Series of Crop Specific Biology Documents

BIOLOGY OF *BRASSICA JUNCEA* (INDIAN MUSTARD)

Phase II Capacity Building Project on Biosafety

सत्यमेव जयते

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Ministry of Environment, Forest and Climate Change
Government of India

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BIOLOGY OF *Brassica juncea* (INDIAN MUSTARD)

Phase II Capacity Building
Project on Biosafety

Ministry of Environment, Forest and Climate Change
Government of India

Biology of *Brassica juncea* (Indian Mustard)

Prepared by

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under UNEP/GEF supported Phase II Capacity Building Project on Biosafety

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सत्यमेव जयते

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पर्यावरण, वन एवं जलवायु परिवर्तन
ENVIRONMENT, FOREST & CLIMATE CHANGE
भारत सरकार / GOVERNMENT OF INDIA

Message

I am happy to learn that the Ministry of Environment, Forest & Climate Change (MoEFCC) as part of the initiative under the UNEP GEF supported "Phase II Capacity Building Project on Biosafety" has developed eight crop specific biology document on Chickpea, Mustard, Papaya, Pigeon-pea, Potato, Rubber, Sorghum, and Tomato.

I am happy to note that the documents have been prepared with support from seven research institutions namely Indian Institute of Pulses Research, Directorate of Rapeseed and Mustard Research, Indian Institute of Horticulture Research, Central Potato Research Institute, Rubber Research Institute of India, Indian Institute of Millets Research and Indian Institute of Vegetable Research.

While Bt cotton is the only genetically modified (GM) crop approved for commercial cultivation in India, there are several crops under various stages of research, development and field trials. The present set of crop specific biology documents aims to provide scientific baseline information of a particular plant species that can be used as credible source of information for conducting safety assessment of GM plants.

I would like to congratulate all those who were involved in preparing these documents and those involved in steering this initiative.

I am confident that these biology documents will serve as a valuable tool for regulators, scientists, crop developers, policymakers, academicians and other stakeholders who are involved in the safety assessment of GM plants. I am also hopeful that baseline information provided in the biology document would further enhance awareness on biosafety aspects of GM crops.

(Prakash Javadekar)

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सत्यमेव जयते

PREFACE

India is an agriculture based economy with abundance of genetic base, diverse agro-climatic zones and highly qualified manpower which provides a rich scope for technological advances in agricultural biotechnology. The shortage of healthy seeds/planting material, lack of disease resistant clones, crop damage by insects, pests etc. have often affected the Indian agricultural economy adversely and therefore the role of new technologies assumes significant importance for Indian economy.

With significant advances in the field of agricultural biotechnology the regulatory system has to deal with multiple crops integrated with multiple traits. In order to streamline the process of safety assessment, the Ministry of Environment, Forest & Climate Change (MoEF&CC) under the UNEP-GEF supported "Phase II Capacity Building Project on Biosafety" has prepared a set of crop specific biology documents namely Chickpea, Mustard, Papaya, Pigeon-Pea, Potato, Rubber, Sorghum, Tomato with support from six Indian Council of Agriculture Research (ICAR) institutions and Rubber Research Institute of India.

The biology documents provides an overview of baseline biological information of a particular plant species such as taxonomy, the centres of origin, its related species including wild relatives, general description of their morphology, reproductive biology, biochemistry, potential for gene introgression, biotic and abiotic interactions. Such species specific information is expected to serve as a guiding tool for use in risk assessment of genetically modified (GM) plants.

The documents has been prepared through a consultative approach and comments received from several organizations have been extremely useful in validating this

document. I express my deep appreciation for the support provided by Indian Institute of Pulses Research, Directorate of Rapeseed and Mustard Research, Indian Institute of Horticulture Research, Central Potato Research Institute, Rubber Research Institute of India, Indian Institute of Millets Research and Indian Institute of Vegetable Research in preparing these documents. I would also like congratulate Dr. Ranjini Warrier, Advisor, (MoEFCC) and Dr O.P Govila (Former Professor, Department of Genetics, IARI) for their sincere efforts and the consultative approach adopted in finalizing the biology documents.

I am confident that these crop specific biology documents would be of immense value for researchers, regulators and industry in planning for the safety assessment of GM crops.

Hem Pande

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BIOLOGY OF *Brassica juncea L.* (Czern & Coss.) (INDIAN MUSTARD)

INTRODUCTION

1.1 General Distribution

Brassica juncea (L.) Czern & Coss., also known by the name of Indian mustard, belongs to the plant family *Brassiceae* (*Cruciferae*) or the mustard family. In the trade, it is commonly referred to as Rapeseed-mustard along with four other closely related cultivated oilseed species viz. *B. rapa*, *B. napus*, *B.carinata* and *Eruca sativa*. Over the past couple of decades, these crops have become one of the most important sources of vegetable oil in the world. Continuous improvement in rapeseed-mustard has resulted in nutritionally superior edible oil, and meal as an important source of protein in animal feed. Rapeseed-mustard crops are commercially cultivated in more than 60 countries and major produces include China, Canada, India, Australia, France, Germany, United Kingdom, Poland, Ukraine, Russia, USA and Czech Republic (Fig 1). In the past the area under Rapeseed-mustard globally increased from 6.3 million hectare in 1961 to 34.3 million hectare in 2012 with a mean increment of 0.56 million hectare per annum (Fig 2). Production in the same period increased from 3.68 to 65.1 million tonnes at mean increment of 3.68 mt/annum. These crops occupy a prominent position as the second important oilseeds in the world as well as in India.

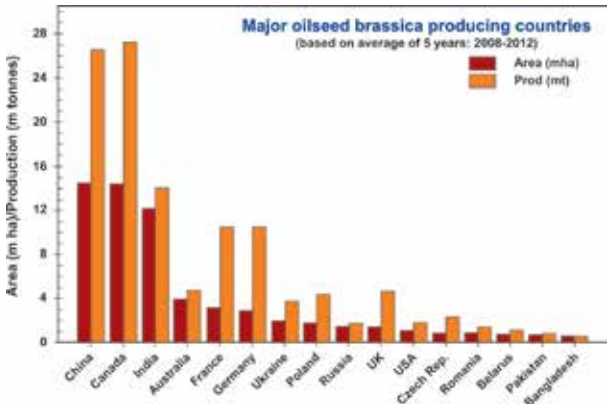

Fig 1. Area (m ha) and Production (mt) of Rapeseed-mustard crops in major producer countries (2008-2012)

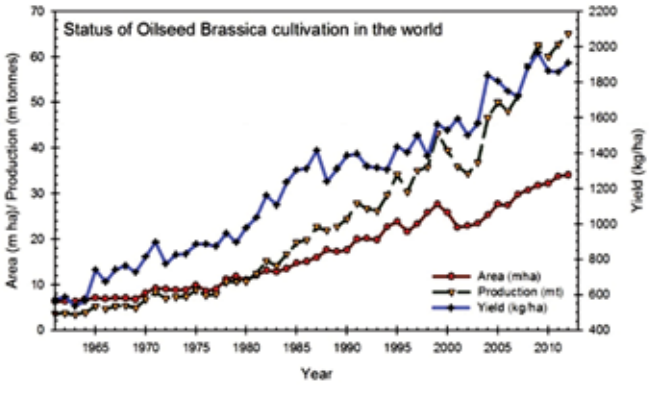

Fig 2. Area (m ha), Production (m t) and Seed Yield (kg/ha) of Rapeseed-mustard in the world

1.2 Distribution

Rapeseed-mustard crops have two major cultivated groups, winter type and spring type. The

productivity of winter type long duration (8-10 months) cultivars grown in temperate region is higher than spring type short duration (3-5 months) cultivars grown in semi-arid conditions of the Indian subcontinent, USA and Australia with a global average yield of 1896 (kg/ha) (Fig 3). The mean productivity of long duration cultivars is 4467 (kg/ha) in Mexico, 4176 (kg/ha) in Belgium and 3965 (kg/ha) in Netherland, while it is 1346 (kg/ha) in Pakistan, 1264 (kg/ha) in India and 1185 (kg/ha) in Australia for short duration cultivars (FAOSTAT, 2013). However, the per day productivity of both types is competitive.

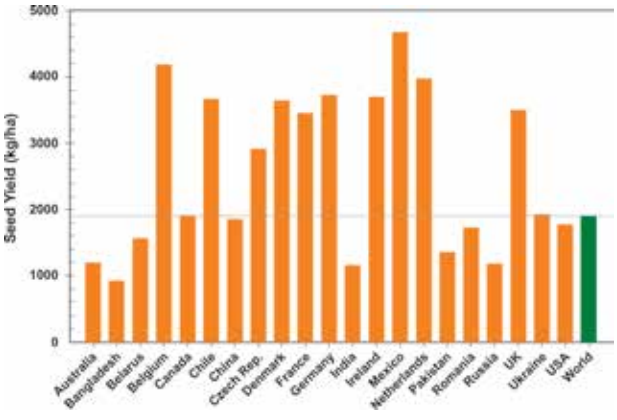

Fig 3. Average Seed Yield (kg/ha) of Rapeseed-mustard crops in important producer countries
(**Source:** FAOSTAT, 2013)

Brassica juncea is the most predominant crop out of Rapeseed-mustard crops in India and accounts for more than 90% of the area. *B. juncea* is grown in marginal and sub - marginal lands either as pure crop or as a mixed crop with wheat, lentil, chickpea, pea, sugarcane, linseed etc. Its cultivation, which was confined to the Northern belt earlier has now spread to non-traditional areas in Eastern, Western and Southern regions of the country. The crop grows well under both irrigated and rainfed conditions. Being more responsive to fertilizers, it gives better return under irrigated conditions.

Rapeseed-mustard is cultivated during rabi season in about 5.9 million hectare area and 6.78 million tonnes seeds are produced with an average productivity of 1.26 (tonnes/ha) (DAC, 2013). Although it is being cultivated across the country, 7 states (Rajasthan, MP, UP, Haryana, WB, Assam and Gujarat) contribute significantly to its production (> 90%) and acreage (>80%). Rajasthan alone contributes almost 50% to acreage in the country (Table 1).

Table 1. Area (m ha), Production (m t) and Yield of Rapeseed - mustard during 2013-14 in Major Producing States.

State	Area (m. Ha.)	Production (m. tonnes)	Yield (Kg/ha)
Rajasthan	2.83	3.81	1346
Madhya Pradesh	0.78	0.92	1172
Haryana	0.56	0.96	1721
West Bengal	0.45	0.47	1062
Uttar Pradesh	0.66	0.84	1263
Gujarat	0.21	0.36	1695
Assam	0.28	0.17	610
Bihar	0.08	0.09	1132
Punjab	0.03	0.04	1281
Others	0.28	0.21	741

(**Source:** www.drmr.res.in)

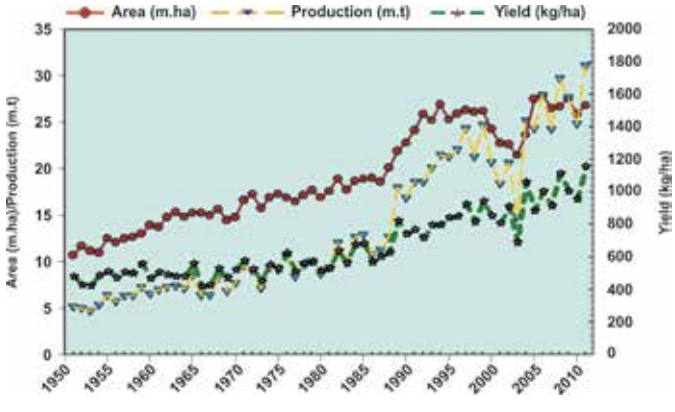

Fig 4. Area (m ha), Production (m t) and Seed Yield (kg/ha) of Rapeseed-mustard in India

1.3 Uses

B. juncea is used as sources of oil, vegetable, condiments and fodder. Some important uses are described below:

a) The oil content of the seeds ranges from 38-46%. The conventional varieties of B. juncea are high in Erucic acid (~40-50%) as well as in glucosinolates (180-200 micro moles). Internationally erucic acid is included as one of the hazardous constituents in food material in the OECD-WHO Food Safety Standards and FAO's Manual on Food Safety Assessment (2008).

b) The seed and oil are used in the preparation of pickles and for flavouring curries and vegetables. Whole seeds, ground or in powdered form, prepared pastes, sauces and oil are all used in cooking. The aroma and pungent flavour of mustards come from the Sulphur containing glucosinolates. Mustard paste is used in salad dressings, sandwiches etc. Mustard oil is used in many recipes of North and East India.

c) The oil cake is rich in protein and is mostly used as cattle feed. However, it is also used as concentrated organic manure.

d) The leaves of young plants are used as green vegetables. The use of leaves (also referred as mustard green) is particularly popular in cuisine of Punjab where a famous disch called 'sarson ka saag' is prepared.

e) Mustard seeds and oil have been traditionally used to relieve muscle pain, rheumatism and arthritic pain. In India, mustard oil is applied over scalp and is believed to stimulate hair growth. Ground mustard seeds act as a laxative, stimulant to gastric mucosa and increase intestinal secretion.

Table 2 gives the nutritive value of mustard leaves and seeds and Table 3 gives the fatty acid composition of mustard oil.

Table 2: Nutritive value of mustard leaves and seeds (per 100 gms of edible portion)

S. No.	Food Component	Mustard leaves	Mustard seeds
1.	Moisture	89.8 g	8.5 g
2.	Protein (NX 6.25)	4.0 g	20.0 g
3.	Fat	0.6 g	39.7 g
4.	Minerals	1.6 g	4.2 g
5.	Crude fibre	0.8 g	1.8 g
6.	Carbohydrates	3.2 g	23.8 g
7.	Energy	34Kcal	541 Kcal
8.	Calcium	155 mg	490 mg
9.	Phosphorus	26 mg	700 mg
10.	Iron	16.3 mg	7.9mg

Source: Gopalan *et al.*, 2007

Table 3: Fatty acid composition of mustard/rapeseed oil (in percentage of total methylester of fatty acid)

S. No	Fatty acids	Composition
1.	Palmitic	2.9
2.	Stearic	0.9
3.	Arachidic	6.9
4.	Behenic	-
5.	Lignoceric	-
	Total saturates	**10.7**
6.	Palmitoleic	0.6
7.	Oleic	8.9
	Total monounsaturates	**56.0***
8.	Linolenic	18.1
9.	Linolenic	14.5
	Total polyunsaturates	**32.6**
*Includes 46.5% of Erucic acid		

Source: Gopalan *et al.*, 2007

1.4 Nomenclature and Classification

B. juncea belongs to the family *Brassicaceae* (Syn. *Cruciferae*). The family currently includes 3709 species and 338 genera (Warwick *et al.*, 2006) and is one of the ten most economically important plant families (Rich, 1991). The genus *Brassica* is one of the ten core genera within *Brassicaceae*. Taxonomic classification of *B. juncea* is presented in Table 4.

Table 4. Taxnomic classification of *B. juncea*

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Capparales
Family	Brassicaceae
Genera	*Brassica*
Species	*Brassica juncea*

The genera *Brassica* display enormous diversity and a range of wild and weedy species related to the genus occur in nature. However, most of these species in the wild germplasm belong to secondary and tertiary gene pools, reproductively isolated and invariably show strong incompatibility barriers. A list of economically important species of genus *Brassica* and its close allies along with their uses is presented in Table 5 and those grown in India are given in Table 6.

Table 5: Economically important *Brassica* species and their close allies

Botanical name	Common name	Genome	Chromo-some No.	Usages
Brassica rapa (syn. B. compestris)		AA	20	
spp. Oleifera	Turnip rape			Oilseed
var. brown sarson	Brown sarson			Oilseed
var. yellow sarson	Yellow sarson			Oilseed
var. toria	Toria			Oilseed
spp. *Rapifera*	Turnip			Fodder, vegetable (root)
spp. *Chinensis*	Bok choi			Vegetable (leaves), fodder (head)
spp. *pekinensis*	Chinese cabbage			Vegetable (leaves)
spp. *nipposinica*	-			Vegetable (leaves)
spp. *Parachinensis*	-			Vegetable (leaves)
Brassica nigra	Black mustard	BB	16	Condiment (seed)
Brassica oleracea		CC	18	
var. *acephala*	Kale			Vegetable, fodder (leaves)
var. *capitata*	Cabbage			Vegetable (head)
var. *sabauda*	Savoy cabbage			Vegetable (terminal buds)
var. *gemmifera*	Brussels sprouts			Vegetable (head)
var. *gongilodes*	Kohlrabi			Vegetable, fodder (stem)
var. *botrytis*	Cauliflower			Vegetable (inflorescence)
var. *italic*	Broccoli			Vegetable (inflorescence)
var. *fruticosa*	Branching bush kale			Fodder (leaves)

var. *alboglabra*	Chinese kale			Vegetable (stem, leaves)
Brassica juncea	Mustard	AABB	36	Oilseeds, vegetable
Brassica napus		AACC	38	
spp. oleifera	Rapeseed, gobhi sarson			Oilseed
spp. Rapifera	Rutabaga, swede			Fodder
Brassica carinata	Ethiopian mustard	BBCC	34	Vegetable, oilseed
Eruca sativa	Rocket, taramira	EE	22	Vegetable, non-edible oilseed
Raphanus sativus	Radish		18	Vegetable, fodder
Sinapis alba	White mustard	SS	24	oilseed

(**Sourcce:** Prakesh, s. *et al.*, 2009)

Table 6: Rapeseed-mustard crops grown in India

Botanical Name	Common Name	Botanical Name	Common Name
Brassica juncea	Indian mustard, Rai, Raya, Laha, Rayda, Banga sarson	*B. tournefortii*	Panjabi rai, Jangali rai
B. juncea var. *Cuneifolia*	Vegetable mustard, Rai	*B. nigra*	True mustard, black mustard, Banarasi rai
B. rapa spp. *Oleifera*	Turnip	*B. pekinensis*	Chinese cabbage-heading
B. rapa var. *brown sarson*	Brown sarson, Kali sarson	*B. napus*	Gobhi sarson
B. rapa var. *yellow sarson*	Yellow sarson, Pili sarson	*B. carinata*	Karan rai, Ethiopian mustard
B. rapa var. *toria*	Toria, Rai, Lahia, Magni achara rai	*Eruca sativa*	Taramira, Rocket salad

(**Source:** Mishra, A.K. and Kumar, A. 2008)

2. GEOGRAPHIC ORIGIN, GENOMIC EVOLUTION AND CHROMOSOME NUMBER

2.1 Centres of Origin and Diversity

Brassica juncea (2n=36) is an amphidiploid species derived from interspecific cross between *Brassica nigra* (2n=18) and B. rapa (2n=20). Wild forms of *Brassica juncea* have been found in the near East and Southern Iran.

There are conflicting views about the origin of *B. juncea* (Bhowmik, 2003). During the late 19th century it was believed that *B. juncea* probably originated in China and entered India through a North Eastern route independent of any Aryan incursion. According to Vavilov (1949) Afghanistan and its adjoining regions (Central Asia) were the primary centre of its origin while central and western China, Eastern India and Asia minor with Iran comprised the secondary centres of origin.

Others have proposed multiple centres of origin for *B. juncea* where the putative progenitors, *B. campestris* (syn rapa) and *B. nigra* had geographic sympatry. Middle East has been proposed as the

most probable place of origin of *B. juncea* as wild forms of its progenitor species *B. rapa* and *B. nigra* occur together in this region (Olsson, 1960a,b; Mizushima and Tsunda, 1967; Prakash and Hinata, 1980). The regions of south western China and North Western Himalayas may constitute two secondary centres where there is enormous diversity in *B. juncea* forms. Biochemical studies support this finding about the diversity in these regions (Vaughan et al., 1963) and further provide evidence for the existence of two geographical races of *B. juncea*, the Chinese pool and the Indian pool (Vaughan and Gordon, 1973). This evidence is supported by Song et al. (1988) through RFLP studies which suggest two centres of origin (i) Middle East and (ii) China. However Rakow (2004) had opined that China cannot be considered as a centre of origin for *B. juncea* because the two parent species *B. nigra* and *B. rapa* (syn campestris), were never found as wild species in that country.

2.2 Genomic Evolution

An evolutionary relationship exists among the six crop *Brassica* species exist. This involves three basic diploid species *B. rapa*, *B. nigra* and *B. oleracea*. Pairwise hybridization between these diploid species followed by chromosome doubling led to the evolution of the basic diploid level and development of the three amphiploid species *B. napus, B. carinata and B. juncea*.This evolutionary relationship is depicted in Fig.5. This relationship was confirmed through a high degree of homology and regular meiotic pairing between the similar genomes in the F1 hybrids produced as a result of artificial hybridization between these species. For example, when *B. juncea* (AABB; 2n = 36) is crossed with *B. rapa* (AA; 2n = 20), 10 chromosomes of *B. juncea* are seen to pair with the 10 of *B. rapa* leaving the other 8 as univalent at metaphase-1. Likewise, when *B. juncea* is crossed with *B. nigra* (CC; 2n = 18), eight bivalents are commonly observed at meiosis and the remaining 10 chromosomes exhibit their identity as univalent. This evidence clearly suggests that *B.juncea* arose through hybridization between these two diploid progenitor species.

Similar cytogenetical observations for *B. carinata* and *B. napus* have proved their amphidiploid origin. Apart from pairing between homologous chromosomes of similar genomes coming from related species, a loose secondary association is also seen between two or more bivalents. The secondary association has led to the hypothesis that all the three elementary genomes have evolved from a common ancestor. The haploid number of this ancestral genome was first thought to be 5 but later studies indicated it as n = 6.

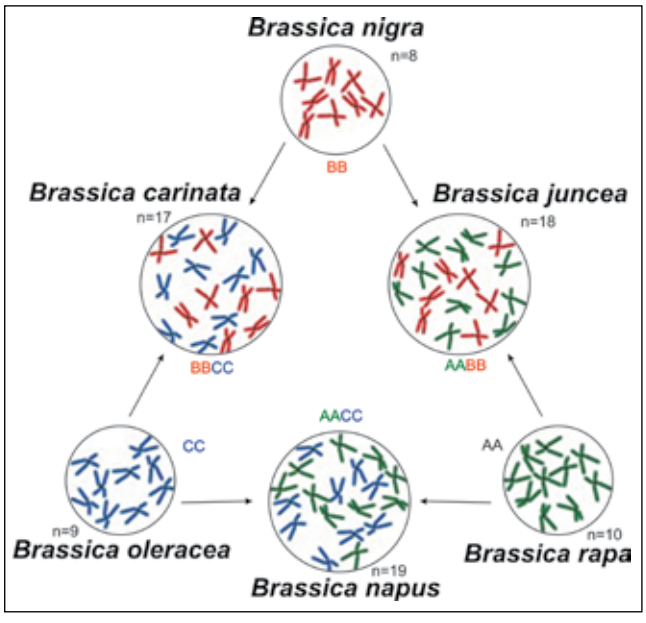

Fig 5: The "Triangle of U", showing the genetic relationships between the six species of the enus Brassica. Chromosomes from each of the genomes A, B and C are represented by different colors
(**Source:** viswiki.com/en/Triangle_of_U)

2.3 Germplasm Conservation

Germplasm conservation is necessary to preserve

the great biodiversity available in Brassicas. Worldwide, there are more than 90,000 accessions of *Brassica* conserved in 140 germplasm banks. It has been reported that five countries share nearly 60% of *Brassica* germplasm holdings (Boukema and Hintum, 1999) led by China (17%) and followed by India (15%), UK (10%), USA (9%) and Germany (8%).

India presents rich diversity of Rapeseed-mustard group of crops. A large number of indigenous Rapeseed-mustard collections have been made in the country by National Bureau of Plant Genetic Resources (NBPGR), Indian Agricultural Research Institute (IARI), Haryana Agricultural University and Directorate of Rapessed Mustard Research (DRMR). Several *Brassica* specific explorations were undertaken in the drier parts of Gujarat, Rajasthan, Bundelkhand region of Uttar Pradesh, parts of Bihar, West Bengal, Orissa, hilly areas of Jammu and Kashmir, Himachal Pradesh and the North Eastern Himalayas. As a result of these explorations, 3677 collections of different species of *Brassica* were made from different states during 1976-1999. Local land races of *B. juncea* such as *Jatai rai, desi rai*, and *maghi rai* were collected from farmer's fields in the areas bordering Bangladesh. In yellow sarson dwarf and early types with pendulous siliqua were collected from Indo-Bangladesh border whereas tall, robust, multi-locular types were mainly collected from Eastern Uttar Pradesh. Diversity of *B. tournefortii* and *B. nigra* was collected from drier parts of Haryana and Rajasthan. Explorations for wild crucifers in Pauri Garhwal hills of Uttar Pradesh added 22 accessions of *Capsella*, *Crambe*, *Lepidium* and *Sisymbrium* spp. Some unique collections were also made, which include yellow seeded toria, dwarf mustard, dwarf and early toria, white flowered yellow sarson etc. Exchange Division of NBPGR introduced 3401 exotic accessions of Rapeseed-mustard during 1985–2006 (Sharma and Singh, 2007) and the National Research Centre on Rapeseed Mustard (NRCRM) through NBPGR received 853 exotic accessions from Canada, USA, Germany, Sweden, Belgium, Australia and China during 2001–07. National Gene Bank at NBPGR conserved 10259 accessions till December 2010 (NBPGR 2011).

3. REPRODUCTIVE BIOLOGY

3.1 Reproduction

B. juncea is an annual herbaceous plant. The plants are tall (90-200 cm), erect and heavily branched. The leaves are dilated at the base, are stalked, broad and pinnatified. The fruits (siliquae) are slender and only 2 to 6.5 cm long, strongly ascending or erect with short and stout beaks. The colour of the seed is brown or dark brown. The seed coat is rough. Fig. 6 (a-c) provides the structure of leaf, flowers and silique of *B. juncea*.

Fig 6. (a) Leaf of *B. juncea* (b) Flowers of *B. juncea* (c) Siliquae of *B. juncea*

The leaves are alternate (rarely opposite), and may be coriaceous, very often pinnately incised and do not have stipules. The inflorescence is of corymbose raceme type. Flowering is indeterminate, beginning at the lowest end on the main shoot and continues upward. Flowers are ebracteate, pedicillate, complete, hypogynous and actinomorphic. Calyx comprises four sepals in two whorls each. Antero-posterior sepals form the outer whorl, whereas as lateral ones form the inner whorl. These are pale green in colour. Corolla comprises of four cruciform petals. These are clawed and regular. Two functional nectaries are located at the base of the short stamens and two non functional nectaries at the base of the pair of long stamens. Androecium is tetradynamous and consists of six stamens arranged in two whorls. The longer four stamens form the inner whorl and are arranged in antero-posterior pairs. The two shorter stamens form the outer whorl and are present in lateral position. Anthers are bithecus and basifixed. Gynoecium is usually bicarpellary, syncarpus and superior with carpels transversely placed. It is bilocular due to the presence of false septum. Plancentation is parietal; ovary is usually sessile with many ovules, short style and bifid stigma (Fig.7-8).

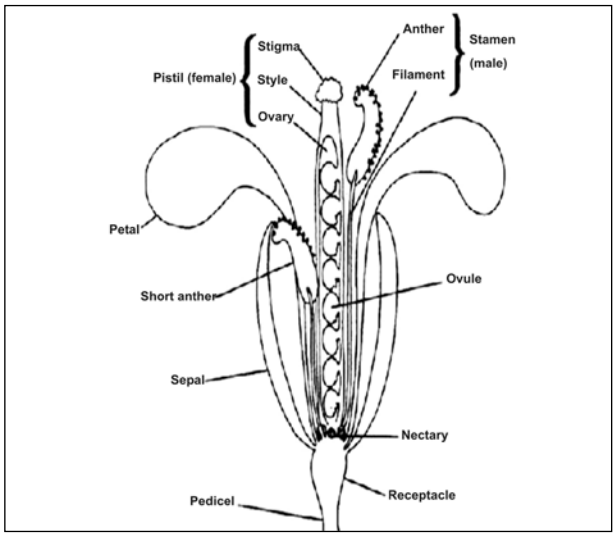

Fig 7. A typical flower of family *Brassicaceae*

Fig. 8. Inflorescence is corymbose raceme type
Source: Wisconsin Fast Plants program, University of Wisconsin Madison

The mature bud flowers within two hours after sun rise. The stigma becomes receptive two to three days before the flower opens and thus facilitates selfing by bud pollination (Kumar, 2001). The dehiscing side of anther sacs faces the stigma, but as the time of dehiscence approaches, the inner whorl of two anthers undergoes a twist of 60° to 180° which results in extrose dehiscence in the case of the self incompatible types. The dehiscence of all the anthers in self compatible types is introse.

3.1.1 Stages of growth and development

B. juncea, as a part of family *Brassicaceae* is distinguished on the basis of the presence of conduplicate cotyledons, (i.e. the cotyledons are longitudinally folded around the radicle). Normally within 3 to 5 days of sowing, epigeal germination and emergence takes place. The radicle (embryonic root) emerges first. Two cotyledons (seed leaves) appear and the hypocotyl (embryonic stem) extends upward. From 6 to 12 days cotyledons enlarge, true leaves (4 to 5) emerge and develop. Stem elongates between the nodes (points of leaf attachment). Flowering and reproduction take place between 30 to 60 days

and plants attain maturity normally between 120 to 150 days depending upon the genotype and environmental conditions (Figs.9 -11)

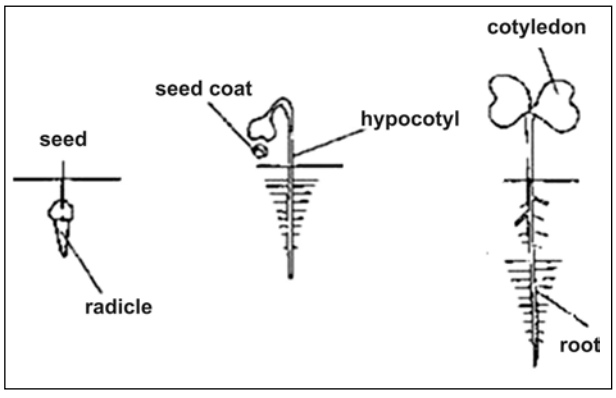

Fig. 9.Germination and Emergence 3 to 5 days

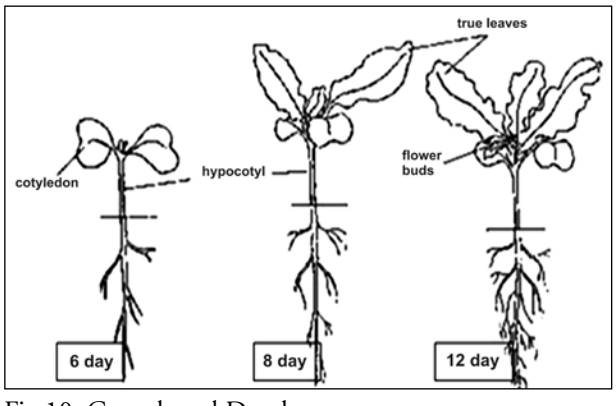

Fig.10. Growth and Development:

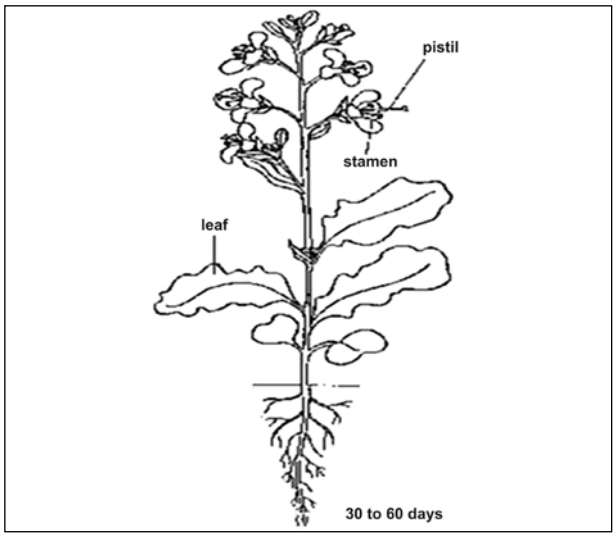

Fig. 11 Flowering and Reproduction
Source: Re drawn from Wisconsin Fast Plants program, University of Wisconsin Madison

3.2 Methods of Pollination, Known Pollinators and Pollen Viability

B. juncea is a predominantly self pollinated crop (Labana *et al.* 1992). However, in some environments out crossing varies from 7.6% to 22% (Dhillon and Labana, 1988; Ram Bhajan *et al.* 1991; Abhram, 1994). *B. juncea* is self compatible and largely self pollinated.

Pollen can live up to 4 or 5 days when temperature is low and humidity is high. With warm temperatures and low humidity, survival time may drop to 1 or 2 days (Mayers, 2006). *Brassica* pollen is viable even after 4 hours of stress at 60° C. (Rao *et al.*, 1992). However under experimental conditions, it has been observed that pollen could remain functional for a year or more in dry storage at -20° C (Brown* and Dyer, 1990).

Pollination is carried out in nature both by insects and wind; bees however are the primary pollen vector because the pollen is heavy and sticky and is not carried to great distances in the absence of wind. Wind can carry pollen over long distances as pollen counts of upto 22 pollen grains/m^3 were observed 1.5 km away from the source field and were sufficient to effect the seed set on bait plants (Timmons *et al.*, 1995).The extent of wind pollination was recorded up to 11 - 17.5% (Singhal *et al.*, 2005) However, insect pollination is an important component of reproductive biology of *B. juncea* (Labana and Banga, 1984). Bees visit flowers for nectar; the positioning of nectaries is such that in self incompatible types, the body of bee gets smeared with pollen and in self compatible types, the bee affects self pollination by pressing the inner whorl of introrsely dehisced anthers while extracting nectar thus bringing them in contact with the stigmatic surface (Kumar, 2001). The stigmas remain receptive 3 days before opening to 3 days after opening of the flowers (Singh and Rai, 2004). Bees may carry pollen over long distance

and have been found foraging in fields more than four Km away from the hive (Esthamn and Sweet, 2002), resulting in outcross seed set. Besides physical carrying of pollen grains, bee visitation also cause pollens to become air borne. Air borne pollen grains can then be carried by wind, leading to cross pollination. Cross pollination of nearby plants can also result from physical contact of the flowering racemes.

The extent of wind pollination in *B. juncea* cv. Pusa Bold was studied in New Delhi, India for three years (1996-96 to 1998-99). Dispersal of pollen grains by wind was noticed up to 35 metres from the pollen source. Air borne pollen grains may pass through insect proof nets and effective pollination may occur. The extent of wind pollination was recorded up to 11 to 17.5% (Singhal *et al.* 2005). The commonly used method of reproductive isolation in case of *B. juncea* is spatial isolation. The recommendations made on isolation distance for production of foundation seed and certified seed of 96% purity of self fertile *B. juncea* are 200m and 50m, respectively (Tunwar and Singh, 1988, Kumar, 2001).

3.3 Seed Production and Dispersal

B. juncea seeds virtually show no signs of dormancy at maturity. However, non-dormant seeds may enter dormancy if environmental conditions are unfavourable for germination. Induction of secondary dormancy in *B. juncea* occurs in response to sub optimal germination conditions such as large temperature fluctuations, low available soil moisture, long exposure to darkness and suboptimal oxygen supply. Persistence of *B. juncea* seeds is considerably longer in undisturbed soils compared to cultivated soils. Persistence will also vary between soil types.

So *B. juncea* may escape harvest and remain in the soil until the following season when they commonly germinate either before or following seeding of successive crop. As a result, *B. juncea* volunteers could grow and become weedy in subsequent crops. However, despite a long history of cultivation, *B. juncea* is not considered a weed, and therefore there is good reason to conclude that it does not have the weedy characteristics of wild mustard and may be less prone than *B. napus* and *B. rapa* to become a volunteer weed (Biology Document BIO2007-01). Although there are no free living populations of *Brassica* species in India.

3.4 Potential for Vegetative Propagation

Normal means of *B. juncea* propagation is through seeds. There are no reports of vegetative propagation under field conditions. However, *B. juncea* can be grown through transplanting under normal field conditions. Organogenesis is another important tool for plant regeneration using tissue culture techniques. *Brassica* species have been widely exploited for tissue culture purposes. Regeneration protocols have been developed for most of the *Brassica* species. Regeneration of plants via organogenesis has been accomplished using various tissues such as cotyledons (Hachey *et al.* 1991, Ono *et al.* 1994), hypocotyls (Das *et al.* 2006), peduncle segments (Eapen and George, 1997), leaves (Radke *et al.* 1988), thin cell layers of epidermal and sub-epidermal cells (Klimaszewska and Keller, 1985) and roots (Xu *et al.* 1982). Hypocotyl segments, however remain the most desirable explants for tissue culture and have been used for most *Brassica* species because of their ability to regenerate.

4. HYBRIDIZATION AND INTROGRESSION

4.1 Naturally Occurring Interspecific Crosses

Published reports on naturally occurring interspecific crosses among cultivated or wild species of *Brassica* in India are not available in literature. However, a very low frequency of natural hybridization among *B. rapa*, *B. juncea* and *B. napus* does occur if they are cultivated closely. *B.juncea* x *B.rapa* and *B.napus* x *B.juncea* hybrids are partially fertile and can set a few open pollinated seeds. *B. rapa* and *B. juncea* are major candidate recipients of introgression from *B. napus* (OECD 2012). *B. juncea* shows the second highest crossability with *B. napus* after *B. rapa*. The maximum spontaneous hybridization between *B. juncea* and *B. napus* was reported to be 5.91% in mixed planting (Heenan *et al.* 2007), but spontaneous hybrids were not detected among plants separated at distances of 20 m (Tsuda *et al.* 2012).

Bing (1991) reported that *B. juncea* and *B. nigra* had relatively high cross compatibility in hand-crossing, especially when *B. juncea* was used as the female. Extensive back-crossing to *B. nigra* failed to produce seed. No interspecific hybrids were found with field crossing. It was concluded that there are strong natural barriers to gene flow from *B. juncea* to *B. nigra*. The hybridization frequency decreased drastically with distance from the pollen source, and was lower under field conditions than estimated from the high crossability some wild species (like *B.tournefortii*) may also occur where *B. juncea* is cultivated. However, these interspecific hybrids do not set seed on open pollination because of the genomic constitution.

4.2 Experimental Interspecific/ Intergeneric Crosses

Successful hybrids have been obtained in both the directions by crossing *B. juncea* and *B. rapa*, although *B. juncea* is a tetraploid species as compared to *B. rapa* which is diploid. It has been reported that frequency is higher when *B. juncea* is used as a female parent (Choudhary and Joshi, 1999, Oslon, 1960, Ahmed,1991). The success of cross fertility of three ecospecies of *B. rapa* with *B. juncea* was in order of Yellow sarson > toria > brown sarson. Differences were also observed at varietal level. Successful transfer of genes from *B. rapa* to *B. juncea* has been reported (Love *et al.*, 1990) as well as transfer of genes from *B. juncea* to *B. carinata* (Getinet *et al.*, 1994).

Over 45 *Brassica* wild relatives were reported in reviews of the literature on the production of F1 hybrids (Prakash *et al.* 2009; Kaneko and Bang 2014). Numerous novel F1 hybrids have been produced through embryo culture, ovary culture, ovule culture and placenta culture. Ovary culture followed by embryo or ovule culture, placenta culture followed by embryo culture, and successive ovary, ovule, and embryo culture have also been used. Many *Brassica* species show a high degree of relatedness, which allows crossing to occur across species and even genera. Inter crossing occurs with varying degrees of difficulty. While many interspecific and intergeneric crosses have been made between *B. juncea* and its relatives in the mustard family, most have required human intervention in the form of ovary culture, ovule culture, embryo rescue, or protoplast fusion. Examples of interspecific/intergeneric hybrids obtained between *B. juncea* and its relatives have been published in review papers (Prakash *et al.* 2009; Kaneko and Bang 2014) and one list in Table 7.

Table 7. Reports of interspecific / intergeneric hybridization between *B. juncea* and related species.
(Symbols: BC - Backcross; F1-F1 hybrid; Rs - the reciprocal cross has been successful; Rt - the reciprocal cross has been tried and not been successful; SEXL - hybrid was obtained sexually; EMBR - hybrid obtained with embryo culture).

Parental combination	Cross type	Reference
B. juncea x B. carinata	Rs, SEXL	Alam *et al.* (1992)
B. juncea x B. carinata	SEXL	Barcikowska *et al.* (1994)
B. juncea x B. carinata	Rs, SEXL	Gosh Dastidar &Varma (1999)
B. juncea x B. carinata	Rs, SEXL	Gupta (1997)
B. juncea x B. carinata	Rs, SEXL	Katiyar & Chamola (1995)
B. juncea x B. carinata	Rs, SEXL	Krishnia *et al.* (2000)
B. juncea x B. carinata	Rs, SEXL	Kumar *et al.* (2002)
B. juncea x B. carinata	SEXL	Rao & Shivanna (1997)
B. juncea x B. carinata	SEXL	Sharma & Singh (1992)
B. juncea x B. carinata	SEXL	Singh *et al.* (1997)
B. juncea x B. maurorum	SEXL	Bijral *et al.* (1995)
B. juncea x B. napus	Rs, SEXL	Alam *et al.* (1992)
B. juncea x B. napus	Rs, SEXL (field)	Bing *et al.* (1991; 1996)
B. juncea x B. napus	Rs, SEXL	Choudhary & Joshi (1999)
B. juncea x B. napus	Rs, SEXL	Gosh Dastidar & Varma (1999)
B. juncea x B. napus	Rs, SEXL	Gupta (1997)
B. juncea x B. napus	EMBR	Shen *et al.* (2006)
B. juncea x B. napus	Rt, SEXL	Warwick (2007)
B. juncea x B. napus	SEXL	Rao & Shivanna (1997)
B. juncea x B. napus	Artificial pollination	Mason *et al.* 2011, Tsuda *et al.* 2011)
B. juncea x B. napus	SEXL	Sharma & Singh (1992)
B. juncea x B. napus	SEXL	Vijayakumar *et al.* (1994)
B. juncea x B. nigra	Rs, SEXL	Bing *et al.* (1991)
B. juncea x B. nigra	SEXL	Prasad *et al.* (1997)
B. juncea x B. nigra	SEXL	Rao & Shivanna (1997)
B. juncea x B. oleracea	Rs, SEXL	Gupta (1997)
B. juncea x B. oxyrrhina	SEXL	Bijral & Sharma (1999b)
B. juncea x B. rapa	SEXL	Choudhary *et al.* (2002)
B. juncea x B. rapa	SEXL	Gupta (1997)
B. juncea x B. rapa	Rs, SEXL	Gupta *et al.* (2006)
B. juncea x B. rapa	SEXL	Katiyar & Chamola (1995)
B. juncea x B. rapa	Rs, SEXL	Rhee *et al.* (1997)
B. juncea x B. rapa	SEXL	Sharma & Singh (1992)
B. juncea x B. rapa	Rs, SEXL	Choudhary & Joshi (1999)
B. juncea x B. rapa	Rs, SEXL	Gosh Dastidar & Varma (1999)
B. rapa x B. juncea	SEXL	Prasad *et al.* (1997)
B. juncea x Diplotaxis muralis	SEXL	Bijral & Sharma (1995)
D. muralis x B. juncea	SEXL	Gupta (1997)
B. juncea x Eruca sativa	SEXL	Bijral & Sharma (1999a)

B. juncea x Eruca sativa	Rt, SEXL	Gosh Dastidar & Varma (1999)
B. juncea x Orychophragmus violaceus	SEXL, BC	Li *et al.* (1998, 2003)
B. juncea x Raphanus sativus	Rt, SEXL	Gupta (1997)
B. juncea x Raphanus sativus	Rs, SEXL	Rhee *et al.* (1997)
B. juncea x Sinapis alba	SEXL	Bijral *et al.* (1991)
B. juncea x Sinapis alba	SEXL	Sharma & Singh (1992)
B. juncea x Sinapis arvensis	Rt, SEXL	Bing *et al.* (1991)
B. tournefortii x B. juncea	SEXL	Gupta (1997)
Diplotaxis siifolia x B. juncea	SEXL	Gupta (1997), Ahuja *et al.*(2003)
D. catholica x B.juncea	SEXL	Banga *et al.*(2003)
D. tenuifolia x B. juncea	Rt, SEXL	Salisbury (1989)
(B.fruticulosa x B. rapa F1) x B. juncea	SEXL,BC	Garg *et al.*(2010)
(Erucastrum cardaminoides x B.rapa F1)x B.juncea	SEXL,BC	Garg *et al.*(2010)
(D. erucoides x B. rapa F1) x B. juncea	SEXL, BC	Malik *et al.* (1999),Garg *et al.*2007
(B. juncea x B. napus F1) x B. juncea	SEXL, BC	Frello *et al*. (1995)
(B. carinata x B. juncea F1) x B. carinata	SEXL, F1 & BC	Getinet *et al.* (1994; 1997)
(B. napus x B. juncea F1) x B. juncea	SEXL, F1 & BC	Kirti *et al.* (1995)

(Update on the the Biology of *Brassica juncea*. Biology Document BIO2007-01)

Seeds from interspecific crosses should be checked for hybridity, as matromorphic seeds are often produced rather than true hybrid seed (Salisbury, 2006). For a trait to become incorporated into a species genome, recurrent backcrossing of plants of that species by the hybrid intermediates and survival and fertility of the resulting offspring would be required.

4.3 Genetic Introgression

There are several prerequisites for a successful gene transfer to occur between species. Pre-fertilization factors include physical proximity, pollen movement, and pollen longevity, synchrony of flowering, breeding system, floral characteristics and competitiveness of foreign pollen. Post-fertilization factors include sexual compatibility, hybrid fertility, viability and fertility of progeny through several generations of backcrossing and successful introgression of the gene into the chromosomes of the recipient species (Salisbury, 2006).

Successful sexual hybrids between *B. juncea* and Sinapis arvensis were reported by Bing *et al.*, (1991). Hybrids (2.5% frequency) were obtained between *B. juncea* and *S. arvensis* in controlled greenhouse studies, when emasculated plants of *B. juncea* served as the female parent, but not for the reciprocal cross. Seed produced on backcross F_1 x *B. juncea* failed to germinate and the one seed from F_1 x *S. arvensis* developed into a weak, male sterile plant which produced no seed on open pollination. No gene flow was detected from *B. juncea* to 45 plants of S. *arvensis* grown together in a small field plot experiment in Saskatchewan (Bing *et al.*, 1991, 1996); however hybrid detection was based primarily on morphological characters and very small sample sizes. In a field

co-cultivation experiment between *B. juncea* and *S. arvensis* (pollen recipient), where use of a herbicide resistance marker allowed for screening of larger numbers of seedlings, hybrid plants were detected but at a very low frequency (Warwick, 2005). Only two hybrids were obtained from 109,951 screened seedlings, i.e frequency of 1.8 x 10-5. One F_1 hybrid was able to set seed when selfed, and many of the subsequent selfed F_2, F_3 and F_4 hybrid generation plants derived from this plant showed vigorous growth and high pollen fertility levels. Herbicide resistance persisted in the F_2, F_3 and F_4 hybrid generations. However, no backcross progeny were produced when neither the F_1 hybrid nor when self-derived hybrids were backcrossed to *S. arvensis*, confirming the results obtained by Bing *et al.*, (1991). The likelihood of introgression of traits from *B. juncea* to *S. arvensis* appears to be low to negligible.

Lefol *et al.*, (1997) investigated the production of hybrid seeds between *B. juncea* and *Erucastrum gallicum* or *Raphanus raphanistrum* using reciprocal crosses. They did not use embryo rescue so their measurements were of seed production that might occur under natural conditions. The *R. raphanistrum* x *B. juncea* cross failed to produce any seed and the viable seed produced from all the other crosses were not considered to be hybrids. Therefore, the probability of intergeneric crosses between these two weedy species and *B. juncea* also appears to be low.

However, four complete sets of introgression lines developed following hybridization of four wild crucifers (*viz. Erucastrum cardaminoides, Diplotaxis tenuisiliqua, E. abyssinicum and Brassica fruticulosa*) have been reported in *B. juncea* (Garg *et al.*2010, Banga unpub).The wild crucifer, *Brassica fruticulosa* is known to be resistant to mustard aphid. An artificially synthesized amphiploid, (*B. fruticulosa* × *B. rapa* var. brown sarson) was developed (Chandera *et al.* 2004) for use as a bridge species to transfer fruticulosa resistance to *B. juncea* as reported by Atri *et al.*, (2012).

4.4 Gene to Other Organisms

The only means by which genes could be transferred from non plant organisms is by horizontal gene transfer (HGT). Such transfers have not been demonstrated under natural conditions (Nielson et al., 1997, Nielson *et al.*, 1998, Syvanen, 1999) and deliberate attempts to introduce them have so far failed (Schluter et al., 1995, Coghlan, 2000). Thus gene transfer from *B. juncea* to organisms other than plants, is extremely unlikely.

4.5 Free Living Populations

The term "free living" is assigned to plant populations that are able to survive, without direct human assistance, over long term in competition with the native flora. This is a general ecological category that includes plants that colonize open, disturbed prime habitat that is either under human control (weedy populations) or natural disturbed areas such as river banks and sand bars (wild populations). There are no such free living populations of *Brassica* species in India.

5. KNOWN INTERACTIONS WITH OTHER ORGANISMS IN MANAGED AND UNMANAGED ECOSYSTEM

5.1 Interactions in Unmanaged and Managed Ecosystem

Effects of ecosystem on agriculture or more precisely on insect pests and diseases of *B. juncea* are multi dimensional. A managed ecosystem is one in which societies take steps to ensure the efficient and sustainable use of a resource. The increase in infection rate of *Alternaria blight* (AB), white rust (WR) and *Sclerotinia* rot (SR) diseases and infestation rate of aphid attack are directly proportional to delay in planting of the crop in most mustard growing areas in India. The same is true with respect to powdery mildew infection and severity in non traditional areas in the central and southern states of India. An unmanaged ecosystem is one that operates largely without human intervention. It might be unmanaged because humans choose to leave it alone. Little self pollination occurs in most species and cultivars, and insect pollination is essential to produce good crops of seed. The flowers of most *B. juncea* plants are attractive to honey bees. *B. juncea* and other related *Brassica* species can be used for cover crops because they grow rapidly, provide erosion control, produce large amounts of biomass (up to 9070 kg/ ha) and are excellent at scavenging nutrients (up to 159 kg of nitrogen/ ha). Antagonists *Trichoderma harzianum, T. viride* (G R isolate), *Streptomyces rochei*, and *Bacillus subtilis* strain (UK-9) are very effective against *Alternaria brassicae*, *A brassicicola* and *Plasmodiophora brassicae)*. Low solar radiation and short-day periodicity could result in higher infections by *Fusarium*, *Sclerotinia* and *Verticillium* (Nagarajan and Muralidharan, 1995). Root rot is an emerging threat for rapeseed-mustard production system, as recently reported from the farmers' field in some pockets of the country (Meena *et al*., 2010a), which was initially identified as stand-alone bacterial or fungal incidence or in combinations (*Erwinia carotovora* pv. *Carotovora, Fusarium*, *Rhizoctonia solani* and *Sclerotium rolfsii*). Keeping in view the facts that some isolates of *A. brassicae* sporulated at 35°C and several isolates had increased fecundity under higher Relative Humidity, it seems that as per recent changes towards warmer and humid winters, being in line with current projections for future climate change (Waugh *et al*. 2003), existence of such isolates could pose more danger to the Rapeseed-mustard due to *Alternaria blight* in times to come. The immense variation available among only twenty five representative isolates of *A. brassicae* also indicates their ability to adapt to varied climatic situations (Meena *et al*., 2012). Similaly, *Sclerotinia* rot (*Sclerotinia sclerotiorum*) which emerged as a new threat in Brassicas during 1998-99, is now the major constraint in enhancing the production of the crop in India. Recently, the stem blight (*Nigrospora oryzae*) disease has been reported as the new challenge for rapeseed-mustard (Sharma *et al*., 2013). Similar other pathogens have also been reported which may become established as major challenges for the crop in the coming years. Rapeseed-mustard crop is being continuously cultivated as a monocrop in certain pockets of the country and within that cropping system, pathogens are increasing under changed pest scenario).

5.2 Major Insect Pests of *Brassica juncea*

About 50 insect pests are known to *B. juncea* and related species and among these, the mustard aphid (*Lipaphis erysimi*) is the key pest while saw fly (*Athalia lugens proxima*), painted bug (*Bagrada hilaris*), pea leaf miner (*Chromatomyia horticola*) and Bihar hairy caterpillars (*Spilosoma obliqua* (Walker)) are also serious pests. Several newer pests like *Myzus persicae, Agrotis segetum, Crocidolomia binotalis, Plutella xylostella, Odontotermus obesus* and *Monomorium sp.* are minor probable threats to Rapeseed-mustard.

Fig 12. *Coccinella septempunctata*

i) Mustard Aphid (*Lipaphis erysimi* (Kaltenbich)

The mustard aphid (*Lipaphis erysimi*) (Homoptera: Aphididae) commonly called Chenpa, Mahoo, Moyala or Tela is the key pest of Rapeseed-mustard crops in India. Other species of aphids associated with these crops are *Myzus* persicae Sulzer and *Brevicoryne brassicae* (Linn.).

Distribution in India: The mustard aphid is widely distributed in all parts of India wherever the Rapeseed-mustard crops are grown. In south India, the pest is of minor importance.

Identification of Pest: The mustard aphid (Fig 13) is a small, globular, pear shaped, delicate insect with a soft and fragile body. Adult aphid is found in two forms i.e. winged (alate) and wingless (delate). Wingless adult aphid is varying in colour mostly green or pale green and 2 mm long in size. Winged form has transparent homogenous wings and yellowish abdomen. Nymphs are like wingless forms but smaller in size. Two tubular structures (cornicles or siphunculi) are present on the posterior region of the body.

Symptoms of Damage: The pest sucks the cell sap from the plants by inserting its needle like stylets into the tissue and can produce a large number of offspring. The infested plants exhibit yellow, curled, wrinkled, and withered leaves, the plant growth remains stunted, and they fail to produce seeds. Generally, the mustard aphid infests the parts of the plant above ground including leaves, stem, inflorescence and pods.

Fig 13. Mustard Aphid

Extent of damage: The pest causes reduction of about 9 to 96% in seed yield and up to 10% reduction in oil content.

ii) Painted Bug (*Bagrada hilaris* (Burm.)

Painted bug is a highly polyphagous pest kown as Chitkabra in Hindi. (Fig. 14)

Distribution in India: This pest infests Rapeseed-mustard crops all over India, and has been found to cause serious damage in the states of Rajasthan, Punjab, Haryana, Uttar Pradesh and Madhya Pradesh.

Fig 14. Painted bug

Identification of pest: Adult bugs are pretty looking sub ovate, grey to dark brown or black in colour having many orange/ brownish spots on the dorsal side of the body. Adults measure 6.5 – 7.0 mm in width and full grown nymph measures 4 mm long and 2.6 mm wide with brown markings. The first and second instar nymph is bright orange in colour while third and fourth instar is red. It has piercing and sucking type of mouth parts with hypognathous position.

Symptoms of Damage: Both adult and nymphs suck cell sap from the leaves and shoots. Some times, in case of tender two leaf plant, the infested tender shoot falls down and the plant dies. The infestation of this pest in the vegetative growth stage results in whitening of leaves, wilting, and complete drying of the plant. In both the cases re-sowing of the crop becomes necessary. The pest also attacks the crop at pod formation and maturity, which results in curling of the pods and shrivelling of grains. Bugs can be seen feeding on the harvested material lying on the threshing floor.

Extent of damage: Painted bug may lead to yield losses of 31.1% and reduction of oil content by 3-4%.

iii) Pea Leaf Miner (*Chromatomyia horticola* Goureau)

The pea leaf miner (Fig 15), known as Patti Ka Surangi Keet in Hindi, is a highly polyphagous pest found in all the mustard growing areas of the country.

Identification of pest: Adult is a small black coloured fly with yellow head, 1.5 mm long with about 4 mm wing span and resembles the house fly, but is smaller in size. Young maggot is dirty white in colour with smoky brown mouth parts. Full grown maggot is greenish yellow about 3 mm long and 0.7 mm broad with thickest region in middle and tapering interiorly. Maggots remain inside the mine and also pupate there.

Fig 15. Pea Leaf Miner

Damage symptoms: The damaged leaves present zig zag silvery lines with black pupae at the end of the mines.

Extent of damage: Crops suffer severely during their vegetative and flowering phases. Yield losses vary from 4.4 to 15.5%.

iv) Mustard Sawfly (*Athalia lugens proxima* Klug.)

The pest is locally known as Ara Makhi.

Distribution: The insect is found in the pest form in Uttar Pradesh, Bihar and West Bengal.

Identification of pest: Adult sawfly measures 8-11 mm long with orange yellow coloured wasp having smoky wings with black veins. Its ovipositor is serrated and saw like hence called sawfly. Head and legs are black. The larvae are yellowish green to dark green with five lateral longitudinal stripes. Freshly hatched first instar larvae are 2 mm long, cylindrical, violet or greenish grey or green in colour. Full grown larvae measure about 15-18 mm and look like pseudocaterpillars.

Damage symptoms: It appears in early stages of the crop, i.e. October and November. The larvae make irregular holes in the leaves. Grown up larvae feed from the margins of leaf. The crop is attacked at seedling stage and three to four weeks old crop is most preferred.

Extent of damage: In severe infestation the crop looks as if it has been grazed by animals.

v) Bihar Hairy Caterpillar (*Spilosoma oblique* (Walker)

The Bihar Hairy Caterpillar (Fig 16) is commonly known as Katra, Kambal-Keera, Balon Wali Sundi, etc.

Distribution: The pest is highly ploy phagous and sporadic in nature and is found throughout Rapeseed-mustard growing areas of the country.

Identification of pest: Adult moth is dull yellow coloured and measures about 40-45 mm across the wings. The orange tiny wings have black spots. The abdomen is crimson or red in colour with black spots.

Fig 16. Bihar Hairy Caterpillar

Damage symptoms: First two larval instars feed gregariously on chlorophyll content of leaves. Leaves become papery devoid of chlorophyll and almost transparent. The larvae feed from the margin of leaves and defoliate the entire plant. Fourth and fifth instar larvae are voracious feeders and consume much more food per day than their own body weight. Larvae have the habit of migrating from one field to the other.

Extent of damage: In severe attack re-sowing has to be done.

vi) Cabbage Butterfly (*Pieris brassicae* L.)

This is a very destructive pest of *Brassica* vegetables.

Distribution: Cabbage butterfly (Fig 17) is found in temperate parts of India. It also infests Rapeseed-mustard crops specially Karan Rai (*Brassica carinata*).

Identification of pest: Adult butterfly is pale white in colour having the wing expanse of 55-66 mm. The tip of anterior wing has conspicuous black marks. The female butterfly has two extra black marks on the anterior wings.

Damage symptom: The pale coloured first instar larvae feed gregariously on leaves. Larvae migrate

Fig 17. Cabbage Butterfly

to whole field for food and then to weed bushes for pupation. Karan rai (*Brassica carinata*) suffers more damage in comparison to other Rapeseed-mustard.

Extent of damage: Under severe infestation the whole plant except the main stem is eaten up.

vii) Flea Beetle (*Phyllotreta cruciferae* (Goeze)

Distribution: Different species of this pest are found all over the country wherever the Brassica crops are grown.

Identification of pest: The insect is a blue-black coloured beetle having greenish reflection. The shape of the insect is anteriorily conical and posteriorily round. The length of ranges from 1.8 to 2.0 mm. Grubs are dirty coloured, about 5 mm in length.

Damage Symptoms: The grubs feed on the root part of the plant by making a tunnel. The adults feed on leaves and pods of the plant by making typical "shot holes" in the leaf and scraping the chlorophyll. Adults are found in large numbers on the plant and on disturbance, jump to other plants. The early crop may also suffer damages.

Extent of damage: Causes considerable damage to the crop by feeding on leaves and pods of the maturing crop and some times also in the initial stage of the crop.

viii) Leaf Webber (*Crocidolomia binotalis* Zeller)

Commonly known as Patti Modak the leaf webber (Fig 18) is a common insect pest of *Brassica* crops.

Distribution: It is a minor pest found throughout the country, causes considerable damage to Rapeseed-mustard in sub tropical parts.

Fig 18. Leaf Webber

Identification of pest: The moth is small and light reddish brown in colour. Forewings have black specks whose margin is green with white spots. Hind wings are light yellow in colour. The larvae are about 13-16 mm long and greenish in colour with red colour perpendicular strips. The head of larvae is reddish in colour. The full grown larva change to cocoon in soil.

Damage symptoms: Freshly hatched larvae feed on the chlorophyll content of tender leaves. Later on the upper canopy leaves, flower buds and inflorescence are webbed together resulting in stunting of growth. Damage is more severe when entire inflorescence is webbed tightly and eaten by the larvae. Severely attacked plants are completely

defoliated. Larvae also bore inside the pods and feed on the developing seeds. *Toria* crop suffers the most.

Extent of damage: The pest is of minor importance but under favourable conditions causes considerable damage.

ix) Diamondback moth (*Plutella xylostella* (Linn.)

Distribution: This is a serious sporadic pest of *Brassica* vegetables and found on *Brassica* crops all over the country.

Identification of pest: Adult moth is grey or brown, measures 8-10 mm in body length with wing span of about 10-15 mm. Anterior wings are light brown in colour having three yellow spots. The anal margin of forewing has white triangular spot when the moth sits with wings lying over the body. These spots look like a row of diamonds; hence the name, diamond back moth. Young larvae are dirty white in colour and make mines in the leaf and then come out to feed on the leaves. Adult larvae are about 8 mm long, yellow green in colour with fine black hairs all over the body.

Damage symptoms: First two instar larvae tunnel into the leaves and feed on the mesophyll. Third instar feed on leaves outside the tunnel. Full-grown larvae feed on the leaves by making holes. Attack of this pest is confined to late sown crop.

Extent of damage: It is a minor and sporadic pest of Rapeseed-mustard.

x) Termites or White Ants (*Odontoterms obesus* Rambur)

The local name of termite is Deemak.

Distribution: The pest is found on the *Brassica* crops all over the country under rainfed conditions.

Identification of pest: Wings appear during nuptial flight. They have biting and chewing type of mouth parts. They are social insects with many castes *viz.*, king, queen, soldiers and workers. Highly polyphagous in nature & look like ants but are dirty white in colour hence called "white ants".

Causal Symptoms: Yellowing of plants finally drying up due to damage of roots. There are wilt-like symptoms due to termite damage.

Extent of damage: This is a minor pest of Rapeseed-mustard. Termite damage the crop soon after sowing and near maturity. The damaged plants dry up completely and are easily pulled out. Plants damaged at later stages, give rise to white foliage.

5.3 Major Diseases, Causal Agents and their Control in Managed Ecosystem

Diverse plant pathogens are reported to distress the crop. Among them, 18 are considered to be economically important in different parts of the world. Among various diseases, 4 diseases *viz*; *Alternaria* blight (*Alternaria brassicae*), white rust + downy mildew complex (*Albugo candida* + *Hyaloperonospora brassicae*), white rot (*Sclerotinia sclerotiorum*) and powdery mildew (*Erysiphe cruciferarum*) are of great economic importance. Among a number of other relatively less important diseases, seedling blights/ damping-off (*Rhizoctonia solani, Sclerotium rolfsii* and *Fusarium solani*), phyllody (caused by Sesame phytoplasma), bacterial rot (*Xanthomonas campestris pv campestris*), club root (*Plasmodiophora brassicae*), mosaic (Turnip Mosaic Virus) and Orobanche (a phanerogamic parasite) appear to become important only under specific agroecological conditions in certain geographical areas and hence are assumed to be of regional and sporadic importance (Kolte, 1985).

i) Damping off and Seedling Blight

Many fungal species such as *Alternaria, Fusarium, Phoma*, and *Rhizoctania solani* are involved in causing seed rot and seedling blight. Among them, *Rhizopus stolonifer* is reported to be more important (Petrie, 1973).

Distribution: Disease occurs all around the world in Rapeseed-mustard.

Causal pathogens: Post emergence mortality is not frequent in *Pythium aphanidermatum* (Mahmud, 1950), *P. butleri* (Aulakh, 1971), *Rhizoctonia solani* (Srivastava, 1968), *Sclerotium rolfsii* (Upadhyay and Pavgi, 1967), *Macrophomina phaseolina* (Srivastava and Dhawan, 1979), *Fusarium* spp., *Verticillium* spp., and *Phoma* (Neupane *et al.*, 2013). They mostly survive on crop debris and soil as different resting structures to infect the following crop. Damping-off can produce many symptoms ranging from pre-emergence rot (failure of plants to emerge) to post emergence damping-off (plants emerge and collapse at ground level). If affected plants survive, they are normally stunted and may flower and mature prematurely.

Extent of damage: The pathogens involved in India, cause 6-15% incidence (Khan and Kolte, 2002).

ii) *Alternaria* Blight

Alternaria blight (Fig 19) or black spot, the most common, widespread and destructive disease is caused mostly by *Alternaria brassicae* (Berk.) *Sacc.* infecting all above ground parts of the plant.

Distribution: It has been reported from all the continents of the world. In India, the disease is severe mainly in the states of Himachal Pradesh, Haryana, Rajasthan, Uttar Pradesh, Uttara Khand, Bihar and Madhya Pradesh, but appears in almost all the parts of the country.

Fig 19. *Alternaria* blight affecting the above ground plant parts.

Identification of pathogen: Pathogens of the disease, *A. brassicicola* and *A. raphani* are also encountered but rarely. *Alternaria* produce large, multicellular, dark-coloured (melanized) conidia with longitudinal as well as transverse septa. These conidia are broadest near the base and gradually taper to an elongated beak, providing a club like appearance. They are produced in single or branched chains on short, erect conidiophores.

Disease symptoms: The symptoms of disease are formation of brown to black spots with concentric rings on leaves, stem and siliquae (Meena *et al.*, 2010a). Generally, the disease appears at 40 - 45 days after sowing and the most critical stage has been reported at 45 and 75 days of plant growth (Meena *et al.*, 2004).

Extent of damage: Though total destruction of the crop due to the disease is rare and usually yield losses at harvest are 5-15%, they can reach up to 47% (Kolte *et al.*, 1987) accompanied by reduction in seed quality *viz.*, seed size, viability etc. Severity

of Alternaria blight on Rapeseed-mustard differs in various seasons and regions as also between individual crops within a region.

iii) White Rust

White rust, caused by *Albugo candida* (Pers. Ex Fr.) Kuntz. is an obligate pathogen of all cruciferous crops.

Distribution: Plants of 241 species in 63 genera of cruciferae family have been reported to be infected by *A. candida* (Biga, 1955) all over the world.

Fig 20. White rust caused in leaves by *Albugo candida*

Identification of pathogen: The non septate and intercellular mycelium of *Albugo* species (Fig 20) feeds by means of globose or knob shaped intracellular haustoria. The mycelium soon organizes the characteristic groups of sporangiophores which develop beneath the epidermis, raising it to make whitish pustules or extended blister-like areas due to the merging of adjacent sori. The sporangiophores are short, basally branched, club shaped and give rise to simple chains of sporangia. The number of sporangia produced is indefinite in basipetal succession; that is, the sporangiophore forms a cross-wall or septum, cutting off that portion which is to become a sporangium. The size of sporangia range between 13.6-21.8 µM. The unique mode of oospore germination is by producing zoospores without prior formation of a germ tube (de Bary, 1863; Schröter, 1893; Verma and Petrie, 1979).

Causal symptoms: Disease appearing on leaves is characterized by the appearance of white or creamy yellow raised pustules up to 2 mm in diameter, which later coalesce to form patches. The part of upper surface corresponding to the lower surface is tan yellow, which enables recognition of the affected leaves (Parui. and Bandyopadhyay, 1973; Saharan and Verma, 1992; Verma, 2012).

Extent of damage: Can result in yield loss up to 47% (Kolte, 1985) with each per cent of disease severity and staghead formation causing reduction in seed yield of about 82 kg/ ha and 22 kg/ ha respectively (Meena *et al.*, 2002).

iv) *Sclerotinia* Rot or White Rot

Rot of mustard caused by *Sclerotinia sclerotiorum* (Lib.) de Bary has become important in recent times in India (Fig 21).

Fig 21. *Sclerotinia sclerotiorum* affective leaf & stem

Distribution: Sclerotinia rot is also a serious threat to oilseed rape production with substantial yield losses worldwide. The first record of its occurrence on Rapeseed and mustard appears to have been made from India (Shaw and Ajrekar, 1915). The pathogen is reported to have a wide host range, known to infect about 408 plant species (Boland and Hall, 1994) with no proven source of resistance against the disease reported till date in any of the hosts.

Identification of pathogen: The pathogen is *Sclerotinia sclerotiorum* (Lib.) de Bary. Mycelium is thin, 9-18µm in diameter with lateral branches of smaller diameter than the main hyphae. The vegetative hyphae are multi nucleate (n=8). The sclerotia are black, round or semi spherical in shape measuring 3-10 µm. The sclerotial germination is mycelogenic (by mycelium) or carpogenic (by formation of apothecia). On germination, the sclerotia form stalked apothecia. One to several apothecia may grow from a single sclerotium. Ascospores discharged from the apothecia at the base of the plants in soil constitute important primary sources of infection.

Causal symptoms: Symptoms on the stem become visible as elongated water soaked lesions, which are later covered by a cottony mycelial growth of the fungus. Infected plants are at times overlooked until the fungus grows throughout the stem to rot it.

Extent of damage: The damage is significant, with high (up to 66%) disease incidence and severe yield losses (up to 39.9%) leading to discouragement of growers of the crop (Chattopadhyay *et al.*, 2003).

v) Powdery Mildew

Distribution: Occurrence of powdery mildew (Fig 22) on Rapeseed-mustard is reported from various parts of the world. Recent reports on the occurrence of powdery mildew of Rapeseed-mustard deal with *Erysiphe cruciferarum* (Sharma, 1979). In certain states of India such as Gujarat, Haryana and Rajasthan, the disease has been found to occur quite severely, resulting in considerable loss in yield.

Fig 22. Powdery Mildew in Rapeseed Mustard Plants

Extent of damage: Though the exact data on yield losses are not available, considering the differences in disease intensity from year to year, it appears that yield loss is proportional to the disease intensity, which varies considerably depending on the stage at which it occurs.

Disease symptoms: The symptoms appear in the form of dirty-white, circular, floury patches on both sides of lower leaves of the infected plants. The floury patches increase in size and coalesce to cover the entire stem and leaves under environmental conditions favourable to the pathogen.

vi) Club Root

Clubroot (Fig 23) disease of the Brassicaceae has been a major threat to the crop caused by *Plasmodiophora brassicae* Woronin. Incidence and severity are greater in regions of extreme winters

Fig 23. Club root diseases

than in regions with spring type climates. It occurs more frequently in soils, which are acidic and poorly drained. (Woronin 1878) was the first to study the disease in a systematic manner. Walker (1952) has described the disease in more detail on cabbage.

Distribution; On oilseeds Brassica, the disease is reported to occur in East Germany, Malaya, New Zealand, Poland, Sweden, United Kingdom and the U. S. In India, the disease has been reported from hills of Darjeeling (Chattopadhyay and Sengupta, 1952) and Nilgiri (Rajappan *et al.*, 1999) on vegetable *Brassicas*. On *B. rapa* var. *yellow sarson* and var. *toria* (Das *et al.*, 1987), the disease has been reported from West Bengal and Orissa, respectively with losses in yield being up to 50% (Laha *et al.*, 1985; Chattopadhyay, 1991).

Extent of damage: Exact information on losses caused by the disease on Rapeseed-mustard is not available.

Causal symptoms: At the initial stages, affected plants show normal healthy growth, but as the disease develops, the plants become stunted showing pale green or yellowish leaves. The plant is then dies within a short time. When the dead plants are pulled out, overgrowth (hypertrophy/ hyperplasia) of the main and lateral roots become visible in the form of small spindles or spherical-shaped knobs, called clubs. Depending on the type of root of a species, the shape of the club varies. When many infections occur close together, the root system is transformed into various shaped malformations. The swollen roots contain large numbers of resting spores. The older, more particularly, the larger, clubbed roots disintegrate before the end of the season.

vii) *Fusarium* Wilt

Rapeseed-mustard is affected by *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *conglutinans* (Wr.) Snyder and Hansen.

Distribution: The first authentic report of *F. oxysporum* f. sp. *conglutinans* as the cause of the disease in *B. juncea* was made from India by Rai and Singh (1973). Later, it was reported to occur quite severely on *B. nigra* also in India (Kannaujia and Kishore, 1981).

Extent of damage: Yield losses of greater than 30% are common.

Causal symptoms: The affected plants show drooping, vein clearing and chlorosis of leaves, followed by wilting and drying, resulting in the death of the plant. The expression of the disease symptoms progress from the base upward and vary with the age of the plants (Rai and Singh, 1973). Plants affected in pre-flowering and early-flowering stages show defoliation, and stem of such plants develop longitudinal ridges and furrows externally, which are generally not observed in the later stages. Diseased plants often show stunting, which is more pronounced when the plants are attacked

in pre-flowering stages. Unilateral development of the disease is also observed in some of the cases when only one side of the plant shows symptoms of the disease. Roots of the diseased plants show no external abnormality or decay of the tissue until the plants are completely dried. Vascular tissues of stem and root show the presence of the mycelium and/or microconidia of the pathogen. Such tissues show browning of their walls and their plugging with a dark gummy substance, which is one of the characteristic symptoms of vascular wilts. At later stages of the disease, epidermis of roots sloughs off.

viii) Bacterial Stalk Rot

The occurrence of stalk rot caused by *Erwinia carotovora* (Jones) Holland was reported first by Bhowmik and Trivedi (1980). Vigorously growing succulent plants, due to a heavy nitrogen application, as well as those growing poorly in drained soil are affected more severely (Fig24).

Distribution: Emerging as a threat for mustard in states *viz.*, Rajasthan, Haryana, Uttar Pradesh, Madhya Pradesh and Bihar.

Extent of damage: On an average, about 60-80% of plants were affected by the disease in a farmer's field in Mahua Simpani village of Bharatpur district of Rajasthan (Meena *et al.*, 2010b). The bacterium can infect *Brassica oleracea* var. *botrytis, Daucus carota, Lycopersicon esculentum* and *Nicotiana tabacum*.

Disease symptom: The disease is characterized by the appearance of water-soaked lesions on the collar region of plants, which are usually accompanied by a white frothing. The tender branches are also affected as the lesions advance further to cover larger areas. The leaves show signs of water stress, and wither. The affected stem and branches, particularly the pith tissues, become soft, pulpy, and produce dirty white ooze with a foul smell. The infected collar region becomes sunken and turns buff white to pale brown. Badly affected plants topple down at the basal region within a few days.

Identification of pathogen: The bacterium is gram negative, rod-shaped with blunt ends, capsulated, and motile with peritrichous flagella. It forms grayish, circular, translucent, shining, smooth colonies on nutrient agar with raised centres and wavy margins.

ix) Bacterial rot

The pathogen is *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson.

Fig 24. Bacterial rot

Distribution: The black rot symptom on *B. juncea* was first observed in India by Patel *et al.* (1949). The disease is now reported to occur in a severe form in the State of Haryana (Vir *et al.*, 1973; Gandhi and Parashar, 1978). Occurrence of the disease has also been reported in Brazil, Canada

(Conners and Savile, 1946), Germany, Sweden and the U.S. (Bain, 1952). In certain years, the disease has been reported to take a heavy toll of the crop in Haryana with records up to 60% incidence in certain varieties of mustard (Vir *et al.*, 1973).

Causal symptom: Disease appears when the plants are two-months old. In the initial stages, dark streaks of varying length are observed either near the base of the stems or 8 to 10 cm above the ground level. These streaks gradually enlarge and girdle the stem. Finally the diseased stem becomes very soft and hollow due to severe internal rotting, and this often results in a total collapse of the plant. Sometimes cracking of the stem is observed before the toppling down of the plant. Lower leaves show the symptoms first, which include midrib cracking and browning of the veins; when extensive, it brings about withering of the leaves. The affected plants, on stripping, show a dark brown crust full of bacterial ooze. The black rot does not cause any disagreeable odor.

Identification of pathogen: Profuse exudation of yellowish fluid from affected stems and leaves may also occur.

x) Mosaics

Some of the more common crucifer mosaic diseases are caused by viruses including *Sarson mosaic* virus (SMV), Turnip Virus I group (Larson *et al.*, 1950),etc.

Distribution: The earliest report of occurrence of a virus disease on Rapeseed was made by Chamberlain (1936) from New Zealand. On Rapeseed-mustard, the mosaic diseases caused by this virus group are described under different names *viz.*, (i) rape mosaic in China (Ling and Yang, 1940) and Canada (Rao *et al.*, 1977), (ii) mustard mosaic in the U.S (Zhu *et al.*, 2012) and Trinidad (Dale, 1948) (iii) Chinese sarson mosaic in India (Azad *et al.*, 1963) (iv) *Brassica nigra* virus in the U.S. (Sylvester, 1954) and (v) turnip mosaic in China (Shen and Pu, 1965), Germany, Hungary, the Soviet Union and the United Kingdom (Rawlinson and Muthyalu, 1975).

Extent of damage: Over 30% of the crop has been reported to be destroyed by the disease in China (Ling and Yang, 1940) resulting in 37-85.5% loss in yield. Wei *et al.* (1960) reported 90% loss in yield due to the disease in eastern China. Shen and Pu (1965) have described infection of rape by a necrotic strain of turnip mosaic virus (TuMV) as lethal to the crop.

Causal symptoms: Symptoms appear as vein clearing, green vein banding, mottling, and severe puckering of the leaves. The affected plants remain stunted and do not produce flowers, or produce very few flowers. When siliquae are formed, they remain poorly filled and show shriveling (Azad and Sehgal, 1959). During the later stages of infection, numerous raised or non raised dark-green islands of irregular outlines appear in the chlorotic area between the veins, giving rise to a mottled look. Curvature of the midrib and distortion of the leaf blade on affected leaves can also be a prominent symptom. Plants infected early are usually stunted and are killed, but those infected late show marginally reduced growth. More or less similar symptoms have been described by Dale (1948), Rao *et al.* (1977).

xi) Phyllody

Distribution: The disease has been reported to occur in India on toria (*B. rapa* var. *toria*), and yellow sarson (*B. rapa* var. *yellow sarson*) in the states of Punjab (Vasudeva and Sahambi, 1955), Haryana (Sandhu *et al.*, 1969) and Uttar Pradesh.

Fig 24. Phyllody

The disease is reported to be caused by the jassid transmissible mycoplasma like organism (Fig 24).

Causal symptom: The characteristic symptom is the transformation of floral parts into leafy structures. In addition, there are some leafy structures attached to the false septum.

Extent of damage: The average yield reported is only 0.63 g per diseased plant as compared to 5.62 g per healthy plant. Thus, yield loss may go up to 90%. The loss in yield in ITSA, synthetic 65, and Shyamgarh varieties of toria appears to be 78.8, 90.8 and 69.3%, respectively. Losses in yield over a large area would be tremendous if the average percentage of diseased plants is high.

Causal Symptoms: The disease is reported to be caused by the jassid transmissible phytoplasma-like organism, which causes phyllody disease of sesamum (Klein, 1977). Transmission, detection and identification of potential vector plant hopper (Laodelpax striatellus) for phyllody disease of toria (*B. rapa* subsp. *dichotoma*) have been reported by Azadvar *et al.* (2011). Molecular characterization and phylogeny of a phytoplasma associated with phyllody disease of toria have also been reported (Azadvar and Baranwal, 2010).

6. HUMAN HEALTH CONSIDERATIONS

The nutritional quality of oil and seed meal derived from *Brassica* seeds are determined by the quality and quantity of fatty acids, proteins and essential amino acids. *B. juncea* has high levels of erucic acid and glucosinolates.

Erucic acid

The oil extracted from *B. junce*a has substantial amount of unsaturated fatty acids and the lowest concentration (approx. 7%) of saturated fatty acids. Of the total fatty acids, there is predominance of erucic acid fraction (35.7–51.4%) (Chauhan *et al.* 2007), in mustard cultivars that are sown in India. The effects of erucic acid from edible oils on human health are controversial. Although, no negative health effects of any exposure to erucic acid have ever been reported in human beings in studies based on laboratory animals during early 1970s, (Amy McInnis, 2004) erucic acid appears to have shown toxic effects on the heart at high doses (Food Standards Australia, New

Zealand, 2003). Mustard oil was once considered to be unsuitable for human consumption due to high content of erucic acid in United States, Canada and Europian Union. Subsquent studies on rats have shown that they are less able to digest vegetable fats (whether they contain erucic acid or not) than humans and pigs.

Glucosinolates:

The seed meal from Rapeseed-mustard contains high quality proteins with well balanced amino acids composition. However, the presence of high levels of Glucosinolates (49.9–120.3 µmole/g defatted seed meal) (Chauhan *et al.* 2007), limits its utilization as livestock and poultry feed. The intact glucosinolates are harmless but the myrosinase enzyme, chemically splits the glucosinolates into glucose and nutritionally undesirable toxic isothiocynates, oxazolidinethione or nitriles. Higher consumption of meal of such seeds in the diet causes enlargement of thyroids in poultry and low palatability for cattle etc. Mustard meal is therefore unusable for non-ruminants like pigs and poultry and can be fed only in a limited way to cattle.

7. AGRONOMIC PRACTICES

7.1 Soil

B. juncea can be grown under a wide range of soil conditions varying from sandy loam to clay loam soils, but thrive best on medium loam soils. It can not tolerate water logging and heavy clayey soils, but can tolerate moderate salinity reasonably well. However a soil having neutral pH is ideal for proper growth and development.

7.2 Sowing Season

B. juncea requires comparatively higher temperature (30-32°C maximum and 20-22°C minimum) for germination and vegetative phases and cool temperature during reproductive phase. The production efficiency of genotypes therefore, greatly differs under different planting dates. Soil temperature and moisture influence the sowing time of rapeseed *B. juncea* in various zones of the country. However, weather conditions in October are conducive for better crop establishment, growth and higher yields in major crop zones.

7.3 Raising Nursery

B. juncea is sown directly through well calibrated seed drill; seedlings are generally not raised. Generally 4-5 kg seed is sufficient for one hectare area. However, under rainfed conditions the seed rate should be increased by 15-20%.

7.4 Soil Preparation

Sound soil preparation as per crop requirement is a prerequisite for good growth and development of OSB. Good seed bed supports in general, one deep ploughing and 3-4 harrowings are recommended to prepare good seed beds for OSB. However, reduced tillage practices of one ploughing and 1-2 harrowings were found to be equally effective after 3-4 years of continuous practice.

7.5 Spacing and Transplantation

Optimum plant population is the next most important low cost agro-technique after sowing time. Although mustard plants are highly plastic so spacing has no absolute value in its cultivation. But under Indian sub-tropical condition the availability of limited growing season restricts the spread of the crop and the yield is greatly influenced by the growth habit of variety, date of sowing, manuring and irrigation practices.Under Indian conditions, depending upon the plant structure and maturity duration, the planting geometry of 30 - 45 cm x 10 - 15 cm is recommended. Seed-drills should preferably be used for sowing to ensure even distribution and placement of seeds for uniform germination and plant population. About 4-5 kg seeds/ha is sufficient for a good crop stand under normal sowing conditions. However, seed rate should be increased by 25% under rainfed and salt affected conditions.

7.6 Manures and Fertilizer Requirement

The nutrient requirement of of mustard is higher than the cereals. For producing one tonne of seeds almost 55 kg N, 11 kg P, 47 kg K, 42 kg Ca, 9 kg Mg 17 kg S, 10 g Zn, 1120 g Fe, 95 g Mn and 17 g Cu are generally removed from the soil (Tandon, 1995). The recommended fertilizer doses for one hectare field are 80-100 kg N, 30-40 kg P_2O_5, 20-30 kg K_2O depending upon the soil-site and cropping conditions. Half dose of nitrogen and full doses of rest of the fertilizers should be applied basally at last ploughing or through ferti-seed drill. Fertilizer should be placed at least 5 cm below the seeds. Remaining nitrogen should be applied at the time of first irrigation. Application of 20-40 kg S/ha, 5 kg Zn/ha and 1 kg B/ha in deficient soils improve the vigour and seed yield. Crop gives excellent response to organic fertilizers therefore besides chemical fertilizers, 2.5 t/ha FYM must be applied basally at last ploughing and well mixed in the field. Sesbania green manuring must be adopted under fallow mustard sequence.

7.7 Water Management

Rapeseed-mustard is usually raised under limited water availability conditions but it responds very well to moisture availability through irrigation or winter rains. Generally, two irrigations at pre-bloom and pod filling stages are recommended for higher seed yield.

7.8 Intercultural and Weed Control

Thinning, hoeing and weed control are important intercultural operations to ensure higher yield of OSB. Weeds in OSB compete for nutrients, water and light, and cause up to 63% loss in seed yield annually. The most important weed flora of OSB includes *Chenopodium album, Cynodon dactylon, Anagalis arvensis, Cyperus rotundus, Phalaris minor, Melilotus alba, Convolvulus arvensis, Fumaria parviflora, Euphorbia helioscopia, Polygonum hydropiper, Asphodelus tenuifolius, Avena fatua/ludovicinia, Medicago denticulata, Vicia sativa, Carthamus oxycantha, Parthenium hysterophorus, Ranunculus arvensis, Rumex acetosella/sp., Tridax procumbens* and parasitic weed *Orobanche* spp. Care should be taken to remove all the weeds in the early stages of the crop growth to avoid the competition for reserve moisture. One interculture operation with hand hoe is sufficient. Besides creating a soil mulch to reduce moisture lossess through evaporation, this also helps in better growth and development

of crop plant. Thinning of the crop should be accompanied with interculture at three weeks after sowing to provide the plants proper space within the rows.

Herbicides can also be used to control the weeds effectively. Pre-planting incorporation of 1.0 kg a.i./ha fluchloralin into surface soil before sowing of crop or pre-emergence spray of 0.75-1.0 kg a.i./ha Pendimethalin control many annual grasses and some broad leaved weeds BLW). Pre-emergence or early post emergence of 0.75-1.0 kg a.i. /ha Isoproteuron, 0.5 kg a.i./ha Oxadiazon, 40-50 g a.i/ha. Quizalofop or 0.75 kg a.i./ha Pendimethalin (Stomp Extra 38.7 CS) also control many annual grasses and BLWs (AICRP-RM, 2013).

7.9 Harvest and Post Harvest Practices

Harvesing of *B. juncea* is a critical operation. Losses can be heavy due to the small size of seeds and unsynchronised maturity of the crop. Early harvesting can reduce seed quality and late harvesting enhances pod shattering. Timely harvesting reduces harvesting losses and improves quality of the produce. From a practical point of view, the crop is mature when all seeds are black and seed moisture content is less than 15%. The crop should be harvested when almost 75% pods turn yellowish brown. The harvested crop should be stacked on the threshing floor for five to six days before threshing. The threshed and cleaned seed must be dried in the sun for four to five days or till the moisture content comes down to 8%. The properly dried seeds should be stored in special storage structures that are free from moisture, insect-pests and diseases.

7.10 Seed Production

Production of genetically pure and otherwise good quality pedigree seed is an exacting task requiring high technical skills and comparatively heavy financial investment. During seed production strict attention must be given to the maintenance of genetic purity and other qualities of seeds. The plot selected for seed crop must be free from volunteer plants, weed plants and have good soil texture and fertility. The soil of the seed plot should be comparatively free from soil borne diseases and insects pests. In addition, the selected fields should be well levelled and suitable for cultivation of the crop. An isolation distance of 200 meters for foundation seed class and 50 meters for certified seed class from field of other OSB varieties/ crop must be maintained. Roguing and weeding at different crop stages are equally essential to maintain genetic purity of the variety.

7.11 Weediness Potential

Typically weeds are plant species that spread easily in disturbed areas or among crops. Weeds generally have a range of life history characters in common that enable them to rapidly colonise and persist in an ecosystem.

B. juncea siliquae on maturity show shattering habit if harvesting is delayed and temperature is high. Therefore, seeds may escape harvest and remain in soil until the following season or even second season when they germinate with the seeds of successive crop (Scoggan, 1957). As a result *B. juncea* volunteers could grow and become weedy in the crop sown in the next winter season. The lossess in *B. juncea* may be somewhat less due to greater pod shatter resistance but are assumed to be substantial.

8. CROP IMPROVEMENT

8.1 Breeding Objectives

B. juncea breeders aim to make simultaneous improvement of agronomic performance, disease resistance and quality traits. Agronomic performance include yield, lodging, maturity, herbicide tolerance, drought tolerance, shattering resistance and seed size. Disease resistance efforts may include White rust (*Albugo candida*), *Alternaria* blight (*Alternaria brassicae*), *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*), Downy mildew (*Pernospora parasitica*) and Powdery mildew (*Erysiphe cruciferarum*) resistance. Improvements in quality traits will depend on whether the aim is to develop canola or conventional mustard varieties. For canola, high oil content, low glucosinolate content(20µmoles/g), high protein content, and a fatty acid profile that is canola quality with low erucic and low saturated fatty acid content are desired. For conventional mustard varieties, low oil content, high glucosinolate content and a fatty acid profile with a moderate level of erucic acid are desired.

8.2 Varietal Improvement

Up to 1970, mass and pure line selection were the main breeding methods used in breeding programmes and 26 varieties were developed. The first variety was released in 1936. After 1980, varieties developed through hybridization increased and 22 varieties were released in each of the 8th and 9th decade of 20th century. This number further increased to 41 during the first decade of the 21st century. Simultaneously, 12 varieties had been developed through mutation breeding. Chauhan and Singh (2004) opined that most of the Indian mustard varieties were the pure line selections derived from a few common ancestors and limited number of donors were utilized in the breeding programme resulting in a narrow genetic base.

Since the early 80's, systematic and vigorous recombinant breeding has been followed and a large number of varieties have been identified and released. The first notified variety was ITSA of toria (*B. rapa*) in 1973 after the adoption of official notification of varieties in 1969 under the Seed Act 1966 (Section 5). The major objectives of the varietal improvement programme have been genetic enhancement for seed and oil yield through developing varieties for early, timely and late sown conditions to cater to the need of diverse agro ecological situations of the country, improvement of oil (low erucic acid) and seed meal (low glucosinolate) quality, introgression of resistance/tolerance against major biotic (white rust, *Alternaria* blight, *Sclerotinia* rot diseases and aphid and painted bug insects) and abiotic stresses (drought, high temperature, frost tolerance and salinity). Many trait/ situation specific varieties have been developed under ther programmes.

A total of 142 varieties (Indian mustard-91; toria-16; yellow sarson-11; gobhi sarson-11; brown sarson-3; karan rai-4; taramira-5 and black mustard-1) of Rapeseed mustard have been released after inception of All India Coordinated Research Project on Rapeseed mustard (AICRP-RM) in 1967 till 2013. These include six hybrids. Rapeseed-mustard varieties having tolerance to biotic (white rust, Alternaria blight, powdery mildew) & abiotic stresses (salinity, high temperature) and quality traits have been recommended for specific growing conditions.

Fifty novel genetic stocks of rapeseed-mustard

(CMS, restorer, low erucic acid & low glucosinoltes, high oil content, high oleic acid and low linolenic acid, dwarf, earliness, long main shoot, bold seed, yellow seed, tetralocular siliquae, white rust resistance, tolerance to high temperature and salinity during juvenile stage, high temperature tolerance during terminal stage and high water use efficiency) have been registered with NBPGR, New Delhi till April 2013.

8.3 Hybrid Development

Unlike in most other crops, no natural cytoplasmic male sterility fertility restoration(CMS-FR) was available in *B. juncea*. Major emphasis during the initial phase of hybrid breeding was towards the development of CMS-FR systems using alloplasmic variation. Cytoplasmic male sterile lines could be developed by backcross substitution of *B. juncea* genomes in the cytoplasmic background of wild crucifers. To facilitate this sexually synthesized allopolyploids or somatic hybrids between wild and crop species were used as the bridging species. CMS lines originating from sexual hybridizations possess unaltered organelle genomes because of exclusive maternal inheritance. Since organelle assortment and intergenomic mitochondrial recombinant is of frequent occurrence in *Brassiceae*, the cytoplasmic constitution is entirely different in those originating from somatic hybrids, and different combinations of mitochondrial and chloroplast genomes have been reported in different CMS lines (Prakash *et al.* 2009). A number of such CMS systems are now available (Table 8).

Many of these CMS systems exhibited developmental and floral abnormalities as a consequence of altered nucleo-cytoplasmic interactions. Varying degrees of leaf chlorosis were associated with *Raphanus/Ogu, Oxyrrhina, Tournefortii, Moricandia*, and *Enarthrocarpus* systems. Floral abnormalities in male sterile plants included: petaloid anthers (*nigra, muralis, trachystoma, raphanus, tournefortii, canariense*); poor or absent

Table 8: Alloplasmic male sterile systems developed for *B.juncea*

CMS - System	Cytoplasm donor	Reference
ogura	*Raphanus sativus*	Kirti *et al.*1995a
oxyrrhina	*B.oxyrrhina*	Prakash and Chopra 1988, 1990; Kirti *et al.* 1993
siifolia	*Diplotaxis siifolia*	Rao *et al.* 1994; Rao and Shivanna 1996
		Kirti *et al.* 1995b
		Prakash *et al.* 1998, Kirti et al.
trachystoma	*Trachystoma ballii*	1998; Kaur *et al.*2004
moricandia	*Moricandia arvensis*	Malik *et al.* 1999; Prakash 2001; Bhat *et al.* 2006
erucoides	*D.erucoides*	Malik *et al.* 1999; Bhat *et al.*2008 Prakash *et al.* 2001
berthauti	*D.berthauti*	
canariense	*Erucastrum canariense*	
catholica	*D.catholica*	Pathania *et al.*2007
lyratus	*Enarthrocarpus lyratus*	Deol *et al.* 2003; Janeja *et al.* 2003
fruticulosa	*Brassica fruticulosa*	Atri *et al.*(2016)

nectarines (*tournefortii* and *raphanus*); crooked style (*tournefortii, raphanus*); thick pistil (*raphanus*); and low seed fertility (*raphanus, tournefortii, enarthrocarpus* and *trachystoma*). Fertility restorers for *moricandia, ogura, catholica*, and *erucoides* and *lyratus* CMS systems could be developed by introgressing gene(s) for fertility restoration from cytoplasm donor wild species (Banga and Banga, 2009; Prakash et al. 2009). Fertility restorer for the mori CMS could also restore fertility of eru CMS system (Bhat *et al.* 2005).

At present, *ogura* and *mori* CMS systems are being used to develop hybrids. Sustained efforts resulted in the release of five CMS based hybrids, out of which, NRCHB 506 and DMH 1were released in 2009 and Coral432 (PAC 432) in 2010. DMH 1 is based on a novel CMS system(126 I), developed by the University of Delhi.

8.4 Varietal Testing and Zonalization

Research work on the improvement of rapeseed-mustard was started at the turn of 20th century at Pusa, Bihar with emphasis on collection and purification of land races . The organized research programme, however, began with the launching of a comprehensive multi disciplinary research project for the improvement of oilseeds in the country under the banner of the All India Coordinated Research Project on Oilseeds (AICRPO) at the Indian Council of Agricultural Research (ICAR) in 1967. Setting up separate Project Coordinating Units in the V Plan further strengthened the research programmes. The Unit of the Project Coordinator (Rapeseed-Mustard) was accordingly established on January 28, 1981 at Haryana Agricultural University, Hisar. During VIII Plan the (ICAR established the NRCRM at Sewar, Bharatpur on October 20, 1993. The mandate is to carry out basic, strategic and applied research on Rapeseed-mustard AICRP-RM was also brought under its ambit. In February 2009, the ICAR re-designated NRCRM as DRMR. The DRMR functions as a fulcrum to support the production system researches on Rapeseed-mustard through 11 main, 12 sub and 22 verification centres of AICRP-RM spread across the country and grouped into the following six zones to harness the vast variability in the climatic and edaphic conditions in the mustard growing areas of India for enhancing OSB production (Table 9)

8.5 Quality breeding

Breeding efforts have been underway in India since 1970 to reduce glucosinolate content in the seeds of Rapeseed-mustard varieties up to 30 micro moles/g defatted seed meal (low or 0) and erucic acid up to 2% (low or 0) as well as combining both to develop double zero or double low varieties to meet the internationally acceptable standard of oil and seed meal. First low erucic acid variety, PusaKarishma of Indian mustard and first double low variety, GSC 5 of gobhi sarson were released in 2004 and 2005, respectively. Presently, eight low erucic varieties have been released in *Brassica juncea*. The challenges include:

- Efficient utilization of rapeseed-mustard genetic resources.
- Genetic enhancement of heterosis in mustard for further enhancing the yield potential.
- Developing high yielding varieties/hybrids with improved oil and seed meal quality for food, feed and industrial uses using conventional as well as biotechnological approaches.

Table 9: Siz zones of Mustard growing areas in India

Zone I:	Kashmir, Himachal Pradesh, Hills of Uttarakhand
Zone II:	Jammu, Punjab, Haryana, Rajasthan (Navgaon, Sriganganagar, Bikaner), Delhi
Zone III:	Uttar Pradesh, Uttarakhand (plains), Madhya Pradesh, Rajasthan (Kota, Bharatpur)
Zone IV:	Rajasthan (Jobner, Mandor, Bikaner), Gujarat, Maharashtra
Zone V:	Chhattisgarh, Bihar, Jharkhand, Odisha, West Bengal, Assam, Manipur, Meghalaya, Tripura, Nagaland, Arunachal Pradesh, Sikkim, Mizoram
Zone VI:	Andhra Pradesh, Tamil Nadu, Karnataka *B. juncea* is primarily grown in Zone II and Zone III

- Development of thermo-photo-insensitive genotypes for diverse cropping systems under varied agro-ecological situations.
- Development of cultivars with high water and nutrient use and photosynthetic efficiency for different situations.
- Development of designer Brassica for different fatty acids profile & value-added products.
- Development of rapeseed mustard genotypes tolerant to various biotic (*Alternaria* blight, *Sclerotinia* rot, White rust, *Orobanche*, Mustard aphid, Painted bug) and abiotic stresses (drought, temperature, salinity and herbicides)

9. BIOTECHNOLOGICAL DEVELOPMENTS IN *BRASSICA*

New emerging challenges are being addressed by the biotechnological tools and techniques available. Biotechnological interventions have also been made in Indian mustard breeding programme. Many attempts have been made by the scientists to improve *Brassica* using molecular markers like Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Single Nucleotide Polymorphism (SNP) based maps (Ramen *et al.*, 2014) etc. Abraham *et al.* (2000) at BARC Mumbai, reported somaclonal variants from mesophyll protoplast in *B. juncea* cv Rai 5 which showed 3-5 days early flowering. Commonly, some techniques e.g. tissue culture techniques to create somaclonal variation, anther culture to produce haploids and homozygous lines, protoplast culture and somatic hybridization, marker- assisted selection, development of transgenics for biotic and abiotic stresses have yielded promising results.

Optimization of regeneration protocols have been achieved for most of the Brassica species using different explants such as cotyledons, hypocotyls, leaf segments and protoplasts, cotyledonary petiole and shoot apex (Narasimhulu and Chopra 1989, Kirti and Chopra 1989, Verma and Singh 2007). Somaclonal variation as a tool for creating *in vitro* variability offers a unique opportunity for desirable attributes. A somaclone of Varuna, BIO-902, has been released as a variety which possesses shattering resistance along with high yield (Katiyar and Chopra, 1995). Prakash *et al.*, (2004) reported regeneration of normal plants by culturing anthers of CMS line of *B. juncea* carrying *Diplotaxis erucoides* cytoplasm.

Somatic cell fusion of sexually incompatible species has also been made possible through production of somatic hybrids which have been utilized for transfer of desirable traits from parents to hybrids. Inter-specific hybrids were produced by fusing mesophyll protoplast of *B. juncea* and *B. spinesceens* (Kirti *et al.*, 1991). Prakash *et al.*

(1998) developed a male sterility and fertility restoration system in *B.juncea* by protoplast fusion with Moricandia arvensis.) These CMS lines were found to be chlorotic. Protoplast fusion of chlorotic male sterile *B. juncea* with green male sterile *B. juncea* resulted in green male sterile plants (Kirti *et al*., 1998).

Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), AFLP and SSR have been used for improving selection efficiency and selecting plant genotypes with the desired combinations of traits. Markers linked with white rust resistance (Prabhu *et al*., 1997), fatty acids, oil content, yellow seed colour and fertility restoration have been reported. Transgenic approaches have been followed to develop the transgenic for aphid resistance, male sterility, AB tolerance, herbicide resistance and drought tolerance. Bar, Barnase and Barstar based herbicide resistance and genetic male sterility have been used in the development of experimental hybrids (Jagannath *et al*,. 2002). Transgenics expressing Cod A (from *A. glabiformis*) gene for tolerance against abiotic stresses (salt and drought) have also been reported (Singh, 2003). Lectin gene for aphid resistance and DREB gene construct for drought tolerance are being used. Osmotin (from tobacco) for drought and salt tolerance, annexin gene for stress tolerance, Chitinase and Glucanase (from Arabidopsis) for tolerance to *Alternaria* blight disease and FAE1 gene for low erucic acid mustard cultivar are other transgenes being used in Rapeseed-mustard.

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Note

Note

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