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Rapeseed-Mustard Breeding in India: Scenario, Achievements and Research Needs

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Abstract

Brassica spp., commonly known as rapeseed-mustard, plays a significant role in the Indian economy by providing edible oils, vegetables, condiments and animal feed. Globally, India holds second and third position in rapeseed-mustard area under cultivation and production, respectively. However, anthropogenically accelerated climate change thwarts yield potential of rapeseed-mustard by employing abiotic (drought, flood, temperature variation and salinity) and biotic (disease and insects) stresses. Various approaches such as molecular breeding, pre-breeding, –omics and biotechnological interventions have been used to develop varieties for improved yield and oil quality, climate resilient and resistance or tolerance to abiotic and biotic stresses. In this context, this chapter highlighted the different cytoplasmic male sterility (CMS) sources and their potential use for hybrid development. At the end, this chapter also enlisted salient achievement by the government and non-government institutes and briefly described the future perspective for improvement of rapeseed-mustard in India.

Keywords: rapeseed-mustard, hybrid breeding, oil quality, pre-breeding, biotic and abiotic stress

1. Introduction

Brassica spp., commonly known as rapeseed-mustard, plays an important role in the Indian economy by providing edible oils, vegetables, condiments and animal feed [1]. Nine oilseeds are the primary sources of vegetable oil in India. Among them soybean (39%), groundnut (26%) and rapeseed-mustard (24%) contribute more than 88% of total oilseeds production in the country. However, rapeseed-mustard (31%) contributes maximum in terms of edible oil production followed by soybean (26%) and groundnut (25%) in the country [2].

Rapeseed-mustard is the third major edible oilseed crop of the world after soybean and palm oil. Globally, as per USDA during 2018-2019, it was grown over 36.6 million hectares and produced 72.4 MT with a productivity of 19.8 q/ha. Globally, India accounts 19.8% of total acreage and 9.8% of total production.

Species	Common name	Type of Pollination	Chromosome No. (2n)	Genome	Genome size (Mb)
<i>B. juncea</i> (L.) Czern.	Indian mustard	Often-self	36	AABB	~922
<i>B. carinata</i> A. Braun	Karan rai or Ethiopian mustard	Often-self	34	BBCC	—
<i>B. napus</i> L.	Gobhi sarson	Self and cross	38	AACC	~1130
<i>B. nigra</i> (L.) Koch	Black mustard	Cross	16	BB	~558
<i>B. oleracea</i> L.	Cabbage, cauliflower etc.	Cross	18	CC	~630
<i>B. rapa</i> L.	var. <i>brown sarson</i>	Lotni type: Cross Tora type: Self	20	AA	~485
	var. <i>toria</i>	Cross			
	var. <i>yellow sarson</i>	Self			
<i>Eruca sativa</i>	Taramira	Self	22	EE	—
<i>B. alba</i> Rab. (Syn. <i>Sinapis alba</i>)	White mustard	Self	24	SS	—

Table 1.
List of limited and importantly cultivated species of Brassica species.

Rapeseed-mustard (8.3 MT) is the third most important annual oilseed crop in India, next to soybean (13.6 MT) and groundnut (9.1 MT) [2]. In India, rapeseed-mustard is widely grown in diverse agro-climatic environments from North-East, North-West, Central to Southern states under different conditions such as sole crop/mixed crop, early/timely/late, rainfed/irrigated and saline or alkaline soils [3]. Based on average of 2014-2015 to 2018-2019 area and production data, major rapeseed-mustard growing states are Rajasthan (producing 44.9% of total rapeseed-mustard from 40.7% area), Madhya Pradesh (producing 11.3% from 11.9% area) and Uttar Pradesh (producing 10.6% from 11.2% area). Rapeseed-mustard crops in India comprise eight species *viz.*, Indian mustard, toria, black mustard, yellow sarson, brown sarson, gobhi sarson, karan rai and taramira (**Table 1**).

2. Origin

Historically, the cultivation of *Brassica* spp. has been quoted in numerous ancient scriptures and believed to be cultivated on or prior to 5000 BC. It has also been reported that mustard crop had cultivated in Channhu-daro of Harrapan ancient civilization during 2300-1750 BC [4]. There is ambiguity in the history as the origin of *B. juncea* is concerned. It had been believed that center of origin for *B. juncea* is Middle-East, where putative parents *i.e.* *B. nigra* and *B. rapa* would have crossed with each other. Later on, it had been disseminated to other parts of the world such as Europe, Asia, and Africa etc. [5]. Today, there are two centers of diversity *i.e.* China and Eastern India based on the prevalence of their wild progenitors and relatives. At present, it has been proved that there are two geographical races *i.e.* Chinese and Indian of *B. juncea* based on molecular and biochemical studies [6].

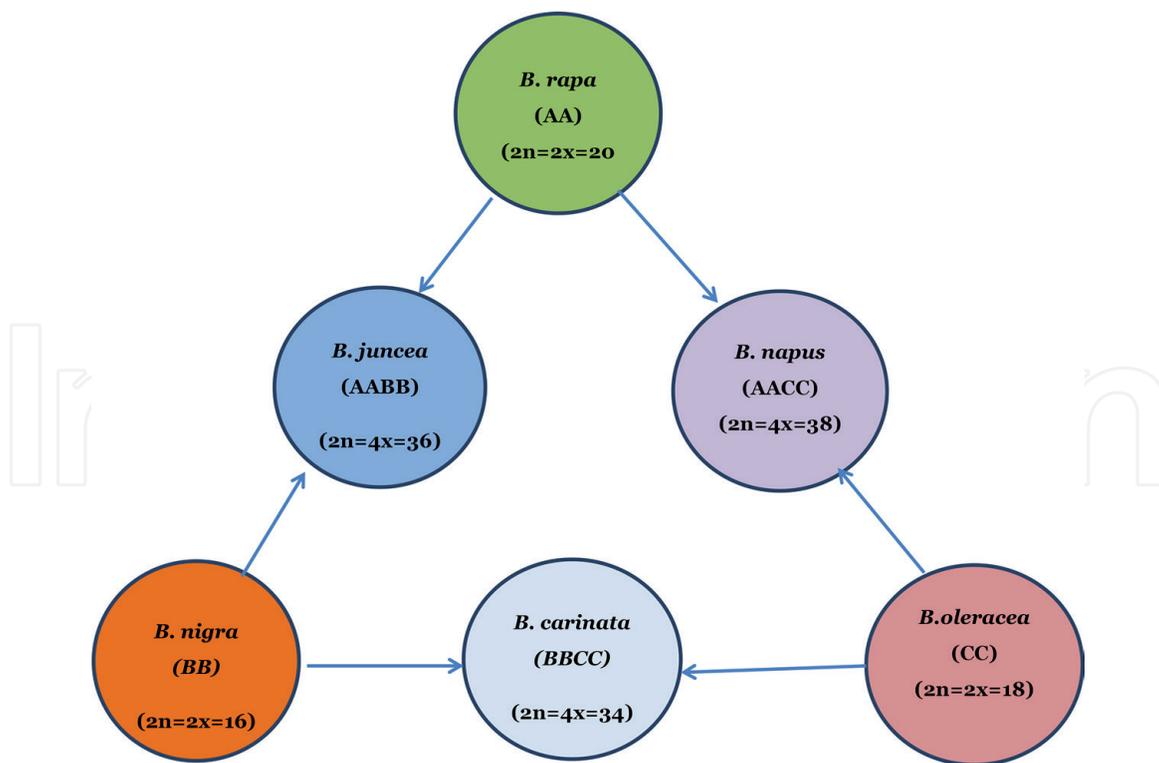


Figure 1.
U's triangle showing genetic relationship among six Brassica species [7].

In 1935, Nagaharu U [7] proposed a theory known as U's triangle to show genetic relationships based on artificial inter-specific hybridization experiments among six species, namely; *B. rapa*, *B. nigra*, *B. oleracea*, *B. carinata*, *B. napus* and *B. juncea*. As per theory, three allotetrapolyploid species (*B. napus*, *B. juncea* and *B. carinata*) were derived by natural hybridization of three basic diploid species (*B. rapa*, *B. nigra* and *B. oleracea*) followed by genome doubling (**Figure 1**). Nowadays, with the accomplishments of genome sequencing of *Brassica* taxa, this hypothesis has been increasingly accepted. Furthermore, it has been scientifically proved that allotetraploid *B. napus* and *B. juncea* had been derived from their diploid parents based on comparative genomic analysis and the results were in accordance with 'U' triangle [8].

3. Distribution

*Brassicac*s include large number of crops under cultivation. Among them, the Indian mustard occupies maximum area (> 90%) and predominantly cultivated in North-Western states followed by some nontraditional areas of Central and Southern states of the country [1]. The lotni (cross-pollinated) and tora (self-pollinated) are two different ecotypes of brown sarson. Earlier one is mainly cultivated in temperate regions of the country such as parts of Jammu, Kashmir and hilly areas of Himachal Pradesh, whereas later one is cultivated in parts of Eastern Uttar Pradesh [3]. However, yellow sarson is predominantly cultivated in parts of Bihar, West Bengal and Orissa. Toria is mainly used as short period crop in parts of Bihar, West Bengal, Orissa and Assam. Whereas, it is grown as a catch crop in Haryana, Himachal Pradesh, Madhya Pradesh, Punjab, Uttarakhand and Western Uttar Pradesh. Taramira, relatively more drought tolerant, is cultivated in drier parts of Rajasthan, Uttar Pradesh and Haryana. However, karan rai and gobhi sarson have limited area under cultivation in India [1].

4. Breeding approaches in rapeseed-mustard

4.1 Abiotic stresses

Plant stress factors can be elucidated as any adverse condition or substance that affects the growth, reproduction, metabolism and development of the plant [3]. Acclimatization or hardening refers to exposure of unfavorable environmental circumstance to the plant and thereby results into physiological adjustment that protects it from injury or impaired growth which is mostly occurred due to environmental stresses [9]. There might be fixed genetic changes if plant faces several generations under constant stress condition by selective environmental pressure and thereby population show adaptation to changed environment. Abiotic factors are the main yield-limiting factors for crop plants including rapeseed-mustard. The major abiotic factors are- moisture variation (drought and flood), temperature variation (heat, cold and frost), salinity and heavy metal that adversely affect the metabolic pathways and thereby result into yield penalty.

4.1.1 Drought stress

Globally, rapid climate change under anthropogenic accelerated interventions crafts drought a major menace to the agricultural production system and consequently has a great challenge to the global food and nutritional security. Plants have different ways to synergies with drought stress such as modifications in plant growth, behavior, morphology, and physiology. In *Brassica*, drought tolerance is a complex trait and thereby associated with different traits; and can be evaluated by various indicators. Moreover, it is difficult to choose all the exiting indicators at a time to use in breeding programs for crop improvement. Drought can adversely affect plant growth at various stages from seed germination to reproduction and flowering to harvesting, and ultimately results into oil and yield penalty [3]. Prolonged drought reduces chlorophyll content mostly due to impaired functioning of thylakoid membrane and heavy loss of pigments [10]. In the context, the pattern of gene expression of those traits which are associated with osmotic balance, water transport, damage repair and oxidative stress will be altered by prolonged drought stress (Table 2). Thus, drought is one of the major factors to reduce potential yield of crop plants and introgression of traits from wild relatives can be used for the development of drought resilient cultivars in rapeseed-mustard.

4.1.2 Salt stress

Recent advances in molecular breeding have been characterized and genetically mapped various salt related genes in plants. Gradual increase of the understanding of several biochemical, and physiological mechanisms and pathways of salt related genes has made it easy to develop genetically improved varieties which are more resilient and high yielding under salinity stress. In this context, transgenic approaches have also been used to know the effect of salt tolerant genes into the different genetic background by up-regulating or down-regulating genes under salt stress [33]. The progress under salt tolerance is great in major agricultural crops such as wheat, rice, mustard and tomato. A large number of gene (s)/QTLs have been mapped as well as cloned [33]. As *Brassica* crops are concerned, there are limited studies on salt regulating genes or QTLs across the world. In India, only limited salt tolerant varieties have been developed so far such as “CS56” and breeding approaches are not as much successful as to other stresses [3]. It is need of the hour to understand the mechanism of salt tolerance and to identify stable salt tolerance genotypes from available genetic resources

Species	Gene/s	Function	Tolerance	References
<i>Arabidopsis</i>	<i>DREB1A</i>	Dehydration response element binding protein	Drought, salt and freezing	[11]
	<i>SOS1</i>	Plasma membrane-bound Na ⁺ /H ⁺ antiports	Salt	[12]
	<i>AtNHX1</i>	Vacuolar Na ⁺ /H ⁺ antiporter	Salt	[13]
	<i>AtHKT1</i>	Na ⁺ transporter	Salt	[14]
	<i>FTA</i>	Farnesyltransferase	Drought	[15]
	<i>AtFTB</i>	β-subunit of Farnesyltransferase	Drought	[16]
<i>Arthrobacter globiformis</i>	<i>codA</i>	Choline oxidase	Salt	[17]
<i>B. rapa</i>	<i>BrERF4</i>	Ethylene-responsive factors	Drought and salt	[18]
	<i>BrGI</i>	Reduced expression of GI, enhanced salt tolerance	Salt	[19]
<i>B. napus</i>	<i>AtDWF4</i>	Enhanced defense gene expression	Drought and heat	[20]
	<i>BnNHX1</i> and <i>BnHKT</i>	Salt-responsive genes	Salt	[21]
	<i>BnLEA4-1</i>	Late-embryogenesis abundant proteins in group 4	Salt	[22]
	<i>BnLAS</i>	Transcriptional regulator members in GRAS family	Drought	[23]
	<i>DREB</i>	Improving the abiotic stress tolerance	Salt	[24]
	<i>BnSIP1-1</i>	Played roles in ABA synthesis and signaling	Salt and Osmotic	[25]
	<i>AnnBn1</i>	Membrane-binding proteins for Ca ²⁺	Drought	[26]
<i>B. oleracea</i> var. <i>botrytis</i>	<i>APX, SOD</i>	Protect from oxidative stress	Salt	[27]
<i>B. juncea</i> cv. <i>varuna</i>	<i>Glyoxalase I</i> <i>Lectin</i>	Catalyze the detoxification of a highly cytotoxic metabolite methylglyoxal to d-lactate	Drought and salt	[28]
<i>B. juncea</i>	<i>BrECS</i>	Glutamylcysteine synthetase	Salt	[29]
	<i>AtLEA4-1</i>	AtLEA4-1 LEA4 protein	Salt	[30]
	<i>Gly I</i>	Detoxification of methylglyoxal	Salt	[31]
	<i>AnnBj2</i>	Upregulated expression of ABA-dependent (<i>RAB18</i>) and ABA independent (<i>DREB2B</i>) genes	Salt	[32]

Table 2.
 Brief summary of abiotic stress tolerance associated genes and their functions.

by extensive screening methods to use them in breeding programs. Researchers have done excellent work on ion homeostasis and osmolytes regulation by using transgenic approach in *Brassica* crops [34] and identified few candidate genes (Table 2).

Apparently, both drought and salinity stress have few similarities in plants. Both stresses are primarily responsible for cellular dehydration, which removes water from the cytoplasm into the intercellular space [35]. Based on the functional similarity of both the stresses in plants, it can be concluded that plants have almost identical mechanism to deal with both stresses. In the present scenario, researchers are extensively working on model plant *i.e.* *A. thaliana* to understand the genetics of salt and drought stress tolerance, which can positively help to develop tolerance cultivars in *Brassica* spp. and will improve agronomically important traits [36].

4.1.3 Heat stress

As the global warming is increasing due to unwarranted human activities, heat stress has become a major factor to hamper plant growth and development in agricultural crops including rapeseed-mustard. Early sowing of Indian mustard, have various advantages as enlisted by Kaur and coworkers [37] but high temperature during the germination stage leads to reduction in the plant emergence and poor plant stand. The yield potential of Indian mustard was significantly reduced under late sown condition compared to timely sown due to terminal heat stress [38]. The reduction in emergence of Indian mustard due to hot soils can lead to substantial economic losses [39]. Where irrigation is available and multiple cropping system followed, especially in Central and North-Western plain zones, sowing of the mustard crop is delayed up to end of November due to late vacation of *Kharif* crop, leads to exposure of the crop to high temperature at maturity.

Rapeseed-mustard is adversely affected by heat stress (35/15 °C) at the early stage of flowering. Moreover, yield penalty can be avoided if high temperature occurs during early pod formation. In this context, *B. rapa* is more sensitive to high temperature whereas *B. juncea* and *B. napus* are equally affected [40]. It has been reported that optimal temperature for *B. napus* is lower than *B. juncea* and *B. rapa* [41]. Generally, as temperature increased, the number of pods produced by the plants increased and seed weight decreased. High temperature has a direct effect on the formation of reproductive organs. More research is needed under controlled environments to identify the critical temperature, sensitive reproductive organ stage, source-sink relationship, and genotypic variations for heat stress tolerance and must be verified under natural conditions [42].

4.1.4 Low temperature stress

Freezing injury has adverse effect on plant growth and development, and thereby leads to yield penalty. Seed germination is seriously affected by low temperature. Plant stress hormones such as Brassinolide (BR) regulate plant physiological pathways and helps in plant protection to combat low temperature stress [43]. Exogenous application of BR increased cold stress tolerance in *A. thaliana* and *B. napus* [44]. In this context, BR increases chlorophyll content, PS-II, antioxidant enzymatic activities and protect photosynthetic membrane system from oxidative damage [45]. It has been reported that accumulation of reactive oxygen species such as superoxide anion, hydrogen peroxide, singlet oxygen and hydroxyl radical is high under cold stress, and thereby causes oxidative stress in plants which leads to cell death [46]. The *B. rapa* has been reported more cold tolerance than *B. napus*. The impact of heat stress is high than cold stress because of inactivation of RuBisCO and/or other associated enzymes under heat stress. Intriguingly, *B. oleracea* is cold tolerant due to its acclimatization in cold regions of Europe, where summer temperature is also low and crop had domesticated since long back.

Thus, acclimatization, domestication, adaptive trans-generational plasticity and genetic adaptation phenomenon can work simultaneously to abiotic stress tolerance in *Brassica* species.

4.2 Biotic stresses

A number of biotic stresses adversely affect the yield potential of rapeseed-mustard in India. The major diseases are- *Alternaria* blight (*Alternaria brassicae* and *A. brassicicola*), white rust (*Albugo candida*), stem rot (*Sclerotinia sclerotiorum*), Rhizoctonia rot and downy mildew (*Peronospora brassicae*); and major insect pests are- aphid (*Lipaphis erysimi*), mustard saw fly (*Athalia proxima*) and painted bug (*Bagrada hilaris*). There are several methods to control insect and disease incidence such as application of pesticides, fungicides, biological agents and other non-chemical techniques. However, the most economic, eco-friendly and cheap way to mitigate these menaces are to use of resistant or tolerant cultivars through convention and molecular breeding approaches.

4.2.1 *Alternaria* blight

The yield potential of *Brassica* spp. is adversely affected by *Alternaria* blight [*Alternaria brassicae* (Berk) Sacc.] disease. The pathogen can affect the host plant at all stages of growth and highest disease severity was observed during rainy season. The *B. juncea* and *B. rapa* are more susceptible than *B. carinata* and *B. napus* to *Alternaria* blight. The researchers have reported several sources of disease tolerance such as *B. juncea* cv. Divya, and wild species such as *Sinapis alba* L., *B. maurorum*, *Diploaxis berthautii* and *D. eruroides* etc. [47]. Higher concentration of phenolic compounds (polyphenol peroxidase, oxidase and catalase), low N content, higher leaf sugar content, and more leaf wax deposition have been reported to deliver resistance to plants against *Alternaria* blight disease [48]. Pre and post fertilization barriers are major concern while using wild relatives and progenitors as donor source in rapeseed-mustard breeding programs. However, limited sources of *B. juncea* (PHR 2, RC781, Divya, PAB 9534, and EC 399301) have been reported tolerance against this disease and extensively being used in breeding programs [3].

4.2.2 White rust

White rust [*Albugo candida* (Pers.) Kuntze] is a destructive disease in *B. juncea* and *B. rapa*; and significantly reduces potential yield up to 60% in mustard [49]. Forty-nine races of *A. candida* have been reported in India based on their infectivity on different *Brassica* spp. and their cultivars [50]. Most of the varieties under Indian mustard are susceptible to white rust whereas *B. carinata* and *B. napus* demonstrate high degree of resistance. Thus, gene introgression from *B. carinata* and *B. napus* to *B. juncea* through interspecific hybridization is essential for development of resistant or tolerant cultivars in the country [51]. The varieties bred for disease tolerance are- JM-1, JM-2, DMH-1 and Basanti etc.

4.2.3 *Sclerotinia* rot

In rapeseed-mustard, *Sclerotinia* rot disease is triggered by *Sclerotinia sclerotiorum* and adversely affects plant growth and development. The disease has turned form minor significance to major one since last decade due to change in climatic condition. Pre-mature ripening is the cause of the disease. The pathogen has an array of alternate host therefore breeding for disease resistant is difficult [3].

4.2.4 Insect (Aphid)

Mustard aphid (*Lipaphis erysimi*) is one of the major insect pests in rapeseed-mustard and adversely affects plant growth, development, and reproduction; and thereby results into yield penalty. They are also act as vector for plant viral diseases such as turnip mosaic virus. There are several methods to identify resistant source for aphid resistance/tolerance in *Brassica* family such as based on seedling survival, aphid fecundity, and aphid infestation index etc. Some genotypes of *B. juncea* such as Glossy B-85, RH 7847, and T 6343 were reported more tolerant to aphid infestation. *B. campestris* is more susceptible to aphid infestation than *B. juncea* and *B. carinata* [3].

4.3 Oil quality improvement

The oil quality for human consumption is determined by its fatty acid composition and concentration. Seed oil with high proportion of unsaturated fatty acid, particularly 16 and 18 carbon chain, is considered suitable for human consumption as edible oil. Rapeseed-mustard is mostly used as oilseed crop in India and its seed contain 35-45% oil content with 92-98% triacylglycerol of fatty acids (C16-C22). Seed oil contains lowermost saturated fat and possesses high proportion of essential fatty acid such as linoleic (C18:2) and linolenic (C18:3) which are not synthesized by human body. Linolenic acid is an essential dietary fatty acid; however, its higher concentration reduces shelf-life of oil because of auto-oxidation [3]. Erucic acid (C22:1) comprises almost 50% of total seed oil fatty acid in rapeseed-mustard and is undesirable for human consumption due to its adverse role in myocardial conductance and increase the level of blood cholesterol. The level of detrimental saturated fatty acid is less in rapeseed-mustard compared to other edible oilseed crops. The major constrains in seed oil are- erucic acid and glucosinolates [52]. Therefore, reduced concentration of glucosinolates and erucic acids is one of the important objectives in quality amelioration of Indian mustard seed oil. It has been reported that genetic inheritance of glucosinolates is complex and mostly are aliphatic (methionine derived) in nature in *B. juncea*. Genetic control of total glucosinolates in *B. juncea* has been reported to be under two major genes [53], multiple additive alleles at a single locus with maternal effects involved [54], six to seven genes [55] and up to five major QTLs [56] based on molecular mapping information.

The rapeseed-mustard varieties with low erucic (<2%) and glucosinolates (<30 μ mole/g of defatted cake) are termed as double zero ("00"). The term single zero ("0") is used when variety contains only one factor either low erucic (<2%) or glucosinolates (<30 μ mole/g of defatted cake). In this context, several efforts have been made to improve oil quality of rapeseed-mustard in India since last three decades. In India, first low erucic acid ("0") variety was LES-39 (Pusa Karishma) followed by LES-1-27 (Pusa Mustard 21), LET-18 (PM 24), and LET-17 (PM-22) in *B. juncea*, whereas double zero variety was Pusa Double Zero Mustard 31 (PDZM-1).

4.4 Hybrid breeding

Rapeseed-mustard exploits high level of heterosis but employ difficulty in seed production due to complex flower structure, presence of self-compatibility and thereby self-pollination in nature, however crop also enjoyed cross-pollination (30%) by pollinators such as honey bees. The extent of heterosis was reported by Sun [57] in rapeseed-mustard during early forties and was pioneer to begin with hybridization for exploitation of hybrid vigor. Subsequently, Ogura

[58] had successfully transferred male sterile cytoplasm from radish (*Raphanus sativus* L.) to *B. juncea*. In this context, several cytoplasmic male sterility systems have been reported such as *tour* [59] in *B. napus*, *oxyrrhina* [59], *siifolia* [60], *trachystoma* [61], *moricandia* [62], *catholica* [63], *alba* [62], *lyratus* [64], *canariense* [65], *erucoides* [66], 126-1 [67] and *barthauti* [68]. Transgenic male sterility (barnase-barstar system) system was also used for exploitation of heterosis and development of hybrid varieties [69, 70]. It has been reported that large number of sterile cytoplasm is available, however only few can be utilized in heterosis due to lack of adequate and efficient fertility restoration system. Therefore, ICAR sponsored project (1989) "Promotion of Research and Development Efforts on Hybrids in Crops" which aimed for systematic and coordinated efforts for hybrid development in rapeseed-mustard in India with two CMS systems (*ogu* and *tour*) in *B. juncea* while *polima* in *B. napus*.

In India, heterosis was first reported in brown sarson (*B. rapa*) by Singh and Mehta [71]. It has been reported that the extent of heterosis is 13 to 99% in *B. juncea*, 10 to 72% in *B. napus*, 25 to 110% in *B. rapa*. Generally, hybridization between genetically distinct groups exploits high level heterosis than within group. Exploitation of high level of heterosis in plants necessitates large and usable heterosis, effective pollination control mechanism, and profitability of seed production [70]. Thus, there is urgent need to improve genetic gain and heterosis in rapeseed-mustard; genetic variability, in terms of variety, can be tested for 2-3 years across the centers in the country through All India Coordinated Research Project [72] and by result of high yielding, stress tolerance and stable variety would be produced.

4.4.1 Cytoplasmic male sterility and hybrids

A large number of CMS systems are available in rapeseed-mustard such as *Raphanus/ogu*, *tour*, *oxyrrhina*, *siifolia*, *trachystoma*, *moricandia*, *catholica*, *lyratus*, *canariense*, *erucoides*, and *barthauti* (Table 3). All the CMS sources cannot be directly used in hybridization programme due to their negative effects on plant growth and development such as chlorosis (*ogura*, *oxyrrhina* and *moricandia*), impaired flower opening (*tour*, *trachystoma* and *lyratus*), and also absence of fertility restoration. The chlorosis of three systems (*ogu*, *oxyrrhina*, *moricandia*) had been cured through somatic hybridization by fusing protoplast of chlorotic sterile and normal green plant [74]. The fertility restorer genes (*Rfs*) were identified in five CMS systems viz. *trachystoma*, *moricandia*, *catholica*, *canariense* and *lyratus* in their respective cytoplasmic donor species and restorer can be isolated simultaneously during transfer of sterile cytoplasm.

The success of hybridization programme, by using CMS system, depends upon availability of efficient fertility restoration. In rapeseed-mustard, the utmost used CMS system in India are-*Raphanus/ogu* CMS system, *B. tournefortii* CMS system, *Moricandia arvensis* CMS system, and *Erucastrum canariense* CMS system. In India, the first commercial hybrid PGSH 51 (*B. napus*) was released in 1994 based on *tour* CMS and yield was increased by 18% over the best hybrid check. The other hybrids are as follow- Hyola 401 hybrid (2000) was based on *pol* CMS system, NRCHB-506 (2008) on *mori* cytoplasm, DMH-1 (2008) on 126-1 CMS, and PAC-432 (2009) on *ogu* cytoplasm etc. The genetic engineering techniques had also utilized for the development of male sterile system to exploit the heterosis in rapeseed-mustard and develop the barnase-barstar male sterile system [69, 70]. Hybrid DMH-11 was developed by Delhi University in India which became India's first transgenic hybrid through barnase-barstar system. But DMH-11 was not released for commercial cultivation due to resistance from environmental activist in thought of its harm to environment.

CMS system	Discovered by	Year	Fertility restoration
<i>Raphanus/ogu</i>	Ogura [58]	1968	Restorer gene is available in <i>B. juncea</i>
<i>tour</i>	Rawat and Anand [59]	1979	Available in <i>B. napus</i>
<i>oxyrrhina</i>	Prakash and Chopra [73]	1988	No restoration available
<i>siifolia</i>	Rao and coworkers [60]	1994	No restoration available
<i>trachystoma</i>	Kirti and coworkers [61]	1995	Single dominant gene available for restoration
<i>moricaudia</i>	Prakash and coworkers [62]	1995	Single dominant gene reported for restoration
<i>catholica</i>	Kirti and coworkers [63]	1995	Reported but not in use
<i>alba</i>	Prakash and coworkers [62]	1995	Available in <i>B. napus</i>
<i>lyratus</i>	Banga and Banga [64]	1997	Reported but not in use
<i>canariense</i>	Prakash and coworkers [65]	2001	Reported but not in use
<i>erucoides</i>	Bhat and coworkers [66]	2006	Reported but not in use
<i>126-1</i>	Sodhi and coworkers [67]	2006	Reported in <i>B. napus</i>
<i>barthauti</i>	Bhat and coworkers [68]	2008	Reported but not in use

Table 3.
Important sources of CMS in rapeseed-mustard for hybrid seed production.

4.5 Pre-breeding

Wild progenitors and wild relatives are to be known as repository of valuable traits (quality, agronomic, biotic and abiotic stress tolerance) in crop plants but cannot be introgressed into the cultivated ones due to linkage drag, and cross-incompatibility barriers. Pre-breeding helps to identify the useful traits in wild germplasm and employ its use in breeding programs. The major objective of pre-breeding is to introduce new variation into the species of interest with minimum linkage drag. Molecular markers would play a great role to accelerate the breeding cycle, reduction in cost and time, and increase in the efficiency of introgression in pre-breeding programs [75].

Globally, India (15%) ranked second after China (17%) in terms of repository of *Brassica* germplasm. In India, National Bureau of Plant Genetic Resources (NBPGR) has contributed 4095 indigenous and 3401 exotic rapeseed-mustard accessions from 1986-2006 [76]. All the efforts have resulted into the collection of a total of 14,722 accessions of cultivated, wild relatives, wild progenitors and related species [3]. There is a wide gap between available germplasm in gene banks and its utilization in the breeding programs due to lack of available identified traits. Thus, there is urgent need to broaden the plant genetic diversity to combat anthropogenically accelerated climate change in the near future.

5. Biotechnological approaches

Rapeseed (*B. napus*), cultivated in temperate climate, have been believed to originate by natural hybridization between *B. oleracea* and *B. rapa*. *B. napus* was resynthesized by protoplast fusion of *B. oleracea* and *B. rapa* to widen genetic diversity and alter oil content. The biotechnical intervention was used either to

increase of genetic variability or transfer of desirable traits from other related species such wild relatives, wild progenitors or other unrelated crops to improve yield potential of crop which were not possible due to conventional or classical breeding methods.

5.1 Anther culture

Pollen culture can be used to develop stable homozygous lines by double haploid (DH) technique to improve agronomic traits in *B. juncea*. Improvement in culture condition and associated factors, which are limiting factor for embryo production, tend to increase efficiency of microspore culture or anther culture in *B. juncea* [77]. It has been reported that microspore culture is more successful than anther culture due to better response of genotypes for embryo culture. Microspore culture can be used for gene transfer, biochemical studies, and modification of fatty acid profile through mutagenesis [77]. The major factors which affect doubled haploid production are- isolation of microspore, culture media, embryo selection, plant regeneration, and chromosomal duplication. In India, there is no variety under cultivation of this technique.

5.2 Somaclonal variation

Somaclonal variation can be defined as genetic variation in somatic cells due to chromosomal rearrangement and regeneration of variable plants from callus by plant tissue culture. Furthermore, *B. juncea* variety Prakash produced multiple shoots in cotyledonary callus when high cytokinin and low IAA concentration was used in MS media [78]. A large genetic variation has been created in *B. juncea* by tissue culture through induced somaclonal, chemical mutagens, and gamma rays induced variation. For example, somaclone- SC-122 was developed with improvement of five traits which were associated with yield improvement [79]. In India, Pusa Jai Kisan (Bio-902) was first somaclonal derived variety in 1993 by using Varuna as a parent and yield was improved by 17.4% over the parent.

5.3 Protoplast culture

Protoplast, cell without cell wall, culture induces protoclonal variation and creates stable genetic variability in rapeseed-mustard by using tissue culture technique. This technique was used *B. juncea* cv. RLM-198 by using V-47 media for production of somatic embryo and organogenesis. This method can be used for those *Brassica* species where hybridization is not possible and will help to create genetic variability for betterment of crop improvement.

5.4 Transgenic plants

In crop species, transgenic plants have been developed by using the recombinant DNA technology. It has been widely used to transfer alien gene/chromosomal segment to the recipient parent where naturally gene of interest is absent for betterment of mankind. Various direct and indirect methods have been used for gene transfer in crop plants including rapeseed-mustard and mostly used direct method is *Agrobacterium* mediated gene transfer for seed yield, seed quality, biotic and abiotic stress tolerance and desirable agronomic traits [80]. As earlier mentioned, transgenic male sterility system was used for production of hybrids in India. Thus, these biotechnological interventions can solve the problems of conventional breeding which are mainly associated with hybridization and selection.

5.5 -Omics approaches

The world of -omics is vast and covers several disciplines such as genomics (total DNA content of organism), transcriptomics (deals with total RNA content), proteomics (deals with total proteins), and metabolomics (total metabolites of an individual). Being amphidiploid and tetraploid in nature, both *B. juncea* and *B. napus* need -omics approaches to understand the trait based genetics for improvement of these crops.

5.6 Genomics

Linkage mapping and association studies were used to identify the genomic locations of a particular trait of interest. Genomic locations were identified based on molecular markers in *Brassica* spp. For example, Mukherjee and coworkers [81] mapped genes governing white rust resistance using BSA in *B. juncea*. Padmaja and coworkers [82] mapped seed coat color gene and identified microsatellite markers, *Ra2-A11*, *Na10-A08* and *Ni4-F11* linked to seed coat color in *B. juncea*. Furthermore, Liu and coworkers [83] dissected genetic architecture for glucosinolates accumulation in seed and leaves using GWAS in *B. napus*. Kaur and coworkers [84] carried out genome wide association mapping and candidate gene analysis for pod shatter resistance in *B. juncea*. Comparative mapping was also used in rapeseed-mustard for different agronomic and quality traits. For example, Cai and coworkers [85] identified candidate gene- *BnAP2* for seed weight in *B. napus* by using comparative mapping with *A. thaliana*. Bisht and coworkers [86] identified candidate genes, *BjuA.GSL-ELONG.a*, *BjuA.GSL-ELONG.c*, *BjuA.GSL-ELONG.d*, *BjuA.GSL-ALK.a* and *BjuA.Myb28.a* for glucosinolates biosynthesis through comparative mapping among *A. thaliana*, *B. oleracea* and *B. juncea*. Genomics has been extensively used for evolutionary studies in *Brassica* spp. Couvreur and coworkers [87] used *nad4 intron 1* marker for phylogenetic analysis to study temporal diversification and establishment of evolutionary pattern in the mustard family. Furthermore, Augustine and coworkers [88] isolated four *BjuCYB83A1* genes from *B. juncea*, which involved in glucosinolates synthesis and through phylogenetic and divergence analysis they have revealed that these genes have evolved via duplication and hybridization of two diploid *Brassica* genomes i.e. *B. rapa* and *B. nigra*.

5.7 Transcriptomics

Transcriptomics contributes the comprehensive understanding about the gene expression, through which it is easy to allocate gene function and its effect on any organism. It has been used for expression studies, gene silencing, and genome editing in *Brassica* spp. for example, Heng and coworkers [89] identified *orf288* gene associated with male sterility in *B. juncea* through expression analysis of *orf288* transcript. Bhattacharya and coworkers [90] studied down regulation of BjAGPase and seed specific expression of *AtWRI1* gene of *Arabidopsis* in order to increase seed lipid content in *B. juncea*. Savadi and coworkers [91] increased seed weight and seed oil content in Indian mustard through seed specific overexpression of *DGAT1* gene of *A. thaliana*. Zhao and coworkers [92] carried out RNAi mediated gene silencing of *mutS homolog1* which results in male sterility in *B. juncea* due to sub-stoichiometric shifting in *ORF220*. Zheng and coworkers [93] carried out gene knockout experiment through CRISPR/Cas9 in *BnaMAX1* homologs of *B. napus*, which resulted in reduction in plant height and increase in branch number.

5.8 Proteomics

Proteins are the ultimate products which confer the gene function and govern the phenotypic expression to an individual. Proteomics approaches such as protein expression profiling and comparative proteomics analysis were used to study the gene function in *Brassica* spp. For example, Mihr and coworkers [94] used “*Tournefortii*” CMS system of *B. napus* to study protein content of mitochondrial compartments in male sterile and fertile NILs. Mohammadi and coworkers [95] performed comparative proteome analysis in rapeseed seedlings for root traits under draught stress and concluded that proteins such as H⁺ ATPase, HSP 90 and EF2 play a key role in draught tolerance. Yousuf and coworkers [96] identified salt stress responsive proteins in the shoots of Indian mustard genotypes through comparative proteome analysis approach. Yousuf and coworkers [97] studied different protein expression profiles of N₂ efficient and N₂ inefficient Indian mustard in response to elevated CO₂ and low N₂.

5.9 Metabolomics

Recent efforts in metabolomics have been directed to improve quality and yield of any crop. An integration of metabolomics with other approaches establishes an important relevance in crop improvement. However, metabolomics has not exploited much in mustard breeding, so it would be an emerging field of research for *Brassica* improvement. Few studies have been carried out in *B. juncea*. For example, Sinha and coworkers [98] performed metabolic engineering of fatty acid biosynthesis in order to improve nutritional quality of seed oil in Indian mustard. Kortensniemi and coworkers [99] investigated seed metabolomics using NMR in *B. napus* and *B. rapa* and found that unsaturated fatty acids, sucrose and sinapine were most discriminating metabolites.

6. Achievements

In India, 189 rapeseed-mustard varieties (118 Indian mustard; 7 karan rai; 14 gobhi sarson; 24 toria; 15 yellow sarson; 3 brown sarson; 1 black mustard; 7 taramira) were developed and released and some of them are enlisted in **Table 4**. Several CMS based hybrids were developed by government and non-government institutes. A total of 7029 accessions comprising toria (508), Indian mustard (4,600), yellow sarson (548), gobhi sarson (146), brown sarson (108), karan rai (232), taramira (67), *B. caudatus* (04), *R. caudates* (01), *B. rugose* (30), *B. nigra* (22), *S. alba* (01), *Crambe* spp. (02), and *Lapidium* spp. (02) were maintained through appropriate mating system at various coordinated centers in the country [100]. As seed oil quality is concerned, low glucosinolates content was transferred from agronomically poor exotic genetic stock of *B. juncea*, BJ-1058 to the genetic background of high yielding mustard varieties. Genetics of fatty acid profile and glucosinolates content has been worked out and gene pool for high oil content and disease resistance were developed.

7. Future outlook and strategy

To fulfill the demand of edible oil for ever increasing population, constant efforts are needed for higher production and productivity by conventional, molecular or biotechnological approaches in the country. Genetic variability is the prerequisite for crop improvement program. Moreover, there is imperative need to

Stress/situation/condition	Recommended varieties
Salinity	Indian mustard: CS-54, Pusa Vijay, NRCDR 2, CS 234-4, CS-52, Narendra Rai-1, NRCDR 601
High temperature	Indian mustard: Urvashi, RGN 13, Pusa Agrani, Kanti, PM 26, PM 27, DRMR 1165-40, NRCDR 2, NRCDR 601
High oil content	Indian mustard: Narendra Swarna Rai 8
Earliness	Indian mustard: Kanti, Narendra Ageti Rai 4, Pusa Agrani, Pusa Mahak, DRMR 150-35; Yellow sarson: NRCYS 05-01
Intercropping	Indian mustard: RH-30, RH781, Vardan
Non-traditional areas	Indian mustard: Pusa Agrani, Pusa Jai kisan, Gujarat Mustard 2, Pusa Mahak (for north-east only)
Late sown	Indian mustard: Ashirwad, RLM 619, Swaranjyoti, Vardan, Navgold, NRCHB 101
Frost tolerance	RGN13, RH-781, Swaranjyoti
Drought (Rainfed)	Indian mustard: RH-819, RH-781, GM1, Pusa Bahar, Pusa Bold, Aravali Mustard, Sej-2, JD-6, Geeta, RGN-48, RL-99-27, Shivani, PBR-9 Karan rai: Pusa Aditya, DRMR 150-35, Pusa Swarnim
Irrigated	Indian mustard: PM-28, DRMRIJ 31
Low erucic acid /glucosinolates	Indian mustard: Pusa Karishma, Pusa Mustard 21, PM 22 Gobhi Sarson: Hyola 401, GSC 5, GSC 6, NUDB 26-11, Teri Uttam Jawahar, PM 24
White rust	Indian mustard: Basanti, JM 1, JM 2, Maya, Pusa Jagannath
Powdery mildew and Alternaria blight	Indian mustard: DRMR 150-35, NRCDR 2, NRCDR 601
Wider adaptability	Indian mustard: Pusa Bold

Table 4.
Improved varieties of Indian mustard for specific environmental conditions.

diversify the genetic base of varieties by utilization of exotic germplasm as well as other wild and related species. In this context, combination of conventional plant breeding with biotechnological tools can be used for development of high yielding varieties with good oil quality and tolerance against biotic and abiotic stresses. Global warming and the climate change are very critical challenges in the near future. Efforts to develop climate resilient crop cultivars are the need of the hour. Marker assisted selection (MAS), functional genomics, phenomics, proteomics and metabolomics are the next step to develop varieties for drought and heat tolerance and breeding programs must be reoriented to meet the future challenges. Nowadays, omics breeding has emerged as a novel concept in crop improvement and upcoming era will be dominated by this approach as it is more robust and rapid as compared to conventional breeding.

Conflict of interest

“The authors declare no conflict of interest.”

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