6 Application of Genomics and Breeding Technologies to Increase Yield and Nutritional Qualities of Rapeseed-Mustard and Sunflower

Vivek K. Singh, Pratik Istari Bhoyar, Anu, and Vijay Sharma

Abstract

The oilseed crop plays a pivotal role in the agricultural economy. The main breeding objectives of the oilseed crop are to increase the yield of seeds and oil, improve the quality of the oil and meal according to its use, and developing stable biotic and abiotic-resistant/tolerant varieties. Adding to the yield potential of varieties is a natural way to enhance the quantity of both oil and meal. The fatty acid composition determines the oil's quality, whereas the desired fatty acid profile is determined by the oil's consumption. Reduced erucic and eicosenoic acid content has significantly improved the quality of edible oil in rapeseed and mustard species, whereas high oleic acid and tocopherol content has been achieved in sunflower. With the advancement in genetics, genomics and availability of sequenced information of genomes, the traditional breeding is replaced by genomics-assisted breeding which will lead to the effective development of new-generation high-yielding oil genotypes. In this review, we aim to provide an overview of the various advanced approaches for genomics-assisted breeding to enhance genetic gain in yield and nutritional quality.

Keywords

Genomics · GWAS · Conventional breeding · Oil qualities · Rapeseed-mustard · Sunflower

V. K. Singh · Anu
CCS Haryana Agricultural University, Hisar, Haryana, India

P. I. Bhoyar (✉)
Indian Agricultural Research Institute, Regional Station, Indore, Madhya Pradesh, India

V. Sharma
Banda University of Agriculture and Technology, Banda, Uttar Pradesh, India

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6.1 Introduction

Oil-bearing crops like soybean, rapeseed-mustard, sunflower, groundnut, sesame, safflower, niger (for edible oil) and linseed, castor (non-edible oil) are mainly cultivated for the oil preserved in their seeds. In addition to this, a wide variety of minor oilseed crops and oil-bearing trees have also been cultivated in India and other countries. Next to the United States of America (USA), China and Brazil, India is the world's fourth-largest vegetable oilseed producers. Oilseeds are predominantly used as a source of dietary oil and also as a high-protein feed for livestock but are also used in the manufacturing of industrial products, for example lubricants, emulsifiers, plasticizers, detergents, cosmetics, pharmaceutical products etc. Subsequently, the oils of the crops like rapeseed-mustard, sunflower and soybean have been identified as potential oils for biodiesel (Qiu et al. 2011). Globally, rapeseed-mustard (12.5%) and sunflower (9.2%) ranked third and fourth, respectively, in total vegetable oil economy after palm (36.5%) and soybean oil (27.4%). Vegetable oils are in high demand across the world due to the increase in per capita oil intake in our diets and their use as biofuels. The global market for vegetable oils is estimated to more than double by 2050, compared to current production (Savadi et al. 2017). The nutritional and functional properties of the oil are determined by the fatty acid composition and distribution pattern within the triacylglycerol molecule (Pham and Pham 2012). From a nutritional point of view, oils of sunflower and rapeseed-mustard are believed to be high-quality oil owing to the existence of unsaturated fatty acids, essential fatty acids and tocopherols which reduce the risk of cardiovascular diseases and lower blood cholesterol (Iocca et al. 2016). However, the oil of rapeseed-mustard also contains a high amount of erucic acid which is undesirable for health; therefore, there is a need to develop more varieties like Pusa mustard 24, RLC 1 and RLC 2 etc. which have erucic acid $<2\%$. Furthermore, the possibility of using its oil as a raw material for the production of biodiesel has piqued the interest of farmers, agricultural experts and companies across the globe. Thus, to meet the increasing demands, there is a need to increase oil yields. One option is to enhance the oil content in oilseeds crop to improve oil yields without expanding the area under cultivation and to save the inputs used to increase the additional crops needed to meet the upcoming global oil crises.

Improvement in oil yields can be achieved by enhancing the amount of oil per seed, higher test weight or improvement in seed yield per plant. The development of varieties/hybrids with higher seed yield along with increased oil content has been led by classical plant breeding approaches such as pure-line selection, heterosis and mutation breeding. In terms of optimism to improve traditional breeding approach constantly, modern tools are required to considerably improve efficacy, accuracy and save time, resource and efforts. Still, the level of effectiveness relies on the objectives and key points in species-specific pathways and the techniques used to exploit those targets. The seed yield is complex and relies on many other morphological features, which are mostly quantitatively inherited and strongly influenced by the environment, so direct selection for seed yield alone decreases the efficacy of the selection and ultimately results in limited success in improving it. The genome

regions containing certain genes linked with a specific quantitative trait are referred to as quantitative trait loci (QTL). DNA markers may be used for the identification of allelic disparity for QTL or major gene underlying the trait of interest due to genetic linkage. In many oilseeds crop, several QTLs regulating seeds oil have been reported (Savadi et al. 2017). Moreover, there is a need for stacking of new gene controlling traits like increased oil content, seed yield and oil yield with each improved variety. This chapter addresses developments in the genetic improvement of oilseed crops and various techniques that can be used in the cultivation of oilseeds to achieve sustainability.

6.2 Origin and Distribution

6.2.1 Sunflower

Botanically the term "sunflower" is originated from the Greek words "Helios" = "sun" and "anthos" = "flower". Archaeological and botanical findings suggested that it has been domesticated by American tribes in the temperate region of North America, probably about 1000 years BC; it was then transported to America's eastern and southern parts (Putnam et al. 1990). However, evidence procured from San Andres archaeological site in Mexico suggested that it may have been grown as far back as 2600 BC. It was brought from North America to Europe in 1510 and has been used exclusively as an ornamental plant by Spanish explorers for over two centuries (Putt 1997). In the middle of eighteenth century, sunflower cultivated as an oilseeds crop when it reached Russia (USSR) from Europe, where it was easily domesticated in local environments and was exploited as food, source of oil (28–50%) and for medicinal purposes (anti-inflammatory effects). Thereafter, sunflower reintroduced in the United States of America (USA) from Russian immigrants (Davey and Jan 2010). Russia, Argentina, Ukraine, United States, China, India and Turkey are the major sunflower-producing countries in the world. Sunflower has acquired the rank of an important commercial oilseeds crop in India after the introduction of Russian varieties such as Peredovick (EC 68414) and Armavirskii (EC 68415) during the 1960s and due to its day length neutrality, broader adaptability and responsiveness to added inputs, it is cultivated in a variety of climatic and geographical regions.

6.2.2 Rapeseed-Mustard

The family Brassicaceae comprises almost 3500 species and 350 genera. *Brassicas* are one of the most primitive crops domesticated by man since 5000 BC. The findings of Allchin (1969) suggested that mustard was in cultivation since Channhu-daro of Harrapan civilization ca. 2300–1750 BC. Mediterranean basin and south-western Asia are considered as the origin of the genus *Brassica* and its wild relatives. However, it is geographically distributed in the south-western Mediterranean region.

It is evident from earlier reports that *Brassica* originated from the Miocene's genus *Sinapidendron* through *Diplotaxis-Erucastrum*. Among the various species of oilseeds brassica, four species namely *B. juncea* (Indian mustard), *B. napus* (winter and spring rape), *B. rapa* (*B. compestris*) and *B. carinata* (Ethiopian mustard) are of economic importance. The wild form of *B. juncea* thought to be evolved in the Middle East, since its putative parental species, viz., *B. nigra* and *B. rapa* coincided geographically in wild. In the Indian subcontinent, it is grown as an oilseeds crop whereas in China, as a leafy vegetable. Vavilov (1949) suggested that Central Asia and Afghanistan are the primary centres of origin, whereas Asia minor, eastern India and western China included as a secondary centres of origin. The *B. nigra* (black mustard), also known as table mustard, consumed as spices and widespread in southern Europe. *B. rapa* also known as *B. compestris* (L.) is probably native of uplands near the Mediterranean Sea. From here, it was introduced into Germany and Europe. It spread into China as an agricultural crop from Mangolia. In India, it is grown as an oilseeds crop without any known wild relatives. Bengal region of the Indian subcontinent exhibited large diversity of yellow sarson (Chauhan et al. 2011). It is believed that the wild form of *B. napus* has been native to the coastal region of Sweden, Gothland, the Netherlands and Britain, whereas *B. carinata* (Braun) is native to the Ethiopian plateau. *Eruca sativa* (taramira) is widespread in southern Europe/northern Africa and recently introduced in India. Indian mustard, toria, yellow sarson, brown sarson, gobhi sarson, karan rai, black mustard and taramira are eight distinct varieties of rapeseed-mustard that are grown in 53 countries around the world.

6.3 Taxonomical Classification

6.3.1 Sunflower

Botanically, sunflower is known as *Helianthus annuus* (L.), belongs to tribe: *Heliantheae* (Table 6.1), diploid ($2n = 34$), interfertile, an annual herb, 60–300 cm in height with hairy stem, broad and rough leaves, circular head called as disk of diameter of 7.0–30 cm, widely adopted due to short growing season among other oilseeds crops. The cultivated sunflower is usually unbranched, solitary head with several ray florets and bigger achenes. *Helianthus annuus* L. consist of three main races: (1) *H. annuus* ssp. *Macrocarpus*, cultivated for seed or fodder purpose; (2) *H. annuus* ssp. *Annuus*, weed sunflower, grown as ornament plant and (3) *H. annuus* ssp. *Lenticularis*, wild and uncultivated sunflower.

6.3.2 Rapeseed-Mustard

Rapeseed-mustard belongs to *Brassicaceae* (Syn. *Cruciferae*) family of plants which holds above 338 genera and 3709 species. The genus *Brassica* of *Brassicaceae* family comprises over 150 species; however, there is conflict about the exact group

Table 6.1 Botanical classification of *H. annuus* L. and *B. juncea* L.

Taxa		
Domain	Eukaryota	Eukaryota
Kingdom	Plantae	Plantae
Subkingdom	Tracheobionta	Tracheobionta
Superdivision	Spermatophyta	Spermatophyta
Division	Magnoliophyta	Magnoliophyta
Class	Magnoliopsida	Magnoliopsida
Subclass	Asteridae	Dilleniidae
Order	Asterales	Capparales
Family	Asteraceae	Brassicaceae
Tribe	Heliantheae	Brassiceae
Genera	*Helianthus*	*Brassica*
Species	*annuus*	*juncea*

and nomenclature of various species. It includes several cultivated vegetables such as cabbages, cauliflower, broccoli, turnip and rutabaga, along with cultivated oilseeds species. Species such as *B. juncea*, *B. napus*, *B. rapa* and *B. carinata* are grown as oilseeds crops whereas *B. oleracea* and *B. nigra* for seed condiments. Among them, *B. juncea* contributes significantly in terms of the area and production of edible oilseeds. The taxonomic classification of *B. juncea* is given in Table 6.1. Massive diversity in the company of a wide range of wild relative unveils by the genera *Brassica*. However, most of the wild accessions show incompatibility barriers due to the availability of these species in secondary and tertiary gene pools. Table 6.2 contains some of the economically important species of the genus *Brassica*.

6.3.2.1 Genomic Evolution of Brassica

The naturally occurring polyploidy relationship found in the *Brassica* species is interesting and we will discuss it in detail here. This includes three collective *Brassica* diploid species, namely *B. rapa*, *B. nigra* and *B. oleracea*, having haploid chromosome numbers of 10, 8 and 9, respectively. Genome designations A, B and C have been assigned to these (Fig. 6.1). Pairwise crossing among these diploid species after the chromosome doubling led to the evolution of the three amphidiploid species *B. juncea*, *B. napus* and *B. carinata*. *B. juncea* (AABB) contains the genomes of the two species, *B. rapa* (AA) and *B. nigra* (BB). *B. napus* (AACC) share the genomes of *B. rapa* (AA) and *B. oleracea* (CC), whereas *B. carinata* (BBCC) combines the genomes of *B. nigra* (BB) and *B. oleracea* (CC).

The cytological studies of Morinaga (1934) and his associates fundamentally explained the evolutionary relationship among cultivated *Brassica* species. For example, when *B. juncea* is hybridized with *B. rapa*, ten chromosomes of *B. juncea* are seen to pair with the ten of *B. rapa* leaving the other eight as univalent; however, when *B. juncea* is hybridized with *B. nigra*, eight bivalents are usually detected at meiosis which showed that *B. juncea* evolved from these two ancestor species. Similar results were also observed for *B. carinata* and *B. napus*. The

Table 6.2 List of some economic important species of genus *Brassica*

Botanical name	Chromosome No.	Genome	common name	Usages
Diploid species				
Brassica rapa	20	AA		
B. rapa spp. *Oleifera*	20	AA	Turnip rape	Oilseed
B. rapa var. *brown sarson*	20	AA	Brown sarson	Oilseed
B. rapa var. *yellow sarson*	20	AA	Yellow sarson	Oilseed
B. rapa var. *toria*	20	AA	Toria	Oilseed
B. rapa spp. *Rapifera*	20	AA	Turnip	Fodder, vegetable (root)
B. rapa spp. *japonica*	20	AA	Curled mustard	
B. rapa spp. *Chinensis*	20	AA	Chinese mustard	Vegetable (leaves), fodder
B. rapa spp. *pekinensis*	20	AA	Chinese cabbage	Vegetable (leaves)
B. rapa spp. *nipposinica*	20	AA	–	Vegetable (leaves)
B. rapa spp. *Parachinensis*	20	AA	–	Vegetable (leaves)
Brassica nigra	16	BB	Black mustard, Banarasi rai	Condiment (seed)
Brassica oleracea	18	CC		
B. oleracea var. *acephala*	18	CC	Kale	Vegetable, fodder (leaves)
B. oleracea var. *capitata*	18	CC	Cabbage	Vegetable (head)
B. oleracea var. *sabauda*	18	CC	Savoy cabbage	Vegetable (terminal buds)
B. oleracea var. *gemmifera*	18	CC	Brussels sprouts	Vegetable (head)
B. oleracea var. *gongilodes*	18	CC	Kohlrabi	Vegetable, fodder (leaves)
B. oleracea var. *botrytis*	18	CC	Cauliflower	Vegetable (inflorescence)
B. oleracea var. *italic*	18	CC	Broccoli	Vegetable (inflorescence)
B. oleracea var. *fruticosa*	18	CC	Branching bush kale	Fodder (leaves)
B. oleracea var. *alboglabra*	18	CC	Chinese kale	Vegetable (stem, leaves)
Eruca sativa	22	EE	Taramira, rocket salad	Vegetable, non-edible oilseed
Raphanus sativus	18	RR	Radish	Vegetable, fodder

(continued)

Table 6.2 (continued)

Botanical name	Chromosome No.	Genome	common name	Usages
Sinapis alba	24	SS	White mustard	Oilseed
Brassica tournefortii	20	DD	Asian/African mustard, Jangali rai	–
Tetraploid species				
Brassica juncea	36	AABB	Indian Mustard, rai	Oilseeds, vegetable
B. juncea var. *Cuneifolia*	36	AABB	Vegetable mustard	Vegetable
Brassica napus	38	AACC	Rape	Oilseed
B. napus spp. *oleifera*	38	AACC	Rapeseed, gobhi sarson	Oilseed
B. napus spp. *Rapifera*	38	AACC	Rutabaga, swede	Fodder
B. napus spp. *pabularia*	38	AACC	Siberian kale, leaf rape	–
Brassica carinata	34	BBCC	Ethiopian mustard, Karan rai	Vegetable, oilseed

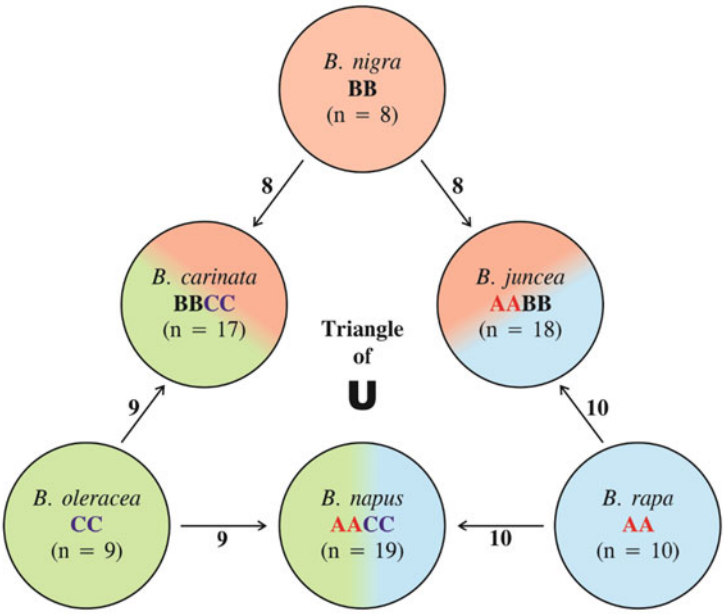

Fig. 6.1 The "Triangle of U", demonstrating the genomic connections between the six *Brassica* species. AA, BB and CC are the genome symbols of *B. rapa*, *B. nigra* and *B. oleracea*, respectively. AABB, AACC, BBCC are the three tetraploid species (*B. juncea*, *B. napus* and *B. carinata*). The numbers on the lines connecting two genomes indicate the maximum of bivalents possible among the respective tetraploid species

genomic evolutionary relationship among six species of Brassica may be represented in triangular form, known as "U" triangle (Fig. 6.1).

6.4 Floral Biology

The inflorescence of sunflower is a capitulum or head which may be convex, concave or flat attached to the main stem at different angles (Fig. 6.2a). The capitulum is enclosed by an involucral bract of variable shape and size (Fig. 6.2a). Each capitulum is composed of two distinct forms of flowers. The peripheral flowers are ray florets, usually yellow and sterile or pistillate type which attract the insect pollinators. The central/inner part of the capitulum is disc florets, hermaphrodite and fertile, which are organized as curves radiating from the centre of the capitulum. The two papus scales are modified form of calyx, and the corolla tube is made up of five petals that are joined together. The stamens are free and connected to the corolla's base. Five anthers come together to form an anther tube, and the style is inside the tube, with a bilobed stigma. Stigma remains receptive for 2–4 days. The flowering process begins from periphery to centre of the capitulum (Fig. 6.2a). Generally, anthesis starts in the morning hours between 07:00 and 10:00 A.M.

In the case of rapeseed-mustard, the basic floral biology of all the *Brassica* species is almost the same, except for varying flowers size (Fig. 6.2b). The inflorescence of brassica is typically a corymbose raceme; indeterminate in flowering starts at the base of the main shoot and continue upward. Flowers of *Brassica* species are regular, bisexual and hypogynous. The calyx is pale green, contains four sepals in two whorls each. Corolla consists of four free cruciform petals which are clawed and regular. Tetradynamous androecium comprises six stamens arranged in two whorls, viz., two stamens with shorter filaments on the lateral side and four median stamens with longer filaments. Two carpels combine to create a superior ovary with a "false" septum, two rows of campylotropous ovules, and a short style and stigma. Stigma remains receptive for 2–3 days. Generally, anthesis starts in the mid of the day between 11:00 and 02:00 P.M.

6.5 Crossing Techniques

Buds of flower that will open the next day are selected for emasculation. The remaining buds, flowers and flowering branches are detached. Emasculation takes place in the evening and pollinates them the next morning. The petals along with the stamens of the picked buds are detached with the help of forceps and emasculated buds are bagged. In the next morning, ripe anthers are collected from desired male parent, and pollination is carried out by dusting pollen over the stigma. The buds are again bagged after pollination. Pollen grains remain viable up to 7 days, whereas stigma is receptive up to 3 days after emasculation. Moreover, sunflower is predominantly a cross-pollinated crop due to its protandrous nature, and the position of stigma above the anther makes it self-incompatible. Emasculation is carried out in

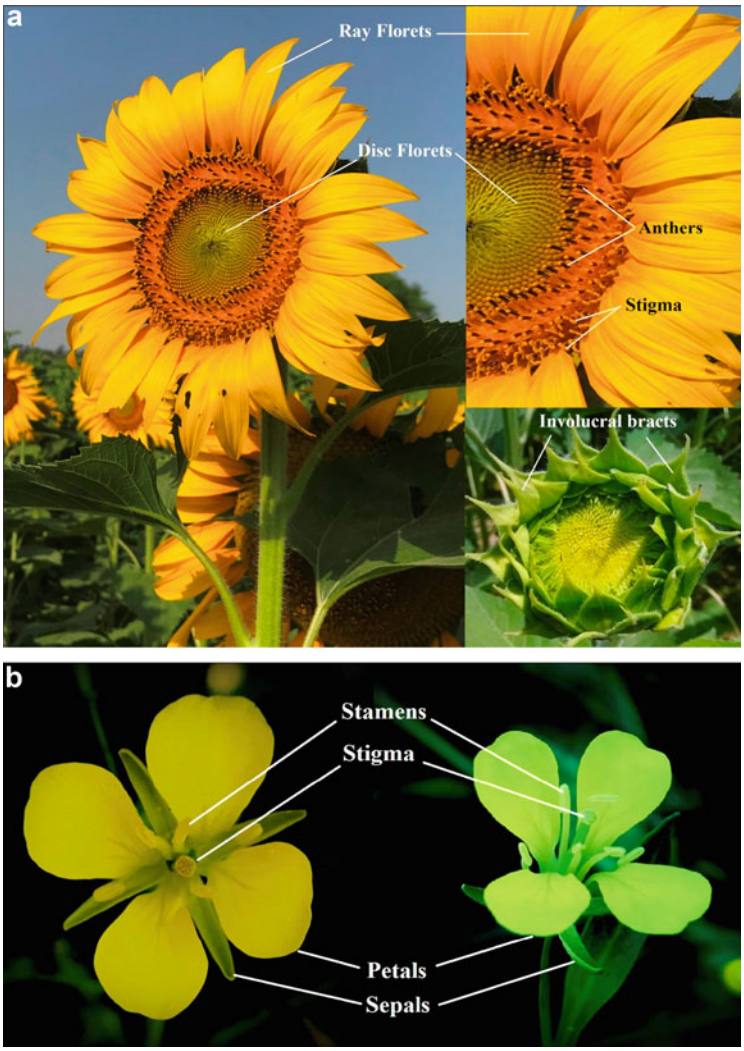

Fig. 6.2 (**a**) Floral biology of *H. annuus* (**b**) Floral biology of *B. juncea*

the early morning by using forceps to remove the anthers of the disc florets in 2–3 whorls. The pollen from the preferred male parent is collected the next morning and dusted on the emasculated head with a small piece of cotton. This procedure is repeated for another 2–3 days. Undeveloped central florets are removed. To avoid undesirable cross-pollination, hands and forceps have to be surface sterilized with ethanol. Emasculation can also be carried out by spraying 100 ppm GA_3 at the bud-initiation stage consecutively for 3 days in the morning.

6.6 Breeding History and Breeding Objectives

6.6.1 Breeding History

Breeding in sunflower began around 1912 in the former Soviet Union and the most successful early breeding programme was that of V.S. Pustovoit at VNIIMK. The concerted efforts for four decades resulted in increasing oil content from 30% to almost 52%. High oil sunflower varieties, such as Peredovik, Armavirski 3497, Mayak, VNIIMK 8931, VNIIMK 6540 and Smena developed by V.S. Pustovoit and his associates enabled the spread of sunflower crop not only in the Soviet Union but also in other continents. In the 1940s, Putt in Canada developed shorter, early maturing cultivars (Miller et al. 1992). Rust resistance was incorporated from wild species. Argentina and so many other countries began breeding programmes at the same time. In the USA, Kinman began a breeding programme around 1950. Intensive breeding programmes were pursued in several countries around the world, as a result of which sunflower is now grown over a large area in many countries. On another hand, at the beginning of the nineteenth century, research work started for the improvement of rapeseed-mustard through a collection of landraces and their purification at Pusa (Bihar). Scientific work began in Lyallpur (now Faisalabad, Pakistan), then in Punjab (India), for the varietal development of Indian *oleiferous* Brassiceae (NRCRM 2000). At the initial efforts, RL18, a variety of Indian mustards, was identified and released for cultivation in 1937 and L1, a variety of yellow sarson through selection, whereas RT11, another strain of Indian mustard from Uttar Pradesh demonstrated considerably higher yield than local check and released in 1936. After independence, high-yielding varieties of Indian mustard (Laha 101, Varuna, Durgamani, Patan Pustard); yellow sarson (T 151, Patan sarson, YSPb 24, T 42); brown sarson (BSA, BSG, BSH 1, BS 2, BS 65, BS 70); toria (Abohar, BR 23, M 27, T 9, T 36, DK 1) and taramira were developed from 1947 to 1967 (NRCRM 2007). In January 1981, a separate unit of All India Coordinated Research Project (AICRP) on rapeseed-mustard was accordingly established at the campus of the CCS Haryana Agricultural University, Hisar. However, Australia started the first public rapeseed breeding programme in 1970 in Victoria, followed by New South Wales and Western Australia in 1973. The two species *B. napus* and *B. rapa* were initially developed for Australia, with short duration *B. rapa* directed especially at lower rainfall environments. In the late 1970s and early 1980s, breeding programmes of *B. juncea* for canola quality were started in Canberra, Victoria and Western Australia. Kirk and Oram recognized *ZEM 1* and *ZEM 2*, low erucic acid line of *B. juncea*, which were widely distributed to mustard breeder everywhere in the world, including India.

The development of hybrids in maize and other crops in the 1930s stimulated sunflower and rapeseed-mustard breeders to work towards developing hybrids in sunflower and rapeseed-mustard. Although a high percent of hybrid seed could be obtained on the female line, the *percent* seed set varied with the lines and environmental conditions. The discovery of cytoplasmic male sterility (*PET-1*) in the progeny of a cross between *Helianthus petiolaris* Nutt and cultivated sunflower by

Leclercq (1969) was a turning point in the development of commercial sunflower hybrids. Evolving hybrids using genetic male sterility was carried out by Varanceanu and Stoenescu (1982) in Romania. Kinman in USA, Putt in Canada and several other sunflower workers started a hybrid production scheme taking advantage of the self-incompatibility system in the crop (Fick and Miller 1997). Male fertility (MS ms) was linked with red anthocyanin pigmentation enabling rouging of fertile plants in the female line in the seed production plots. Hybrid seed production cost was high because of the labour required to remove fertile plants. The system was stable as evidenced by sterility obtained in the progeny of male sterile plants crossed with fertile cultivated sunflower plants. In 1970, Kinman discovered genes for genetic restoration of fertility (*Rf1*) in wild species. The first commercial hybrid based on cytoplasmic male sterility was made available in 1972 in the USA. Subsequently, the cultivation of sunflower hybrids spread throughout the globe. In India, with the initiation of AICRP on Sunflower in 1972–1973, the importance of hybrids and heterosis breeding was recognized. In 1974 and 1975, experimental hybrids have been developed in Bangalore with the help of four CMS lines (CMS2, CMS124, CMS 204 and CMS234) and two restorer lines (RHA 266 and RHA 274) introduced from the USA. The achene and oil yield in all the hybrids were significantly higher than the check variety EC 68415. Thus, BSH-1 (CMS 234A X RHA 274), the first sunflower hybrid was released in 1980 for commercial cultivation.

Moreover, the heterosis breeding strategy may be a feasible alternative for crop variety improvement. Singh and Mehta (1954) were the first to report heterosis in brown sarson. In *B. juncea*, 13–91% heterosis has been reported by many workers for seed yield (Verma et al. 1998). A significant level of heterosis for yield contributing parameters was observed among the crosses obtained from the genetically diverse group as compared to the same group. Heterosis for increased branch number, siliqua number and 1000-seed weight are the major contributors to the heterosis for yield (Chauhan et al. 2011). Besides this, in *B. juncea*, there have been many reports of *genetic male sterility* (Banga and Labana 1985). The majority of them are originated spontaneously and have a monogenic inheritance. Due to the lack of linkage between a morphological marker and GMS controlling gene, we could not distinguish male fertile plants before flower initiation. Therefore, the GMS system could not be economically feasible for the development of a commercial hybrid.

Out of various male sterility systems, *cytoplasmic male sterility* (CMS) is maternally inherited and most effective due to its easier maintenance. This male sterility system remains intact in a variety of environmental conditions. Rawat and Anand (1979) gave the first report on CMS in *B. juncea*. The most significant drawback of this system is the depletion of sterility at higher temperatures. Later on, in 1985, Raphanus-based CMS was introduced into *B. juncea* using *Ogura* system (Banga and Labana 1985) resulting in highly thermostable male sterile lines. However, it has two major limitations, viz., (1) chlorophyll deficiency at low temperature (<13 °C) and (2) lack of fertility restoration. Kirti et al. (1993) fixed the chlorosis from *Ogura*, *Mori* and *Oxy* system through protoplast fusion between chlorotic sterile and normal

green plant. Fertility restorers for most of the male sterile systems are not available. *Ogura*-based CMS line of *B. juncea* (RLM 198) has been obtained from male sterile *B. napus* through repetitive backcrossing and selection (Kirti et al. 1995). To overcome the problem of the male sterility/restoration system of *B. juncea*, transgenic lines having the Barnase/Barstar gene (both from *Bacillus amyloliquefacieus*) have been developed using spacer DNA and CaMV35S promoter (Jagannath et al. 2001).

6.6.2 Breeding Objectives

Traditional goals for crop improvement primarily focus on endeavours to produce, preserve and modify the biomass as per human and industrial requirements. In Asia, higher seed/achne yield is the major breeding goal, although breeding for oil quality trait and meal quality received greater attention in European countries. In India, improvement in seed yield of rapeseed-mustard is largely determined by the number of primary and secondary branches per plant, number of siliquae on the main shoot, number of seeds per siliqua and 1000-seed weight; however, head diameter, seed filling percent and 100-seed weight are the major yields contributing traits in sunflower. Short-duration and high-yielding cultivars/varieties are necessary for the relay, multiple and intercropping systems to work. Hybrid development and deployment are some of the utmost feasible alternatives for overcoming yield barriers. At present, there is almost 15–20% yield enhancement in the case of *Brassica*, while in sunflower two times higher achene yield is recorded than open-pollinated varieties (Yadava et al. 2012). The diversity of parental lines must also be improved to reach a higher degree of heterosis. Under the three-line system (i.e., A, B and R) of hybrid development programmes, diverse CMS sources may offer high-heterotic hybrids. The CMS/restorer system introgression should be carried out by marker-assisted backcross breeding to save time. The development of cultivars resistant to disease and insect pests is also an important goal of oilseeds breeder. To achieve the stable yield, source of resistance should be identified and their introgression in parental stocks against major diseases namely powdery and downy mildew, Alternaria blight and rust and insects like head borer, Bihar hairy caterpillar, green semilooper, cut worms, leafhoppers, mustard aphid and mustard sawfly for their exploitation in hybrid breeding programmes. It is important to explore techniques of plant biotechnology such as the gene pyramiding/QTLs for different biotic and abiotic stresses. Besides this, enhancing oil content and improving oil quality such as “double zero” in rapeseed-mustard and “tocopherols” in sunflower is also a major breeding objective of oilseeds crops.

6.7 Breeding Strategies

6.7.1 Exploitation of Genetic Resource

The exploitation of prevailing or newly created genetic variation is a matter of plant breeding. The plant breeder can use naturally existing genetic variation through germplasm collections, typically placed in germplasm banks. For the initiation of a breeding programme, assessment of germplasm based on a novel trait is necessary. Utilizing indigenous collections of *B. juncea*, several donor parents have been identified for various yield components traits, quality components in addition to different biotic and abiotic stresses (Kumar and Chauhan 2005). Though, the least variability existed for oil quality (Chauhan et al. 2011). Besides, exotic lines introduced from Canada (L4 and L6, white rust-resistant) and Australia (ZEM 1 and ZEM 2, low erucic acid) have been exploited in the development of rust-resistant (JM 1 and JM 2) and low erucic acid (LES 1-27, LET 17, LET 18 and Pusa Karishma) varieties of Indian mustard through hybridization. The crops wild relatives were being commonly used as a resistance source of diseases such as powdery mildew, downy mildew, *Sclerotinia* wilt, *Verticillium* wilt, charcoal rot and *Phoma* black stem in the sunflower breeding programme (Seiler 2010). Resistant to broomrape was identified in germplasm collections of the sunflower which broaden the genetic base for resistance to new races (Seiler and Jan 2014). Interspecific and intergeneric crosses may be attempted if such desirable traits are not present in the same gene pool but present in related species. *H. argophyllus* is commonly known as silver sunflower because of extreme hairiness and lathery leave which support them to adapt in drought conditions; however, *H. paradox* has been used as a genetic source for salinity tolerance. To upsurge genetic diversity, *H. annuus* (cultivated sunflower) were crossed with *H. argophyllus*, *H. annuus*, *H. petiolaris* and *H. debilis* (Sujatha et al. 2008). Based on the existence of the various genomes in the species, diploid annual and perennial species of *Helianthus* did not cross easily. However, interspecific hybridization is more effective for *Brassica* if a female parent is an amphidiploid that shares one similar genome with a parent of pollen (Zhang et al. 2006). The blackleg-resistant gene has been successfully transferred from *B. juncea* to *B. napus* due to apparent recombination among the A and C genome in *B. juncea* crosses (Sacristan and Gerdemann 1986). Similarly, the resistant gene for triazine was successfully introgressed from *B. napus* to *B. oleracea* (Ayotte et al. 1987). If those approaches fail, an alternative method is to create new variation in the absence of natural variation or where its transition to the cultivated species is impracticable. Generally, identification of tightly linked QTL, mapping of gene and gene transfer, despite regulatory hurdles, is the last possibility.

6.7.2 Breeding for Yield

The success of yields enhancement depends on the ability to substitute more desirable alleles in a genotype. Rapeseed-mustard includes several species that

have breeding mechanism varied from self-pollination to cross-pollination. Moreover, the sunflower is a highly cross-pollinated species. So, from a breeding point of view, these are quite interesting material. In the case of cross-pollinated species selection procedure ranging from mass selection to recurrent selection, whereas in self-pollinated species, desirable plants are chosen from germplasm collections, gene pools and segregating populations. The recurrent selection programmes are designed to upsurge the frequency of desirable alleles in the population. The end product can be utilized in two ways: (1) the improved population can be used for commercial cultivation if it is superior to the existing cultivated varieties and (2) it forms the reservoir of genotypes for developing inbred lines for heterosis breeding programme. The efficacy of recurrent selection to improve various traits has been well documented in sunflower, but a very limited attempt has been made to exploit this technique in *B. compestris*. In the self-pollinated *Brassicas*, the yield improvement in classical breeding programmes has been achieved by various types of pedigree methods. Handling segregating populations from bi-parental or multiple crosses as bulk populations, before extraction of pure lines, is a common practice. Visual selection in F_2 on a distinct plant basis for seed yield and oil content has proved inefficient. However, the influence of environment on yield component relationship modulates the response to selection (Thurling 1993). In India, several high-yielding varieties (namely RH 725, RH 749 and RH 761) have been released through the pedigree method. Chauhan and Singh (2004) argued that most of the *B. juncea* varieties were pure line and had a narrow genetic base due to a common ancestor, and a limited number of donors were exploited in breeding programmes. Transfer of desirable gene or new traits from one strain to another line or well-adapted cultivars has been achieved by backcross breeding methods. Dihaploid techniques have helped to shorten the breeding cycle in *B. napus* but not successful in *B. compestris*, due to inbreeding depression. Since most agronomic traits have a quantitative inheritance pattern, their identification, mapping and characterization would be essential for heterosis' ability to exploit favourable genes for achieving future production goals.

6.7.3 Hybrid Breeding

Hybrids are superior in yield potential and uniform maturity over synthetic and open-pollinated varieties. It is mainly because of the manifestation of heterosis. Mostly, heterosis is higher in crosses involving parents with diverse genetic background (Hladni et al. 2018). Sunflowers have been shown to have a high degree of heterosis in addition to *Brassicas*. Efforts to generate desirable hybrids are stimulated by the availability of a broad range of CSM sources. Of the various CMS source *PET 1*, *PET 2* (in sunflower) and *Mori*, *Ogura* (in Indian mustard) have been extensively used in the development of hybrids in India. Several sunflower hybrids, viz., KBSH-53, PSFH-569, DCS-107, RSFH-130 and CO-2 have also been released for commercial cultivation in different parts of India. PGSH-51, the first CMS-based gobhi sarson hybrid has recommended for cultivation in Punjab by the

Punjab Agricultural University, Ludhiana (India). Thereafter, two hybrids, viz., NRCHB-560 and DMH-1, have been identified for release by CVRC. Also, to overcome the limitation associated with CMS systems of *Brassicas*, a transgenic system of pollination control has been advocated. Of these, *barnase-barstar* appears to be very promising.

6.7.4 Breeding for Quality Traits

The concept of quality oil in vegetable oil is assessed by their fatty acid content and level of tocopherols, sterols, glucosinolates and other nutrients which rely on the ultimate use of the oil, i.e., food and industrial purpose. Hence, the key goal of oilseeds breeders in the development of improved quality oil producing lines and hybrids. Oils of rapeseed-mustard comprised of two major anti-nutritional factors namely erucic acid (C22:1) and a sulphur-containing compound, i.e., glucosinolate (Agnihotri et al. 2007). Intake of mustard oil with high erucic acid can cause myocardial fibrosis, lipidosis as well as enhanced blood cholesterol (Ackman et al. 1977). Most of the varieties of Indian mustard have erucic acid content. Glucosinolate is the major anti-nutritional factor found in seed meal of rapeseed-mustard which reduces the feed palatability and iodine uptake in animals, particularly in non-ruminants (Fenewick et al. 1983). A polish *B. napus* strain, "Bronowski", is the only known source of low glucosinolate ($\leq$12 μmol/g of the defatted meal) till 2015 (Gupta 2016). In India, breeding efforts have been carried out since 1970 for the development of canola type (erucic acid $<$2% in oil and glucosinolate $<$30 μmol/g in the defatted meal) cultivars, i.e., "00" type. Pusa Karishma (LES-39), the first low erucic acid variety of Indian mustard and GSC 5, the first "00" variety of *B. napus* (gobhi sarson) were released in 2005. Successively, five more, low erucic varieties have been released from IARI, New Delhi. Recently, PDZ-1 commonly known as Pusa Double Zero Mustard (PDZ-31), the first "00" variety of *B. juncea* developed by crossing Pusa Mustard-21 (LES-1-27) $\times$ NUDHYJ-3 following the pedigree method of selection was released in 2017 for Zone-II. Nowadays, breeding efforts have been carried out in the development of "00" varsities with improved oil content and enhanced vitamin levels.

On the other hand, achenes of sunflower have high oleic acid (C18:1) and tocopherols (a component of vitamin E) which makes it desirable for cooking (Škoric 2012). The biosynthesis of oleic and linoleic acids is significantly affected by genetic factors along with environmental factors such as high temperature during the seed filling period (Škoric 2012). Soldatov (1976) developed a mutant having a high level of oleic acid (80%) through induced mutation using dimethyl sulphate solution. Later on, it has also been extensively used as a source of the gene by sunflower breeders throughout the world. The inheritance of high oleic acid is controlled by one to three major genes with multiple alleles (Velasco et al. 2000); however, two non-allelic unlinked genes namely *Tph-1* (increased β-tocopherol) and *Tph-2* (increased γ-tocopherol) controlled the composition of tocopherol. Of the four derivatives of tocopherols, α-tocopherol (90%) showed maximum vitamin E activity

while γ-tocopherol is the best antioxidant (Fernández-Martínez et al. 2007). Velasco et al. (2004) identified few lines which have high γ-tocopherol, δ-tocopherol (>65%) and β-tocopherol (>75%). Moreover, combining genes for high oleic acid and derivatives tocopherols endorsed the development of hybrids with different oil quality types (Skoric et al. 2008).

6.8 Genomics-Assisted Breeding

For the past plant, breeding has always aided with the latest technologies in biology and genetics to accelerate crop improvement. Phenotypic selection has been employed for several centuries to harvest and increase the productivity of crop varieties. With the discovery of DNA and its structure and properties, the technology has advanced at a nucleic acid level in every aspect. In the same way, the identification of DNA markers such as RFLP, RAPD, SSR, DArt, SNP etc., has led to the foundation of marker-assisted breeding. With the advancement of DNA technologies, the generation of markers also evolved with their efficiency and efficacy in breeding and dissecting the genes. The linkage map gave the tool to identify and locate the genes through the markers which are linked and segregate together on the chromosome. The linkage mapping helps to identify the QTL for different traits of interest and to locate them on the chromosome and use them efficiently in the breeding programme (Perez-de-Castro et al. 2012). The bi-parental mapping has overshadowed by the association mapping in the present years due to its advantages of large-scale selection on the germplasm, which consider prehistoric hierarchy in the germplasm. The marker has aided several breeding studies in different crops, proving their efficiency for breeding for different traits of interest. With the advancement in sequencing technologies and reduction in sequencing, cost leads to several sequencing programmes in different crops and an increase in available information on different crops, which accelerates the precision in breeding strategies at the nucleotide level (Kole et al. 2015; Bevan et al. 2017). These new technologies are capable of comprehensive large-scale gene studies and at a high pace parallelly these can allow studying several genes at a time.

Genomic-assisted breeding has emerged as the advanced breeding strategy with the advancement in the DNA technologies such as makers, DNA sequencing and reduced cost of these technologies. Genomics-assisted breeding is an integrated approach in which genomic information is applied to the breeding to improve the genotypes for different traits (Bevan et al. 2017). The selection has been employed at the genomic level in addition to phenotype and the traits were studied throughout the germplasm, and variation for the specific traits were identified and are employed for improvement of the candidate genotypes (Kole et al. 2015). The sequencing has given insight into the genome of the several crop plants in the same way the sequencing of the genome of rapeseed-mustard and sunflower will provide the information about the genome which can help to identify the variation present in the germplasm for the specific nutritional quality or yield traits. The identified variation can be integrated into the genotypes which are lacking in the quality of

oil but having the yield potential (Perez-de-Castro et al. 2012). The genomics-assisted breeding approaches include marker-assisted selection, genotyping by sequencing (GBS), association mapping and genomic selection which are discussed in the context of rapeseed and sunflower.

6.8.1 Marker-Assisted Selection

Marker-assisted selection (MAS) evolved with the identification of different molecular markers and played a very significant role in accelerating the traditional breeding programmes. MAS has made a selection at the genomic level with a specific gene combination of desirable traits possible. The linkage mapping and QTL mapping has proved very beneficial in several crops and can be employed to enhance the oil quality in rapeseed-mustard and sunflower. The linkage map gives an insight into DNA sequences concerning these important traits which can help to plant breeder improve the present genotypes (Rauf et al. 2020). Several QTL responsible for the oil quality have been identified and mapped which can be further used for improvement in other genotypes which need to be improved (Table 6.3). The genetic map has been constructed using different markers such as RFLP, AFLP, SSR and DArt in both crops and can be applied to identify other important QTL for oil quality and yield traits (Chen et al. 2010; Pushpa et al. 2015). The advancement in sequencing technology and increased available data on the genomics of species helps to carry out a rigorous examination to identify the candidate genes responsible for oil quality. The available sequence data help to identify the SNPs available for traits and correlate with the quality of oil and further employed in the improvement of different genotypes for the quality.

Sunflower was also studied for marker association with different traits such as yield, head diameter, and hull content. Also, to yield traits, oil composition traits like

Table 6.3 Summary of linkage map and QTL identified for different oil quality traits in sunflower and rapeseed

Sr. No.	Trait	Marker	Reference
Rapeseed-mustard			
1	Oil content and seed yield	SSR and SRAPs	Chen et al. (2010)
2	Erucic acid content	AFLP	Saini et al. (2016)
3	Oil composition	AFLP	Singh et al. (2013)
4	Oil content	SSR and SRAPs	Huang et al. (2016)
5	Glucosinolate content	SSR	Pushpa et al. (2015)
Sunflower			
1	SSR markers	SSR	Tang et al. (2002)
2	Fatty acid composition	SSR	Pérez-Vich et al. (2004)
3	Oil content	SSR and SRAPs	Wang et al. (2013)
4	Oleic acid content	SLAFseq	Zhou et al. (2018)
5	β-Tocopherol	SSR	Vera-Ruiz et al. (2005)

oleic acid content, omega acid content, tocopherol content, linoleic acid content and linolenic acid content linkage maps were constructed with different types of markers such as SSR, RFLP and AFLP (Table 6.3). The investigated linkage map has given the interrelationship between marker, and traits of interest as well as QTL were mapped for the same. Primer sets such as NI-3F/N2-IR serve to identify defective FAD2-ID gene which is responsible for high oleic acid in sunflower seed (Rauf et al. 2020). A fine-genetic map of 5019 SNP via RAD sequencing was constructed (Talukder et al. 2014). The oil properties of most of them are quantitative, whereas Oleic Acid Content (OAC) could be considered as a semi-qualitative trait due to its dependency on the genetic background of the receiver in addition to the environment (Ferfuia et al. 2015). Recently, Premnath et al. (2016) identified two additional QTL for OAC on LG8 and LG9. OAC is the choice of study for the identification of different linked markers due to the importance of oleic acid content in the seeds of sunflower (Bilgen 2016). F4-R-1 is most effective in MAS for OAC (Dimitrijević et al. 2017). Three SSR, viz., ORS-1093, ORS-222, and ORS-598, found tightly linked with Tph1 suggesting a role in β-tocopherol accumulation in the seed. Rauf et al. (2020) successfully parted the lines having low β-tocopherol from high β-tocopherol using ORS716. Recently developed fine linkage maps in sunflower triggered the sunflower breeding at the molecular level and allow the identification of the genes responsible for these traits. These linkage maps help to link the markers with different traits, which helps in achieving precision in sunflower breeding for these traits.

In rapeseed, seed yield was directly correlated with test weight, the number of pods/plant and the number of seeds/pod (Cai et al. 2014). Cai et al. (2014) also suggested the indirect role of biomass yield, plant height, first effective branch height, first effective branch number, length of the main inflorescence and the number of pods on the main inflorescence in seed yield. Several attempts were made for the identification of QTL associated with yield and yield-related traits in rapeseed (Chen et al. 2010; Saini et al. 2016; Huang et al. 2016). The nutritional quality depends on the fatty acid composition of the seed which includes oleic acid, erucic acid, glucosinolate, linoleic acid and linoleic acid. The oleic acid content in the seed decides the oil quality, and several markers linked to oleic acid content were identified (Singh et al. 2013), percent of glucosinolate in oil plays a key role in the seed oil composition, several markers including AFLP, RFLP, SSR and other were mapped on the rapeseed-mustard genome (Pushpa et al. 2015). Erucic acid is an unwanted component of the oil, its increased percentage in seed oil is harmful to human and cattle as well for consumption. Markers that are linked with erucic acid content in the rapeseed were mapped (Saini et al. 2016), use of the makers in breeding for selection can help to reduce the genotypes which have higher erucic acid. Linoleic acid is another important component of the seed oil, its quantity is the other parameter for the oil quality and the increased linoleic acid content is favoured over low linoleic acid content. The linkage maps were constructed for the linoleic acid content of rapeseed-mustard (Huang et al. 2016). The identified linkage maps for different traits have significant use in plant breeding for the nutritional quality of the seed oil in rapeseed-mustard. These can be employed for the selection of the

traits in the population with the desirable composition of the oil quality. QTL mapping with the help of different DNA markers like RAPD, AFLP, SSR and SNP discloses that the oleic acid and linolenic acid content linked to loci present on A04 and C10 chromosome of *B. napus* (Yang et al. 2012). The identified allele-specific DNA markers play a key role in the selection of homozygous genotypes for the content of oleic acid in rapeseed breeding.

The traditional bi-parental mapping has a weakness for identifying QTL with small effect, and methods applied for the identification of QTL may also hinder crop improvement. The bi-parental mapping in addition to these also have few limitations: (1) bi-parental population do not possess the same level of allelic diversity throughout the breeding programme which makes them unsuitable as representative of the populations, (2) developing population and its maintenance become costly affair, (3) identified QTL are needed to be validated which require further efforts and (4) QTL with small effects entirely missed due to stringent significant threshold. With the availability of the NGS, the bi-parental mapping slowly replaced association mapping which is more cost-effective as well as precise for QTL mapping and trait investigations (Table 6.3).

6.8.2 Genotyping by Sequencing

The genome-wide association studies require many molecular markers distributed throughout the genome of the plant. The GBS become a tool to identify new molecular marker which accelerates the breeding through the use of such markers present throughout the genome (Celik et al. 2016). With a gradual reduction in the sequencing cost, GBS has triggered genomics breeding in the present era. GBS is an innovative strategy where SNPs discovered and genotyped using NGS in different crop genome or population (Mondon et al. 2018). GBS helps in constructing the SNP array for the crops using NGS which widely employed in genome-wide studies which allow the breeder to characterize the available germplasm of any crops in a very short time to identify the available allele diversity. In GBS, the genomic DNA is digested using restriction enzymes, the digested fragments ligated with barcode adaptor and multiplex libraries generated which subjected to NGS further. The GBS can produce more than thousands of molecular markers. The GBS is flexible to species, populations and research objective which makes it the ideal tool for plant genomics studies. GBS stands as an ideal MAS tool due to its cost-effectiveness, extensively employed for genome-wide association study (GWAS), genomic diversity study, genetic linkage analysis, molecular marker discovery and genomic selection (Fig. 6.3).

Pioneered use of GBS for large-scale SNP detection in sunflower was carried by Celik et al. (2016); they produced a linkage map of 817 SNP-markers covering all 17 LG by analysing an F_2 obtained from the cross RHA 436-H08 M1. Mondon et al. (2018) used GBS using SNP for genotyping of 182 samples of sunflower from 11 sites in Argentina to find the actual population source of the Argentinian samples and to find admixture. However, they surprisingly come across two distinguishing

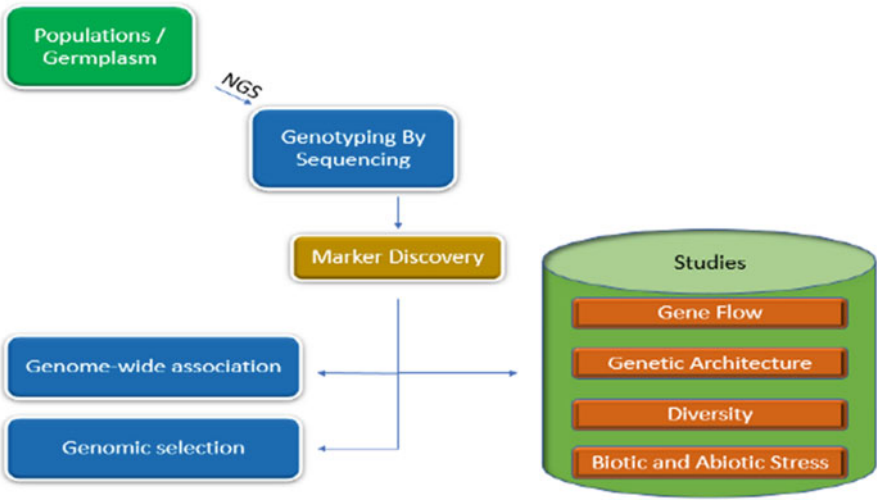

Fig. 6.3 Applications of genotyping by sequencing (GBS)

forms of *H. petiolaris* in Argentina, one from *H. petiolaris* subsp. *petiolaris* as expected, but the other from an unknown source. Ma et al. (2017) conducted a GBS study which suggested that Pl_{20} gene located on linkage group 8 of the sunflower genome belongs to wild *Helianthus argophyllus* which can be used for marker-assisted selection of resistance gene. Ma et al. (2018) used GBS approach to identify new rust resistance gene R_{15} in cultivated sunflower HA-R8. SNP markers closely linked to R_{15} were identified, facilitating marker-assisted selection of resistance genes. Baute et al. (2016) conducted genome-wide GBS to study wild *Helianthus* diversity, genetic structure and interspecies gene flow. The GBS has widely adapted for study gene flow, genetic architecture, diversity or disease resistance in sunflower which makes it a tool for future use in the study of genes responsible for yield and its components in addition to oil quality.

In rapeseed, several genome-wide studies have conducted to study the genetic architecture of different stresses, diversity and disease resistance (Lee et al. 2016; Fu et al. 2020; Ding et al. 2020). The scope of acceleration of the GBS in rapeseed-mustard due to *Brassica* 60K Illumina Infinium™ array, which can genotype about 52, 157 SNPs of *B. napus*, opens the door to study the gene architecture for yield and its components and oil quality responsible alleles (Mason et al. 2017). A genome-wide study conducted on Canadian canola/rapeseed to identify resistance loci for blackleg disease caused by fungus *Leptosphaeria maculans* (*Lm*) using GBS. They identified 32 and 13 SNPs from the Canadian and Chinese accessions, respectively, which are tightly associated with blackleg resistance with p values $<1 \times 10^{-4}$ (Fu et al. 2020). In another study, waterlogging tolerance QTL were identified using GBS in rapeseed (Ding et al. 2020). Lee et al. (2016) characterize the 79 genotypes of *B. napus* using GBS in association with the neighbour-joining clustering method. Zhai et al. (2020) identified 148 SNP loci significantly associated

with fatty acid content traits and 20 orthologs of the candidate genes regulating the fatty acid biosynthesis out of 201,187 SNP markers developed from SLAFseq (specific locus amplified fragment sequencing). In the future, GBS has given the pace to genomic-aided breeding in crop species and will help to conduct GS on new genotypes with or without prior molecular tools available and to find the population structure without known diversity.

Association Mapping The association mapping approach has picked up the pace in crop improvement before it was widely adopted in human disease to identify responsible genes. Recently, association mapping (AM) approach is the choice of genetic study to identify QTL in several major crops like maize, wheat, barley, rice and beans (Nambeesan et al. 2015). AM has advantages over bi-parental mapping as it uses the variation present throughout the germplasm or natural population which helps to construct the fine genetic map with the precise marker-trait association. AM involves establishing marker-trait association using several thousand markers throughout the genome which used to find out the allelic diversity for different traits present in germplasm or natural population; henceforth, it makes more beneficial than traditional bi-parental mapping. The identified allelic diversity can be used to improve the genotypes further as per need. In general, AM has been two approaches, viz., GWAS and candidate gene (CG). The AM has greater power over the bi-parental mapping as loci form similar parents contributing to the traits that are unable to map. AM considers the LD event throughout the germplasm which helps to map the allele at a higher resolution to associated with traits (Nambeesan et al. 2015). Previously the bi-parental mapping was widely applied in many crop species, as the whole genome sequences have become available recently. The high-throughput marker system provided the whole genome information which made the recent advances possible such as GWAS, QTLSeq and genome selection.

In sunflower, LD decays rapidly which affects the resolution level of QTL for genes of interest; henceforth, the population structure plays a key role in establishing correct marker-trait association (Kolkman et al. 2007). Mandel et al. (2013) pioneered the GWAS in sunflower whereas most of the studies conducted were candidate gene-based (Cadic et al. 2013; Nambeesan et al. 2015). GWAS was performed on a population of 271 lines for flowering time, branching and heterotic groups using 5359 SNP marker from the Illumina Infinium Beadchip (Mandel et al. 2013). The variability for LD throughout the genome was observed but the marker-trait association was significant suggesting that initial domestication and variety selection for the disease might be the cause of variable LD profile throughout the genome. The candidate gene-based approaches have been well documented in sunflower for branching (Nambeesan et al. 2015) and flowering time (Cadic et al. 2013). The use of CG approach for flowering time and branching is well notified but its implication to trace quality traits is a matter of future study in sunflower. In recent years, AM has proved a very efficient breeding approach to study linkage mapping especially for quantitative traits in different crop species, in the same manner, it is needed to explore sunflower in future.

In rapeseed, several attempts were made to study linkage disequilibrium; however, the homologous nature of subgenomes and limitation of earlier technologies was not enough to develop the high-density molecular marker. With the development of SNP-array in rapeseed, the availability of higher density marker has increased at a genome-wide level. About 4300 SNP markers were developed from 313 inbreeds of *B. napus* (Delourme et al. 2013). A high-density 26841 SNP was developed from 472 accessions of *B. napus* (Li et al. 2014a). A GWAS reported a novel locus for seed oil content on chromosome A05 of *B. napus* (Liu et al. 2013). The AM has two prerequisites, the extent of LD and population or family structure in diverse genetic panel (Sorkheh et al. 2008). A comprehensive study revealed a high-density SNP-based genetic map in four different segregating DH populations using 5764 SNP markers in oilseed rape. GWAS can be performed with a low number of SNP but evenly spaced in the genome of oilseed rape (Delourme et al. 2013). Even in rapeseed, the use of GWAS and CG has been limited, the other quality traits investigation in the area of future research in this crop.

AM has disclosed significant marker-trait association in several important crops such as maize, wheat, rice and barley which is well documented. The GWAS has been used to identify the marker associated with morphological traits (Honsdorf et al. 2010) and seed quality traits (Li et al. 2014b). CG is used to trace the gene polymorphism for glucosinolate biosynthesis (Hasan et al. 2008), tocopherol content and composition gene (Fritsche et al. 2012). Two types of these AM, viz., GWAS and CG, have separate advantages but for quantitative characters, GWAS has the potential to find out the allelic diversity, and in CG, it can help to identify the allelic diversity for the single gene itself (Honsdorf et al. 2010). With the advancement in NGS platforms, the cost and time of sequencing have reduced substantially which increases the number of available markers for GWAS. Both these AM approaches have been applied in both crops which are considered of importance here.

Genomics Selection (GS) Genomic selection (GS) is widely used in crops nowadays which explore available molecular markers to predict genomic-estimated breeding values based on new marker-based models (Bhat et al. 2016; Xu et al. 2020). GS approach comprises two populations, viz., training population (reference population) and breeding population (testing population). Training population is used to predict the genomic-estimated breeding values for testing population based on a marker-based statistical model developed using phenotypic and genotypic information of the training population (Xu et al. 2020). GS for self- and cross-pollinated crops follows the different skim (Fig. 6.4) as the training population and breeding population varies. The GS has two advantages over traditional MAS as there is no need to unearth the QTL related to target traits, and phenotyping for a breeding population can be exempted which reduce the time for GS. GS provides the opportunities to enhance the genetic gain of multigenic traits per unit time and cost. The high-throughput techniques of the genome-wide association have become cheaper and several new markers have been developed in a large population with or without the reference genome sequence is the most important consideration for GS implementation in crop species (Bhat et al. 2016). The next-generation

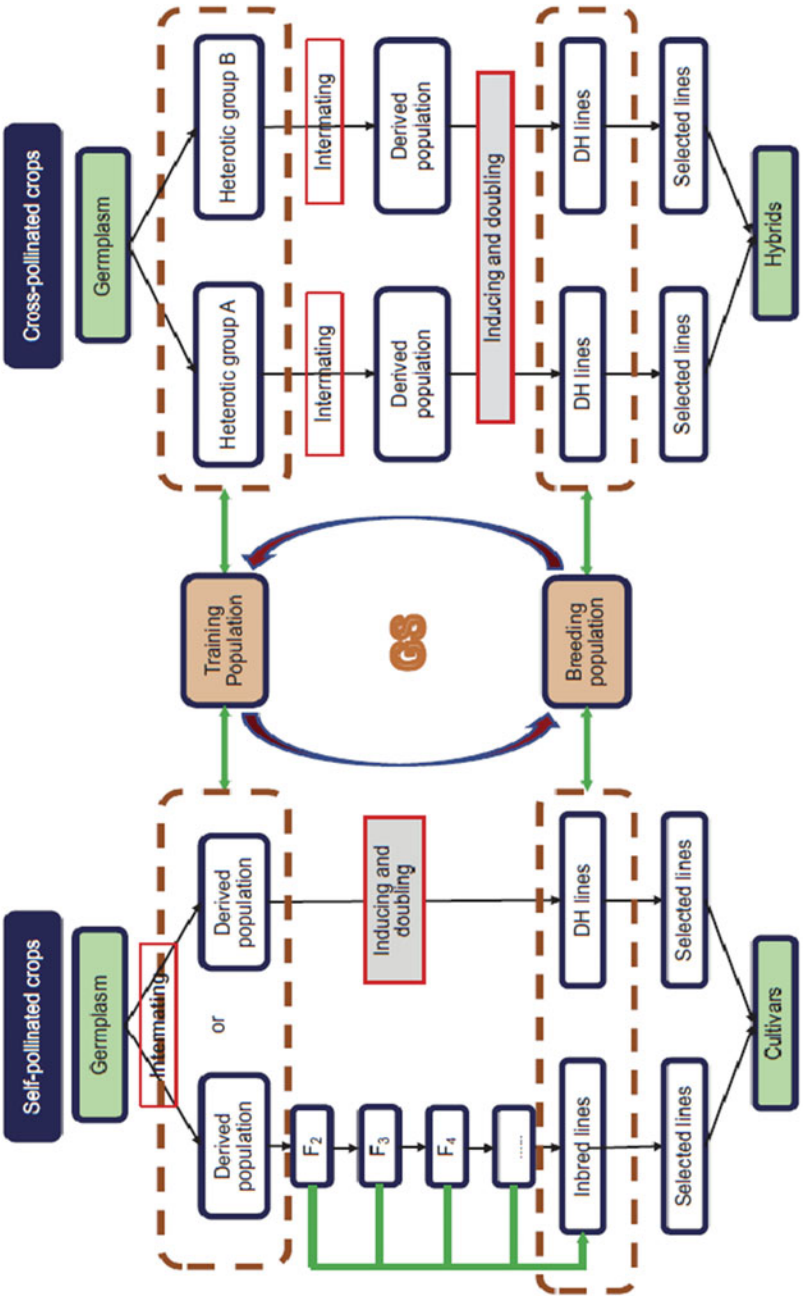

Fig. 6.4 A flow chart representing the GS for self-pollinated (left) and cross-pollinated (right) crops (Xu et al. 2020)

sequencing has provided an SNP genotyping platform through GBS; hence, the availability of the SNP markers for genome-wide studies has increased, so the precision in the marker-trait relation has increased. Availability of such high precision molecular marker and its platform made the GS routine work for crop improvement in both model and non-model crop species. The GBS using NGS has increased the precision in the prediction of the genomic-estimated breeding values (Xu et al. 2020). The GS must combine with high-throughput phenotyping to acquire maximum genetic gain from complex traits. The gradual decrease in sequencing cost has made sequencing of complete genome possible for all important crops which will accelerate the genomic selection in present and future also.

The genomic selection has employed widely in a variety of major crops due to the generation of vigorous SNP markers through sequencing data for the SNP array construction and development of a suitable statistical model for prediction (Würschum et al. 2014). Owing to its advantages, the GS is getting the attention of breeders in crops like rapeseed-mustard and sunflower. GS has been used to study the performance of hybrid in sunflower (Mangin et al. 2017). GS can predict the performance of the hybrid for oil content in sunflower using GCA-based model for prediction from genotypic and phenotypic data which strongly suggest that GS increases the breeding efficiency, although the parents are not phenotyped when compared to classical GCA modelling (Mangin et al. 2017). Würschum et al. (2014) reported the potential of the GS for complex agronomic traits in rapeseed breeding. The traits which are quantitative and regulated by several QTL, the GS allows the breeder to capture the effects of the small-effect QTL in addition to large-effect QTL. A high-density SNP array is the basis of GS in many crops but crops with a narrow gene pool with a low-density SNP array can be employed for better prediction. Even a few hundreds of low-density marker set can enable prediction with precision in breeding population with strong LD in Asian rapeseed. GS becomes a choice of breeder due to the efficiency of NGS which made high-throughput genotyping more easy and economical; henceforth, it is widely adopted by breeders in many crop species.

6.9 Conclusion

The modern-day genomic tool has given a plant breeder the power to exploit the available plant genetic resources which could lead to improvements in rapeseed-mustard and sunflower concerning yield-related traits and oil quality and whatnot. The available allelic diversity present in the natural germplasm can be used to improve the traits. The GBS helps to develop new markers which can be further used for genome-wide studies and genomic selection. The genome-wide studies add the available allelic diversity for the traits with their position in the genome. Genomic selection can help to predict the genetic values of the population which enhances the breeding approach and its advantages. These will give the inside of genetic architecture of the trait and available positive and negative diverse allele.

Such variation can be incorporate in the genotypes further to make the next-generation crops with better climate-resilient, better yield and better oil quality.

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