

## MUSTARD BREEDING IN INDIA -A REVIEW

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The *Brassica* spp., which is widely recognized as rapeseed-mustard, holds considerable importance within the Indian economy owing to its substantial contributions to the cultivation of edible oils, vegetables, condiments, and animal feed. India ranks as the second largest nation worldwide in terms of land area allocated to the cultivation of rapeseed-mustard, and it occupies the third position globally in terms of production. Nevertheless, the rapeseed-mustard crop experience a decline in productivity due to the influence of anthropogenic climate change, exacerbated by the existence of abiotic stressors including drought, floods, temperature variations, and salinity. Moreover, the impact is further intensified by biotic stressors, including diseases and insects. Several methodologies, including molecular breeding, pre-breeding, -omics, and biotechnological interventions, have been employed to enhance the yield and oil quality of cultivars. Furthermore, these methodologies have the objective of augmenting the resilience of cultivars in response to climate-related challenges, while also bolstering their resistance or tolerance to both abiotic and biotic stresses. Within the current context, this chapter has presented a thorough elucidation of the various sources of cytoplasmic male sterility (CMS) and their potential application in the progression of hybrid cultivars. In conclusion, this chapter has provided a thorough overview of notable accomplishments attained by both governmental and non-governmental entities. Moreover, it has provided a succinct evaluation of the prospective strategies for advancing rapeseed-mustard cultivation in India. This paper aims to provide an overview of the current status, notable accomplishments, and research gaps in the field of Rapeseed-Mustard breeding in India.

The term *Brassica* spp. refers to a group of different species of rapeseed-mustard that play a crucial role in the Indian economy. These plants contribute to the production of edible oils, vegetables, condiments, and animal feed, making them economically significant in India (Jat *et al.*, 2019). India is home to a variety of oilseeds that play a significant role as the main sources of vegetable oil. The primary oilseed crops cultivated in the nation consist of soybean, groundnut, and rapeseed-mustard, collectively representing more than 88% of the overall oilseed production. Based on the referenced literature, it is evident that the dominant contributors to the production of edible oil in the nation are rapeseed-mustard, accounting for 31% of the total production. Subsequently,

soybean and groundnut make up 26% and 25% of the production, respectively.

Rapeseed-mustard is widely acknowledged as a prominent oilseed crop on a global scale, occupying the third position in terms of its importance as an edible oilseed, preceded only by soybean and palm oil. Based on the data provided by the United States Department of Agriculture (USDA) for the time frame of 2018-2019, the worldwide cultivation of the specific crop encompassed an area surpassing 36.6 million hectares. The intensive cultivation practices employed led to a cumulative output of 72.4 million metric tons, achieving a productivity rate of 19.8 quintals ha<sup>-1</sup>. India accounts for approximately 19.8% of the total land area on a global scale, while its contribution to the overall agricultural output stands at 9.8%. Rapeseed-mustard, boasting an annual production volume of 8.3 million metric tons, occupies the esteemed rank of being the third most prominent oilseed crop in India. According to the data from MAFW 2018-19, soybean exhibits a production volume of 13.6 million metric tons, while groundnut demonstrates a production volume of 9.1 million metric tons. The cultivation of rapeseed-mustard in India is widespread in various agro-climatic regions, encompassing the North-East, North-West, Central, and Southern states. This particular crop is cultivated under diverse conditions, including sole crop or mixed crop systems, employing different planting timings (early, timely, or late), and in both rainfed and irrigated environments. Furthermore, the cultivation of rapeseed-mustard is observed in soils exhibiting diverse degrees of salinity or alkalinity (Chauhan *et al.*, 2011). Based on the aggregated data spanning from 2014-2015 to 2018-2019 pertaining to the geographical area and crop production, it is evident that the prominent states involved in the cultivation of rapeseed-mustard are Rajasthan, Madhya Pradesh, and Uttar Pradesh. Rajasthan holds the predominant portion, contributing 44.9% of the overall rapeseed-mustard production, encompassing an area of 40.7%. The state of Madhya Pradesh exhibits a production contribution of 11.3% from an agricultural area encompassing 11.9%. Similarly, Uttar Pradesh demonstrates a production output of 10.6% from an agricultural area spanning 11.2%. In the Indian context, the rapeseed-mustard crops encompass a collective of eight distinct species, specifically Indian mustard, toria, black mustard, yellow sarson, brown sarson, gobhisarson, karan rai, and taramira (as illustrated in Table 1).

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**Table 1. *Brassica* spp. genetic information**

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

### 1. Origin

Numerous ancient manuscripts mention the cultivation of *Brassica* spp., which is estimated to have begun as early as 5000 BC. According to reports, mustard crops were cultivated in Channhu-daro of the Harrapan ancient civilization between 2300-1750 BC (Allchin, 1969). The historical origins of *B. juncea* are subject to ambiguity. The prevailing belief is that the Middle-East serves as the center of origin for *B. juncea*, where it is hypothesized that the putative parents, namely *B. nigra* and *B. rapa*, would have undergone a cross-breeding event. Subsequently, the dissemination of this phenomenon extended to various regions across the globe, including Europe, Asia, Africa, and others (Hemingway, 1979). Currently, there exist two primary regions characterized by a significant level of diversity, namely China and Eastern India. This diversity is primarily attributed to the abundance of wild progenitors and closely related species found in these areas. Currently, empirical evidence has demonstrated the existence of two distinct geographical races, namely Chinese and Indian, within the *B. juncea* species. This conclusion has been reached through the application of molecular and biochemical research methods (Song, 1988).

In 1935, Nagaharu U. (Nagaharu, 1935) introduced a conceptual framework referred to as U's triangle to elucidate genetic associations. This was accomplished through the implementation of controlled inter-specific hybridization experiments involving six distinct *Brassica* species: *B. rapa*, *B. nigra*, *B. oleracea*, *B. carinata*, *B. napus*, and *B. juncea*. The emergence of three allotetrapolyploid species (*B. napus*, *B. juncea*, and *B. carinata*) can be elucidated by the occurrence of natural hybridization involving three fundamental diploid species (*B. rapa*, *B. nigra*, and *B. oleracea*), followed by genome duplication, in accordance with theoretical principles. The hypothesis currently under examination has witnessed a growing level of acceptance in recent years, primarily attributed to the advancements made

in the field of genome sequencing of *Brassica taxa*. Moreover, the scientific demonstration of the derivation of allotetraploid *B. napus* and *B. juncea* from their diploid progenitors has been achieved by employing comparative genomic analysis. The results presented in this research are consistent with the theoretical framework referred to as the 'U' triangle, as postulated by Yang *et al.* (2016).

### 2. Distribution

The taxonomic classification known as Brassica encompasses a wide array of cultivated plant species, exhibiting significant diversity. Indian mustard, scientifically known as *Brassica juncea*, is a widely prevalent agricultural crop, occupying a significant portion of cultivated land, exceeding 90%. The cultivation of this particular crop is predominantly practiced in the North-Western states of India, with some cultivation also observed in non-traditional regions of the Central and Southern states (Jat *et al.*, 2019). The pollination mechanisms employed by the lotni and tora ecotypes of the brown sarson plant exhibit dissimilarities. The lotni ecotype exhibits cross-pollination, while the tora ecotype demonstrates self-pollination. The cultivation of the former variety is primarily concentrated in the temperate areas of the country, such as J&K, and the mountainous areas of Himachal Pradesh. In contrast, the latter type is cultivated in particular areas of eastern Uttar Pradesh, as noted by Chauhan *et al.* (2011). The cultivation of yellow sarson is primarily concentrated in the regions of Bihar, West Bengal, and Orissa. Toria is primarily cultivated as a short-term crop in various regions of Bihar, West Bengal, Orissa, and Assam. The cultivation of this specific crop has been employed as a capture crop in several Indian states, namely Haryana, Himachal Pradesh, Madhya Pradesh, Punjab, Uttarakhand, and Western Uttar Pradesh. Taramira, a plant known for its comparatively heightened tolerance to drought, is cultivated in regions that are distinguished by arid climatic conditions, specifically Rajasthan, Uttar Pradesh, and Haryana. Based on a study conducted in India,

Jat *et al.* (2019) discovered that the cultivation of karan rai and gobhisarson exhibits constraints in terms of land utilization.

### 3. Breeding approaches in rapeseed mustard

#### 3.1. Abiotic stress

Plant stress factors can be defined as any detrimental condition or substance that has an impact on the growth, reproduction, metabolism, and development of plants (Chauhan *et al.*, 2011). Acclimatization, also known as hardening, is the process by which a plant is exposed to adverse environmental conditions. This exposure leads to physiological adjustments that protect the plant from potential harm or hindered growth, primarily caused by environmental stresses (Crisp *et al.*, 2016). It is possible for plants to undergo fixed genetic changes when exposed to constant stress conditions over multiple generations, as a result of selective environmental pressure. This can lead to the population adapting to the altered environment. The primary factors that restrict crop plant yields, such as rapeseed-mustard, are abiotic in nature. The primary abiotic factors encompass moisture fluctuations, such as drought and flooding, temperature variations including heat, cold, and frost, as well as salinity and heavy metal presence. These factors have a detrimental impact on metabolic pathways, leading to a reduction in crop yield.

##### 3.1.1. The impact of drought stress

Globally, the accelerated interventions caused by human activities have led to rapid climate change, which

poses a significant threat to the agricultural production system. As a result, drought has emerged as a major challenge to global food security and nutrition. Plants exhibit various mechanisms to effectively respond to drought stress, including adaptations in plant growth, behaviour, morphology, and physiology. Drought tolerance in *Brassica* is a multifaceted trait that is linked to various traits and can be assessed using diverse indicators. Furthermore, the selection of all available indicators simultaneously for implementation in breeding programs aimed at enhancing crop improvement poses a challenging task. Drought has the potential to negatively impact plant growth across multiple stages, including seed germination, reproduction and flowering, and harvesting. Ultimately, this can lead to reduced oil production and yield (Chauhan *et al.*, 2011). The chlorophyll content is significantly diminished during extended periods of drought primarily as a result of compromised thylakoid membrane functionality and substantial pigment loss (Champolivier *et al.*, 1996). In the given context, prolonged drought stress is expected to induce alterations in the pattern of gene expression related to osmotic balance, water transport, damage repair, and oxidative stress, as indicated in Table 2. Therefore, the occurrence of drought is a significant determinant in diminishing the potential crop yield of plant species. In order to address this issue, the incorporation of genetic traits from wild relatives can be employed as a strategy for the cultivation of drought-tolerant cultivars in the rapeseed-mustard crop.

**Table 2. Major genes along with their function**

Species	Gene/s	Function	Tolerance	References	
<i>B. rapa</i>	<i>DREB1A</i>	DREB protein	Drought, salt and freezing	(Kasuga <i>et al.</i> , 1999)	
	<i>SOS1</i>	Plasma membrane Na <sup>+</sup> /H <sup>+</sup> antiports	Salt	(Shi <i>et al.</i> , 2000)	
	<i>AtNHX1</i>	Na <sup>+</sup> /H <sup>+</sup> antiporter in vacuoles	Salt	(Zhang <i>et al.</i> , 2001)	
	<i>AtHKT1</i>	Na <sup>+</sup> transporter	Salt	(Berthomieu <i>et al.</i> , 2003)	
	<i>FTA</i>	Farnesyltransferase	Drought	(Wang <i>et al.</i> , 2005)	
	<i>AtFTB</i>	Farnesyltransferase $\alpha$ -subunit	Drought	(Wang <i>et al.</i> , 2009)	
	<i>codA</i>	Choline oxidase	Salt	(Wang <i>et al.</i> , 2010)	
	<i>BrERF4</i>	Ethylene-responsive factors	Drought and salt	(Seo <i>et al.</i> , 2010)	
	<i>BrGI</i>	Reduced GI, increased salt tolerance	Salt	(Kim <i>et al.</i> , 2016)	
	<i>B. napus</i>	<i>AtDWF4</i>	Increased defensive gene expression	Drought and heat	(Maqbool <i>et al.</i> , 2002)
		<i>BnNHX1</i> and <i>BnHKT</i>	Salt-responsive genes	Salt	(Agarwal <i>et al.</i> , 2006)
<i>BnLEA4-1</i>		Late-embryogenesis large proteins in group 4	Salt	(Dalal <i>et al.</i> , 2009)	
<i>BnLAS</i>		Members of the GRAS family act as transcriptional regulators	Drought	(Yang <i>et al.</i> , 2011)	
<i>DREB</i>		Improving the abiotic stress tolerance	Salt	(Lata <i>et al.</i> , 2011)	
<i>BnSIP1-1</i>		ABA synthesis and signaling.	Salt and Osmotic	(Luo <i>et al.</i> , 2017)	
<i>AnnBn1</i>		Calcium-binding proteins	Drought	(Xiao <i>et al.</i> , 2012)	
<i>APX, SOD</i>		Antioxidant protection	Salt	(Ali <i>et al.</i> , 2016)	
<i>B. oleracea</i> var. <i>botrytis</i>	<i>Glyoxalase I Lectin</i>	Detoxify highly cytotoxic methylglyoxal to d-lactate.	Drought and salt	(Shinwari <i>et al.</i> , 1998)	
<i>B. juncea</i> cv. <i>varuna</i>	<i>BrECS</i>	Glutamylcysteine synthetase	Salt	(Bae <i>et al.</i> , 2013)	
	<i>AtLEA4-1</i>	AtLEA4-1 LEA4 protein	Salt	(Saha <i>et al.</i> , 2016)	
	<i>Gly I</i>	Detoxification of methylglyoxal	Salt	(Rajwanshi <i>et al.</i> , 2016)	
	<i>AnnBj2</i>	The expression of ABA-dependent gene RAB18 and ABA-independent gene DREB2B was found to be upregulated.	Salt	(Ahmed <i>et al.</i> , 2017)	

### 3.1.2. Salt stress

In the past few years, notable advancements have been achieved in the domain of molecular breeding, specifically in the realm of identifying and genetically mapping genes linked to salt tolerance in plants. The comprehension of diverse biochemical and physiological mechanisms and pathways associated with genes related to salt has facilitated the creation of genetically modified cultivars that exhibit enhanced resistance and productivity in the presence of stress induced by salinity. In the present context, transgenic methodologies have been employed to investigate the impact of salt-tolerant genes on diverse genetic backgrounds through the manipulation of gene expression in response to salt-induced stress (Ren *et al.*, 2005). Significant advancements in salt tolerance have been observed in important agricultural commodities, including wheat, rice, mustard, and tomato. In their study, Ren *et al.* (2005) effectively conducted gene mapping and cloning, resulting in the identification of a significant number of genes and quantitative trait loci (QTLs). The current state of research on salt-regulating genes or quantitative trait loci (QTLs) in Brassica crops is characterized by a dearth of global studies. The advancement of salt-tolerant varieties in India has been somewhat constrained, resulting in a limited number of favorable outcomes, notably the “CS56” variety. Nevertheless, the efficacy of breeding strategies in augmenting salt tolerance has been comparatively less successful in comparison to other stressors (Chauhan *et al.*, 2011). In order to successfully incorporate pre-existing genetic resources into breeding programs, it is imperative to acquire a comprehensive comprehension of the underlying mechanism that governs salt tolerance, as well as to identify genetically stable individuals that demonstrate this desirable trait. The successful execution of this task necessitates the adoption of rigorous screening protocols. The researchers Zhang *et al.* (2004) have conducted extensive investigations into the mechanisms of ion homeostasis and osmolyte regulation in Brassica plants, employing transgenic technology as a key tool in their studies. As illustrated in Table 2, a restricted quantity of potential genes has been effectively identified.

There exists a limited number of similarities between arid stress and salt stress in plants. The transport of water from the cytoplasm to the intercellular space, leading to cellular dehydration, is primarily caused by two stressors, as identified by Farooq *et al.* (2001). The presence of functional similarity in the stresses experienced by plants implies that plants possess comparable mechanisms for effectively managing these stresses. Presently, researchers are actively engaged in the examination of the model organism *Arabidopsis thaliana* with the aim of gaining insights into the genetic mechanisms underlying salt and drought tolerance. The knowledge that has been obtained possesses the potential to make a substantial contribution towards the advancement of cultivars belonging to the Brassica species. This contribution would manifest in the form of enhanced tolerance to a wide range of stressors.

Consequently, the upregulation of agronomically significant traits would be enhanced (Roze and Flowers, 2008).

### 3.1.3. Heat stress

The rise in global warming caused by human activities has led to a notable augmentation in heat stress, which has emerged as a significant impediment to the cultivation and maturation of agricultural crops, such as rapeseed-mustard. Kaur *et al.* (2009) assert that the early sowing of Indian mustard during the growing season offers numerous benefits. It is imperative to acknowledge, nevertheless, that heightened temperatures during the germination phase can impede plant emergence and lead to a suboptimal plant establishment. According to Patidar *et al.* (2020), the yield potential of Indian mustard was found to be considerably diminished when sown late as opposed to being sown at the appropriate time. This decline in yield can be attributed primarily to the occurrence of terminal heat stress. The reduced emergence of Indian mustard caused by higher soil temperatures can lead to substantial economic losses (Azharudheen *et al.*, 2013). In areas where irrigation infrastructure is present and a multi-cropping system is employed, particularly in the Central and North-Western plain regions, the commencement of mustard sowing is often postponed until the conclusion of November. The primary cause of this delay can be attributed to the delayed harvesting of the *kharif* crop. As a result, mustard plants experience increased temperatures throughout their maturation phase.

The early stage of flowering in rapeseed-mustard is negatively impacted by heat stress occurring at temperatures of 35/15 °C. Furthermore, the occurrence of high temperatures during the early stage of pod formation presents an opportunity to prevent yield penalties. In the given context, it has been observed that *B. rapa* exhibits a higher degree of sensitivity to elevated temperatures, while *B. juncea* and *B. napus* demonstrate comparable levels of susceptibility (Angadi *et al.*, 2000). According to a report, the optimal temperature for *B. napus* has been found to be lower as compared to *B. juncea* and *B. Rapa* (Young *et al.*, 2004). In general, an increase in temperature was observed to correspond with an increase in the number of pods produced by the plants, while simultaneously resulting in a decrease in seed weight. Elevated temperatures exert a direct influence on the development of reproductive structures. Further investigation is required within controlled settings in order to ascertain the crucial temperature, the stage of reproductive organs that is particularly susceptible to heat stress, the relationship between source and sink, and the variations in genotype that contribute to heat stress tolerance. These findings must then be validated in natural conditions (Kumar *et al.*, 2013).

### 3.1.4. Low temperature stress

The occurrence of freezing injury has a detrimental effect on the growth and maturation of plants, leading to a decrease in crop yield. The germination of seeds is significantly impacted by low temperatures. Brassinolide (BR), a phytohormone involved in plant stress response,

plays a pivotal role in regulating various physiological processes and serves as a key component in plants' defense mechanisms against low temperature stress (Chen *et al.*, 2018). A previous study conducted by Kagale *et al.* (2007) observed that the application of exogenous BR resulted in an enhanced tolerance to cold stress in *Arabidopsis thaliana* and *Brassica napus*. The supplementation of brassinosteroids (BR) has been observed to result in an elevation of chlorophyll content, PS-II activity, antioxidant enzymatic activities, and a safeguarding effect on the photosynthetic membrane system against oxidative damage within the present context (Zhang *et al.*, 2020). During episodes of cold stress, there is a significant increase in reactive oxygen species (ROS), such as the superoxide anion, hydrogen peroxide, singlet oxygen, and hydroxyl radical. Consequently, plants undergo oxidative stress, leading to cellular demise (Marcec *et al.*, 2019), as a consequence of heightened accumulation of reactive oxygen species (ROS). Prior studies have indicated that *Brassica rapa* exhibits a greater degree of cold tolerance in comparison to *Brassica napus*. The inactivation of *RuBisCO* and other associated enzymes due to elevated temperatures results in more severe consequences of heat stress compared to cold stress. The cold resistance observed in *Brassica oleracea* is of great importance due to its adaptation to cold regions in Europe, where it has experienced prolonged exposure to high temperatures during the summer and undergone domestication for agricultural purposes.

Hence, the concomitant mechanisms of domestication, acclimatization, trans-generational plasticity, and genetic adaptation collectively contribute to the development of abiotic stress tolerance in Brassica species.

### 3.2. Biotic stress

The rapeseed-mustard production in India is subject to substantial influence from various biotic stress factors. The most common diseases that affect brassica crops include Alternaria blight, caused by *Alternaria brassicae* and *A. brassicicola*, white rust, caused by *Albugo candida*, stem rot, caused by *Sclerotinia sclerotiorum*, *Rhizoctonia* rot, and downy mildew, caused by *Peronospora brassicae*. Furthermore, it is imperative to highlight the presence of a diverse array of noteworthy insect parasites, including the aphid species *Lipaphis erysimi*, the mustard sawfly species *Athaliaproxima*, and the painted bug *Bagrada hilaris*. Each of these insects is taxonomically classified as an aphid, a mustard sawfly, or a painted bug. To effectively manage the occurrence of pests and diseases, a diverse range of strategies can be employed, encompassing the utilization of pesticides, fungicides, biological agents, and alternative methods that do not rely on chemical substances. The utilization of cultivars that exhibit resistance or tolerance, achieved through traditional or molecular breeding methods, represents the most economically efficient and ecologically sustainable approach for addressing these challenges.

#### 3.2.1. Alternaria blight

The agricultural production of various Brassica species is adversely affected by Alternaria blight, a plant

disease caused by the pathogen *Alternaria brassicae* (Berk) Sacc. The observed phenomenon can be attributed to the infection of the plant by a fungus, resulting in a noticeable alteration in the morphology of the plant's foliage. The pathogenic agent possesses the ability to induce harm to the host plant at any point in its life cycle; nevertheless, the manifestations of the disease tend to be most pronounced during periods of increased precipitation throughout the year. The susceptibility of *Brassica carinata* and *Brassica napus* to Alternaria blight is higher compared to that of *Brassica juncea* and *Brassica rapa*. However, it is more probable for *Brassica juncea* and *Brassica rapa* to be susceptible to Alternaria blight compared to *Brassica juncea* and *Brassica napus*. The researchers achieved success in identifying the origins of disease resistance. In addition to a variety of wild species, including *Sinapis alba* (L.), *B. maurorum*, *Diplotaxis berthautii*, and *D. eruroides*, these sources also encompass *B. juncea* cv. *Divya* (Sharma *et al.*, 2002). The study conducted by Kumar and Chakravarty (2008) revealed that plants exhibiting elevated levels of phenolic compounds, including polyphenol peroxidase, oxidase, and catalase, along with reduced nitrogen content, higher leaf sugar content, and increased deposition of leaf wax, demonstrated enhanced resistance against the Alternaria blight disease. The incorporation of wild relatives and progenitors as potential donors in rapeseed-mustard breeding programs encounters significant challenges arising from pre-fertilization and post-fertilization barriers. As a consequence of these impediments, the plants are incapable of engaging in reciprocal fertilization. Nevertheless, prior studies have effectively identified numerous distinct sources of *B. juncea* (PHR 2, RC781, Divya, PAB 9534, and EC 399301) that exhibit resistance to this specific disease. Chauhan *et al.* (2011) reported that several other breeding programs have extensively incorporated the sources referenced in this study. The citations can be located within the confines of this scholarly article.

#### 3.2.2. White rust

White rust, also known as *Albugo candida* (Pers.) Kuntze, is a pathogen that poses a significant threat to the agricultural cultivation of *Brassica juncea* and *Brassica rapa*. It has been demonstrated that mustard plants can experience yield reductions of up to sixty per cent as a direct consequence of the diseased condition that was discussed earlier (Dev *et al.*, 2020). The occurrence of this event has been documented. In India, a database of forty-nine different genotypes of *A. candida* has been created. This record includes information on the infectivity of each genotype on a number of different cultivars and species of Brassica (Chauhan and Sharma, 2001). This record was assembled in the country of India. White rust is a disease that has the potential to harm a considerable number of the cultivars that are used to create Indian mustard. On the other hand, *B. carinata* and *B. napus* show exceptionally high levels of resistance to the disease. The process of gene introgression from *Brassica carinata* and *Brassica napus* into *Brassica juncea* through interspecific hybridization has a significant influence in the development

of cultivars in the United States that are resistant or tolerant to a variety of environmental conditions, according to research that was conducted by Ramos *et al.* (2009) published in the journal *Molecular Breeding* that several disease-resistant cultivars have been established through the process of selective breeding, which has led to their widespread availability. JM-1, JM-2, DMH-1, and Basanti are some of the cultivars that fall under this category.

### 3.2.3. Sclerotinia rot

The occurrence of Sclerotinia rot disease in rapeseed-mustard is initiated by the pathogenic fungus *Sclerotinia sclerotiorum*, leading to detrimental consequences on the overall growth and development of the affected plants. The disease has transitioned from having minor significance to assuming a more prominent role over the past decade, primarily attributed to shifts in climatic conditions. The disease is caused by premature ripening. The presence of multiple alternate hosts for the pathogen complicates the breeding process aimed at developing disease-resistant varieties (Chauhan *et al.*, 2011).

### 3.2.4. Insect (Aphid)

The *Lipaphis erysimi*, commonly referred to as mustard aphids, pose a significant threat to rapeseed and mustard crops as a highly destructive insect pest. The deleterious effects of this phenomenon on the physiological mechanisms governing plant growth, development, and reproductive processes culminate in a consequential decline in overall crop productivity. Additionally, they bear the responsibility of disseminating viral infections that impact plant species, such as the turnip mosaic virus. Multiple methodologies can be employed to ascertain the presence of aphid-resistant or aphid-tolerant genetic resources within the *Brassica* genus. Included in the repertoire of techniques are the assessment of seedling survival, the quantification of aphid fecundity, and the evaluation of the aphid infestation index. There exists empirical evidence suggesting that specific genotypes of *B. juncea*, namely Glossy B-85, RH 7847, and T 6343, exhibit elevated levels of tolerance in the face of aphid infestation. The genotypes under investigation are encompassed within the scope of this study. According to a study conducted by Chauhan *et al.* (2011), *B. campestris* exhibits a higher susceptibility to aphid infestation when compared to *B. juncea* and *B. carinata*.

## 3.3. Oil quality improvement

The assessment of the appropriateness of oil for human consumption relies on its fatty acid composition and concentration. Seed oil that possesses a notable quantity of unsaturated fatty acids, particularly those characterized by carbon chains of 16 and 18, is considered a feasible alternative for human consumption as a food-grade oil. Rapeseed-mustard is primarily employed as an oilseed crop in India, distinguished by the oil content of its seeds, which typically falls within the range of 35% to 45%. The oil derived from these seeds is predominantly composed of triacylglycerols, which are comprised of fatty acids

primarily ranging from carbon chain lengths of C16 to C22. These fatty acids make up approximately 92% to 98% of the overall composition. Seed oil exhibits a diminished quantity of saturated fat and a notable concentration of essential fatty acids, namely linoleic acid (C18:2) and linolenic acid (C18:3), which are not naturally produced within the human body. The classification of linolenic acid as an essential dietary fatty acid is well-established in academic literature. However, it has been observed in various studies, such as the one conducted by Chauhan *et al.* (2011), that an increased concentration of linolenic acid can lead to a decrease in the longevity of oil. This decrease is primarily attributed to the occurrence of auto-oxidation. Approximately 50% of the total fatty acid content in rapeseed-mustard seed oil is attributed to the occurrence of erucic acid (C22:1). Nevertheless, erucic acid is deemed inappropriate for human consumption due to its adverse effects on myocardial conductance and its capacity to raise levels of blood cholesterol. The rapeseed-mustard plant demonstrates a comparatively reduced level of detrimental saturated fatty acids when compared to other oilseed crops that are cultivated for human consumption. The main constraints linked to seed oil pertain to the presence of erucic acid and glucosinolates (Yoshie *et al.*, 2008). Therefore, a primary objective in improving the overall quality of Indian mustard seed oil is to reduce the concentrations of glucosinolates and erucic acids. Based on available reports, the genetic transmission of glucosinolates in *B. juncea* exhibits a complex nature, primarily featuring the prevalence of aliphatic glucosinolates that originate from methionine. The influence of genetic factors on the regulation of total glucosinolates in *B. juncea* has been extensively studied. Previous research has identified two prominent genes (Love *et al.*, 1990a), multiple additive alleles at a single locus with the contribution of maternal effects (Love *et al.*, 1990b), as well as six to seven genes (Sodhi *et al.*, 2002) and up to five major quantitative trait loci (QTLs) (Mahmood *et al.*, 2003), as evidenced by molecular mapping data.

The rapeseed-mustard cultivars commonly known as double zero (“00”) varieties are characterized by their low levels of erucic acid (less than 2%) and glucosinolates (less than 30 micromoles g<sup>-1</sup> of defatted cake). The term “single zero” is employed to denote a specific type of variety characterized by the presence of only one trait. In a more precise manner, this particular variety exhibits either a reduced erucic acid concentration, measuring below 2%, or a glucosinolates content that is less than 30 mole g<sup>-1</sup> of defatted cake. Both of mole g<sup>-1</sup> these values are regarded as having negligible levels of glucosinolates. In the current context, India has implemented various endeavours in the past thirty years to improve the quality of oil derived from rapeseed and mustard seeds. The initial stride in the development of a low erucic acid (“0”) variety within the context of Indian agriculture was made through the introduction of LES-39 (Pusa Karishma). Subsequently, in *Brassica juncea*, the subsequent introduction of LES-1-27 (also known as Pusa Mustard 21), LET-18 (PM 24), and

LET-17 (PM-22) occurred. Furthermore, the significance of the Pusa Double Zero Mustard 31 (PDZM-1) cannot be overstated in the successful attainment of the objective to effectively introduce a cultivar possessing a double zero genotype.

### 3.4. Hybrid breeding

The rapeseed-mustard plant demonstrates a notable level of heterosis, but faces difficulties in seed production due to its complex flower structure and the presence of self-compatibility, which results in self-pollination. Nevertheless, this particular crop also experiences advantages from the process of cross-pollination, which is made possible through the assistance of pollinators like honey bees. It is estimated that these pollinators contribute to approximately 30% of the overall pollination for this crop. Sun (1943) documented the extent of heterosis in rapeseed-mustard during the 1940s, which represented a significant advancement in the field of hybridization aimed at exploiting the benefits of hybrid vigor. Subsequently, Ogura (1968) accomplished the effective transfer of male sterile cytoplasm from radish (*Raphanus sativus* L.) to *B. juncea*. Numerous instances of cytoplasmic male sterility systems have been documented within the context of this specific framework. The aforementioned species encompass *tour* (Rawat and Anand, 1979) in *B. napus*, *oxyrrhina* (Rawat and Anand, 1979), *siifolia* (Rao *et al.*, 1994), *trachystoma* (Kirti *et al.*, 1995), *moricaudia* (Prakash *et al.*, 1995), *catholica* (Kirti, 1995), *alba* (Prakash *et al.*, 1995), *lyratus* (Banga and Bnaga, 1997), *canariense* (Parkash *et al.*, 2001), *erucooides* (Bhat *et al.*, 2006), 126-1 (Sodhi *et al.*, 2006), and *barthauti* (Bhat *et al.*, 2008). The application of the transgenic male sterility system, specifically the barnase-barstar system, has been utilized in the endeavour to exploit heterosis and generate hybrid varieties (Jagannath *et al.*, 2002, Chand *et al.*, 2018). Based on available reports, it has been observed that there exists a notable surplus of sterile cytoplasm. However, the application of this cytoplasm in heterosis is constrained by the inadequate presence of a viable and efficient fertility restoration mechanism. The project titled "Promotion of Research and Development Efforts on Hybrids in Crops," sponsored by the Indian Council of Agricultural Research (ICAR) in 1989, aimed to facilitate coordinated and harmonized efforts towards the development of hybrids in rapeseed-mustard crops within India. The primary objective of this study was to investigate two cytoplasmic male sterility (CMS) systems, *ogu* and *tour*, in *Brassica juncea*, along with the *polima* CMS system in *Brassica napus*.

Singh and Mehta (1954) initially documented the occurrence of heterosis in brown sarson (*B. rapa*) within the Indian context. Based on the existing literature, it has been observed that the extent of heterosis in *Brassica juncea* ranges from 13% to 99%. Similarly, in *Brassica napus*, the magnitude of heterosis varies from 10% to 72%, and in *Brassica rapa*, it spans from 25% to 110%. Typically, the phenomenon of hybridization between genetically disparate groups tends to yield a greater degree of heterosis in

comparison to hybridization occurring within a singular group. In order to effectively harness the considerable benefits of heterosis in plants, several crucial elements must be taken into consideration. These include the presence of a substantial and feasible degree of heterosis, the implementation of an efficient pollination control mechanism, and the economic feasibility of seed production (70). Hence, it is crucial to augment genetic progress and heterosis in the cultivation of rapeseed-mustard. The assessment of genetic variability, particularly in relation to diversity, can be carried out over a period of 2-3 years at multiple research institutions throughout the country via the All India Coordinated Research Project (Chand *et al.*, 2020). This methodology has the potential to generate plant varieties that exhibit superior performance, resilience to stress, and consistent traits.

#### 3.4.1. Cytoplasmic male sterility and hybrids

Rapeseed-mustard exhibits a diverse range of CMS (cytoplasmic male sterility) systems, which encompass *raphanus/ogu*, *tour*, *oxyrrhina*, *siifolia*, *trachystoma*, *moricaudia*, *catholica*, *lyratus*, *canariense*, *erucooides*, and *barthauti*, as outlined in Table 3. The presence of CMS sources has been observed to induce chlorosis in *ogura*, *oxyrrhina*, and *moricaudia*, as well as restrict flower opening in *tour*, *trachystoma*, and *lyratus*. Furthermore, these CMS sources have been found to result in a loss of fertility, rendering them unsuitable for hybridization endeavors. Somatic hybridization was employed as a method to address chlorosis in *ogu*, *oxyrrhina*, and *moricaudia*. The technique described in the study conducted by Kirti *et al.* (1993) involved the fusion of regular green and chlorotic sterile plant protoplasts. The plant species *Trachystoma*, *Moricaudia*, *Catholica*, *Canariense*, and *Lyratus* were employed in the identification of fertility restorer genes (*Rfs*). The process of transferring sterile cytoplasm involves the isolation of restorative genes. The aforementioned genes were discovered within the cytoplasmic donor species.

Hybridization programs that utilize the cytoplasmic male sterility (CMS) method necessitate the implementation of efficient fertility restoration techniques. The predominant CMS (Cytoplasmic Male Sterility) systems employed in rapeseed-mustard agriculture in India include the *Raphanus/ogu*, *B. tournefortii*, *Moricaudia arvensis*, and *Erucastrum canariense* systems. In the year 1994, India implemented the commercial hybrid PGSH 51, which is derived from *Brassica napus*, utilizing the *tour* cytoplasmic male sterility (CMS) system. The hybrid in question exhibited a notable increase in yield, with a growth rate of 18% when compared to the standard hybrid variety. Hybrids encompass a variety of entities. The Hyola 401 hybrid, introduced in 2000, employed the *pol* CMS system. The studies conducted by NRCHB-506 (2008), DMH-1 (2008), and PAC-432 (2009) involved the utilization of the *mori*, 126-1, and *ogucytoplasm*s. The *barnase-barstar* male sterile system was created through the utilization of genetic engineering techniques, specifically in the context of rapeseed-mustard heterosis (Jagannath *et al.*, 2002; Chand *et al.*, 2018).

Researchers from Delhi University successfully produced India's inaugural transgenic hybrid, DMH-11, by employing the *barnase-barstar* system. The commercial production of

DMH-11 was impeded due to the environmental concerns raised by various environmental groups.

**Table 3. CMS systems introduction**

Year	CMS system	Fertility restoration	Discovered by	Reference
1968	<i>Raphanus/ogu</i>	Restorer gene is available in <i>B. juncea</i>	Ogura	(Ogura, 1968)
1979	<i>tour</i>	Available in <i>B. napus</i>	Rawat and Anand	(Rawat and Anand, 1979)
1988	<i>oxyrrhina</i>	No restoration available	Prakash and Chopra	(Parkash and Chopra, 1988)
1994	<i>siifolia</i>	No restoration available	Rao and coworkers	(Rao <i>et al.</i> ,1994)
1995	<i>trachystoma</i>	just one dominant gene is restoreable	Kirti and coworkers	(Kirti <i>et al.</i> ,1995)
1995	<i>moricaudia</i>	just one dominant gene is restoreable	Prakash and coworkers	(Prakash <i>et al.</i> ,1995)
1995	<i>catholica</i>	Reported but not in use	Kirti and coworkers	(Kirti, 1995)
1995	<i>alba</i>	Available in <i>B. napus</i>	Prakash and coworkers	(Prakash <i>et al.</i> ,1995)
1997	<i>lyratus</i>	Reported but not in use	Banga and Banga	(Banga and Bnaga, 1997)
2001	<i>canariense</i>	Reported but not in use	Prakash and coworkers	(Parkash <i>et al.</i> ,2001)
2006	<i>erucoides</i>	Reported but not in use	Bhat and coworkers	(Bhat <i>et al.</i> ,2006)
2006	<i>126-1</i>	Reported in <i>B. napus</i>	Sodhi and coworkers	(Sodhi <i>et al.</i> ,2006)
2008	<i>barthauti</i>	Reported but not in use	Bhat and coworkers	(Bhat <i>et al.</i> ,2008)

### 3.5. Pre-breeding

It is generally accepted that wild progenitors and wild relatives serve as reservoirs of beneficial properties in crop plants. These attributes include quality, agronomic characteristics, and tolerance to both biotic and abiotic challenges. However, their incorporation into cultivated varieties is hampered by linkage drag and cross-incompatibility barriers. Linkage drag is the unwanted co-inheritance of unpleasant traits, and cross-incompatibility barriers are barriers that prevent certain traits from being passed on to offspring. Pre-breeding is done with the intention of identifying desirable characteristics present in wild germplasm and incorporating those characteristics into subsequent breeding efforts. The basic objective of pre-breeding is to minimize the detrimental effects of linkage drag while simultaneously increasing the amount of unique genetic variation within the target species. According to Kumawat *et al.*(2020),the exploitation of molecular markers possesses a tremendous potential for accelerating the breeding cycle, cutting expenses and saving time, and improving the efficacy of introgression in pre-breeding programs (Kumawat *et al.*,2020),

In a global context, India holds the second position, with a repository of Brassica germplasm amounting to 15%,

following China which holds the first position with 17%. The National Bureau of Plant Genetic Resources (NBPGR) in India has made a substantial contribution by offering a combined total of 4095 native and 3401 foreign rapeseed-mustard accessions between 1986 and 2006 (Sharma and Singh, 2007).According to Chauhan *et al.* (2011), a comprehensive collection of 14,722 accessions has been obtained, which includes cultivated varieties, wild relatives, wild progenitors, and associated species. This significant accumulation of accessions is the result of multiple efforts and endeavors. The utilization of germplasm in breeding programs is hindered by a significant disparity between the available resources in gene banks and the lack of identified traits. Hence, it is imperative to expand the genetic diversity of plants in order to address the accelerated climate change caused by human activities in the foreseeable future.

### 4. Biotechnology techniques

Rapeseed (*Brassica napus*), a crop commonly grown in temperate regions, is widely hypothesized to have emerged as a result of spontaneous hybridization between *Brassica oleracea* and *Brassica rapa*. The synthesis of *Brassica napus* involved the fusion of protoplasts derived from *Brassica oleracea* and *Brassica rapa*. This process was undertaken with the objective of enhancing genetic

diversity and altering the oil content of the resulting plant. Biotechnological interventions have been utilized to augment genetic diversity and expedite the transmission of advantageous characteristics from closely related species, such as wild relatives, wild progenitors, or even unrelated crops. The primary objective of these interventions is to enhance the productivity potential of crops, a task that was previously unachievable using traditional or conventional breeding techniques.

#### 4.1. Anther culture

The utilization of pollen culture in the double haploid (DH) technique is employed to enhance agronomic traits in *B. juncea* by developing stable homozygous lines. The enhancement of cultural conditions and the corresponding factors, which serve as constraints for embryo production, have been observed to have a positive impact on the efficacy of microspore culture or anther culture in *B. Juncea* (Watts *et al.*, 2020). According to reports, microspore culture has demonstrated higher success rates compared to anther culture, primarily attributed to the superior response of genotypes in relation to embryo culture. Microspore culture has been employed as a technique for gene transfer, biochemical investigations, and the alteration of fatty acid profiles via mutagenesis (Watts *et al.*, 2020). Several key factors that have a significant impact on doubled haploid production include the isolation of microspores, the composition of the culture media, the selection of embryos, the process of plant regeneration, and the subsequent chromosomal duplication. In the context of agricultural practices in India, it is observed that there is a lack of diversity in the cultivation of this particular technique.

#### 4.2. Somaclonal variation

Somaclonal variation refers to the occurrence of genetic variation within somatic cells, which arises as a result of chromosomal rearrangements and the subsequent regeneration of diverse plants from callus through the process of plant tissue culture. In addition, it was observed that the *B. juncea* variety Prakash exhibited the ability to generate multiple shoots in cotyledonary callus under conditions of high cytokinin concentration and low indole-3-acetic acid (IAA) concentration in Murashige and Skoog (MS) media (Jain *et al.*, 1989). Tissue culture techniques have been employed to induce somaclonal variation, as well as chemical mutagens and gamma rays, resulting in the generation of a substantial genetic diversity in *B. juncea*. An instance of genetic modification, specifically somaclone-SC-122, was created to enhance five specific traits that have been linked to an increase in crop yield (Anuradha *et al.*, 1992). The initial somaclonal derivation of the Pusa Jai Kisan (Bio-902) variety took place in India in 1993, utilizing Varuna as a progenitor. This process resulted in a 17.4% enhancement in yield compared to the parent variety.

#### 4.3. Protoplast culture

The induction of protoclonal variation and the establishment of stable genetic variability in rapeseed-mustard are achieved through the utilization of tissue culture

techniques, specifically by culturing protoplasts, which are cells lacking a cell wall. The technique employed in this study involved the utilization of V-47 media for the production of somatic embryos and organogenesis in *B. juncea* cv. RLM-198. This approach can be employed in Brassica species that are not amenable to hybridization, thereby facilitating the generation of genetic diversity to enhance crop enhancement efforts.

#### 4.4. Transgenic plants

Transgenic plants have been created in crop species through the application of recombinant DNA technology. The technique of transferring alien genes or chromosomal segments to recipient parents, in cases where the desired gene of interest is naturally absent, has been extensively employed for the advancement of humanity. Several different techniques have been employed to transfer genes in crop plants, such as rapeseed-mustard. The most commonly utilized approach is the direct method of gene transfer mediated by *Agrobacterium*. This method has been predominantly employed to enhance seed yield, seed quality, tolerance to biotic and abiotic stresses, as well as to introduce desirable agronomic traits (Walden *et al.*, 1990). As previously stated, the transgenic male sterility system was employed in India for the purpose of generating hybrids. Therefore, biotechnological interventions have the potential to address the challenges encountered in conventional breeding, particularly those related to hybridization and selection processes.

#### 4.5. Omics approach

The field of -omics encompasses a wide range of disciplines, including genomics (which focuses on the entirety of an organism's DNA content), transcriptomics (which examines the complete RNA content), proteomics (which investigates the total proteins present), and metabolomics (which analyses the complete set of metabolites within an individual). Given their amphidiploid and tetraploid nature, both *Brassica juncea* and *Brassica napus* require -omics methodologies in order to comprehensively investigate the genetic basis of traits and facilitate the enhancement of these crop species.

##### 4.5.1. Genomics

The ability to identify genetic loci that are associated with a specific trait of interest has been facilitated by the utilization of linkage mapping and association studies. The genomic positions of Brassica spp. were ascertained through the utilization of molecular markers. Mukherjee *et al.*, (2001) employed the technique of bulked segregant analysis (BSA) to investigate the genetic loci that are accountable for imparting resistance to white rust in *Brassica juncea*. The gene responsible for the color of the seed coat in *B. juncea* was successfully identified in a study conducted by Padmaja *et al.* (2005). The researchers additionally discovered three specific microsatellite markers, namely Ra2-A11, Na10-A08, and Ni4-F11, which exhibited a significant association with the characteristic of seed coat color. Moreover, the study conducted by Liu *et al.* (2020)

employed genome-wide association studies (GWAS) to examine the genetic foundations of glucosinolate accumulation in both the seeds and leaves of *B. napus*. The research conducted by Kaur *et al.* (1984) utilized candidate gene analysis and genome-wide association mapping to examine the phenomenon of pod shatter resistance in *B. juncea*. Comparative mapping was employed to investigate a range of agronomic and qualitative traits within the rapeseed-mustard domain. Cai *et al.* (1985) employed comparative mapping techniques to ascertain the existence of the candidate gene *BnAP2*, which is associated with seed weight in *B. napus*. Bisht *et al.* (2009) conducted a comparative mapping study involving *Arabidopsis thaliana*, *Brassica oleracea*, and *Brassica juncea* to identify potential candidate genes associated with glucosinolate production. The researchers have identified several potential genes, namely *BjuA.GSL-ELONG.a*, *BjuA.GSL-ELONG.c*, *BjuA.GSL-ELONG.d*, *BjuA.GSL-ALK.a*, and *BjuA.Myb28.a*. The utilization of genomics has been extensively employed in investigations pertaining to the evolutionary history of *Brassica spp.* Couvreur *et al.* (1987) employed the nad4 intron 1 marker in their phylogenetic investigation, aiming to examine the temporal diversification and establishment of evolutionary patterns within the Brassicaceae family. In addition, the four *BjuCYB83A1* genes of *Brassica juncea* were effectively isolated by Meenu *et al.* (2015). The impact of these genes on glucosinolate biosynthesis has been observed to be significant. The researchers utilized phylogenetic and divergence analysis tools to ascertain that these genes underwent evolutionary processes characterized by the duplication and hybridization of the two diploid Brassica genomes, namely *B. rapa* and *B. nigra*.

#### 4.5.2. Transcriptomics

Transcriptomics plays a crucial role in facilitating a comprehensive understanding of gene expression, allowing for the identification of gene function and its effects on diverse organisms. The utilization of expression studies, gene silencing, and genome editing techniques has been implemented in the context of *Brassica spp.* For example, Heng *et al.* (2018) conducted a study in which they utilized expression analysis of the *orf288* transcript to ascertain the association between the gene *orf288* and male sterility in *B. juncea*. The study conducted by Bhattacharya *et al.* (1990) examined the process of down regulating *BjAGPase* and the seed-specific expression of the *AtWRI1* gene in *Arabidopsis*. The objective of this investigation was to enhance the lipid content in *B. juncea* seeds. In a study conducted by Savadi *et al.* (2016), the researchers aimed to increase the seed weight and seed oil content in Indian mustard. This was achieved by introducing the *DGATI* gene from *Arabidopsis thaliana* into the seeds of Indian mustard through overexpression. The manifestation of male sterility in *B. juncea* was achieved through the utilization of RNA interference (RNAi) to silence the *mutS homolog1* gene, as demonstrated in a study conducted by Zhao *et al.* (2016). The lack of fertility observed in this case was ascribed to a

sub-stoichiometric shift that took place in the *ORF220* region. In their study, Zheng *et al.* (2020) performed a gene knockout experiment utilizing CRISPR/Cas9 technology on the *BnaMAX1* homologs found in *Brassica napus*. The aforementioned manipulation resulted in a reduction in the vertical dimension of the plants and a concomitant augmentation in the quantity of lateral appendages.

#### 4.5.3. Proteomics

Proteins play a crucial role in conferring gene functionality and governing the expression of phenotypic traits in organisms. The investigation of gene functionality in *Brassica spp.* involved the utilization of proteomics methodologies, such as protein expression profiling and comparative proteomics analysis. To provide an example, Mihrand co-workers (Mihr *et al.*, 1994) utilized the "Tournfortii" cytoplasmic male sterility (CMS) system of *Brassica napus* in order to examine the protein composition of mitochondrial compartments in male sterile and fertile near-isogenic lines (*NILs*). Mohammadi *et al.* (1995) conducted a study wherein they performed a comparative proteome analysis on rapeseed seedlings. The objective of the study was to examine the effects of drought stress on root traits. The proteins H<sup>+</sup> ATPase, *HSP 90*, and *EF2* were identified by the researchers as being essential in imparting drought tolerance in the seedlings. In their study, Yousuf and co-workers (Yousuf *et al.*, 1996) employed a comparative proteome analysis methodology to ascertain the proteins that exhibit a response to salt stress in the shoots of Indian mustard genotypes. The study conducted by Yousuf *et al.* (1997), aimed to investigate the differential protein expression patterns in Indian mustard plants with varying nitrogen efficiency, specifically in response to increased carbon dioxide levels and reduced nitrogen availability.

#### 4.5.4. Metabolomics

In contemporary metabolomics research, there has been a concerted focus on enhancing the overall quality and yield of various crops. The incorporation of metabolomics into other methodologies demonstrates a significant pertinence in enhancing crop enhancement. Nevertheless, the utilization of metabolomics in mustard breeding has been relatively limited, thus presenting an emerging area of investigation for enhancing *Brassica* cultivars. Limited research has been conducted on *Brassica juncea*. Sinha *et al.* (2007) conducted metabolic engineering on the fatty acid biosynthesis pathway to enhance the nutritional composition of seed oil in Indian mustard. In a study conducted by Kortensniemi *et al.* (1999), the authors examined seed metabolomics in *B. napus* and *B. rapa* using NMR techniques. Their findings revealed that unsaturated fatty acids, sucrose, and sinapine were the metabolites that exhibited the highest discriminatory power.

### 5. Germplasm collection

According to AICRP-RM 2023 annual report Currently examining a comprehensive collection of 8229 accessions, including various species such as toria, Indian mustard, Yellow sarson, Gobhi sarson, brown sarson, karan

rai, taramira, *B. caudatus*, *B. nigra*, *B. tournifortii*, *Crambe abyssinica*, *S. alba*, *Crambe* sp. *B. Chinensis*, *B. fruticulosa*, *Camelina sativa*, *Capsella bursapastoris*, *Diplotaxis assurgens*, *Diplotaxis tenuisiliqua*, *Erucastrum* spp, and *Lepidium* sp. Currently maintaining accessions at various locations including Bhubneshwar, Dholi, Hisar, Pantnagar, Ludhiana, Kanpur, Hisar, IARI, New Delhi, Jobner, Morena, Chatha-Jammu, Jagdalpur, and SK Nagar. Gathered a total of 453 fresh accessions! We have got toria (95), Indian mustard (231), yellow Sarson (79), gobhi sarson (07), Brown Sarson (08), Taramira (22), and Karan rai (09) among the various species. Currently comprehensive evaluation was done on collection of accessions from the year 1783. Currently, there are 1299 Indian mustard accessions, 162 toria accessions, 123 yellow sarson accessions, 37 gobhi sarson accessions, 35 brown sarson accessions, 97 taramira accessions, and 23 *B. rugosa* accessions in the collection.

## 6. Current research

### 6.1. Transgenic mustard (DMH-11)

DMH-11 refers to a type of transgenic mustard that has been developed within the country. The product being referred to is a type of mustard called Herbicide Tolerant (HT) mustard, which has been genetically modified. The DMH-11 mustard is a hybrid variety that was created by crossing the Indian mustard variety called 'Varuna' with the East European mustard variety known as 'Early Heera-2'. The genetic makeup of this organism includes two genes, known as 'barnase' and 'barstar', which were obtained from a bacterium called *Bacillus amyloliquefaciens* found in soil. These genes play a crucial role in the development of high-yielding commercial mustard hybrids. The presence of Barnase in Varuna causes a temporary sterility, preventing it from undergoing natural self-pollination. In Heera, the presence of Barstar blocks the activity of barnase, which enables the production of seeds. The DMH-11 variety has been found to have a higher yield compared to the national check by approximately 28%. Additionally, it has shown a yield that is approximately 37% higher than the zonal checks. The use of DMH-11 has been claimed and approved by the GEAC. The "Bar gene" is responsible for preserving the genetic integrity of hybrid seeds (Sodhi et al., 2007 and Jayaraman, 2017).

### 6.2. Public sector research programme

The development of hybrid varieties was carried out through the collaborative research platform known as the "Consortia Research Platform on Hybrids" at ICAR-DRMR Bharatpur, ICAR-IARI New Delhi, PAU Ludhiana, and CCS HAU Hisar during the period of 2022-23. In this study, a total of 20 experimental Indian mustard hybrids were evaluated across four different centers. The experiment followed a duplicated block design, with five hybrids being assessed at each center. In a study conducted across four locations (Bharatpur, New Delhi, Ludhiana, and Hisar), two hybrid varieties, namely DRMRHJ 410 and RHH 2205, exhibited superior performance compared to the best check variety, DMH-1. DRMRHJ 410 achieved a mean seed yield

of 3101 kg ha<sup>-1</sup>, surpassing DMH-1 by 5.26%. Similarly, RHH 2205 recorded a mean seed yield of 2950 kg ha<sup>-1</sup>, outperforming DMH-1 by 0.13%. These findings highlight the potential of these hybrid varieties in enhancing seed yield in multiple locations. In the context of enhanced block design, a total of 40 distinct hybrid varieties were subjected to rigorous testing and evaluation. In terms of average seed yield, the performance of five experimental hybrids, namely DRMRHJ 417, RHH 2214, RHH 2212, RHH 2213, and PHR 8207, surpassed that of the best check hybrid DMH 1. The experimental hybrids achieved yields of 2916 kg ha<sup>-1</sup>, 2883 kg ha<sup>-1</sup>, 2831 kg ha<sup>-1</sup>, 2818 kg ha<sup>-1</sup>, and 2763 kg ha<sup>-1</sup>, respectively, while DMH 1 yielded 2744 kg ha<sup>-1</sup>. The superiority of the experimental hybrids over DMH 1 ranged from 1.0% to 6.26%, with DRMRHJ 417 exhibiting the highest improvement and PHR 8207 showing the smallest enhancement. In the regions of Bharatpur, New Delhi, and Ludhiana, a hybrid DRMRHJ 417 (with a yield of 1227 kg ha<sup>-1</sup>) exhibited superior performance compared to the top-performing check variety DMH- (yielding 1114 kg ha<sup>-1</sup>), with a significant increase of 10.7% in oil yield. In the study, a total of 30 F 1 crosses were evaluated using an enhanced block design across all four centers. Based on the analysis of seed yield across four different locations, it was observed that three specific crosses exhibited a seed yield heterosis of more than 10% when compared to the highest performing check variety, DMH 1, which yielded 2698 kg ha<sup>-1</sup>. In a study conducted across three locations (Bharatpur, New Delhi, and Ludhiana), three F 1 crosses, namely DRMRH 22-10, 22-2, and 22-6, exhibited superior performance in oil yield compared to the best check variety DMH-1. The F 1 crosses achieved oil yields of 1321 kg ha<sup>-1</sup>, 1308 kg ha<sup>-1</sup>, and 1260 kg ha<sup>-1</sup>, respectively, surpassing DMH-1's yield of 1093 kg ha<sup>-1</sup> by 20.9%, 19.7%, and 15.2%, respectively. In this study, a comprehensive analysis was conducted by DRMR, CCSHAU, IARI, and PAU, focusing on a total of 589 experimental hybrids. These hybrids were comprised of 40, 195, 105, and 249 samples, respectively. The discovery of potential hybrids was made by each research center. In this review paper, a comprehensive analysis was conducted on a total of 888 F crosses, 144 ICAR-DRMR crosses, 375 ICAR-IARI crosses, 167 PAU crosses, and 194 HAU crosses. The crosses exhibited exceptional quality at each center. The process of back-crossing involves the conversion of cytoplasmic male sterility (CMS) and restorer lines to nuclear backgrounds of 489 and 160, respectively. In addition, a total of 86 CMS (Cytoplasmic Male Sterile) lines and 172 restorer lines were also maintained in this study. A total of 105 experimental hybrids and 1051 F 1 crossings were generated to facilitate the upcoming evaluation in the following year. Efforts are currently underway to enhance the resistance against white rust, improve the overall quality, and enhance the agronomic qualities of parental lines. A total of 60 inbred lines, consisting of 15 lines from each collaborating center, were carefully maintained as CMS (Cytoplasmic Male Sterility), maintainer, and restorer lines throughout the study. The assessment of genetic diversity

through the utilization of SSR markers resulted in the categorization of 72 inbred lines into two distinct subpopulations (Anonymous,2023).

Heterosis was estimated in Indian Mustard for 12 quantitative characters by Chaudhari *et al.* (2023). Eighteen F<sub>1</sub> crosses were obtained by line x tester mating design using 6 lines and 3 testers. These 18 crosses along with parent (TAM-108-1 and Kranti used as parent as well as check) were grown at AICRP on linseed and mustard experimental farm, College of Agriculture, Nagpur during 2021-22 in randomized block design with three replications. The crosses Bio-902 x *Synapsis alba*, Bio-902 x PC-6, ACN9 x Chhattisgarh Sarson, PM-26 x Chhattisgarh Sarson and Kranti x Chhattisgarh Sarson had high mean performance and exhibited significant useful heterosis over the superior check for yield and most of its contributing characters *viz.*, days to maturity, number of branches plant<sup>-1</sup>, number of siliqua plant<sup>-1</sup>, siliquae density on main branch, oil content, number of seeds siliqua<sup>-1</sup>, 1000 seed weight, point to first siliqua, siliqua length, days to 50% flowering. These crosses identified as superior crosses, which can be utilized for development of hybrid varieties.

Patil *et al.* (2021) studied the effect of crossing efficiency in interspecific hybridization in *Brassica* species. The experiment was carried out in the research farm of Agril. Botany Section, College of Agriculture, Nagpur during *rabi* 2020 by crossing three varieties from *Brassica juncea* (TAM 108-1, ACN-9, Kranti) and one variety from *Brassica carinata* (PC-6) both direct and reciprocal. The results revealed that when *juncea* was used as female parent the mean percentage success in crossing was 76.10 per cent as compared to 48.29 per cent when *carinata* was used as female parent. Maximum per cent success in hybridization was recorded in TAM 108-1 x PC-6 (92.80 per cent), followed by Kranti x PC-6 (77.30 per cent) and PC-6 x Kranti (63.78 per cent) and the least in PC-6 x TAM 108-1 (39.59 per cent). From the pollen studies it is observed that pollen fertility did not show much variation as compared to pollen size and pollen intensity. Maximum pollen size (2.98 um) and fertility (98.1 per cent) was recorded in ACN-9, but it recorded the least pollen intensity (1.99 pollen mm<sup>-2</sup>). Highest pollen intensity was observed in Kranti (6.59 pollen mm<sup>-2</sup>). Thus, it is inferred that to get maximum seed set *juncea* should be used as female parent unless no cytoplasmically governed traits are concerned and in the interspecific hybridization efforts should also be intensified to get adequate F<sub>1</sub> seeds by increasing the frequency of emasculation and pollination so that limitations in seed set can be overcome.

Deshmukh *et al.* (2021) made the forty two F<sub>1</sub> crosses of mustard (*Brassica juncea* L.). Crosses were obtained by full diallel mating design using seven parents and F<sub>1</sub> Hybrids along with parents (Kranti was used as parent as well as check) during *rabi* 2019-20 and were evaluated to estimate the heterobeltiosis and useful heterosis of crosses for seed yield and yield contributing characters. The parents and crosses were grown in

randomized block design replicated thrice at research field of AICRP (Linseed and mustard), college of Agriculture, Nagpur during *rabi* 2020-21 and observations were taken on days to first flower, days to maturity, plant height (cm), number of branches plant<sup>-1</sup>, number of siliqua plant<sup>-1</sup>, siliqua density on main branch, 1000 seed weight (g) and seed yield plant<sup>-1</sup>(g). The crosses Kranti x NRCHB-101, TAM 108-1 x BIO-902, NRCHB-101 x PC-6, TAM 108-1 x PC-6 and PC-6 X TAM 108-1 had high mean performance and exhibited significant standard heterosis over the superior check for yield and most of its contributing characters. These crosses were identified as superior crosses, which can be utilized for development of hybrid varieties after evaluation of yield trial and converting female lines into male sterile lines.

Heterosis was estimated in Indian mustard by Sapkal *et al.* (2021) at research field of AICRP (Linseed and mustard), College of agriculture, Nagpur. Thirty F<sub>1</sub> crosses of Indian mustard (*Brassica juncea* L.) obtained by full diallel fashion using six parents. The parent, crosses and checks were grown in randomized block design replicated thrice and observation were taken on days to first flower, days to maturity, plant height (cm), number of branches plant<sup>-1</sup>, number of siliqua plant<sup>-1</sup>, siliqua density on main branch, 1000 seed weight (g) and seed yield plant<sup>-1</sup>(g). The analysis of variance for experimental design revealed significant genetic variability among them which allowed its exploitation of material for further analysis. The mean square due to parents, crosses and parents Vs crosses exhibited significant difference for all the characters. This indicates the suitability of data for estimation of heterobeltiosis and useful heterosis. The crosses ACNMM 52 x ACNMM29, ACNMM14 x ACNMM9 and ACNMM14 x ACNMM27 had high mean performance and exhibited significant standard heterosis over the superior check for yield and most of its contributing characters. These crosses were identified as superior crosses, which can be utilized for development of hybrid varieties.

Raut *et al.* (2021) made the fifteen F<sub>1</sub> crosses of Indian mustard via half diallel mating design using six parents (excluding reciprocal) to estimate useful heterosis for seed yield and yield contributing characters. The parents, crosses and checks were grown in randomized block design replicated thrice in the year *rabi* 2019 at Research farm of AICRP (Linseed and mustard), College of Agriculture, Nagpur and observations were taken on seed yield and its contributing characters. The crosses RH-406 x Pusa Mustard-31, NRCHB-101 x RH-406, NRCH-101 x RH-749, Giriraj x Pusa Mustard-31 and Rh-749 x Pusa Mustard-31 had high mean performance and exhibited significant standard heterosis over the best check TAM108-1 for yield and most of the characters *viz.*, days to first flower, plant height, number of primary branches plant<sup>-1</sup>, number of siliqua plant<sup>-1</sup>, siliqua density on main branch, 1000 seed weight (g). These crosses were identified as superior crosses, which can be utilized for development of hybrid varieties.

## Future prospective

In order to meet the escalating demand for edible oil resulting from population growth, it is imperative for the nation to consistently enhance its output and productivity through the utilization of conventional, molecular, and biotechnological methodologies. The enhancement of crop quality and yield necessitates the presence of genetic diversity. In order to enhance the genetic diversity of cultivars, it is imperative to incorporate exotic germplasm, as well as untamed and closely related species. The utilization of biotechnology and conventional plant breeding techniques has the potential to augment the quality of oil and improve resistance against both biotic and abiotic stress factors. Climate change and global warming are anticipated to pose significant challenges in the future. The utilization of crop cultivars that possess climate resilience is of utmost importance. Successive utilization of marker-assisted selection (MAS), functional genomics, phenomics, proteomics, and metabolomics is employed to develop cultivars with enhanced tolerance to drought and heat stress. In order to effectively address forthcoming challenges, it is imperative to re-evaluate and adapt existing reproduction strategies. Omics breeding represents an innovative strategy for enhancing agricultural productivity. The longevity and effectiveness of this technology are anticipated to persist as a result of its durability and superior performance when compared to traditional methods of reproduction.

(Integral University MCN Number – IU/R&D/2023-MCN0002164)

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**Rec. on 25.08.2023 & Acc. on 04.10.2023**