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Trombay Phule Mustard 1: a yellow seed coat mutant of *Brassica juncea* with probable pleiotropic effect released as high yielding variety

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Abstract

A yellow seed coat mutant Trombay Mustard 1 (TM1) of Indian mustard (*Brassica juncea* L) was isolated from parent variety Rai 5 using ^{32}P radioisotope. Single plant selection from advance generations of TM1 has resulted into the isolation of high yielding variety TPM1. It was evaluated in national and state agricultural university yield trials. Average seed yield of TPM1 over 17 locations was 1396 kg/ha and oil yield was 631 kg/ha which is 21, 23, and 23 percent higher for seed yield and 37, 31, and 48% higher for oil yield over check varieties Sita, Pusa Bold and Varuna respectively. Therefore, it was released and notified by government of India for non-traditional area of mustard cultivation. Besides yellow seed coat, additional mutated characters are early, reduced plant height, high oil content, reduced erucic acid, powdery mildew and club root tolerance, heat tolerance and higher accumulation of arsenate. Direct mutant with many mutated characters has not yet reported so far. It could be probable pleiotropic effect of same mutated gene which is being reported first time.

Keywords: *Club root, Indian mustard, mutation, powdery mildew, pleiotropic effect, yellow seed*

Introduction

Among oilseed Brassicas, Indian mustard (*B. juncea* L) is the major crop occupying more than 90% area of total 6.23 mha and an average productivity of 1499 kg/ha with total production of 9.34 mt during 2018-19 (Anonymous, 2020). To fulfill increasing demand of edible oil in India, around 13-15 mt of edible oil is being imported every year. It signifies the edible oil deficiency as well as essence of developing high yielding varieties. Among various approaches of crop improvement, induced mutagenesis has played a pivotal role in generation and utilization of variability to develop high yielding varieties (Bhatia *et al*., 1999) in oilseed crops. Mutations for various morphological, biochemical, yield and yield attributes have been isolated in Brassica crops (Jambhulkar, 2007) however, mutants with pleiotropic effect has not yet reported.

Traditional areas of mustard cultivation are central, east, west and north regions of India. Maharashtra and southern part of India are called as non-traditional area of mustard cultivation. If this area is brought under mustard cultivation, nearly 1.2 mha additional area could be increased. In Maharashtra state, mustard is being cultivated in around 10,000 ha but productivity remained stagnated around 300-400 kg/ha which is lower than national average. The main constrains for low productivity are cultivation of mustard as an inter-crop under low input conditions and unlike north India, cold spell in Maharashtra state remains for short duration and when temperature starts increasing, mustard genotypes suffers from powdery mildew disease. Yellow seed coat genotypes have advantage of better oil content, high crude protein and golden yellow oil colour (Stringham *et al*., 1974). Therefore, early maturing yellow seed coat with powdery mildew tolerant variety is suitable for cultivation in Maharashtra state. However, no such high yielding variety has yet been developed for this region. Our efforts have resulted into the development of suitable high yielding variety for non-traditional area of mustard cultivation in India.

Materials and Methods

TM1is a yellow seed coat direct mutant isolated from parent Rai 5 using ^{32}P radioisotope (Nair, 1968) and maintained at Bhabha Atomic Research Centre (BARC) Mumbai, India. Single plant selection in the advance generations and their yield evaluation has resulted into the development of superior strain of TM1. It was evaluated in Initial Varietal Trail (IVT) for late sown condition in All India Co-ordinated Research Project

(AICRP) on Rapeseed-Mustard under the name TNM1 (Trombay Nagpur Mustard 1). The average seed yield of three locations in Maharashtra state under zone IV was 893 kg/ha which was 15.5% more than check variety Varuna (775kg/ha) (Anonymus, 2000). Maharashtra state falls under zone IV. Looking into its superior yield performance, it was decided to conduct its yield trials under Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri and therefore renamed this genotype as 'Trombay Phule Mustard1 (TPM 1) and conducted station trial as well as multilocation yield trials. Seed yield performance of TPM 1 was compared with national check Varuna, zonal check Pusa bold, and local check Seeta for 4 years (2001-02 to 2004-05) over 17 locations. Experiment was laid in randomized block design (RBD) keeping 5 row plot of 3 m row length, 30 cm plant to plant and 10 cm row to row spacing. Standard agronomic practices were followed while conducting experiment. Number of days to maturity was recorded in the field. At maturity, data on seed yield and its components were recorded on the plants harvested from net plot. Powdery mildew (*Erysiphe cruciferarum*) reaction was recorded on the percent leaf area infection in the field condition. Oil content was estimated using Oxford NMR (MQA 6005). Fatty acid analysis was carried out using Gas Liquid Chromatography (GLC). After release of variety, studies on various aspects like club root resistance/tolerance under natural sick plot at Shantiniketan, West Bengal, heat tolerance (Rai *et al*., 2020) and arsenate and arsenite tolerance (Srivastava *et al*., 2010) were undertaken.

Results and Discussion

Genetic variability is the pedestal for crop improvement. Mutation breeding has an advantage of generating desirable traits in an otherwise high yielding variety as well as strengthening of germplasm. Induced mutagenesis has resulted into the isolation of large spectrum of variation in oilseed Brassica crops (Jambhulkar, 2015). Resultant mutants could be used in basic and applied studies, release as direct variety or use in hybridization for trait improvement. Yield evaluation of TPM1 was conducted in national and state agricultural university. Average seed yield of TPM1 in 17 locations was 1396 kg/ha which is 21, 23 and 23% higher over check varieties Sita, Pusa bold and Varuna respectively (Table 1). Similarly, oil yield was 631 kg/ha, which is 37, 31 and 48% higher over check varieties Sita, Pusa bold and Varuna respectively. Higher oil yield is a cumulative effect of high oil content and high seed yield. Based on these superior characters, TPM1 was released for cultivation in the state of Maharashtra which is non-traditional area of mustard cultivation. It has been notified by government of India by gazette notification No.1201, Oct.5, 2007. Mutation breeding approaches have been successful in isolation of high yielding varieties in oilseed crops (Bhatia *et al*., 1999) and in rapeseed-mustard (Das and Rahman 1988; Jambhulkar, 2015). Two high yielding mutant varieties have been developed by Malek et al (2011). Recently, we have released one yellow seed coat high yielding mutant variety TBM204 (Dutta *et al*., 2020) for the state of West Bengal. So far 31 high yielding mutant varieties have been developed using induced mutagenesis approaches among them12 belongs to *B. juncea* (MVD IAEA).

TPM1 is a yellow seed coat mutant (Fig. 1a) reported in *B. juncea* (Nayar, 1968). It was extensively used in crossing programme throughout India to develop yellow seed coat high yielding genotypes. Yellow seed coat mutants have recently been isolated from Variety Varuna and Pusa Bold (Jambhulkar, 2017) too. Yellow seed coat genotypes have thinner seed coat and less fiber compared to brown seed coated genotypes resulting to more oil content (Woods, 1980; Xiao, 1982). TPM1 is early maturing variety (Fig. 1b). Maturity of TPM1 is 95 days compared to 106 days of Varuna and 108 of Pusa bold (Table 1) under Maharashtra condition. Reduction in time to flowering could well be at the expense of yield potential. However, mutation for early flowering in the agronomically superior lines could be useful in the various cropping patterns. Early flowering mutants have been reported in

Table1: Performance of TPM1 over check varieties for seed yield, oil content, oil yield, maturity and powdery mildew score over parent and check varieties

Variety	Seed yield (Kg/ha)	% increase over checks	Oil content (%)	Oil yield (Kg/ha)	% increase over checks	Plant height (cm)	Maturity (days)	% Powdery mildew
TPM1	1396	-	37.0	631	-	120	95	25.0 (29.2)
Rai5 (Parent)	1097	27	35.0	383	64	140	110	72.0 (64.6)
Sita (LC)	1152	21	35.8	462	37	132	94	50.4 (45.4)
Pusa Bold (ZC)	1167	20	36.1	480	31	152	106	56.3 (50.4)
Varuna (NC)	1131	23	35.2	426	48	143	108	75.0 (65.7)

LC – Local Check, ZC – Zonal Check, NC – National Check. Figures in parenthesis for powdery mildew resistance are arc sin values.

B. juncea (Nayar and George, 1969; Khatri *et al.*, 2005) and in *B. napus* (Thurling and Depittayanan, 1992; Shah *et al*., 1999). Plant height of TPM1 was 120 cm compared to parent variety rai5 (140 cm) and check varieties Sita (132 cm) Varuna (151 cm) and Pusa Bold (143 cm). Mutations for reduced height compared to their parents have been isolated using chemical and physical mutagens in *B. juncea* (Khatri *et al.,* 2005; Jambhulkar, 2018) and *B. napus* (Shah *et al*., 1999; Zanewich *et al.*, 1991; Mei *et al*., 2006) however these mutations were not exploited to develop high yielding variety.

TPM1 was tolerant to powdery mildew disease as compared to check varieties (Table 1) under heavily infested powdery mildew plot (Fig. 1c). It could be due to genetic potential or escape due to earliness. In recent years, powdery mildew disease is a major concern in mustard breeding programme more particularly in Maharashtra and Southern part of India where short spell of winter is followed by increasing temperature which is suitable for powdery mildew disease. No defined source of resistance/tolerance has been reported in *B. juncea*. TPM1 could be one of the best sources for breeding powdery mildew resistance/tolerance. When TPM1 was grown along with B9 in natural epiphytotic condition for club root infection, it was found resistant (Fig. 1d).

Quality improvement by reducing erucic acid <2% and glucosinolates <30μM/g of defatted meal are important objectives in Brassica crop improvement programme. TPM1 has reduced erucic acid of 25.74% compared to parent Rai 5 (46.0) and check Varuna (44.2) and corresponding increase for oleic and linoleic acid (Table 2). Reduction of erucic acid to near about 50% indicate that the mutation in one of the alleles either *E1* or *E2* has been taken place as total erucic acid of around 48% is controlled by two dominant genes *E1E1E2E2* (Kirk and Hurlstone, 1983; Bhat *et al*., 2002; Singh *et al*. 2015).

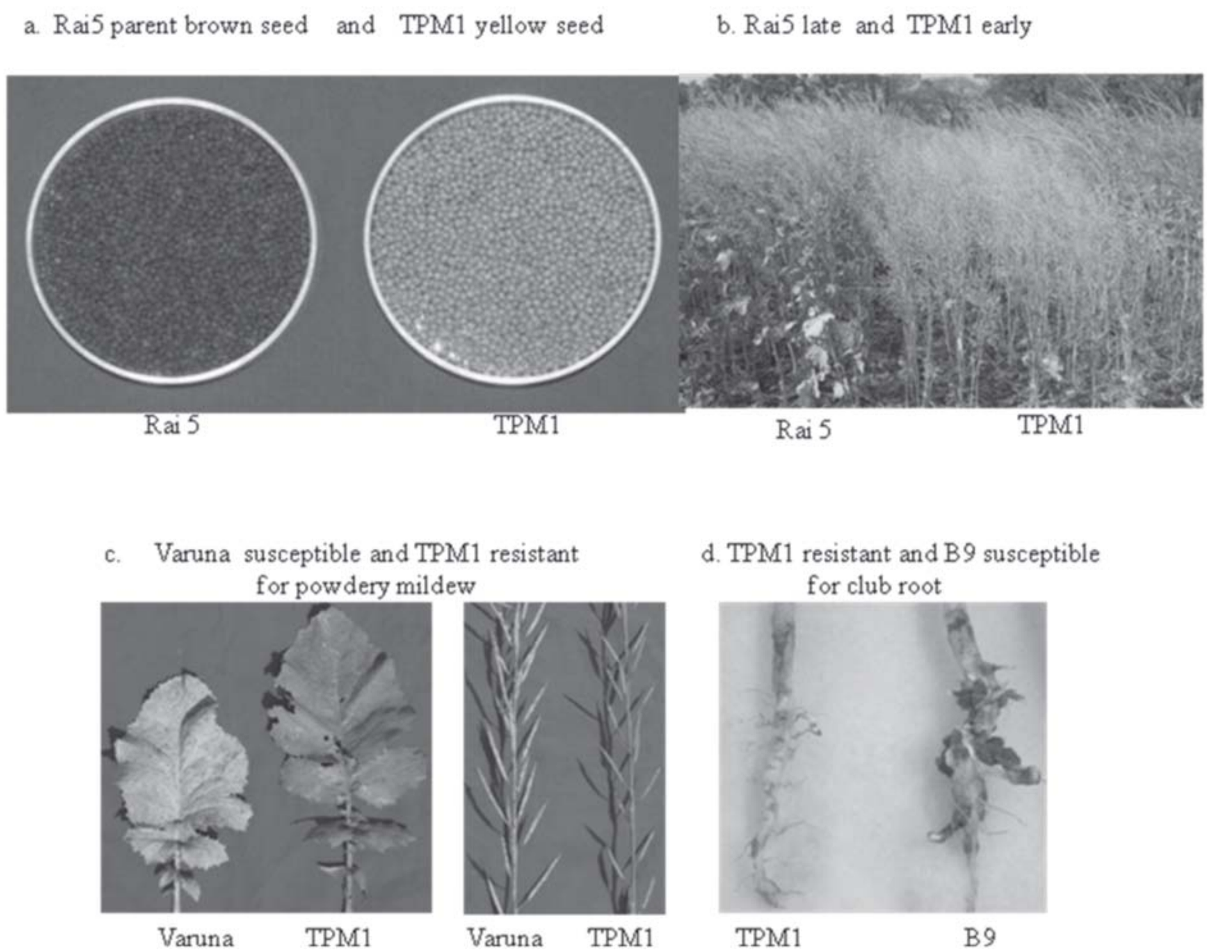

Fig. 1: TPM1 with yellow seed coat, early, powdery mildew and club root resistant along with parent Rai5 and check Varuna

Table 2: Fatty acid composition of TPM1 compared to parent Rai 5 and check Varuna

Variety	Stearic	Oleic	Linoleic	Linolenic	Ecosanoic	Erucic
TPM1	3.77	23.12	28.33	9.52	9.49	25.74
Rai5	2.47	13.58	17.87	13.39	6.67	45.98
Varuna	2.78	14.95	19.78	10.89	7.34	44.22

Mutation breeding has been successful in tailoring oil crops for desirable fatty acid composition (Robbelen, 1990). Olsson (1984) reported natural mutation for low erucic acid in *B. juncea* and Kirk and Oram (1981) found two zero erucic acid natural mutants in Chinese accession of *B. juncea*. Recently, additional feature like heat tolerance and heavy metal uptake were studies in our division. More accumulation of arsenate along with more antioxidant enzyme activity (Srivastava *et al*., 2010) was found in TPM1 compared to TM4. TMP1 was also found as heat tolerance compared to other genotypes. Expression profiling of heat stress-related genes (HSP21 and HSFA7A) showed significant upregulation in the TPM1 (Rai *et al*., 2020).

Mutation induction is character specific as mentioned in most of the articles cited in this paper. However, more than one mutation is likely to occur in same mutated plant but it has not been reported in *B. juncea* so far. TPM1 is a direct mutant isolated for yellow seed coat. Besides yellow seed coat it possesses the mutations for high yield, high oil content, earliness, reduced height, reduced erucic acid, powdery mildew and club root tolerance, heat tolerance and more arsenate accumulation. It showed that mutations have occurred in a greater number of genes responsible for various traits. Among these genes, any one of the gene may affecting expression of other genes resulting into many mutations. Thus, it could be probable pleiotropic effect. Pleiotropic effect of plant height on phenology, morphology, floral scent, color, nectar, and leaf glucosinolates in *B. rapa* (Zu *et al.*, 2017), rugose mutation on plant morphology in sweet clover (Goplen, 1962), barley row type genes on seed size, seed number per spike, thousand grain weight, and tillering (Liller *et al*., 2015), flowering-time genes and their influence on seed germination (Gabriela *et al*., 2019) have been reported. Liu *et al.* (2020) discovered *Pleiotropic Developments Defects* (*PDD^{OL}*) gene responsible to affect plant height, panicles length and inter node length. It is due to chloroplast tRNA modification influencing protein translation leading to altered chloroplast gene expression and thereby affecting communication between the chloroplast and the nucleus and influencing various developmental processes in rice. Such phenomenon needs to be deciphered in TPM1.

Conclusion

Based on seed and oil yield potential, TPM1 was released for cultivation in the state of Maharashtra which falls under non-traditional area of mustard cultivation. Though it was initially isolated for yellow seed coat, additional mutated characters like reduced height, earliness, high seed yield, more oil content, reduced erucic acid, powdery mildew, club root tolerance, heat tolerance and more arsenate accumulation have been observed in the same plant indicating that any one of the mutations could be responsible for pleiotropic effect. This phenomenon needs to be deciphered. This is the first report on probable pleiotropic effect of mutation with high yielding potential in *B. juncea*.

References

Anonymus. 2000. Annual Progress Report: All India Co-ordinated Research Project on Rapeseed-Mustard. pp: PB42.

Anonymous. 2020. Agricultural Statistics. Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, New Delhi,

Bhat MA, Gupta ML, Banga SK, Raheja RK, and Banga SS. 2002. Erucic acid heredity in *B. juncea*-some additional information. *Plant Breed* **121**: 456-458.

Bhatia CR, Nichterlein K, and Maluszynski M. 1999. Oilseed cultivars development from induced mutations and mutation altering fatty acid composition. *Mutation Breed Rev* **11**: 1-36.

Das ML and Rahman A. 1988. Induced mutagenesis for the development of high yielding varieties in mustard. *J Nucl Agric Biol* **17**: 1- 4.

Dutta A, Jambhulkar SJ, Rai AN, Bhujbal S, Banerjee H, Das R, Dewanjee S, Sarkar S, Pramanikand M and Mandal S. 2019. Trombay Bidhan Mustard-204 (TBM-204): A high yielding yellow seed coat mustard [*B. juncea* (L) Czern. & Coss.] variety notified for West Bengal. *J Oilseed Res* **36**: 217-219.

Gabriela AA, Steven P and Kathleen D. 2019. Pleiotropy in developmental regulation by flowering-pathway genes: is it an evolutionary constraint? *New Phytologist* **224**: 55–70.

Goplen BP. 1962. A recessive gene with major pleiotropic effect in sweet clover *Melilotus alba* L. *Can J Genet Cytol* **4**: 141-146.

Jambhulkar S.2018. Isolation of novel mutation for dwarfing genes for enhancing productivity in *B. juncea* L. In: FAO/IAEA International symposium on Plant mutation Breeding and Biotechnology, Vienna Austria: IAEA:CN263: 50.https://www.iaea.org/sites/default/files/18/08/cn-263-abstracts.pdf.

Jambhulkar SJ. 2007. Mutagenesis: generation and evaluation of induced mutations. Advances in Botanical Research (Rapeseed Breeding) Vol. 45 Academic Press: Elsevier Publication, 418p.

Jambhulkar SJ. 2015. Induced mutagenesis and allele mining. Brassica Oilseeds Breeding and Management. CAB International, 53p.

Jambhulkar SJ. 2017. Induced mutagenesis for breaking the yield barriers in *B. juncea* resilient to climate change. Souvenir of *3rd National Brassica Conference,* Feb 16-18, 2017, ICAR-IARI, New Delhi: 51-54.

Khatri A, Khan IA, Siddiqui MA, Raza S and Nizamani GS. 2005. Evaluation of high yielding mutants of *Brassica juncea* Cv. S-9 developed through gamma rays and EMS. *Pak J Bot* **37**: 279-284.

Kirk JTO and Hurlstone CG. 1983. Variation and inheritance of erucic acid content in *B. juncea*. *Z Pûanzenzuchtg* **90**: 331-338.

Kirk JTO and Oram RN. 1981. Isolation of erucic acid free lines of *Brassica juncea*: Indian mustard now a potential oilseed crop in Australia. *J Austral Inst Agric Sci* **47**: 51-52.

Liller CB, Neuhaus R, von Korff M, Koornneef M and, Van Esse W. 2015. Mutations in barley row type genes have pleiotropic effects on hoot branching. *PLoS ONE* **10**: e0140246.

Liu H, Ding Ren L, Jiang XL, Yuan Y, LiminY, Limin M, Wanli C, Aowei M, Ning J, Jinshui Y, Peng C, Hong M, Xiaojin L and Pingli L. 2020. A natural variation in pleiotropic developments defects uncovers a crucial role for chloroplast tRNA modification in translation and plant development. *Plant Cell* **32**: 2345–2366.

Malek MD, Begum HA, Begum M, Sattar MA, Ismail MR and, Rafii MY. 2011. Development of two high yielding mutant varieties of mustard [*B. juncea* (L) Czern & Coss.] through gamma rays irradiation. *Aust J Crop Sci* **6**: 922-927.

Mei DS, Wang HZ, Li YC, Hu Q, Li YD and Xu YS**.** 2006. The discovery and genetic analysis of dwarf mutation 99CDAM in *B. napus* L. *Yi Chuan* **28**: 851-7.

Nayar GG and George KP. 1969. X-ray induced early flowering, appressed pod mutant in *B. juncea* L. *In*: Proc. Symp. Radiation and Radiomimetic Substances in Mutation Breeding. Bombay, 26-29 Sept. 1969: 409-413.

Nayar, GG. 1968. Seed colour mutation in *Brassica juncea* Hook F. and Thomas induced by radioactive phosphorus ^{32}P. *Sci Cult* **34**: 421- 422.

Olsson G. 1984. Selection for low erucic acid in *B. juncea*. *Sveriges Utsadesf Tidskr* **94**: 187.

Rai AN, Saini N, Yadav R and Suprasanna P. 2020. A potential seedling-stage evaluation method for heat tolerance in Indian mustard [*B. juncea* (L) Czern & Coss.]. *Biotech* **10**: 114.

Robbelen G. 1990. Mutation breeding for quality improvement a case study for oilseed crops. *Mutation Breed Rev* **6**: 1- 43.

Shah SA, Ali I, Iqbal MM, Khattak SU and Rahman K. 1999. Evolution of high yielding and early flowering variety of rapeseed (*B. napus* L.) through *in-vivo* mutagenesis. *In*: Proc. 3rd Int. Symp. New Genetical Approaches to Crop improvement-III Nuclear Institute of Agriculture, Tandojam, Pakistan: 47-53.

Singh J, Yadava DK, Sujata V, Singh N, Muthusamy V and Prabhu KV. 2015. Inheritance of low erucic acid in Indian mustard [*B. juncea* (L.) Czern & Coss.]. *Indian J Genet* **75**: 264-266.

Srivastava S, Srivastava AK, Suprasanna P and, D'Souza, SF. 2010. Comparative antioxidant profiling of tolerant and sensitive varieties of *B. juncea* L. to arsenate and arsenite exposure. *Bull Environ Contam Toxicol* **84**: 342–346.

Stringam GR, McGregor DI, and Pawlowski SH. 1974. Chemical and Morphological characteristics associated with seed coat colour in rapeseed. *In*: Proc.4th International rapeseed Congress. Giessen, West Germany: 99-108.

Thurling N and Depittayanan V. 1992. EMS Induction of early flowering mutants in spring rape (*B. napus*). *Plant Breed* **108**: 177-184.

Woods DL. 1980. The association of yellow seed coat with other characteristics in mustard *(B. juncea)*. *Cruciferae Newsletter* **5**: 23-24.

Xiao D. 1982. Analysis of the correlation between seed coat colour and oil content of the *B. napus* L. *Acta Agronomica Sinica* **8**: 24-27.

Zanewich KP, Rood SB, Southworth CE and Williams PH 1991. Dwarf mutant of *Brassica*: responses to applied gibberellins and gibberellin content. *Plant Growth Regul* **10**: 121-127.

Zu, Pengjuan, Schiestl and Florian P. 2017. The effects of becoming taller: direct and pleiotropic effects of artificial selection on plant height in *B. rapa*. *Plant J* **89**: 1009-1019.