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A review on efforts of induced mutagenesis for qualitative and quantitative improvement of oilseed brassicas

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Abstract

Genetic variability is a prerequisite feature of any crop improvement programme and induced mutagenesis has become a proven way of creating new variations within a crop variety. The utility of this method is evident from the fact that in several crops induced mutants have been released as new varieties. Intensive efforts of induced mutagenesis have been also made for the improvement of oilseed brassicas. This review summarizes various efforts of induced mutagenesis, deals with qualitative and quantitative improvement of oilseed brassicas.

Keywords: Induced mutagenesis, Brassicas, Mutagens.

Crop improvement relies on the ability to generate genetic variation and selection of individuals with improved characteristics. Modern crop improvement efforts have relied heavily on the intensive use of favorable alleles present in cultivated germplasm collections, thereby contributing to the narrow genetic base of elite germplasm [1]. Therefore, it is important that attempts be made to expand the genetic diversity by utilizing new and unrelated sources of germplasm.

Induced mutagenesis has become a proven way of creating variation within a crop variety. It offers the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evolution. When no gene, or genes, for resistance to a particular disease, or for tolerance to stress, can be found in the available gene pool, plant breeders have no obvious alternative but to attempt mutation induction. Artificial induction of mutations by ionizing radiation dates back to the beginning of the 20th century. But it took about 30 years to prove that such changes could be used in plant breeding. With the advancement of molecular and biochemical genetics the induced mutations have been found to be indispensable for any breeding programme. Agricultural production has witnessed a sharp rise at the global level due to application of various tools of improvement including induced mutagenesis. Sweden has greatly advanced in mutation breeding since 1929 due to efforts of scientists like Gustafsson at Svaloff Research Station. Induced mutations occur more or less randomly in the genome; even their target cannot be directed. Induced mutations are generally used for genetic improvement for qualitative and quantitative traits in the plants [2-6]. The detailed prospects and utilization of induced mutation have been compiled and reviewed by Gustafsson [7], Konzak [8], Swaminathan [9, 10].

Oleiferous Brassicas (rapeseed and mustard) has been an important conventional oilseed crop of the sub-continent. There are many factors responsible for its low yield, but the most important one is the non-availability of high yielding varieties. It is, therefore, imperative to develop improved varieties of oilseed *Brassica* to bridge the gap between local production and import of edible oil in the country. Induced mutagenesis technique has also been successfully employed in oilseed brassicas including *B. campestris*, one of the extensively cultivated 'AA' genotype containing oilseed crop by the plant breeders to alter the genetic architecture of plant and isolate the possible mutants with desired economic plant characters/traits such as plant height, number of pods per plant, number of grain per pod, 1000- grain weight, grain yield, oil content and disease resistance [11-18]. Although mutagenesis attempts was initiated in oilseed brassicas during 1940's (1940-50) but it gained momentum in 1960's. Many workers have reported mutants for size, texture, type and modification of leaf parts [19]. Singh *et al.* [20] obtained a chlorophyll deficient mutant in *B. juncea*. Hawk [21, 57] identified a mutant for wrinkled leaves and jagged petal margins in *B. campestris*. Orakwue and Crowder [22]

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identified variegated mutants in *B. rapa* by selfing the progenies of normal *B. campestris*. Chauhan *et al.* [51] reported gamma rays induced chocolate bold seeded mutant in *B. campestris* L. cv. yellow Sarson.

Das and Rahman [23] and Shah *et al.* [24] have isolated short statured mutants with high yield potential from mutagen treated populations of rapeseed and mustard respectively. The low linolenic trait traces back to Germany, where mutants ('M57', 'M3', 'M6', 'M8', 'M11') with reduced linolenic acid content were selected after chemical mutagenesis of the Canadian low erucic acid cultivar Oro (*B. napus*, 10% linolenic acid). The mutant M57 with 5.6% linolenic acid was mutagenized again, and a double mutant 'M47' was selected for reduced (up to 3.3%) linolenic acid [13]. Zareen and Pratibha [25] reported mutagenic effectiveness and efficiency of gamma rays, diethyl sulphonate and maleic hydrazide in *B. campestris*. Chauhan and Singh [26] reported the effect of gamma rays on pollen sterility in M₁ and M₂ generation of *B. juncea* var. Pusa bold.

Das *et al.* [27] reported two early maturing and high yielding rapeseed varieties developed through induced mutation. Potts *et al.* [28] reported low glucosinolate content containing germplasm of *B. campestris* developed using spontaneous mutants and intra- and inter-specific hybridization. Bhatia *et al.* [29] studied one hundred and sixty three cultivars of annual oilseed crops, developed using induced mutation, have been officially approved and released for cultivation in 26 countries. Singh *et al.* [30] reported that EMS application on seedling produced higher variability compared to its application on seed in *B. juncea*.

Schnurbusch *et al.* [31] reported a mutant's with increased palmitic acid content was phenotypically characterized and genetically analyzed. Haque [32] studied a mutant of *B. juncea* lines with reduced linolenic acid. Kumar *et al.* [33] reported gamma rays induced high oil percentage containing mutant developed. Schierholt *et al.* [34] studied inheritance of oleic acid mutations in winter oilseed rape (*B. napus* L.). Yadav *et al.* [35] studied the effect of single and combined doses of gamma rays and EMS on four cultivars of Indian mustard (*B. juncea* (L.) Czen & Coss) and reported that type of mutagenic treatment acts as major factor to determine magnitude of variation. Patil *et al.* [36] studied the effect of gamma rays to induce mutation in Indian mustard (*B. juncea* L.) and described 50 KR dose as most effective and efficient dose for inducing wide range of mutations.

Singh *et al.* [37] studied heritability and genetic advance of seed yield and its components in Yellow sarson (*B. campestris*). Javed *et al.* [38] reported two high yielding mutants of *B. campestris* var. toria developed through gamma rays irradiation. The mutant TS96-752 was significantly superior to all other entries in grain yield but at par with FSD 86028-3. Gupta *et al.* [39] isolated small bud mutants in *B. juncea* through ethyl methane sulphonate. Chaudhary *et al.* [40] studied variability in quality parameters (seed size, colour, fiber content, oil content fatty acid profile, protein content and glucosinolate content) in Indian mustard (*B. juncea*). Significant variation was observed for all parameters. Singh *et al.* [41] reported genetics of component of variation for seed yield, harvest index and oil content in Indian mustard. In this study heritability was high for oil content (47.5) and moderate

for seed yield (20.26% and 27.8%) and harvest index (26.6%) more or less similar. Singh and Sareen [42] studied two induced mutants, viz., bunching and apprised pods of *B. juncea* cv. RH 30. One of them, i.e. with bunching pods is dwarf, has more seed weight and oil content. The mutant with apprised pods is superior in respect of plant height, number of primary and secondary branches and seed yield though it is as good as parental stock in respect of oil content. Sonntag *et al.* [43] attempted microspore mutagenesis in transgenic oilseed rape (*B. napus* L.) for the modification of fatty-acid composition. Shikari and Sinhamahapatra [44] studied effect of siliqua angle on seed yield and its component attributes in tetra-ocular *B. campestris* var. Yellow sarson and also the effect of siliqua position and alignment on seed number and weight in *B. campestris* var. Yellow sarson. Khatri *et al.* [45] attempted mutagenesis in *B. juncea* L. cv. S-9 using gamma rays (750 and 1000 Gy) and EMS (0.75% and 1.0%) as mutagen and reported 17 mutants. Out of seventeen mutants, mutants S 97-75/36, S 97-1.0E/20 and S 97-1.0E/21 described as superior to all other entries in grain yield and these were also found early in maturity, short stature and having high seed index. Patel *et al.* [46] reported effect of gamma rays treatment on *B. juncea* and isolated some morphological mutants viz. broad leaf, long and narrow leaf, sterile tricotyledonary seedling, dwarf, tall, early flowering, late flowering, chlorophyll, yellow seed, long siliqua, short siliqua, and high-yielding. Meenu *et al.* [47] evaluated 105 cultivars of Yellow sarson for their oil content. They had observed large range of variation for all the characters studied. Based on these finding they had concluded that there is enough scope for the improvement of quality and quantity of oil through selection. Guan *et al.* [48] studied high oleic acid content rapeseed breeding has great significance, because high oleic acid oil is healthy and nutritious oil, which has a long shelf life and also propitious to producing biodiesel fuel. The high oleic acid content breeding materials of rapeseed (*B. napus*) were obtained by 80-100kR ⁶⁰Co gamma ray ionizing radiation treatment of dry seeds and continuous selection.

Huang *et al.* [49] reported a novel male-sterile mutant which lacks mature pollen, *Brassica campestris* male sterile (bcms), was identified in *B. campestris* L. ssp. chinensis Makino (syn. *B. Rapa* ssp. chinensis). Genetic analysis revealed that bcms was controlled by a single recessive mutation locus. Genome-wide transcriptional profiling was performed on the flower buds of both the bcms mutant and the wild-type from which it originated, and profiling analysis indicated that there were numerous changes in gene expression attributable to the gene mutation.

Ferrie *et al.* [50] developed a protocol for microspore mutagenesis in *B. rapa*, *B. napus* and *B. juncea* for the production of double haploid lines with novel fatty acid profiles in the seed oil. In which freshly isolated *Brassica* microspores were first cultured with ethyl methane sulphonate (EMS) for 1.5 h. then cultured according to the standard *Brassica* microspore culture protocol. Fatty acid analysis of the *B. napus* double haploid lines showed that saturated fatty acid proportions ranged from 5.0% to 7.7%. For *B. juncea*, saturated proportions ranged from 5.4% to 9.5%. Of the 7000 *B. Rapa* lines that were analysed, 197 lines had elevated oleic acid (>55%), 69 lines had reduced α -linolenic acid (<8%) and

157 lines had low saturated fatty acid proportions (<5%), when compared with the parental lines. Siddique *et al.* [51] studied the effect of EMS and gamma rays treatments alone and in combination on rapeseed (*B. napus* L.) cv. Waster and reported that all mutagen treatments showed enhancing effect for siliques per plant and deteriorating effect for grains per silique and oil (%). Combinations of physical and chemical mutagen had shown enhancing effect on primary branches. Syed *et al.* [52] reported two mutant varieties i.e. Abasin-95 of rapeseed and NIFA-Raya of mustard, and one hybrid variety, Durr-e-NIFA, of rapeseed. All the three varieties possess high yield potential, medium-to-high oil content, early maturity and broader adaptability to rainfed and irrigated environments in comparison with the local check varieties and respective parents. Sheikh *et al.* [53] attempted induced mutagenesis in *B. carinata* cv. PC 5 for seed quality traits using ethyl methane sulphonate as mutagen and reported a mutant (EMS 9-56) had about 24 percent erucic acid, 30 per cent oleic acid, and 40 percent oil content as against corresponding values of 46 percent, 11 percent and 34.2 percent in base variety, PC 5. Huapeng *et al.* [54] reported dwarf mutant of *B. napus*, namely NDF-1, which was derived from a high doubled haploid (DH) line '3529' (*B. napus* L.) of which seeds were jointly treated with chemical inducers and fast neutron bombardment, was revealed that dwarfism is under the control of a major gene (designated as *ndf1*) with a mainly additive effect and non-significant dominance effect. Harloff *et al.* [55] developed two mutant populations of oilseed rape (*B. napus* L.) using EMS (ethylmethanesulphonate) as a mutagen. Genes of the sinapine biosynthesis pathway were chosen for determining the mutation frequencies and for creating novel genetic variation for rapeseed breeding. Screening of both populations revealed 229 and 341 mutations within the *BnaX.SGT* sequences (135 missense and 13 nonsense mutations) and the *BnaX.REF1* sequences (162 missense, 3 nonsense, 8 splice site mutations), respectively. Ali *et al.* [56] performed an experiment to assess the effect of induced mutation influence on oil content, oleic acid and erucic acid in rapeseed and reported that the gamma irradiation enhanced synthesis of oleic acid but induced different spectrum of variability for this desirable monounsaturated fatty acid at two locations. The gamma dose 1200 Gys reduced oleic acid at Peshawar but same dose increased oleic acid at Kaghan.

On the basis of above review we are concluded that induced mutagenesis is used as an indispensable tool for the qualitative and quantitative improvement of oilseed brassicas in past as well as in recent years and several outstanding results are obtained. More concerted efforts of induced mutagenesis are able to synergize and boost the oilseed brassica improvement programme. Our collection of scientific records is available to researchers and communicators at all levels to help create new strategy for the improvement of oilseed brassicas.

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References

1. Matus IA, Hayes PM. Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. *Genome* 2002; 45:1095–1106.
2. Konzak CF, Nilon RA, Wagner J, Foster RJ. Efficient chemical mutagenesis. *Rad. Bot* 1965; 5 (suppl.):49-70.
3. Anonymous. Mutagen effects observable in the first generation. Manual of plant breeding. Technical Report Series, International Atomic Energy Agency, Vienna. 1970, 119.
4. Arias J, Frey KJ. Grain yield mutations induced by ethyl methane sulphonate treatment of oat seeds. *Radiation Botany* 1973; 13:73-85
5. Chauhan YS, Kumar K. Gamma rays induced chocolate seeded mutant in *Brassica campestris* L CV Yellow sarson *Current Science*. 1986; 55:410.
6. Sharma JR, Lal RK, Gupta MM, Verma RK, Misra HO. A novel non-narcotic seed variety Sujata of opium poppy (*Papaver somniferum*). *Journal of Medicinal and Aromatic Plant Sciences*. 2002; 24(2):481-485.
7. Gustafsson A. A study of induced mutations in plants. Induced mutations in plants. IAEA, Vienna. 1969; 9-31.
8. Konzak CF. Genetic effects of radiation on higher plants. *Q Rev Bio*. 1957; 32:27-45.
9. Swaminathan MS. Role of mutation breeding in changing agriculture. *Proc Symp. Induced Mutations in Plant*. IAEA, Vienna. 1969; 719-736.
10. Swaminathan MS. Mutational reconstruction of crop ideotypes. In *Induced Mutations and Plant Improvement*, IAEA, Vienna 1972; 155-180.
11. Mahla SVS, Mor BR, Yadava JS. Effect of mutagens on yield and its component characters in mustard. *Haryana Agric Univ J Res*. 1990; 20:259-264.
12. Mahla SVS, Mor BR, Yadava JS. Mutagen induced polygenic variability in some mustard (*Brassica juncea* L.) varieties and their hybrids *J Oilseed Res*. 1972; 8:173-177
13. Robbelen G. Mutation breeding for quality improvement. A case study for oilseed crops. IAEA, 1990; 311-318.
14. Shah SA, Ali I, Iqbal MM, Khattak SU, Rahman K. Evolution of high yielding and early flowering variety of rapeseed (*Brassica napus* L.) through *In vivo* mutagenesis. *Proc. IIIrd Int. Symp. New Genetical Approaches to Crop Improvement*, (Eds.):Arain *et al.*, Nuclear Institute of Agriculture, Tando Jam, Allied Printing Corporation, Hyderabad, Pakistan, 1999; 47-53.
15. Shah SA, Ali I, Rahman K. Induction and selection of superior genetic variables of oilseed rape, *Brassica napus* L. *The Nucleus* 1990; 7:37-40.
16. Shah SA, Ali I, Rahman K. Use of gamma rays induced genetic variability for the improvement of oilseed rape (*Brassica napus* L.). *Proc. 2nd Int. Symp. New Genetical Approaches to Crop Improvement*, (Eds.):Naqvi *et al.*, Nuclear Institute of Agriculture, Tandojam, Pakistan Printing Press Corporation, Karachi, Pakistan, 1998; 229-237.
17. Rahman A. New mutant cultivar. *Mutation Breeding Newsletter* 1996; 42:27.
18. Javed MA, Khatri A, Khan IA, Ahmad M, Siddiqui MA, Arain AG. Utilization of gamma irradiation for the genetic

- improvement of oriental mustard *Brassica juncea* (L.) Coss Pak J Bot. 2000; 32:77-83.
19. Patil SH. Mutations induced in groundnut by x-rays. Indian J Genet. 1969; 26:334-348.
 20. Singh and Srivastava, ANS. A note on the bold seeded mutant in Rai (*Brassica juncea*). Indian Oil Seeds J. 1975; 6:34.
 21. Hawk JA, Crowder LV. The inheritance of four mutants in early flowering *Brassica campestris* LJ Heredity. 1978; 69:125-127.
 22. Orakwue FC, Crowder. Inheritance of variegation in *Brassica campestris* LJ Hered. 1983; 74:65-67.
 23. Das ML, Rahman A. Induced mutagenesis for the development of high yielding varieties in mustard. J Nucl Agric Biol. 1988; 17:1-4.
 24. Shah SA, Ali I, Rahman K. Induction and selection of superior genetic variables of oilseed rape, *Brassica napus* L. The Nucleus 1990; 7:37-40.
 25. Zareen F, Devi P. Induced mutants in yellow sarson. J. Cytol. Genet. 1995; 29(1):23-27.
 26. Chauhan SVS, Singh NK. Gamma rays and EMS induced male sterile mutants in *Brassica juncea*. J Cytol. 1998; 33(1):29-34.
 27. Das ML, Rahman A, Malek MA. Two early maturing and high yielding rapeseed varieties developed through induced mutation. Bangladesh Journal of Botany 1999; 28(1):27-33.
 28. Potts DA, Rakow GW, Males DR. Canola-quality *Brassica juncea*, a new oilseed crop for the Canadian prairies. In: New Horizons for an old crop. Proc. 10th Intl rapeseed Congr, Canberra, Australia 1999.
 29. Bhatia CR, Nichterlein K, Malyszynski M. Oilseed cultivars developed from induced mutations and mutations altering fatty acid composition. Mutation Breeding Review (IAEA, Vienna) 1999; 11:1-36.
 30. Singh VV, Sharma UC. Variation in M₃ for seed and oil yield in *Brassica juncea*. Agriculture Science Digest 2000; 20(3):159-161.
 31. Schnurbusch T, Möllers C, Becker HC. A mutant of *Brassica napus* with increased palmitic acid content. 2000; 119(2):141-144.
 32. Haque A, Sharma G. Mutant *Brassica juncea* lines with reduced linolenic acid. J. Genetics and Plant Breeding 2002; 56:309-316.
 33. Kumar A, Haider ZA, Varade PB. Influence of gamma irradiation on oil quality and quantity in *Brassica juncea*. J Res. and Biotechnology. VCH Verlagsgesellschaft mbH, 2000; 27-36.
 34. Schierholt AB, Rucker, Becker HC. Inheritance of high oleic acid mutations in winter oilseed rape (*Brassica napus* L.). Crop Sci. 2001; 41:1444-1449
 35. Yadav YP. L.B siliqua mutant in taramira (*Euraca sativa* Milli.) Cruciferae News Letter vol 24; 2002:21.
 36. Patil RV, Prema Manapure, Patil SR, Chaudhari SD, Gawande PP. Effect of gamma rays including mustard in Indian mustard (*Brassica juncea* L.). Journal of Oils and Crops 2002; 12(2):241-244.
 37. Singh P, Singh DN, Chakravorty M. Variability, heritability and genetic advance in Indian mustard (*Brassica juncea* L.) (Czern and Coss). Journal of Research BAU 2003; 15:45-47.
 38. Javed MA, Siddique MA, Khan MKR, Khatari A, Khan IA, Dahar NA, Khanzada MH, Khan R. Development of high yielding mutants of *Brassica campestris* L. cv. Toria selection through gamma rays irradiation. Asian J. Plant Sci. 2003; 2(2):192-195.
 39. Gupta KD, Prem MS, Negi, Agnihotri A. ISSRs: An efficient tool to screen interspecific F1 hybrids in *Brassicaceae*. In Proceedings: 4th International Crop Science Congress, 26th Sept - 1st Oct. 2004, Brisbane, Australia.
 40. Chaudhary AK, Singh SP, Pant DP. Variability in quality parameters in Indian mustard (*Brassica juncea* L.). Cruciferae Newsletter 2004; 25:67-68.
 41. Singh AK, Singh B, Sachan JN. Genetics of component of variation for seed yield, harvest index and oil content in Indian mustard. Cruciferae News Letter 2004; 25:59-60.
 42. Singh KP, Sareen PK. Induced podding mutants of Indian mustard (*Brassica juncea*). Cruciferae Newsletter 2004; 25:17-18.
 43. Sonntag Karin, Eicke Rudloff. Microspore mutagenesis in transgenic oilseed rape for the modification of fatty-acid composition Acta Universitatis Latviensis, Biology 2004; 676:227-230.
 44. Shikari AB, Sinhamapatra. Effect of siliqua position and alignment on seed number and weight in *Brassica campestris* var. Yellow sarson. Cruciferae Newsletter 2004; 25:55-56.
 45. Khatri A, Khan AI, Siddiqui MA, Raza S and Nizamani GS. Evaluation of high yielding mutants of *Brassica juncea*. S-9 developed through gamma rays and EMS. Pak. J. Bot. 2005; 37(2):279-284.
 46. Patel JM, Patel KM, Patel CJ, Prajapati KP. Genetic parameters and inter-relationship analysis in Indian mustard (*Brassica juncea* L.) (Czern and Coss). Journal of Oilseeds Research 2006; 23:159-160.
 47. Meenu, Chauhan SVS. Genetic variability and correlation studies in *Brassica campestris* L. Prain. J. Cytol. Genet. 2008; 9:1-5.
 48. Guan CY, Liu CL, Chen SY, Peng Q, Li X, Gaun M. High oleic acid content materials of rapeseed (*Brassica napus*) produced by radiation breeding. Acta Agronomica Sinica 2006; 11:1625-1629.
 49. Huang L, Cao J, Ye W, Liu T, Jiang L, Ye Y. Transcriptional differences between the male-sterile mutant *bcms* and wild-type *Brassica campestris* ssp. *chinensis* reveal genes related to pollen development. Plant Biology. 2008; 10(3):342-355.
 50. Ferrie AMR, Taylor DC, MacKenzie SL, Rakow G, Raney JP, Keller WA. Microspore mutagenesis of *Brassica* species for fatty acid modifications: a preliminary evaluation. Plant Breeding 2008; 127:501-506.
 51. Siddique MA, Khan IA, Khatari A. Induced quantitative variability by gamma rays and EMS alone and in combination in rapeseed (*Brassica napus* L.). Pak J Bot. 2009; 41(3):1189-1195.
 52. Syed AS, Rahman K. Development of Improved Varieties of Rapeseed and Mustard through In Vivo Mutagenesis and Hybridization in Pakistan. QY Shu (ed.) Induced

- Plant Mutations in the Genomics Era, FAO of United Nations, Rome 2009; 376-378.
53. Sheikh FA, Lone B, Najeeb S, Asif B, Shikari, GA, Parray AG, Rather RR, Khudwani RS. Induced mutagenesis for seed quality traits in Ethiopian mustard (*Brassica carinata* A. Braun). ARPN Journal of Agricultural and Biological Science 2009; 4:2-8.
 54. Huapeng Li, Yun Wang, Xiaocheng Li, Yong Gao, Zhijun Wang, Yun Zhao and Maolin Wang. A GA insensitive dwarf mutant of *Brassica napus* L. correlated with mutation in pyrimidine box in the promoter of *GIDI*. Molecular Biology Reports 2010; 38(1):191-197.
 55. Harloff HJ, Lemcke S, Mittasch J, Frolov A, Wu JG, Dreyer F, Leckband G, Jung C. A mutation screening platform for rapeseed (*Brassica napus* L.) and the detection of sinapine biosynthesis mutants. March Theor Appl Genet. 2012; 124 (5):957-69.
 56. Ali I., and Farhatullah. Environmental and induced mutations influences on oil content, oleic acid and erucic acid in rapeseed. Sarhad J Agric. 2013; 29(2):187-193
 57. Hawk JK. Tight linkage of two seedling mutants in early flowering turnip rape (*Brassica campestris*). Canadian Journal of Genetics and Cytology. 1982; 24:475-478.