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Induced Mutagenesis in Urdbean (*Vigna mungo* L. Hepper): A Review

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Abstract: Breeding programmes to improve nutritious pulse crop have not been commensurate with its role in the Indian diet. Induced mutations have played a great role in increasing world food security, since new food crop varieties embedded with various induced mutations have contributed to the significant increase of crop production. Though, the crop improvement work on urdbean (*Vigna mungo* L. Hepper) has been in progress for several years at various state Agriculture universities and ICAR institutes in India, the programmes lacked systematic and coordinated efforts. It was only after 1967 that the crop along with other pulses started receiving thrust under the All India Coordinated Programme on Improvement of Pulses (AICPIP). Plant breeding requires genetic variation of useful traits for crop improvement. Mutagenic agents, such as radiations and certain chemicals, can be used to induced mutations and generate variation from which desired mutants may be selected.

Key words: Mutagenesis, biological damage, cytological aberrations, chlorophyll mutations

INTRODUCTION

Mutation induction offers the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evaluation. Treatment with mutagens alters genes or breaks chromosomes. Gene mutations occur naturally as errors in DNA replication. Most of these errors are repaired, but some may pass to the next cell division to become established in the plant offspring as spontaneous mutations. Gene mutations without phenotypic expressions are usually not recognized. Consequently, genetic variation appears rather limited and breeders have to resort to mutation induction. Artificial induction of mutation by ionizing radiations and chemical agents with mutagenic properties date back to the beginning of the 20th century. Muller (1927) on artificial transmutation of gene hoped that practical breeders need no longer lie on the mercy of the existing limited genetic variability. He found that X-rays considerably enhance mutation rate in *Drosophila*. Success with X-rays was achieved by Stadler (1928) in barley. Indications about the possibility of induction of mutations by the use of chemical mutagens started appearing within a decade after discovery of the phenomenon. The Plant Breeding and Genetic Section of joint FAO/IAEA Division helps plant breeders to develop improved cultivars through the use of induced mutations. The study has not been restricted to any plant species and grain legumes have always had a prominent place (Micke and Swiecicki, 1988).

The word pulse is derived from the Latin *puls*, *pultis*, a thick soup. It is the broad term used to describe the dried, edible seeds of legumes. Pulses also known as grain legumes are important source of vegetable protein for many developing countries but their production has gone down in favour of more profitable crops.

They are also a rich source of energy, minerals and certain vitamins of B-complex group. Further, the amino acid composition of pulse protein is such that a mixed diet of cereal and pulse has superior biological value than either of the component alone. Consequently, pulses help in checking the malnutrition among the children of our country. Besides their nutritional value, pulse crops are endowed with unique property of maintaining and restoring soil fertility through biological nitrogen fixation from the atmosphere as well as of conserving and improving physical properties of soil by virtue of their deep and well spread root system. Genetic improvement of grain legumes is urgently needed. The aim of breeding must be the increase of production of pulses through genetic manipulation and reduction of crop losses.

Although, India has the distinction of being the world's single largest producer of pulses, the difference in production and population ratio is significant. The increase in population has pushed up demand of pulses while the fall in availability has pushed up their prices. Although, a large area of approximately 20-22 million hectares is under different pulse crops, their production is more or less stagnant for the last four decades, which is ranged between 11-13 million tonnes (Ali and Kumar,

Table 1: State wise production of pulses

States	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09
Andhra Pradesh	1138	1062	1239	1019	1376	1347	1697	1390
Bihar	547	561	563	467	447	438	497	378
Chhattisgarh	448	366	581	368	453	494	537	492
Gujarat	380	327	622	479	547	593	743	648
Karnataka	752	694	569	792	964	893	1265	945
Madhya Pradesh	3225	2376	3488	3429	3233	3203	2454	2948
Maharashtra	1881	2058	1960	1664	2005	2304	3024	1710
Rajasthan	1426	485	2278	1337	898	1481	1409	1512

Source: Indiatat

Table 2: Area, production and yield of pulses (India)

Periods	Area (million ha)	Production (million tonnes)	Yield (kg ha ⁻¹)
1999-00	21.12	13.42	635
2000-01	20.35	11.08	544
2001-02	22.01	13.37	607
2002-03	20.50	11.13	543
2003-04	23.46	14.91	635
2004-05	22.76	13.13	577
2005-06	22.39	13.39	598
2006-07	23.19	14.20	612
2007-08	23.86	15.10	638
2008-09	24.54	14.66	597

Source: Agriculture ministry of India, NB Research

2006). This fall in availability of pulses is attributed to many factors; pulses are mostly grown under rain fed conditions where drought is a common feature. Other factors include their low harvest index, prolonged vegetative growth, low yield and their susceptibility to diseases. State wise production and trends in area, production and yield of pulses in India are given in Table 1 and 2.

Until 1970, the varieties were developed by selection among and within landraces. Some of the varieties have made long lasting contributions to urdbean production. T-9, a collection from Bareilly (U.P.) in early fifties, has been used extensively in breeding programme. In combination with LM 151, LU 220 and L 64, it has led to the development of varieties PS 1, UG 218 and KM 2. UPU 1, a selection from T-9, in combination with UPU 2 has led to the development varieties Pant U-19 and Pant U-30. Sincere efforts have gradually been made for hybridization followed by selection with emphasis on desired recombinants. Some improved varieties of urdbean and the varieties developed through induced mutations of urdbean are given in Table 3 and 4.

Blackgram or Urdbean (*Vigna mungo* (L.) Hepper) is a highly self pollinated crop with cleistogamy up to 42% (Puneglov, 1968) Urdbean is grown all over the South East Asia. In India it is mostly grown as a kharif crop. The crop prefers water retentive stiff or heavy soil and does well on both black cotton soils and brown alluviums with pH ranging from 4.7 to 7.5. The average yield of urdbean is very low in comparison to major grain legumes like chickpea and pigeonpea. Trends in area, production and yield of urdbean in India are given in Table 5.

Black gram is a rich protein food. It contains about 26% protein, which is almost three times that of cereals. Black gram supplies a major share of protein requirement of vegetarian population of the country. It is consumed in the form of split pulse as well as whole pulse, which is an essential supplement of cereal based diet. The combination of dal-chawal (pulse-rice) or dal-roti (pulse-wheat bread) is an important ingredient in the average Indian diet. The biological value improves greatly, when wheat or rice is combined with black gram because of the complementary relationship of the essential amino acids such as arginine, leucine, lysine, isoleucine, valine and phenylalanine etc. In addition, being an important source of human food and animal feed, it also plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen. Being a drought resistant crop, it is suitable for dry land farming and predominantly used as an intercrop with other crops.

Due to the lack of sufficient natural variability for yield and its components in urdbean, conventional methods have a limited scope. Gustafsson (1947) advocated that mutation approach was superior to other methods of crop improvement. Mutations provide an opportunity to create hitherto unknown alleles, so that the plant breeder does not remain handicapped due to limited allelic variation at one or more gene loci of interest. Fried (1969) concluded that for increasing food production in the world, induced mutagenesis is important in creating variability in the breeding population to improve yield, earliness and disease resistance. Reconstruction of plant types to improve the productivity in urdbean (Shaikh *et al.*, 1982) through induced mutations stimulates to speculate further and exploit this methodology fully. Thus, mutation breeding technique may have a greater role in crops like pulses where a large part of natural variability has been eliminated in the process of adaptation to the environmental stress. In recent years, a lot of work has been undertaken on induced mutagenesis through physical and chemical mutagens. It has been clearly shown in a number of plant species that the effect induced varies with the varying mutagens and with variation in mutagen dose. Thus, selecting a mutagen and its optimum dose for a genotype in any plant species is an important step in mutation breeding programme.

Table 3: Some improved varieties of urdbean

Variety	Year of release/ identification	Area of adoption zone/state	Ave. yield (Q ha ⁻¹)	Days to maturity	Remarks
Teja (LBG-20)	1991	Andhra Pradesh	14.0	70-75	Tol. To YMV
Vamban-1	1991	Tamilnadu	8.0	65-70	
ADT-4	1991	Tamilnadu	8-9	65-70	Tol. To YMV
ADT-5	1991	Tamilnadu	8.0	65-70	Tol. To YMV
Basant Bahar (PDU-1)	1991	All India except South and Hil Zone	12-13	70-80	Spring, tolerant to YMV
Prabha (LBG 402)	1991	Karnataka andhra Pradesh, Orissa, Tamilnadu	10.0	78	Rabi
Narendra Urd-1 (NDU-88-1)	1993	Uttar Pradesh.	10.0	70-80	Resistant to YMV, black bold seeded
LBG-611	1995	Andhra Pradesh.	14.0	85-90	Resistant to wilt
WBU-108	1996	Punjab, West UP, Rajasthan, Karnataka, Andhra Pradesh, Tamilnadu)	12	83	Resistant to YMV, Kharif
Muabs-338	1996	Punjab	9.0	85-90	Resistant to YMV
Mash-414	1996	Punjab	10	72	Tolerant to root rot
Birsa Urd-1	1996	Bihar	15.0	81	Resistant to YMV
Melghat (AKU-4)	1996	Maharashtra	10.0	93	Tolerant to stress, for Rabi season
KBG-512	1997	Tamilnadu	7-8	70-75	Resistant to Stemfly, pods hairy
Vamban-2	1997	Tamilnadu	8.0	70	Resistant to YMV and drought
KU-301	1998	Tamilnadu, Orissda, A.P. and Karnataka	12	70	Res. To YMV, Rabi Season
Azad Urd-1	1999	UP, Bihar, WB.	10.0	80	Spring
WBG-26	1999	Karnataka, Andhra Pradesh, Orissa, Tamilnadu	10	70	Res. to PM
Barkha (RBU-38)	1999	MP, Maharashtra and Central part of Rajasthan	12.0	75	Bold seeded, Res. to Cercospora leaf spot
Vamban-3	2000				
IPU-94-1 (Uttra)	2000	Punjab, Haryana, Delhi, West UP, North Rajasthan, Gujarat, UP, Bihar, W.B.	11-12	85	Resistant to YMV, Kharif season
KU-300	2001	Punjab, Haryana, Delhi, West UP and North Rajasthan)	11-12	70	Resistant to YMV, spring season
APK-1	2001				
LAM-623	2001				
LAM-685	2001				
KU-91 (Azad Urd-2)	2001				
Sumanjana	2002	Kerala	9-10		
NDU 99-2	2003	NHZ	9.5	85	Res. to YMV, Kharif season
KU 96-3	2003	CZ (MP, Maharashtra and Gujarat)	8.0	73	Res. to YMV, Kharif season

Table 4: Urdbean varieties developed through induced mutations

Variety	Year of release	Country	Mutagenic treatment and parent
Co 4	1978	India	0.02% EMS, Co-1
TAU 1	1985	India	Γ -rays, T-9 × dark green leaf mutant U-196 of variety No. 55.
Manikya	1988	India	40 kR γ - rays, T-9
TAU 4	1992	India	Γ -rays, UM-201 (large seed mutant of variety No.55) × T-9.
TAU 2	1993	India	Γ - rays, T-9 × dark green leaf mutant UM-196 of mutant # 55
BINA MASH1	1994	Bangladesh	600 Gy γ -rays, BINA Acc. No. B-10
TU 94-2	1999	India	Γ -rays, TPU-3 × TAU-5 (early mutant of EC168200).
DU -1	2007	India	200 Gy Γ -rays, TAU 1 (No. 169) × TAU1

Source: The Joint FAO/IAEA Mutant Varieties and Genetic Stocks Database

Table 5: Area, production and yield of urdbean in India

Years	Area (m ha)	Production (m tonnes)	Yield (kg ha ⁻¹)
2000-2001	3.01	1.30	431
2001-2002	3.30	1.50	454
2002-2003	3.55	1.47	415
2003-2004	3.42	1.47	430
2004-2005	3.17	1.33	419
2005-2006	2.97	1.25	463
2006-2007	3.10	1.44	526
2007-2008	3.24	1.46	440

Source: Indian Institute of Pulses Research, Kanpur (UP)

Dose effect / LD-50: The dose required for high mutation efficiency of a physical or chemical mutagen depends on properties of mutagenic agents and of biological system in question. In general, the dose effect of physical and chemical mutagenic treatment comprises several parameters, of which the most important are dose rate,

concentration, duration of treatments, temperature and pH during treatments. In chickpea (Singh, 1988a) reported LD-50 value for gamma rays at 460 Gy (var. G 130) and 483 Gy (var. H208) and for EMS at 0.25% (var. G130) and 0.2% (var. H208). In both the varieties 0.4% EMS treatment was most lethal. Kharakwal (1981a) reported higher lethality in 0.2% EMS in comparison to 400 and 500 Gy gamma rays. Higher LD-50 values for gamma rays in chickpea in comparison to others pulse crops such as 300 Gy in blackgram (Khan, 1988), 200 Gy in lentil (Singh, 1983) and 100 Gy in pea (Singh, 1988b) indicate its greater resistance to the mutagen. Further, differences have been observed for LD-50 values in different chickpea varieties, which are attributed to their differential radiosensitivity. A decline in the survival of a mutated population has been associated with the increase in the dose of mutagen

(Farooq and Nizam, 1979; Singh, 1988b), which has resulted from cytogenic damage and/or physiological disturbances as also reported earlier by Sato and Gaul (1967).

A dose related reduction in seed germination and pollen fertility by both gamma rays and EMS have shown by various workers (Nerker, 1970; Rao and Laxmi, 1980; Khanna and Maherchandani, 1981; Gautam *et al.*, 1992; Wani, 2007). Dose linked effectiveness of EMS and gamma rays were noted in chickpea in terms of germination, reduction in pollen fertility, chlorophyll mutations and seedling height (Kalia *et al.*, 1981; Khanna, 1991; Gumber *et al.*, 1965; Parveen, 2006). Similar effects were also reported in peas (Salim *et al.*, 1974), pearl millet (Singh *et al.*, 1978), *Vigna radiata* (Singh and Chaturvedi, 1980; Khan and Wani, 2004), *Lens culinaris* (Sharma and Sharma, 1981b; Khan, 2002; Wani, 2003), *Arachis hypogea* (Venkatachalam and Jayabalan, 1995) and *Nigella sativa* (Mitra and Bhowmik, 1999).

Mutagenic sensitivity: Same mutagen dose can cause different degrees of effect in different species. Varied mutagenic sensitivity in different genotypes was first reported by Gregory (1955) in groundnut and by Lamprechet (1956) in peas. Similar varietal differences were recorded in production of viable and chlorophyll mutations in *Nigella sativa* (Mitra and Bhowmik, 1999) and in *Vigna mungo* (Rehman, 2000) following gamma rays and EMS treatments. Sharma and Sharma (1981a) observed differential mutagenic response of gamma rays and NMU in microsperma and macrosperma lentils. They observed better viability of chlorophyll mutations like xantha and chlorina in the microsperma than in the macrosperma varieties.

Venkatachalam and Jayabalan (1995) while using EMS, SA and gamma rays found distinct differences in groundnut (*Arachis hypogea*). Distinct varietal differences to SA in *Vigna radiata* was observed by Khan *et al.* (2004). Geeta and Vaidyanthan (1997) observed different phenotypic response of two soyabean cultivars to ethidium bromide and gamma rays. Differences to radiosensitivity were also reported by Khan (1999) in blackgram and Nerker (1976) in *Lathyrus sayivus*. Akbar *et al.* (1976) concluded that differences in radiosensitivity may be due to differences in their recovery process including enzyme activity. In chickpea, Kharakwal (1998) and Parveen (2006) reported that varieties of desi type were more resistant towards mutagenic treatments than kabuli type.

Mutagenic response to cytological aberrations has been reported by many workers (Rao and Laxmi, 1980; Suganthi and Reddy, 1992; Rehman, 2000). Mitra and Bhowmik (1996) observed no varietal differences with regard to mitotic index as well as to meiotic abnormalities

in *Nigella sativa*. Both cultivars of *Nigella sativa* were found equally radiosensitive. Ahmad (1978) and Ahmad and Godward (1981) reported radiosensitivity in nine cultivars of chickpea. Out of these nine, two cultivars CSIMF and F10 were identified as the most radioresistant and radiosensitive, respectively. Kharakwal (1998) reported mutagenic sensitivity in four varieties of chickpea on the basis of total germination rate, seedling damage, pollen sterility and plant survival. The varieties with large assortment of recessive alleles governing traits(s) show greater sensitivity and frequency of M_2 mutants than the varieties having more dominant alleles governing a trait (Gelin *et al.*, 1958; Blixt, 1970). A few members of alkane sulphonate series have been found to be exceptionally mutagenic in a variety of organisms. Freese (1963) and Heslot (1977) gave a detailed account of chemical mutagens like ethylmethane sulphonate (EMS) and diethyl sulphate (DES). The mutagenic action of EMS was studied earlier in *Drosophila* (Fahmy and Fahmy, 1957), bacteriophage (Loveless, 1959), barley and wheat (Gustafsson, 1960; Ehrenberg, 1960; Swaminathan *et al.*, 1962). Gaul (1964) in barley observed that EMS was capable of producing more number of various morphological mutants as compared to gamma rays. At molecular level, EMS is known to react preferentially with guanine and cytosine (Freese, 1963).

Lal *et al.* (2009) studied in mutagenic sensitivity in early generation in black gram on the effect of gamma rays and Sodium azide and their different combination in M_1 generation and observed that an increase in azide concentrations resulted in decrease in M_1 germination. The plant survival was also affected with different doses of gamma rays and SA and was decreased with increasing in doses. The combination treatments of gamma rays and sodium azide had more depressive effect on seedling growth.

Biological damage: The effect of physical and chemical mutagens and their combination treatments to demonstrate different biological parameters such as germination, survival, injury and sterility (Chaturvedi and Singh, 1981; Vandana and Dubey, 1988; Khan, 1990; Khan *et al.*, 1994; Vanniarajan *et al.*, 1993, 1994; Sharma *et al.*, 1995; Khan *et al.*, 1999; Sareen and Kaul, 1999; Verma *et al.*, 1999; Mitra and Bhowmik, 1999; Khan and Wani, 2005). Reduction in seedling height following treatments with gamma rays and EMS was observed in barley (Sharma, 1970). Gupta and Yashvir (1975) reported a radioprotective effect of EMS in *Abelmoschus esculantum*. The combined treatments of gamma rays and EMS showed higher germination percentage than in corresponding EMS treatments. Chaudhary (1983) reported a symmetric reduction in germination in different varieties of wheat with higher doses of gamma rays.

Parveen (2006) reported the effect of seed treatment with different concentration of EMS on germination and growth of seedlings in chickpea. There was a proportionate decrease in germination percentage with the increasing concentrations of EMS.

The effect of gamma rays, EMS and their combination on M_1 parameters in barley was studied by Khalatkar and Bhatia (1975). They observed that the seedling injury, chromosomal aberrations, pollen and seed sterility were less in combined treatments than in separate treatments. Gamma rays were reported to inhibit the uptake of EMS due to the generalized action of radiation on metabolic processes in the cells. Singh and Chaturvedi (1980) reported mutagen induced damage such as plant injury and lethality in M_1 generation arising due to physiological, chromosomal and factor mutations. Khan and Siddiqui (1987) studied the effect of Methyl Methane Sulphonate (MMS) on pollen fertility in the var. T-9 of urdbean. A direct relationship of pollen and ovule sterility with higher doses of gamma rays and EMS doses in *Vigna mungo* was reported by Gautam *et al.* (1992). Increase in pollen sterility and decrease in germination with increasing doses of gamma rays in *Capsicum annum* was reported by Rao and Laxmi (1980).

The mutation rate of NMU was found to be 1.5- 2.0 times higher than gamma rays on plant survival and sterility (Sharma and Sharma, 1981a) in microsperma and macrosperma lentils. Rapoport (1966) has called the super mutagens in view of their higher mutagenic effect. Mutagenic efficiency based on injury and lethality was found higher in combined treatments of gamma rays and NMU than their respective individual treatments (Dixit and Dubey, 1986). Combined treatments also showed greater reduction in seedling survival than the individual treatments. Bhatnagar (1984) reported the adverse effect of combined treatments on germination and survival of plants in chickpea. The pollen sterility increased in combined treatments indicating the additive or synergistic effect. Reduction in seed germination with the increase in dose of gamma rays in chickpea was reported by Khanna and Maherchandani (1981) and Khanna (1991). The EMS treatment was found to cause higher sterility than gamma rays in chickpea (Kharakwal, 1981b).

Cytological aberrations: Auerbach and Robson (1942) presented first elaborate report that mustard gas could induce mutations as well as chromosomal aberrations in *Drosophila*. Urethane was reported to produce chromosomal breaks in *Oenothera* by Oehlker (1946). Formalin was also reported to have mutagenic effect when fed to *Drosophila* (Rapoport, 1946). Sodium azide was found to be a very effective mutagen under certain treatment conditions (Kleinhofs *et al.*, 1974), it made

possible to obtain high mutation frequency, mostly gene mutations, with negligible frequency of chromosomal aberrations.

Gamma rays, MH and gamma rays+MH treatments show disturbed mitotic behaviour which was noticed by Grover and Tejpal (1982) in *Vigna radiata*. The sticky chromosomes, fragments and ring chromosomes at metaphase and the laggards and bridges at anaphase were noticed by these workers. The chromosomal aberrations were found to be significantly co-related with dose. The combined treatment enhanced chromosomal aberrations. Similarly, the meiotic process was also affected. The quadrivalents presumably due to translocations, were occasionally encountered on metaphase-I. Irregular disjunction of chromosome at anaphase-I, accompanied by laggards was also observed. A comparative study on the induction of chromosomal aberrations in the two varieties of mungbean by gamma rays, MNNG, EMS and HA (Grover and Virk, 1986). All the chemical mutagens and gamma rays induced chromosomal aberrations. The maximum frequency was noticed with gamma rays followed by MNNG, EMS and HA. G-65 variety was found to be more sensitive with treatment of EMS and HA. The quadrivalents, trivalents and univalents were encountered at metaphase-I in pollen mother cells. Irregular distribution of chromosomes at anaphase-I accompanied by laggards and chromatin bridges were observed. Mitotic abnormalities like misorientation at metaphase, bridges at anaphase, fragmentation and multinucleolate condition were also observed by Shah *et al.* (1992) in gamma rays treated *Vigna mungo*. Vandana and Dubey (1996) reported the meiotic anomalies induced by EMS and DES in *Vicia faba*. These anomalies were found to increase with the increase in the concentrations of mutagens applied. Overall frequency of meiotic anomalies induced by various concentrations of DES was higher than those of EMS. However, EMS treatments induced higher proportion of anomalies in pairing whereas DES induced higher proportion of anomalies during anaphasic disjunction.

A relative account of cytological and developmental effects of gamma rays, EMS and MMS on meiotic features and pollen fertility in *Vicia faba* L. was provided by Bhat *et al.* (2005). The various kinds of chromosomal abnormalities and reduction in pollen fertility were found to be dose dependent. The induction of meiotic abnormalities was observed to be higher under MMS treatments, followed by gamma rays and EMS, suggesting that MMS could be more effective in inducing chromosomal abnormalities followed by gamma rays and EMS. Precocious migration of univalent to the poles is a very common abnormality among plants (Pagliarini, 1990; Pagliarini and Pereira, 1992; Defani-Scoarize *et al.*, 1995a, b; Consolaro *et al.*, 1996), the other segregational

abnormality (non-oriented bivalents) is rare, but is known to occur in *Chlorophytum comosum* (Pagliarini *et al.*, 1993). The behavior of these and of the laggard chromosomes is characteristic in that they generally lead to micronucleus formation (Koduru and Rao, 1981). The occurrence of univalents, ring and rod bivalents due to the mutagenic treatments was previously reported by Mansour (1994), Bione *et al.* (2002) and Vinita *et al.* (2004). Khan and Tyagi (2009a) reported bridges and laggards on soybean when treated with EMS and gamma rays and their combination. They also reported that laggards were absent in EMS treatment in var. Pusa-16 of soybean.

In maize, sticky chromosomes were first reported by Beadle (1932) and are seen as intense chromatin clustering in the pachytene stage. The phenotypic manifestation of stickiness may vary from mild, when only a few chromosomes of the genome are involved, to intense, with the formation of pycnotic nuclei that may involve the entire genome, culminating in chromatin degeneration. Chromosome stickiness may be caused by genetic or environmental factors. Genetically controlled stickiness has been described in other cultivated plants such as maize (Beadle, 1932; Golubovskaya, 1989; Caetano-Pereira *et al.*, 1995), pearl millet (Rao *et al.*, 1990) and wheat (Zanella *et al.*, 1991). Several agents have been reported to cause chromosome stickiness, including X-rays (Steffensen, 1956), gamma rays (Rao and Rao, 1977; Al-Achkar *et al.*, 1989), temperature (Eriksson, 1968), herbicides (Badr and Ibrahim, 1987) and some chemicals present in soil (Levan, 1945; Steffensen, 1955; Caetano-Pereira *et al.*, 1995). However, the primary cause and biochemical basis of chromosome stickiness are still unknown. Gaulden (1987) postulated that sticky chromosomes may result from the defective functioning of one or two types of specific non-histone proteins involved in chromosome organization, which are needed for chromatid separation and segregation. The altered functioning of these proteins leading to stickiness is caused by mutations in the structural genes coding for them (hereditary stickiness) or by the action of mutagens on the proteins (induced stickiness).

In angiosperms, cytoplasmic connection is a phenomenon widely described by Heslop-Harrison (1966), Risueno *et al.* (1969) and Whelan (1974). The first description was made by Gates (1908), who observed delicate threads of cytoplasm connecting adjacent pollen mother cells in *Oenothera*. Gates (1911) subsequently suggested that these connections must form an important avenue of exchange between PMCs and described the transfer of nuclear material through them from one meiocyte to another, calling the process cytomixis. According to Heslop-Harrison (1966) and Risueno *et al.* (1969), the role of cytoplasmic channels is related to the

transport of nutrients between meiocytes. Investigations in angiosperms have provided evidence that massive protoplasmic connections are formed among microsporocytes. Although, cytoplasmic connections are very common in angiosperms, the movement of nuclear material through them is rare. In general, cytomixis has been detected at a higher frequency in genetically imbalanced species such as hybrids, as well as in apomitic, haploid and polyploid species (Yen *et al.*, 1993). Among the factors proposed to cause cytomixis are the influence of genes, fixation effects, pathological conditions, herbicides and temperature (Caetano-Pereira and Pagliarini, 1997). Cytomixis may have serious genetic consequences by causing deviation in chromosome number and may represent an additional mechanism for the origin of aneuploidy and polyploidy (Sarvella, 1958). In various crops, the abnormal spindles have been reported (Harlan and De-Wet, 1975; Veilleux, 1985). The spindle apparatus is normally bipolar and acts as a single unit, playing a crucial role in the alignment of metaphase chromosomes and their pole ward movement during anaphase. Distortion in meiotic spindles may be responsible for unreduced gamete formation. The formation of unreduced gametes has been investigated in studies of evolution (Harlan and De-Wet, 1975) and in breeding programmes (Veilleux, 1985). It was reported that meiotic abnormalities cause male sterility (Goyal and Khan, 2009). Chromatin bridges and micronuclei were described for the first time in interspecific hybrids of *Glycine max* × *Glycine soja* by Ahmad *et al.* (1977), who found that the extent of abnormalities was influenced by environmental conditions. The same abnormalities were reported by Ahmad *et al.* (1984), who concluded that chromosome behaviour and fertility depends on the percentage of the hybrids and on environmental temperature. Their results, obtained in greenhouse and controlled environmental studies, suggest that genotype, temperature and genotype×temperature interaction influence chromosome behaviour and fertility.

Studies on different plant species have shown that the decline in seed production is correlated with meiotic irregularities (La Fleur and Jalal, 1972; Dewald and Jalal, 1974; Moraes-Fernandes, 1982; Smith and Murphy, 1986; Pagliarini and Pereira, 1992; Pagliarini *et al.*, 1993; Consolaro *et al.*, 1996; Khazanehdari and Jones, 1997). In most of the mungbean varieties, pollen fertility showed a close relationship with meiotic abnormalities (Khan, 1990). The least mutation frequency at higher doses may be attributed to chromosomal aberration or saturation in the mutational events which may result in the elimination of mutant cells during growth (Blixt and Gottschalk, 1975).

Chlorophyll mutations: Chlorophyll mutations are used to evaluate the genetic effects of various mutagens.

Several chlorophyll mutants like chlorina, viriscense, viridis, flavo-viridis, albo-viridis, chlorina-terminalis, chlorina-viriscens, albo-viriscens, chlorotica, albina and xantha were observed following treatments with physical or chemical mutagens or their combinations (Goswami, 1980; Kundu and Singh, 1982; Rao *et al.*, 1975; Vandana, 1991; Singh *et al.*, 1999; Thakare, 1988; Arulbalachandran and Mullainathan, 2009; Khan and Tyagi, 2009b). Vanniarajan *et al.* (1996) treated the two varieties of blackgram with gamma rays and EMS to study the frequency of chlorophyll mutations. Gamma rays were more efficient than EMS in inducing chlorophyll mutations in both the varieties.

Khan and Tyagi (2010) reported four types of chlorophyll mutants viz., albina, xantha, chlorina and viridis in gamma rays and gamma rays+EMS treated population of soybean. Gamma rays were found to be more effective to induce chlorophyll mutations.

Xantha and chlorine types of chlorophyll mutants in soybean were earlier reported by Geeta and Vaidyanathan (2000). Since chlorophyll mutations are easily detectable as they have been extensively used to find out sensitivity of crop plants to mutagens. Hemavathy and Ravindran (2005) reported that the occurrence of albina in the urdbean was less than the other type, when treated with different doses of gamma rays. Maximum frequency of chlorina and xantha was recorded at higher doses of gamma rays.

Mutagenic effectiveness and efficiency: A number of chemical mutagens have been found to be equally and even many times more effective and efficient mutagens (Basu *et al.*, 2008). Thilagavathi and Mullainathan (2009) reported that EMS was more effective and efficient for viable mutants than gamma rays in blackgram. Studies on effectiveness, i.e., the number of mutations produced per unit dose and efficiency, i.e., the ratio of specific desirable mutagenic change to undesired effects like plant damage, sterility or lethality, of the physical and chemical mutagens were carried out in various crops by several workers (Khan and Hashim, 1979; Badami and Bhalla, 1992; Khan *et al.*, 1998a; Mehraj-ud-din *et al.*, 1999; Khan, 1999; Koli and Ramakrishna, 2002). Grover and Virk (1984) found that MNNG, EMS and HA are more effective mutagens than gamma rays whereas gamma rays are more efficient than chemical mutagens in mungbean.

Deepalakshmi and Kumar (2003) studied on the efficiency and effectiveness of physical and chemical mutagens in urdbean and reported that gamma rays were found to be more effective than EMS in producing chlorophyll and viable mutants on M₁ plants and M₂ seedlings bases as well as efficient on lethality and sterility bases. Khan *et al.* (2005) found the order of mutagenic effectiveness in chickpea as HZ>SA>EMS.

They took three criteria (Mf/S) and meiotic abnormalities (Mf/Me) for estimation of mutagenic efficiency. The order of efficiency with regard to Mf/I and Mf/S was: HZ>EMS>SA and EMS>HZ>SA, respectively, while with regard to chromosomal aberrations, the order of efficiency in var. Avrodhi was: EMS>SA>HZ and it was: HZ>EMS>SA in var. BG-256.

Singh (2007) reported mutagenic effectiveness and efficiency of gamma rays and ethylmethane sulphonate in mungbean and found that treatments of the mutagens suggesting the direct relationship with the dose dependent increase.

Dhanavel *et al.* (2008) reported that the effectiveness decreased with increase in concentration of EMS, DES and SA in cowpea. It is obvious that the higher efficiency at lower and intermediate doses of mutagens may be due to the facts that the biological damage (lethality and sterility) increased with the dose at a rate greater than the frequency of mutations (Konzak *et al.*, 1965). Similar findings have also been reported by Dixit and Dubey (1986). Goyal *et al.* (2009) studied a comparison of mutagen effectiveness and efficiency of EMS, SA and gamma rays in mungbean and reported that all the three mutagens were found to be more effective at lower concentrations. The decline in the mutagenic effectiveness recorded at higher dose shows that the increase in mutation rate was not proportional to the increase in the doses of various mutagens. Similar results were obtained by Parveen (2006) in chickpea.

Mutation for quantitative traits: Lower doses of gamma rays and EMS were ineffective for creation of desired variability for yield and yield components in lentil (Singh *et al.*, 2006a). Effect of gamma rays and ethyl methane sulphonate on quantitative and qualitative traits in sunflower has been reported by Selvaraj and Jaykumar (2004). The micro mutations increase variability in yield protein content, plant height, flowering, pod production, seed weight or other yield related traits that are quantitatively inherited. In case of vegetative propagation, mutagen treatment produces chimera, which is basically the mixture of one or more genotypes and hence needs to be dissolved. These chimeras are unstable in clonal crops hence several are needed to extract true morphological mutants (Ahloowalia and Maluszynski, 2001). Though mutation breeding attempts may be made to broaden the variation spectrum to facilitate selection of lines with improved nutritional qualities, especially with respect to protein associated with high yield (Tah, 2006).

The role of mutation breeding in increasing the genetic variability for quantitative traits in various crop plants have been proved beyond doubt (Khan, 1979; Khan *et al.*, 1994, 1998b, 1999; Vyas and Chauhan, 1994; Khan and Siddiqui, 1995; Das and Chakraborty, 1998;

Kumar and Mishra, 2004; Khan and Wani, 2006; Singh *et al.*, 2006b; Wani and Khan, 2006; Khan and Goyal, 2009). Chaudhry (1988), Singh and Yadav (1991) and Parveen (2006) have been reported the improvement in number of branches and pods due to the effect of mutagens in various pulses. Yaqoob and Rashid (2001) reported that various quantitative traits can be improved in various genotypes through variable gamma rays doses.

An increase in mean values for pods as well as yield per plant in M_2 and M_3 generations was observed in the variety T-9 (Kundu and Singh, 1982). Pods per plant exhibited high variability in M_3 and M_4 generations of urdbean varieties Vamban 1 and ADT 3 treated with 20-90 kR gamma rays (Hepziba and Subramanian, 1994). The highest level of variability was observed in pod length after gamma irradiation and in number of pods per plant after EMS treatment in the urdbean cultivars ADT-3 and Vamban 1 (Vanniarajan *et al.*, 1996). Mean values of three characters viz., plant height, pods per plant and yield per plant were negatively affected by gamma rays treatment of TAU 1 (Manapure *et al.*, 1998).

Mutants used in hybridization programme: The large seed mutants, UM 196 and UM 201 were used in hybridization with the elite cultivar T-9 for developing high yielding varieties TAU 1, TAU 2 and TPU 4 (Pawar and Manjaya, 1996). So far, seven varieties have been developed through induced mutation and released for cultivation in India. Four of these varieties are the derivatives of mutants used in cross breeding. Mutation breeding has made significant contribution in increasing the production of urdbean in India. The variety TAU 1, developed at Bhabha Atomic Research Centre (BARC), Mumbai has become the most popular variety in Maharashtra occupying an area over 95% of the total area under urdbean cultivation in Maharashtra.

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