movement (Figure 1I), secondary association, disorientation, and disturbed polarity. In the 7-h treated line of 0.5% EMS, 3 plants out of the 30 randomly selected plants displayed changed morphology. These morphologically distinct plants (plants 1, 2, and 3) were isolated as variants and were found to be more vigorous than control and sib plants of the treated line. Moreover, these plants were characterised by increases in plant height, number of nodes, 100-seed weight, and leaf area, and a decrease in the days to maturity (Table 3).

The cytological analysis of these 3 variants was performed in order to determine whether these phenotypic variations are a result of chromosomal structural changes or point mutations. Cytological analysis of these plants revealed an overwhelming predominance of variety of bridges (Table 4). The frequency of bridges was found to be 52.96% in plant 1, 55.28% in plant 2, and 56.72% in plant 3. At anaphase I various types of bridges like single bridge (Figure 1E), double bridge, multiple bridge, sticky bridge (Figure 1G), and bridge with laggard were found in all 3 plants. At anaphase II also, various types of bridges like lateral bridge (Figure 1J), double bridge (Figure 1K), diagonal bridge (Figure 1L), multiple bridge, sticky bridge, and bridge with laggard were found. Besides these, other chromosomal abnormalities like multivalent formation, secondary association, stickiness, laggards, and precocious movement were also obtained in some of the pollen mother cells (PMCs) of these 3 plants.

It was also worth noting that chiasma frequency increased in these 3 variants of 7-h dose line as compared to the control (Table 5). In the control plants the chiasma frequency was 17.13 ± 0.16. However, in the 3 phenodeviants the chiasma frequency varied from 20.50 ± 0.30 to 22.53 ± 0.56. Pollen fertility seemed to be moderately affected, being 88.42%, 82.19%, and 78.63% in plants 1, 2, and 3, respectively, as compared to control sets, where it was 97.60% (Table 3).

Discussion

The frequency and spectrum of aberrations observed during the present investigation clearly indicated that

Table 1. Comparative cytological abnormalities in the 3 treatment durations of 0.5% EMS.

Treatment duration	T.Ab (%)	Total PMCs observed	No. of abnormal PMCs	Metaphasic abnormalities					Anaphasic abnormalities			
				St	Pr	Mul	Sec	Un	St	Lg	Br	Dis
Control	-	848	-	-	-	-	-	-	-	-	-	-
3 h	3.39	738	25	0.68	0.42	0.32	0.22	0.16	0.79	0.17	0.27	0.36
5 h	10.52	756	80	1.47	0.98	0.83	0.54	0.35	1.62	0.56	0.73	0.90
7 h	19.34	782	151	2.54	1.86	1.34	0.92	0.54	3.12	0.98	1.19	1.72

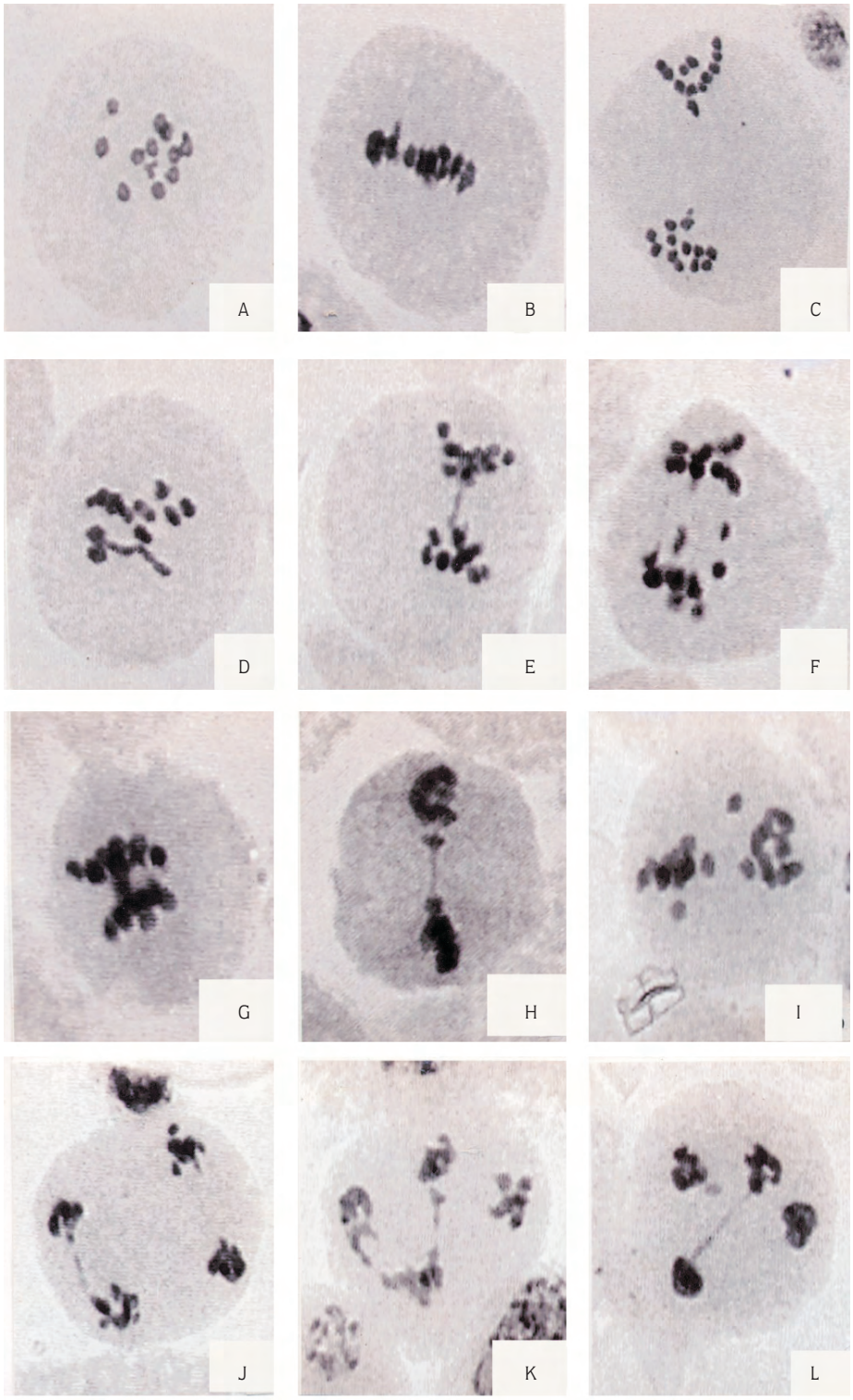

Figure 1. Different stages of meiosis in the pollen mother cells of the control and treated plants.
A) Normal diakinesis (12 bivalents), B) Normal metaphase (12 bivalents), C) Normal anaphase (12:12 segregation), D) Multivalent formation, E) Single bridge at anaphase I, F) Laggards at anaphase I, G) Sticky bridge at anaphase I, H) Sticky anaphase I with bridge, I) Precocious movement at metaphase II, J) Lateral bridge at anaphase II, K) Double bridge at anaphase II, L) Diagonal bridge at anaphase II.

Table 2. Comparative growth and yield parameters in the 3 treatment durations of 0.5% EMS.

Morphological parameters	Control	3 h	5 h	7 h
Plant height (cm)	58.46 ± 1.01	52.23 ± 1.12	47.1 ± 0.87	41.5 ± 0.53
No. of nodes	13 ± 0.57	9 ± 0.59	8 ± 0.68	5 ± 0.86
Leaf area	37.37 ± 0.716	33.10 ± 1.49	28.37 ± 1.02	22.3 ± 1.07
100-seed weight (g)	0.465 ± 0.01	0.416 ± 0.01	0.367 ± 0.012	0.350 ± 0.01
Days to maturity	136 ± 1.15	144.67 ± 1.45	152 ± 1.15	161.67 ± 1.45

Table 3. Comparative growth and yield parameters in the 3 morphologically distinct plants after a 7-h dose of 0.5% EMS.

Morphological Parameters	Control (Mean ± S.E.)	Plant 1 (Mean ± S.E.)	Plant 2 (Mean ± S.E.)	Plant 3 (Mean ± S.E.)
Plant height (cm)	58.46 ± 1.01	68.6	70.2	74.9
No. of nodes	13 ± 0.57	13	15	16
Leaf Area	37.37 ± 0.716	43.24 ± 0.631	53.04 ± 0730	56.71 ± 0.824
100-seed weight (g)	0.465 ± 0.01	0.490	0.525	0.550
Vigorousness	Low	Optimum	Maximum	Maximum
Days to maturity	136 ± 1.15	132	128	125

Table 4. Bridge diversity in the 3 plants isolated after a 7-h dose of 0.5% EMS.

Plant Type	% of PMCs with bridge	Bridge diversity at Anaphase I					Bridge diversity at Anaphase II					
		Single bridge	Double bridge	Multiple bridge	Sticky bridge	Bridge with laggard	Lateral bridge	Diagonal bridge	Double bridge	Multiple bridge	Sticky bridge	Bridge with laggard
Plant 1	52.96	6.84	-	5.14	8.20	4.62	7.56	9.24	-	5.92	5.44	-
Plant 2	55.28	10.37	5.31	4.76	6.67	-	10.53	7.20	2.84	-	4.31	3.29
Plant 3	56.72	8.45	3.17	5.67	6.82	-	9.15	8.19	5.89	4.12	5.26	-

Table 5. Changes in chiasma frequency observed in the 3 variants obtained after 7-h duration of 0.5% EMS.

Plant type	Ring bivalent % (Mean ± S.E)	Rod bivalent % (Mean ± S.E)	Univalents % (Mean ± S.E)	Mean chiasmata per cell (Mean ± S.E)	Pollen fertility (%)
Control	0.03 ± 0.01	11.98 ± 0.30	0.30 ± 0.01	17.13 ± 0.16	97.6
Plant 1	0.17 ± 0.05	11.80 ± 0.15	-	20.50 ± 0.30	88.42
Plant 2	0.21 ± 0.06	11.77 ± 0.10	-	20.51 ± 0.38	82.19
Plant 3	0.37 ± 0.08	11.60 ± 0.09	-	22.53 ± 0.56	78.63

EMS is a very potent mutagen for *C. annuum* (7,8). The results also showed co-linearity between the duration of treatment and percentage of chromosomal anomalies.

The mutagen induced translocations and possibly inversions might be involved in the formation of multivalents. Ignacimuthu and Babu (9), and Stebbins (10) reported secondary associations to be a result of modified chromosome arrangements due to duplication, interchanges, or stickiness. Gaulden (11) attributes chemically induced stickiness to direct action of mutagen on the histone proteins, leading to improper folding of DNA. Precocious movement of chromosomes seems to be a manifestation of improper spindle functioning. The presence of single and multiple bridges may be due to the occurrence of dicentric chromosomes formed as a result of breakage fusion bridge cycles (12-14). The thick sticky

bridges may be due to the stickiness of chromosomes. This stickiness interfered in the normal arrangement of chromosomes at metaphase and further led to their inability to separate, thus leading to sticky bridges. When the spindle fibres pulled the chromosomes towards the poles these bridges were broken into fragments, which either moved towards the poles or formed laggards and micronuclei (15). In the present case EMS seems to be very effective for the induction of bridges. Perhaps the target proteins in this case are those responsible for chiasma terminalisation during diakinesis at meiosis I. Their defective functioning, which may be due to the gene mutation or direct action of the mutagen on the proteins, caused a disturbance during chromosome separation, thus resulting in bridges.

Mutagenic agents are known to bring about changes in chiasma frequency. Rees (16) showed that crossing over and chiasma formation are under genetic control. Bennett and Rees (17) in rye and Umarao (18) in *Delphinium* observed an increase in chiasma frequency after mutagenic treatments. The increase in the chiasma frequency observed in the case of 3 variants may be attributed to the nature and potency of mutagens and also to the underlying factors such as complex structural change or changes in the nature of genes responsible for chiasma formation. According to Stern and Hotta (19), the DNA and proteins have an important role in the crossing over. The effect may be through certain proteins, which have a specific role in chiasma formation. A change in the structure of these proteins might have resulted in the increase in chiasma frequency per cell.

Efficient mutagenesis is defined as the production of desirable changes free from usually associated undesirable changes such as chromosomal aberrations, sterility, and lethality (20). Ehrenberg (21) and Kawai (22) stated that the highest mutation rates also induced a high degree of sterility, lethality, and other undesirable effects. In the present case, the 3 plants isolated at the 7-h dose of 0.5% EMS exhibited an increase in various agronomically and economically important morphological characters like plant height, number of nodes, leaf area, and 100-seed weight. However, the pollen fertility was moderately affected, confirming negligible loss in the seed set.

During the cytological analysis of the 3 phenodeviants isolated with the 7-h dose of 0.5% EMS, predominance of bridges and increased chiasma frequency per cell were recorded. The bridges might have altered the genetic architecture of the 3 plants by interchromosomal mutations or by adding or deleting a segment to the chromosomes, thus giving rise to aneuploidy. This may provide a mechanical basis for creating variations. The increase in chiasma frequency per cell might have resulted in new combinations of genes. Thus it can be presumed that these 2 factors together might have resulted in a variation in the phenotypic traits of the 3 plants. Hence, the 7-h dose of 0.5% EMS can be beneficially utilised to maximise the induction of mutant lines that would be beneficial for crop improvement programmes of *Capsicum annuum* L.

Acknowledgements

The authors are grateful to CSA, Kanpur, for providing the seeds. One of the authors (P.G.) is grateful to UGC for the financial assistance in the form of JRF. Thanks are also due to members of the plant genetics laboratory for their help and support.

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