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Gamma Rays and Ethyl Methane sulphonate Induced Cytotoxicity in Brinjal

(*Solanum melongena* L.)

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Abstract

The better understanding of selecting an effective and efficient mutagen may provide better chances to induce high frequency of desirable mutations in any crop breeding programme. This study was conducted to determine the proper dose/concentrations of gamma irradiation and ethyl methane sulphonate (EMS) for the mutation breeding program of eggplant (*Solanum melongena* L.). To serve the purpose, seeds of brinjal (Var. Pooja selection) were exposed to different dose/conc. of gamma rays (10, 15, 20, 25 and 30kR) and EMS (0.1, 0.2, 0.3 and 0.4%) to induce mutation. The chromosome of a treated and control plants under meiotic stages were observed. The common chromosomal aberrations are univalents, multivalent, stickiness, precocious separation, stray bivalent, unoriented metaphase, laggards and micronuclei etc. As increase in the concentration/dose, the frequency of cells showing chromosomal aberrations shows a linear increase up to a certain level. Compared to EMS, gamma rays produced the highest chromosomal aberrations.

Introduction

Vegetable play a pivotal role in our diet as they are the main source of some important supplements, especially vitamins and minerals. Number of *Solanum* plants are used as vegetables and from these, eggplant (*Solanum melongena* L.) is one of the most common and popular vegetable grown throughout India. Based on production statistics, eggplant is the third most important crop in family Solanaceae, after potato and tomato. Mutation breeding is relatively a quicker method for crop improvement of crops. Many physical and chemical mutagens have been used for induction of useful mutants in a number of crops. Mutation breeding has been extensively followed in other vegetable crops like tomato, chilies etc., (Manuel Gonzalez *et al*., 2002) but only very few studies are reported in brinjal.

Genetic variability for economic traits is the pre-requisite for any successful breeding programme as the degree of response to selection depends on the quantum of variability. In any crop, yield being a complex character influenced by many of its contributing characters controlled by polygenes and the environmental factors. So, an understanding of genetics of yield and its component traits, association between each component trait and yield is necessary for planning effective selection procedure in developing high yielding genotypes. However, the inheritance of quantitative traits is often influenced by variation in other characters which may be due to pleiotropy or genetic linkage. Hence, knowledge of association between yield and its attributes obtained through estimation of genotypic and phenotypic correlation helps in determining the extent of improvement that could be brought about in the characters and also in selecting suitable genotypes.

Mutation breeding is recognized as one of the driving force of evolution. It's relatively quicker method for improvement of various crop species. It is an important tool to create variability for quantitatively inherited traits in different plants and is considered as an alternative method to increase genetic variability in plant breeding. Mutation breeding often used to correct defects in cultivar which has a set of good agronomic characteristics.

Result

Meiotic abnormalities: Meiosis in the control (diploid) was regular and without any irregularities with 12 bivalents (2n=24) observed both at diakinesis and metaphase-I. The data on chromosomal behaviour in control and treated populations is presented in Table 1. Cytological analysis with respect to their mieotic behavior is considered to be one of the most dependable indices to estimate the potency of mutagen. A dose dependent increase in meiotic irregularities was observed with all the mutagenic treatments. Various types of meiotic

abnormalities were scored at stages from metaphase-I/II to telophase-I/II. Unpaired chromosomes were frequent at diplotene. Varying number of univalents was noticed in the treatments but their frequency was higher in EMS (1.92%) as compared to radiation (1.80%) (Table1, Fig E). The frequency of multivalents at metaphase-I (Fig C) was highest at 30kR (5.04%) followed by EMS (4.32%) at 0.4% respectively. Stickiness was a pronounced effect at metaphase-I in gamma rays and in ethyl methane sulphonate (EMS) (Fig. G). Stickiness ranged from 1.42 to 4.20% in 10kR to 30kR and 1.85 to 2.40% in EMS at 0.1% to 0.4% respectively. Precocious segregation ranged from 0.47 to 3.78% in 10kR to 30kR and 1.39 to 4.80% in EMS at 0.1 to 0.4% respectively. The frequency of PMC's showing stray bivalent at metaphase-I (Table 1, Fig B) ranged from 1.90% to 3.36% in case of gamma rays and from 2.31% to 3.84% in case of EMS treatments. The frequency of unoriented at metaphase-I (Table 1, Fig E) ranged from 0.47 to 2.52% in case of gamma rays and from 3.21 to 2.56% in case of EMS treatments. Laggards ranged from 0.95 to 1.26% in 10kR to 30kR and 1.39 to 2.40% in EMS at 0.1 to 0.4% respectively. The frequency of micro-nuclei at metaphase-I (Table 1, Fig C) ranged from 0.91 to 2.94% in case of gamma rays and from 2.32 to 2.88% in case of EMS treatments.

Discussion

In the present investigation similar types of meiotic abnormalities were found in all mutagenic treatments but the frequency of abnormalities was different in different treatments, indicating the different mutagenic potentials of mutagens against *Capsicum annuum* L. Bivalents were found clumped in single or different groups at metaphase I due to stickiness. Jayabalan and Rao (1987) suggested that stickiness of chromosomes might be due to disturbances in cytochemically balanced reactions in the nucleic acids. However, it seems most probable that some kinds of gene mutations lead to incorrect coding of some nonhistone proteins involved in chromosome organization and leads to chromosomes clumping (Gulfishan et al., 2010). It may also be possible that the mutagen itself reacts with the histone proteins and brings about a change in the surface property of chromosomes due to an improper folding of DNA, thereby causing them to clump or stick together (Gaulden, 1987). The occurrence of univalents and multivalents at metaphase-I has been reported in various plants like barley (Kumar and Singh, 2003) and broad beans (Bhat et al., 2005).

Mutagen induced structural changes in chromosomes might be responsible for the failure of pairing among homologous chromosomes and hence the occurrence of univalents. The mutagen induced translocations and possibly inversion might be involved in the formation of multivalents. Precocious chromosome migration to the poles may have resulted from spindle dysfunction or precocious chiasma terminalization at diakinesis or metaphase-I (Kumar and Rai, 2007). Stray bivalents at metaphase-I seem to be caused by spindle dysfunction and clumping of chromosomes (Bhat et al., 2007). The presence of laggards may be attributed to the inability of multivalents to separate properly (Ganai et al., 2005). Laggards may be explained on the basis of abnormal spindle formation and chromosomal breakage. According to Tarar and Dnyansagar (1980), unsynchronized bivalents or laggards might be due to the discrepancies in spindle formation. Laggard at anaphase can be attributed to the delayed terminalization or perhaps to stickiness of chromosomal ends (Minija et al., 1999). Unequal separation of chromosomes at anaphase I/II, observed in the present study, may be due to the occurrence of multivalents and failure of chromosomes to segregate equally. Micronuclei as observed in the present study at telophase I/II may be due to the association of fragments and lagging chromosomes which failed to reach the poles and got included in the daughter nuclei (Kumar and Dubey, 1980).

Cytomixis generally refers to the migration of chromatin from one cell to the other through cytoplasmic connections. The transmigration of chromatin material with cytomictic connections might have resulted in altered numbers of chromosomes. Variation in chromosome number in few pollen mother cells may be due to cytomixis, which is considered as a source of production of aneuploid and polyploid gametes (Koul, 1990; Yeng et al., 1993).

The cytogenetic abnormalities have been regarded as one dependable parameter for estimating the mutagenic potential of a mutagen and it can be judged by the percentage of abnormalities it induces. The genetic changes brought about by the mutagens provide good scope for further improvement of this crop. The present study, thus, envisages the use of gamma rays as compared to EMS, in various concentrations to induce mutations and the positive mutation in economic characters may be selected and tested for the improvement of *Solanum melongena* L. var. Pooja selection.

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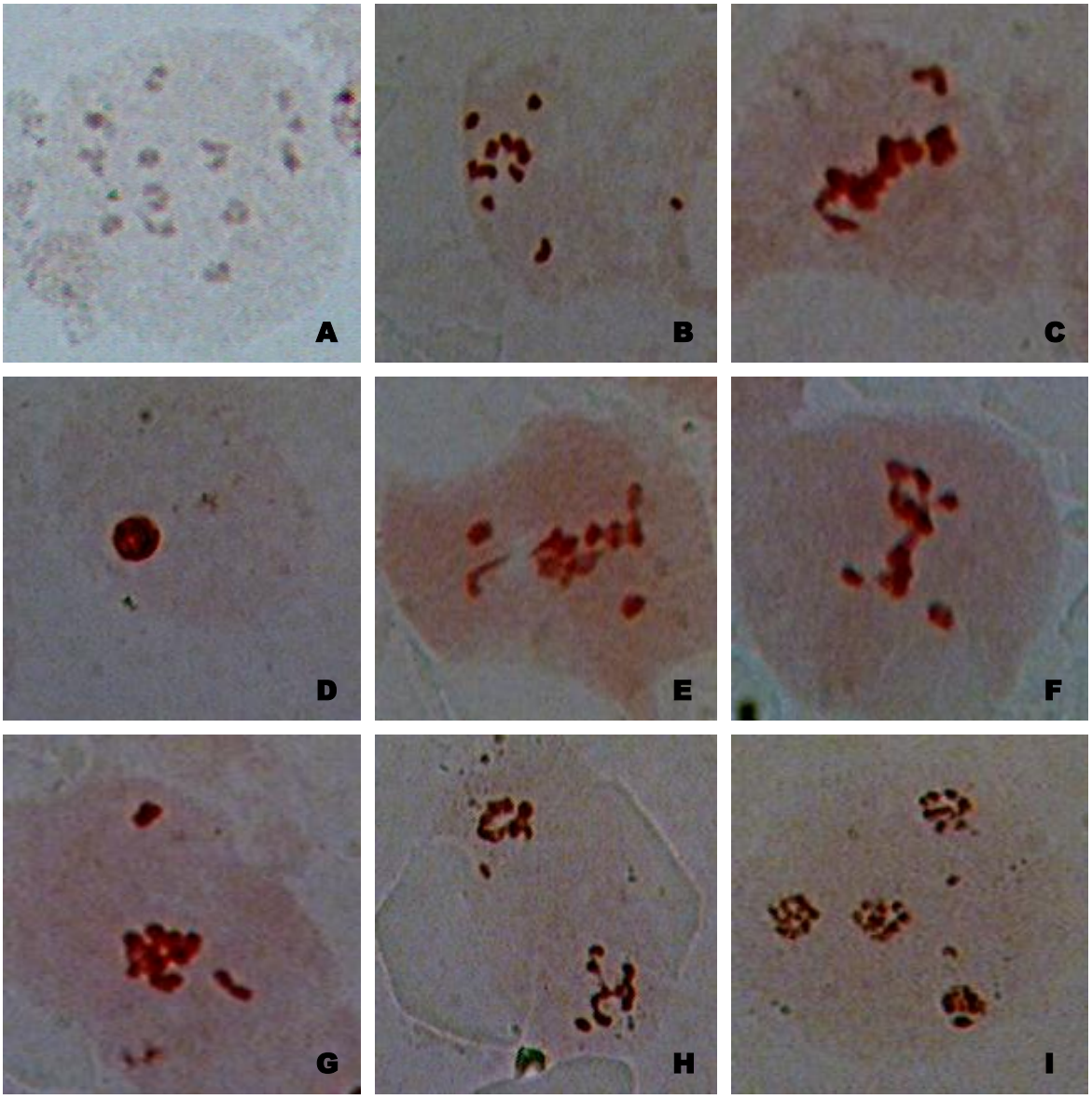

Figs: A Diakinesis (2n=24) B PMCs showing univalents C PMCs showing multivalents D Stickiness E Precocious separation F Stray bivalent G Unoriented metaphase H Laggard at anaphase-I I Micronuclei at telophase-II.

Table 1 Abnormalities (%) at different stages of meiosis induced by Gamma rays and EMS in Brinjal (*Solanum melongena* L.)

Mutagen	Dose/Concentrations	Total PMCs observed	Metaphase-I/II						Anaphase-I/II/Telophase-I/II		Total aberrations (%)
			Uni	Mult	Stic	Pre	Str	Uno	Lag	Micro	
	Control	225	-	-	-	-	-	-	-	-	-
	10kR	210	0.95	2.38	1.42	0.47	1.90	0.47	0.95	-	11.54
Gamma rays	15kR	219	1.36	3.19	1.82	2.28	-	0.91	1.82	0.91	12.29
	20kR	214	1.40	1.86	2.80	2.33	3.27	1.40	0.93	1.86	15.85
	25kR	221	1.80	3.61	4.07	2.71	3.16	2.26	1.80	1.35	20.76
	30kR	238	0.84	5.04	4.20	3.78	3.36	2.52	1.26	2.94	23.94
	0.1%	216	0.47	2.78	1.85	1.39	2.31	-	1.39	2.32	12.51
EMS	0.2%	218	0.92	-	1.38	3.66	2.30	3.21	1.84	2.76	16.07
	0.3%	196	1.53	1.03	2.05	4.08	3.07	2.56	2.56	3.58	20.46
	0.4%	208	1.92	4.32	2.40	4.80	3.84	-	2.40	2.88	22.56

Abb. Uni- Univalents, Mult- Multivalents, Stick- Stickiness, Pre- Precocious segregation, Str- Stray bivalent, Uno- Unoriented metaphase, Lag- Laggard and Micro- Micronuclei