



## Abstracts of papers presented in 2<sup>nd</sup> National Brassica Conference on “Brassicas for addressing edible oil and nutritional security” held at Panjab Agricultural University, Ludhiana, India organized by Society for Rapeseed-Mustard Research during February 14-17, 2014

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### Genetic enhancement of mustard for seed yield & its sustainability

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The contribution of rapeseed-mustard to the total oilseed acreage and production in India is 23.7% and 26% respectively, during the year 2012-13. The yield of rapeseed-mustard was 1176 kg/ha as compared to 955 kg/ha of total oilseeds. Indian mustard (*Brassica juncea* (L.) Czern & Coss.) accounts for about 80 % of the 6.3 m ha area under rapeseed- mustard crops in the country. *Per capita* consumption of edible oil is likely to reach 23.1.43 kg by 2030 from the present level of 13.4 kg. Considering its contribution to the oilseed pool, the production and productivity of rapeseed mustard seed would have to be 16.4-20.5 mt and 2245 kg/ha from the present level of 6.78 mt and 1159 kg/ha, respectively, by the year 2030. Doubling in productivity would have to be achieved under the changing scenario of climate, decreasing and degrading land and water resources, costly inputs and other constraints. As there is very little scope for area expansion, increasing the yield per unit area is the best available option. Considerable increases in the mustard yield level have been achieved in the past by the release of over 100 varieties and by following suitable agronomical practices. The productivity level of these crops varies widely over time and regions due to the influence from the underlying agro-ecological conditions and the limitations posed by the variety of biotic and abiotic stresses. The rapeseed-mustard crops is quite vulnerable to many disease and insect pests and thus potential of the existing cultivars can be sustained/stabilized by insulating the crop by incorporating gene

(s) for resistance to biotic (diseases and insect pests) and abiotic (drought, high & low temperature and salinity) stresses. Further, *per se* ceiling to the genetic potential of the crop could be raised by restructuring plant type or heterosis breeding. There is a need to diversify the genetic base of the varieties by extensive and vigorous utilization of trait specific germplasm available in primary, secondary as well as tertiary gene pools of rapeseed-mustard and related genera. Most importantly, unavailability of donors tolerant to most of these stresses and time and labour-intensive traditional breeding programmes have opened the window for adoption of biotechnological approaches. The QTL analysis of major component traits pertaining to yield could be helpful in locating the favorable alleles and determine their organization and, consequently, help breeders in devising strategies for transferring these loci from one gene pool to the other. Earlier attempts to bring together the desirable traits from exotic Indian mustard gene pool and wild relatives through conventional breeding have been largely unsuccessful as they also brought in poor agronomical genes leading to lesser yields of cross derivatives. Hence, development of more productive purelines lines and hybrids will, therefore, require biotechnological interventions like precise transfers of gene(s)/QTL through marker-assisted selection (MAS) from one gene pool to the other without sacrificing the loci involved in higher yields.

### ● Evolution and divergence in *Brassica juncea*

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Oilseed Brassicas are known to have originated in the countries surrounding the Mediterranean basin and its further extension into southwest and East

Asia encompassing mainly Mediterranean, Irano-Turanian, and Saharo-Sindian phytogeographical regions. Brassicas first entered into domestication as vegetables and only later as edible oilseed crops. The group include three diploid species namely, *B. rapa* (AA; 2n = 20), *B. nigra* (BB; 2n = 16) and *B. oleracea* (CC; 2n = 18) which hybridized in nature to form three oleiferous allopolyploids i.e. *B. juncea* (AABB; 2n = 36), *B. napus* (AACC; 2n = 38) and *B. carinata* (BBCC; 2n = 34). Patterns of chloroplast DNA restriction in *B. napus*, though differed from both *B. rapa* and *B. oleracea*, approached that of *B. oleracea*. It is believed that *B. nigra* and *B. rapa* were cytoplasm donors to *B. carinata* and *B. juncea*, respectively. As inferences drawn from relatively small number of genotypes can prove erroneous when subjected to large scale validation studies, we evaluated genetic diversity in a diverse, global collection of oilseed *B. juncea* and its progenitor species (256 genotypes). cpSSR markers as well as with SSR markers based on sequence information of four nuclear candidate genes (FLC, FT, TFL 1 and SHP). Allelic data of both types were used to infer population structure using STRUCTURE and BAPS software's. Three plasmotypes were detected for *B. rapa* and two for *B. nigra*. Three plasmotypes were also recognized in allotetraploid *B. juncea*. Of these, two were homologous with *B. nigra* and *B. rapa*, the third type differed from both the extant genome donors. East European *B. juncea* was grouped along with *B. nigra*, implying *B. nigra* to be the cytoplasm donor species to *B. juncea* of East European origin. This contradicted previous studies which suggested unidirectional evolution of *B. juncea* with *B. rapa* to be maternal parent. Diversity dendrogram, also confirmed cytoplasmic homology between East European and some Indian *juncea* genotypes with *B. nigra*. Further, the cytoplasm of majority of *juncea* genotypes from India and China was distinct from both *B. nigra* and *B. rapa*. At the same time, there existed many Indian *B. juncea* genotypes (RC accessions), that showed cytoplasmic affinity with *B. rapa*. The nuclear DNA analyses also separated out test genotypes/species into two major groups wherein *B. juncea* and *B. nigra* genotypes were grouped together indicating close homology between the two

species with respect to genes associated with flowering and dispersal characteristics. Our results based on cytoplasmic and nuclear SSR markers indicated that Group I comprised bulk of Indian, Chinese and Australian *B. juncea*. We recognize that most of the genotypes in our group I carried a cytoplasm that was distinct from both *B. rapa* and *B. nigra*. We propose to recognize it as group 'A', mainly because several Indian *B. juncea* genotypes included in this group carried *B. rapa* cytoplasm. Group II comprising East European and few Indian *B. juncea* accessions was cytoplasmically homologous to *B. nigra*. It is proposed to term it as group 'B'. We also characterized an assembled set of 168 accessions comprising natural *B. juncea* (Indian, Chinese, Australian, East European), derived *B. juncea*, introgressed *B. juncea* at the molecular level. Four population groups could be identified. Derived and introgressed *B. juncea* genotypes formed distinct groups, different from those of natural *B. juncea*. Admixturing was indicated in Indian, Australian and Chinese genotypes. This apparently resulted from gene flow in the breeding programmes or in the gene banks. Indian *B. juncea* types showed two lineages. In order to associate gene pools as resolved during population structure with heterosis, very large-scale efforts were initiated for estimating heterosis in a large number of *ogura* CMS based hybrids. This helped to establish tentative heterotic gene pools.

### Challenges for Brassica Breeders from Sub-Specific Pathogen Variation

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The effects of pathogen life cycle, expression of host resistance, disease epidemiology, severity of disease epidemics, and pathogen inoculum production, have all been studied well historically. This is in relation to deploying and managing

Brassica host resistances to pathogens. Similarly, pathogen variation at a sub-specific (i.e., race or pathotype) level has been and remains a strong focus of research and of high relevance for Brassica breeders. For some important pathogens (e.g., blackleg, *Leptosphaeria maculans* and white rust, *Albugo candida*). This pathogen variation is built on the complex changes in crops, rotations, agricultural practices and altering climate scenarios. Despite these efforts, managing pathogen variation at a sub-specific level even in well studied pathogens remains an ongoing challenge for Brassica breeders developing new cultivars with durable resistance. Sub-specific variation in many other Brassica pathogens remains either relatively poorly defined in terms of relevance to breeders (e.g., downy mildew, *Hyaloperonospora parasitica* and white leaf spot, *Pseudocercospora capsellae*), or has attracted little interest in the application of newly available 'systems' that define such sub-specific variation phenotypically (e.g., sclerotinia rot, *Sclerotinia sclerotiorum*). Further, many historical pathogen population biodiversity studies focused solely on genetic variation (e.g., *S. sclerotiorum*) and have not produced the practical information needed from a breeding perspective. Breeders require pathogen sub-specific variation that is clearly defined in terms of host phenotypic responses across different host-pathotype combinations, as recently became available for *S. sclerotiorum*. Some existing environments, like those in Mediterranean-type regions, are historically highly variable in terms of environmental parameters, such as the amount, timing and variability of temperature and rainfall. They also include changes in crops, rotations and farming systems needed to cope with this variability. Such challenging environments provide strong indications of the abilities of pathogens to adapt readily at a sub-specific level to complex changes. They also provide indications of consequent adaptation of pathogen populations to changing crops, rotations, agricultural practices and altered climate scenarios. Together, these offer unique prospects for gaining better insight and understanding of the processes involved with development of pathogen sub-specific variation. Brassicas include diverse species making them an ideal prospect for

use as a 'model' plant group for investigating and modelling the expression of host resistances to pathogen sub-specific diversity. Brassica diversity also allows wider commercial exploitation as a means for coping with current and future agricultural 'system' challenges. Clearly, while pathogen sub-specific variation and consequent disease challenges will remain areas of high priority and significant challenge for breeders, such changes in pathogen sub-specific variation over time can be closely monitored, as done for *L. maculans* in Australia. In conjunction with pathogen population monitoring, there remains significant scope for Brassica breeding to develop more effective and durable host resistances. There is similar scope to deploy them in more effective ways to maximize longevity of such resistances in the face of ongoing sub-specific changes in Brassica pathogen populations.

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### **Translational genomics in target breeding of oilseed mustard (*Brassica juncea*)**

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Targeted breeding is the trait breeding. Efficient trait breeding in crop plants could be achieved by the use of DNA markers. A linked DNA marker to a phenotypic trait acts as an indirect selection marker and the desired plant can be selected in the initial stage of plant development in a segregating population without screening for the phenotype. However, successful trait breeding in crops that needs transfer of traits from ill-adapted lines to elite lines could be achieved by precise marker-assisted introgression. It can be achieved in the following way: (1) region around the gene of interest should be saturated with markers that will help in identifying finer recombinants through background selection in the carrier chromosome(s), and (2) the foreground selection marker should preferably be

from the candidate gene. Oilseed mustard (*Brassica juncea*) is one such crop which requires transfer of several traits such as quality traits (two genes for low erucic acid, 4 genes for low glucosinolates and two genes for yellow seed coat colour), disease resistance (white rust resistance) and some yield enhancing component traits from the ill-adapted east European lines to Indian cultivars. It is a challenging task as both conventional backcross breeding and marker-assisted backcross breeding through foreground selection has been largely unsuccessful due to the retention of large fragment of donor genome around the gene of interest leading to linkage drag in near-isogenic lines.

In today's presentation, first I shall enumerate how we failed in both conventional backcross breeding and marker-assisted backcross breeding through foreground selection for some of the quality traits in developing a productive canola quality *B. juncea* and finally I shall elaborate on how genomics tools are being used to circumvent these problems. The new advancements in the areas of genomics particularly NGS technology and genome sequencing are going to offer unprecedented opportunities to fulfil some of the above impediments particularly saturating the target regions and identification of causal gene(s) underlying the trait even in the recalcitrant genome like *B. juncea*. We have earlier mapped genes controlling seed glucosinolates and erucic acid by candidate gene approach. The seed coat colour trait earlier mapped by anonymous SSR markers has now been mapped by candidate genes. Although these traits have been introgressed from east European line to Indian variety by marker-assisted backcross breeding following foreground selection, all these near-isogenic lines are inferior to their wild type counterpart in yield and yield components due to retention of large areas of donor genomes around the genes of interest. Hence, it was realized that a saturated map of the target regions would be helpful to identify finer recombinants for further elimination of the linkage drag. To fulfil this objective, a SNP-based genome map and target region saturation maps by SNP markers were developed through the use of the RNA-seq data generated in the lab using Illumina paired-end sequencing technology. A

saturated map thus developed is now being used for further reducing the linkage drag around the introgressed genes.

## Genomics of Abiotic Stress Thermal Tolerance

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Climate change and abiotic stress affects agriculture and crop production adversely. Temperate crop are prone to various abiotic stresses therefore mapping and characterizing ESTs offers a manageable approach to understand the functioning of the transcriptome. Tolerance to heat stress is a complex phenomenon and controlled by multiple genes. A program on functional genomics of heat stress has been initiated to understand the transcriptional profile of the sensitive and tolerant cultivars of wheat under heat stress by subtractive hybridization, both at the vegetative and reproductive stages. Heat tolerance related gene transcripts were identified based on their putative functions and validated by cDNA macroarray and northern/RT-PCR analysis, with the aim to unravel the complexity associated with heat stress response in wheat. A common response to high temperature stress is the synthesis of Heat Shock Proteins (HSPs). HSP encoding genes are found to be under the control of Heat Shock Factors (HSF), transcription factors that regulate the expression of HSP genes. One such gene, achloroplastic small heat shock protein (sHSP26) was found essential for tolerance to heat stress. Transgenic *Arabidopsis* plants over-expressing wheat HSP26 were more tolerant to high temperature induced damage to the photosynthetic machinery, and the antisense transgenic plants were found to be severely sensitive to heat stress. Similarly, a newly identified heat shock factor (HSF) possesses the characteristic domains of class A type plant HSFs and shows high similarity to rice OsHsfA2d. The transcription factor activity of TaHsfA2d was confirmed through

transactivation assay in yeast. Transgenic *Arabidopsis* plants overexpressing TaHsfA2d not only possess higher tolerance towards high temperatures but also showed considerable tolerance to salinity stress. They also showed higher yield and biomass under constant heat stress conditions. Analysis of putative target genes through quantitative RT-PCR showed higher and constitutive expression of several abiotic stress responsive genes in transgenic *Arabidopsis* plants over-expressing TaHsfA2d. TaHsfA2d can also functionally compliment the T-DNA insertion mutants of AtHsfA2, although partially. These observations suggest that TaHsfA2d may be useful in molecular breeding of crop plants, especially temperate crops, to improve yield under abiotic stress conditions.

### International trends in hybrid *Brassica* research

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Possibility of development and commercialization of hybrids and herbicide resistance technologies attracted lot of private investments in canola (*Brassica napus*) research in Canada during eighties and nineties. Gary *et al.* (2001) estimated that in 2000, over 100 million dollars of private funds were invested in canola research in Canada. Herbicide resistant open pollinated varieties, resistant to glyphosate, guphosinate and imidazolinone herbicides, were first commercialized in Canada during mid nineties and were quickly adopted by farmers because of ease in weed control. Although, race to commercialize hybrids was underway, large scale hybrid introduction did not occur until 2002 due to problem with either pollination control systems or hybrid performance. The Canadian farmers adopted canola hybrids quickly, and by 2010 over 90% of canola crop was planted with hybrids. Canola has been the most profitable crop in Western Canada and as a result, Canadian farmers grew almost 8 million ha of canola crop and harvested record yields in 2013. Average

Canadian canola yield went up from 1.3 t/ha in 2001 to 2.3 t/ha in 2013, and oil content in commercial crop went up from 42.8% in 2001 to 45.0% in 2013, hugely benefiting to all stakeholders. With more acres occupying canola, crop rotations are being compromised causing disease issues such as blackleg, clubroot and sclerotinia rot. Canola researchers around the world, especially in Australia, Canada and France have been working on adult plant and seedling resistance against blackleg. Host pathogen relation for seedling resistance genes including their position in *Brassica* genome is known today. Most of the hybrids registered in Canada are resistant to blackleg. Clubroot disease first appeared in a very small area in Canada in 2005 and has been spreading since then. Canola breeding companies responded and registered several clubroot resistant hybrids. Sclerotinia stem rot had its widespread appearance in Canada causing major yield losses in 2012. DuPont Pioneer led the development of Sclerotinia resistance and released eight field resistant hybrids since 2008. During last ten years, direct harvesting of commercial canola crops has gain more importance in Canada, increasing chances of yield losses due to pod shatter. Bayer Crop Science launched first shatter tolerant hybrid in 2013.

In Europe, winter rape hybrids have been introduced in most countries with Germany being the leader in hybrid adoption, realizing yield and oil improvements similar to Canada. In Australia, canola hybrids were introduced around 2005 with fast adoption rate in Clearfield® and Roundup® herbicide segments. Recently, Pacific Seed was first to commercialize OGU CMS based Triazine® tolerant hybrids. In China, commercial scale *B. napus* hybrids have been in cultivation, many of them based on *Polima* CMS or genetic male sterility system. In India, DuPont Pioneer commercialized several *B. juncea* hybrids based on OGU CMS system and demonstrated yield advantages to farmers. Private investments in hybrid juncea research will increase in India, producing high performing competitive hybrids, increasing hybrid adoption rate and generating financial benefits to all stakeholders including farmers, traders, crushers, exporters and seed companies.

## Developing and optimizing Cytoplasmic Male Sterility systems in *Brassica juncea*

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Pollination control system is essential for commercial hybrid seed production in crops such as Brassica where flowers are small, bisexual, and only a few seeds are produced per pollination. Cytoplasmic male sterility (CMS) systems of natural origin are widely employed for hybrid breeding in major field cross. Natural CMS system, however, is not available in *Brassica juncea*. Therefore, our lab has been pursuing development of CMS and fertility restorer lines of *B. juncea* through wide hybridization. CMS systems based on *Moricandia arvensis*, *Diplotaxis berthautii* and *D. eruroides* have been found to be suitable for commercial application. Although these CMS lines differ with regard to organelle genome constitution, they appear to share a common molecular mechanism of male sterility. Two other CMS systems based on *D. catholica* and *Trachystoma ballii* also show stable male sterility and fertility restoration but adverse cytoplasmic effects in the female lines prevent their use in practical plant breeding. Similarly, 'Ogura' cytoplasm shows undesirable flower phenotypes, female sterility and leaf chlorosis in *B. juncea*. An improved, chlorosis-corrected CMS involving 'ogura' cytoplasm was developed at NRCPB (called Og-1) through somatic hybridization. However, this CMS is unstable and yields high frequency of male fertile plants. Such male fertile lines fail to confer male fertility to CMS and give rise to only male fertile progeny upon selfing indicating reversion to male fertility. Comparative molecular studies of Og-1, Ogura, and normal *B. juncea* lines have revealed that Og-1 mitochondrial genome is recombinant and carries parts of 'ogura' and *B. juncea* mitochondrial genomes. In revertant lines, orf138, the gene responsible for male sterility in Ogura CMS, is lost through intramolecular recombination. One other CMS line based on *B. oxyrrhina* has all the desirable features but could

not be used so far for heterosis breeding due to non-availability of a restorer line. We have attempted to introgress fertility restorer gene for this CMS line by repeated backcrossing of the amphidiploid (*B. oxyrrhinax B. rapa*) with *B. juncea*. We have recovered some male fertile progenies in the BC1 generation. Preliminary observations in subsequent generations suggest sporophytic mode of restoration. Although our molecular studies have identified genes causing male sterility in different CMS systems, we have not been able to explain differences in flower phenotypes among CMS lines sharing common CMS-inducing genes. We have recently completed the mitochondrial genome and transcriptome sequencing of various CMS lines to gain a better understanding of the molecular basis of CMS phenotypes such as petaloid, anthered sterile, and apetalous fertility restorer lines.

## Genetics and genomics of vegetable Brassicas

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The genus *Brassica* constitutes one of the most diverse and economically important plant groups which are cultivated worldwide for producing vegetables, vegetable oils, condiments and fodders. Of the six cultivated species, the diploid Brassica species i.e. *Brassica oleracea* (CC, n=9) and *Brassica rapa* (AA, n=10) contains maximum diversity of forms and mainly used for vegetables in the form of leaves, flowers, stems, and roots. Apart from providing many nutrients, vitamins, minerals, antioxidants; they are the potential source of a large amount of dietary fibers. Extensive studies done in the past two decades using molecular markers in economically important vegetable and oilseed *Brassica* crops helped researchers to develop several genetic maps, identified QTL/genes for important traits and established synteny relationships through comparative mapping among different *Brassica* genomes. However, only handful of those identified QTL/genetic loci could be applied in breeding program as those studies used mostly

anonymous markers, markers linked to QTL are not close enough with the gene(s) of interest and the presence of multiple QTL in a short chromosomal region. Further, many comparative mapping studies between the closest relative model plant *Arabidopsis thaliana* and Brassicas have revealed the complex organization of the *Brassica* genomes, which have arisen due to extensive segmental duplication and rearrangements, and the presence of many copies of a single *Arabidopsis* gene in *Brassica* genomes. This cautioned the transfer of candidate genes and their flanking sequence information from *Arabidopsis* to *Brassica* species. Therefore, *Brassica rapa*, a representative model species within the *Brassica* genus, was selected for genome sequencing by the Multinational *B. rapa* Genome Sequencing Project Consortium (BrGSP, <http://www.brassica.info/>) in 2003 and the draft genome sequence was published in 2011 (Nature Genetics, Doi:10.1038/ng.919). The recent advances in Next Generation Sequencing (NGS) technologies have supplemented greatly in identifying genes responsible for economically important traits in Brassicas through whole genome and transcriptome sequencing. Our work on *B. rapa*, *B. oleracea*, *B. juncea* (AABB, n=18) and *B. napus* (AACC, n=19) could identify structural and functional evolutionary interrelationships between different *Brassica* genomes besides identifying crucifer building blocks harbouring the most important QTL / candidate genes for yield, leaf, quality, disease and insect resistance traits in *Brassica* genome. We used conventional genetic analysis, different molecular markers and recently developed NGS technology to find some of the candidate genes, and genome wide gene specific SNP markers for genomics-assisted precision breeding of Brassicas.

### Emerging challenges of abiotic stresses in oilseeds Brassicas

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Rapeseed mustard is the third major oilseed crop of

the world after soybean and palm. India's share in the global area planted under rapeseed mustard is 21.45% however, it contributes only 11.92% to the global rapeseed mustard production. Production and productivity of rapeseed mustard in India is affected by number of biotic and abiotic stresses. Adverse climatic factors, such as water scarcity (drought), extreme temperatures (heat, freezing), photon irradiance and contamination of soils by high ion concentration (salt, metals), are the major growth stressors that significantly limit productivity and quality of crop species worldwide. Recent model projections anticipate that the global mean surface air temperatures will increase by 1.4 to 5.8°C by 2100. Problem of heat stress is observed in all major mustard growing countries including China, Australia, Canada and European countries but the heat stress at seedling stage is a problem unique to India (Salisbury and Gurung, 2011). Exposure of the crop to high temperatures at seedling and reproductive stage are the major constraint in realizing the potential yields. Reduced water availability, on the other hand, is projected to reduce crop yields as a consequence of climate change (Singh and Choudhary, 2003; Singh *et al.*, 2011) any abiotic stress hardly comes alone. Therefore, occurrence of more than one stress in the growing area would further complicate and aggravate the problem. Efforts have been made for breeding for abiotic stress tolerance to salt [CS-52, CS-54 (CS-614-4-1-4), CS-234-2-2, CS-56, RH-8814 (IC-401570, BPR-540-6 (INGR13027))]; high temperature [Pusa Vijay (NPJ-93), Pusa Mustard 25 (NPJ-112), Pusa Mustard 27 (EJ-17), Pusa Mustard 28 (NPJ-124), BPR-541, BPR-543, BPR-549-9 (INGR13016)]; frost (RH-819, RGN-48) and drought (RH-819, RN-393, RB-50, RB-24, RGN-48, RGN-229, RH-0406, PBR-91, PBR-210). Resistant varieties are available in public domain. Short duration varieties *viz.*, Pusa Agrani, Pusa Mahak, Pusa Tarak, Pusa Mustard 25, Pusa Mustard 27, Pusa Mustard-28, EJ-20, Kranti, NDRE-4, PR-2006-1, Divya with 100-120 days maturity have been developed and are further being used in breeding early maturing and high temperature tolerant *B. juncea* varieties. Screening methodologies for differentiating genetic variability for drought, salinity and high temperature have been

standardized. A reliable and reproducible protocol for seedling stage heat tolerance has also been developed for rapid screening of genetic resources in *Brassica* (Singh *et al.*, 2012). Five tolerant genotypes viz. EJ-22, NPJ-113, NPJ-124, Pusa Bahar and 5011 (Pusa Agrani x Laxmi) has been identified using this protocol (Azharudheen *et al.*, 2013). *Brassica* species, in general, possess better tolerance against abiotic stresses, however, its growing environments/ adversities still demand higher level of tolerance. Genetic mitigation needs a species-by-species and region by-region analysis of projected environmental issues (Banga & Kang, 2014) on one hand and understanding and utilization of biochemical, physiological and molecular mechanism underlying different abiotic stresses to complement the conventional approaches on the other hand for developing better level of tolerance against different stresses.

### **Breeding *Brassica* vegetables**

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*Brassica* vegetables form an important group of vegetable crops the world over. These include a wide array of crops that span numerous genera and species in the family *Brassicaceae*. However, two species- *B. oleracea* and *B. campestris*-are the source of edible crops. Cole crops, namely cauliflower, cabbage, broccoli, Brussels sprouts, kohlrabi, and kales belong to *oleracea* specie, whereas Chinese cabbage and turnip to *campestris*. Since cauliflower and cabbage are extensively grown vegetables in India, the major breeding efforts, therefore, so far had been only on these. Besides their culinary value, these are rich sources of various health building substances and also of anticancerous compounds. The main criteria for improvement in these crops are higher production and productivity, biotic and abiotic stress resistance, physiologic defect resistance and uniformity. Breeding for appearance, commercial quality, shelf life, taste,

nutraceutical value are parts of product improvement. Development of open pollinated varieties played a significant role in sustaining small and marginal farmers who require staggered harvests to feed small markets continually. Cole crops hybrid breeding, of late, had a shift to *Ogura* sterile cytoplasm from traditional sporophytic self-incompatibility mechanism and further diverse sterile cytoplasm introgression is being carried out to desirable *oleracea* genetic backgrounds. Marker assisted resistance breeding to black rot and downy mildew diseases which take a heavy toll of *Brassica* vegetables has enhanced precision, efficiency and product development in a shorter span of time. Efforts are also on to search new genes for resistance from diverse sources so as to pyramid them into desired genetic backgrounds. Among insect pests, diamondback moth (*Plutellaxyllostella*) is the most notorious causing tremendous problems due to its chemical control; therefore transgenesis through *Bt* genes is being experimented as possible alternative. From quality improvement point of view, the efforts earlier had been only on extrinsic traits, viz. size, shape, colour, firmness and appearance. Now, nutraceutical and bioactive compounds related variety/hybrid breeding is gaining popularity with *Brassica* vegetable breeders, as this aspect besides nutritional and marketing advantage, will enhance industrial value for the development of functional/ fusion food, thus, paving the way for contract farming in near future.

### **Crop management in face of climatic change**

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Climate change is serious man-made environmental threat aggravating vulnerability of key sectors of economy and development. The rising temperature of the earth due to unimpeded growth of greenhouse gases consequences melting glaciers, varied precipitation, extreme weather events and shifting seasons. And no country in the world is as vulnerable



to climate change as India with long coastlines of 7000 kms, vast Himalayan glaciers, 70 mha forests, 143 mha agriculture or 20 river basins. It is estimated that at present pace of activities the atmospheric carbon dioxide level in the country will reach to 410 ppm by 2020 and 500 ppm by 2050. The mean temperature will raise by 1.0-1.5°C by 2030 and 2.0-3.5°C by 2050. The intermittent heat stress periods will be more unpredictable and frequent. The rainfall intensity will increase but rainfall will be more in summers and lesser in winters. It will result into more dry-days, floods and droughts. The receding Himalayan glaciers will gradually decrease blue water availability in Indo-Gangetic Plains and many coastal areas will submerged due to 0-18-0.59 m rise in sea level. Since, Indian agriculture is highly dependent on the vagaries of monsoon as almost 2/3<sup>rd</sup> area is rainfed, the changing climate scenario could further aggravate the risk of farmers and basic livelihood security of the people through direct or indirect effects on crops, soils, livestock, fisheries, diseases and pests. Agriculture is both sufferer and contributor to greenhouse effects primarily through emission and consumption of greenhouse gases such as methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>). But agriculture could be important part of the solutions by understanding Pressure-State-Response relationship of resources and socio-economic attributes. And concerted adaptation and mitigation efforts are required to reduce the vulnerability of Indian agriculture and make it resilient. The adaptation strategies should be at all levels of policy, technology, society and farm. While mitigation efforts should focus on reducing GHG emission through financing, technology, weather forecasting and stringent policies. The adaptive capacity of Indian farmers is often limited due to low formal education and subsistence farming. Thus, simple, economically viable, socially acceptable, environmentally resilient strategies have to be developed and implemented. A win-win solution is to start with simple mitigation strategies that are needed for sustainable development such as increasing soil organic C content. The other mitigation strategies to climate change includes modification of crop production technologies, application of fermented manures like biogas slurry in place of unfermented

FYM, altering feed composition of ruminants, site-specific crop nutrient management, improved carbon sequestration and adoption of best management practices. While potential adaptation strategies to deal with impact of climate change are developing cultivars tolerant to heat and salinity stresses and resistant to flood and droughts, modifying crop management practices, improving water management, adoption of conservation agriculture techniques such as resource conservation technology (RCTs), crop diversification, improve disease and pest management, better weather forecasts, effective crop insurance and harnessing the indigenous technical knowledge of farmers. However, there is a need to develop policy framework for implementing the adaptation and mitigation options so that the farmers are saved from the adverse impacts of climate change. And a rapid building up of capacity is essential to enhance the level of climate change research in the country. In this context scientific cooperation and collaboration is essential in the areas of climate modelling, impact assessment, integrated impact assessments, research on mitigation of climate change concerns and adaption of impacts of climate change.

### **Health benefits of oilseed & vegetable brassicas**

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Various crops are being domesticated as per their seed composition; the proportions of carbohydrate, protein, fats and minerals present therein. About 90% vegetable oils produced in India are used for edible purposes. Mustard oil is one of the major edible oils consumed in the Northern and Eastern parts of the country. The parameters for quality improvement in rapeseed-mustard include high oil content, low / high glucosinolate and fatty acids composition for specific purposes, the crude fiber and protein content, and amino acids composition. Other minor

constituents of importance include phytic acid, sinapine, phenolic compounds, vitamins, minerals etc. Apart from Asian countries, rapeseed- mustard has also been used in traditional medicine system in Iran. Root parts of Colza are used for therapeutic purposes as diuretic, anti-scurvy, anti-inflammatory of bladder. Seeds of Colza are used for treatment of hepatic and kidney colic and have also found use in the Eastern folk medicine as bronchial cathartic. Rapeseed oil has been found to decrease cholesterol absorption by 11%, and increase excretion of cholesterol, bile acids, and their sum as sterols by 9, 32, and 51% as compared to olive oil.

**Fatty Acids Composition** is well known for its nutritional implications. The saturated fatty acids (SFA- such as palmitic acid; 16:0) increase Low Density Lipoprotein (LDL) and thus act as a causative agent of Coronary Artery Disease (CAD) by deposition of cholesterol in arteries. Mono-unsaturated fatty acid (MUFA- such as oleic acid; 18:1) is considered neutral, it is thermo-stable and provides stability to the oil and is preferred for cooking/ deep frying. Poly- unsaturated fatty acid (PUFA- such as linoleic; 18:2 and  $\alpha$ -linolenic; 18:3) are essential fatty acids to be supplemented in diet. These are required for formation of prostaglandin (hormonal or regulatory activity), increase beneficial High Density Lipoprotein (HDL) and thus reduce total cholesterol and LDL. However, linolenic acid is unstable, prone to per-oxidation and flavor change and is thus undesirable for oil storage/ shelf life.

**Very Long Chain PUFAs (VLC PUFA)** are the precursors of prostaglandin synthesis in the mammalian system, but not produced naturally in the animal or plant systems. It is accumulated in fishes that feed on sea flora and is highly valued in nutraceutical. Due to its poor oxidative stability in cooking oil it can be delivered in the form of pills. Multiple transgenic alterations of several new pathways have been reported for production of VLC PUFA in *B. juncea*. It has been achieved by addition of 5 novel desaturase genes from 4 different species (three microbes and one plant), and 3 elongases (two from microbes and one from fish). The resultant transgenic plants accumulated 1.5 % docosahexaenoic acid, 15% eicosapentaenoic acid and 25% arachidonic acid.

**Rapeseed Meal** is a byproduct of the oil removal process comprising of approx. 40% protein and forms valuable products for use in ruminant, swine, poultry, aquaculture and companion animal diets. Rapeseed- mustard meal is a source of vitamins, minerals and proteins. It is also high in sulfur containing amino acids and is a rich source of health benefiting minerals; Ca, Mn, Cu, Fe, Se, and Zn. Calcium help to build bones and teeth, Manganese is an important co-factor for the antioxidant enzyme superoxide dismutase. Copper helps in production of red blood cells while Iron helps in red blood cell formation and cellular metabolism. ACE (Angiotension converting enzyme) is a key enzyme that operates blood pressure regulating pathway called the renin-angiotension system. Rapeseed meal is a source of new **bioactive peptides** having ACE inhibitor activity, and thus it possesses the ability to lower blood pressure after oral administration. It is also a rich source of vitamin E and other important vitamins / precursors such as niacin, pantothenic acid, riboflavin and thiamine.

**Mustard seeds** have highly prized medicinal as well as culinary importance which dates back to ancient times. Perceived as a health benefiting spice, it is a rich source of phytonutrients, minerals, vitamins and anti-oxidants metabolites such as flavonoids, vitamin C, vitamin E, cinnamic acid and carotenoids. Its oil has traditionally been used to relieve muscle pain, rheumatism and arthritic pain. It is also believed to stimulate hair growth. Ground seeds of mustard act as a laxative and stimulant to gastric mucosa.

**Mustard greens** are excellent source of essential vitamin B-complexes involved in enzyme synthesis, nervous system function and regulation of body metabolism. Glucosinolates and their hydrolysis products and phytochemicals possess antitumor activities. The inhibition of carcinogenesis by glucosinolate- derived hydrolysis products (eg., isothiocyanates) has been shown to be by modulation of the antioxidant potential, enhancement of detoxification mechanisms and induction of apoptosis in undifferentiated cells. Consumption of brassica sprouts as raw vegetables provides glucosinolates (GLS) and active plant myrosinase, which enables

the breakdown of GLS into health-promoting isothiocyanates. (ITC) It has been found in edible sprouts of two *B. oleracea* varieties, broccoli and Tuscan black kale, and two *Raphanus sativus* varieties, Daikon and Sango. *Wasabia japonica* (Wasabi) possesses compounds 6-MITC (6-methylsulfinyl hexyl isothiocyanate) and I-7557 that have been shown to have activity against the growth and CSC phenotypes of human pancreatic cancer cells. MITC is known to possess anti-inflammatory, chemo-preventive and anti-melanoma activities. Rapeseed Dithiolethiones (Eg- Oltipraz-Sulphur containing compounds), present in green leafy brassicas such as broccoli and brussels sprouts constitute an important class of chemo-preventive agents that enhance the expression of carcinogen detoxification and antioxidant genes. Oltipraz prevents induction of cancers in skin, breast bladder, lung, colon, pancreas, stomach and liver.

**Phenolics** are potent antioxidants with beneficial effect on radical scavenging. Most significant of these phenolic compounds is Sinapine; the choline ester of sinapic acid which is approximately 80% of total phenolic compounds. Sinapic acid also exists as the glucosidic ester, glucopyranosyl sinapate. Rapeseed phenolic extracts with sinapine as the main phenolic compound have excellent antioxidant activities toward oxidation of liposomes and low density lipid (LDL) particles. Crude rapeseed oil extract with vinyl syringol as the main phenolic compound has shown anti-inflammatory properties. Rapeseed Phytate (such as Myo-inositol hexakisphosphate) has shown to have anticancer and antioxidant activity. It forms an iron chelate that suppresses lipid peroxidation by blocking iron-driven hydroxyl radical generation. Phytate may also contribute to antioxidant defense by increasing the activity of key enzymes that detoxify Reactive Oxygen Species (ROS).

**Tocopherols (TOC)** found in rapeseed- mustard are poly-nutrients having bioactivity as vitamin E-important nutraceutical. They are found as tocopherols or tocotrienols, both acting as anti oxidants. However, both of them have an inverse relationship with oil content, thus selections for high oil content have resulted in corresponding decreased

levels of TOC. Conventional breeding procedures and genetic engineering techniques have been applied to create novel genetic variants for TOC composition. Gamma TOC reduces the oxidation of PUFA providing better shelf life while Alpha TOC is the active Vitamin E necessary for reproductive processes. Increased TOC will have levels for value addition in canola rapeseed oil in the human health sector.

**Phytosterols** are the natural steroid alcohols found in plants. The major phytosterols found in Oilseed Brassica are  $\beta$ -sitosterol, Campesterol, Brassicasterol, Avenasterol and Stigmasterol, in order of decreasing concentrations. They are structurally similar to each other with close molecular weights. Phytosterols have been shown to lower serum cholesterol by reduced LDL cholesterol absorption in gut up to 50%, thus reducing the risk of cardiovascular disorders. They are being used as nutraceuticals and dietary supplements and incorporated by food industry as bioactive components, particularly in margarine & dairy products.

#### The way forward :

- *Brassica* crops-reduced risk of chronic diseases- cardiovascular diseases and cancer
- *Brassica* vegetables-broccoli, cabbage, kale, mustard greens, brussels sprouts, and cauliflower-reduced the risk of several cancers
- *Brassica* foods- provide nutrients and health-promoting phytochemicals: vitamins (carotenoids, tocopherol, ascorbic acid, folic acid), minerals (Cu, Zn, P, Mg etc), carbohydrates, amino acids and different groups of phytochemicals such as indole phytoalexins, phenolics and glucosinolates
- All these phytochemicals contribute to the reported antioxidant, anticarcinogenic, and cardiovascular protective activities of *Brassica* vegetables
- Broccoli (*B. oleracea*) extracts are protective against reactive oxygen species (ROS)

presumably due to the presence of vitamin C, quercetin, kaempferol, lutein, zeaxanthin

- Raw broccoli, cauliflower and cabbage contain folic acid- acts as a coenzyme in many single carbon transfer reactions in the synthesis of DNA, RNA, and proteins
- Folic acid reduces the risk of neural tube defects and may be associated with the reduced risk of vascular disease and cancer
- Among the green leafy vegetables, *B. oleracea* L. acephala (kale) is an excellent source of minerals, accumulating high levels of P, S, Cl, Ca, Fe, Sr, and K.
- Cabbage leaf (*B. oleracea* var. capitata) also contains potentially useful amounts of copper, zinc, iron, and a number of other essential minerals and trace elements
- Awareness generation about health benefits of oilseed and vegetable brassicas, development of commodity-based niche markets for dissemination of value added products at a premium price and participation of private players for marketing of the nutritionally improved oils/ value added products will go a long way in solving health issues at large.