# **Chapter 15 Advances in Classical and Molecular Breeding in Sesame (***Sesamum indicum* **L.)**



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**Abstract** Sesame (*Sesamum indicum* L.) is an important but underexploited oilseed crop of tropical and subtropical region having potential to sustain agriculture under changing climatic conditions. Sesame oils have high nutritional and industrial values due to its desirable fatty acid compositions and high amount of antioxidant components, viz., sesamin and sesamolin. Despite this, still sesame is not grown on large acreage due to unavailability of high-yielding cultivars with inbuilt resistance to various biotic and abiotic stresses. Therefore, serious efforts are necessary to develop cultivars having high adaptive potential to the diverse climatic situations along with high yield potential. Classical plant breeding methods impart considerable improvement in sesame, but still a huge gap is left between realized and actual yield potential of sesame. Therefore, efforts should be made toward modern molecular techniques like marker-assisted plant breeding and omics and modern bioinformatics tools to develop climate adaptive, high yield potential along with excellent oil quality cultivars in sesame.

**Keywords** Sesame · *Sesamum indicum* L. · Nutritional values · Antioxidant · Resistance · Yield potential · Cultivars

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#### **15.1 Introduction**

Many developing countries of the world are facing the problem of malnutrition as major part of population is vegetarian and the availability of good-quality nutritional food is limited. Oilseeds are next to cereals in importance which contain superior quality protein, essential fatty acids, vitamins, and minerals. In India, usually nine oilseeds, i.e., soybean, sesame, castor, niger, rapeseed-mustard, groundnut, safflower, sunflower, and linseed, are cultivated. Out of which, sesame  $(2n = 26)$  is of great importance and widely known as "Queen of Oilseeds" due to high resistance to rancidity and oxidation of sesame oil (Sarwar et al. [2013\)](#page-34-0), and for the same reason, sesame seeds are also known as the "seeds of immortality" (Bedigian and Harlan [1986](#page-28-0)). Tocopherol confers the resistance to oxidative deterioration in sesame oil, bioavailability of which is in turn enhanced by sesamol (Wu [2007](#page-37-0)).

The oil content is abundant in seeds (32.8–62.7%) (Uzun et al. [2008;](#page-36-0) Couch et al. [2017\)](#page-29-0). Sesame oil contains signifcantly high amount of unsaturated essential fatty acids [linoleic acid (37–47%), oleic acid (35–43%)], while the saturated fatty acid content [stearic acid (5–10%), palmitic acid (8–11%), behenic acid, and arachidic acid] is usually low. The seeds also contain 4.3–20.5% carbohydrates, 4.2–6.9% ash,  $2.7-6.7\%$  fiber content, and  $14.1-29.5\%$  proteins as well as vitamin E, minerals, lignans (sesamolin and sesamin), and tocopherols (Fukuda et al. [1985](#page-30-0); Kamal-Eldin et al. [1992](#page-31-0); Ashri [1998;](#page-28-1) Unal and Yalcin [2008](#page-36-1); Hassan [2012](#page-30-1), Couch et al. [2017\)](#page-29-0). In addition, sesame seeds are also rich in a variety of minerals including potassium (K), magnesium (Mg), phosphorous (P), calcium (Ca), and sodium (Na) (Nzikou et al. [2009;](#page-33-0) Couch et al. [2017\)](#page-29-0). Sesame oil is often considered as a good protein source because of its balanced amino acid composition, particularly methionine and tryptophan, and is therefore benefcial to patients suffering from kwashiorkor (Pathak et al. [2014a,](#page-33-1) [b\)](#page-33-2). The various chemical compositions of sesame seed are given in Table [15.1](#page-2-0).

Sesame is being cultivated from ancient times and its oil was used for spiritual purposes (Weiss [1983\)](#page-37-1). It belongs to division Phanerogams, class Dicotyledonae, subclass Gamopetalae, series Bicarpellatae, order Persoriales, family Pedaliaceae, and genus *Sesamum*. It is also named as gingelly, beniseed, sim-sim, and til (Shah [2013\)](#page-35-0). It is grown throughout the tropical and subtropical region from 25°N to 25°S. The origin of sesame can be traced back to Africa as several wild relatives of sesame exist in Africa (Sani et al. [2014\)](#page-34-1). India as well as China, Central Asia, Near East, and Abysinia has been recognized as sesame diversity centers in classical studies which is not at all surprising considering the genotypic variability of sesame in India (Zeven and Zhukovsky [1975](#page-38-0); Hawkes [1983;](#page-31-1) Laurentin and Karlovsky [2006\)](#page-32-0).

Sesame is mostly self-pollinated short-day plant, with the probability of 5–68% of cross-pollination (Langham [1944](#page-32-1); Ashiri [2007](#page-28-2)). Generally, its fower opens at morning and withers after 4–6 h of anthesis. While stigma receptivity lasts for 14–24 h after fower opening (Abdel et al. [1976;](#page-27-0) Yermanos [1980](#page-38-1)) and varies with genotype (Langham [2007\)](#page-32-2). Anther dehisce just after opening of fower and pollen grains remains viable for 24 h after dehiscence at 24–27 °C (Yermanos [1980](#page-38-1)). Plants

Constituents	Concentration (per 100 g dry seeds)
Fat	49.7 g
Saturated fatty acid	7 g
Monounsaturated fatty acid	18.8 <sub>g</sub>
Polyunsaturated fatty acid	21.8 <sub>g</sub>
Energy	573 kcal (2400 kJ)
Carbohydrate	23.4 g
Protein	17.7 g
Dietary fiber	11 <sub>g</sub>
Moisture	4.7 g
Sugar	0.3 g
Minerals	
Potassium	468 mg
Zinc	7.8 mg
Magnesium	351 mg
Sodium	$11 \text{ mg}$
Phosphorous	629 mg
<b>Iron</b>	14.6 mg
Calcium	975 mg
<b>Vitamins</b>	
Vitamin A	9 IU
Thiamine (B1)	$0.79$ mg
Riboflavin (B2)	$0.25$ mg
Niacin $(B3)$	4.52 mg
Pyridoxine (B6)	$0.79$ mg
Folate (B9)	$97 \mu g$
Vitamin E	$0.25$ mg

<span id="page-2-0"></span>**Table 15.1** Sesame seed chemical compositions

Source: <https://ndb.nal.usda.gov/ndb/foods/show/3620>

are erect to semi-erect decided by branching types lanceolate to ovate, having pointed leaves with entire or serrate margins and round stem. Flowers are axillary, solitary, short pedicellate and zygomorphic with fve fused sepals. Corolla is pendulous, campanulate, and tubular, and one petal is longer than others. Each flower has fve (one sterile and four fertile) didynamous and epipetalous stamens with dorsifxed flaments. The gynoecium is multicarpellary with bifd stigma, long style, and a superior ovary. Seeds are very small  $(4 \times 2 \text{ mm with } 1\text{-mm-thick hilum})$ , pearl shaped, ovate, small, and slightly fattened. Indeterminate growth habit is observed in most of the varieties showing continuous growth of new leaves, regular fowering, and formation of capsules, and as long as the environmental conditions are favorable though, distinct varieties and strains differ considerably in size, growth, form, flower color, seed size, and seed color.

Sesame (*Sesamum indicum* L.) is a valuable *Kharif* oilseed crop, mostly grown in light sandy soil as rainfed crop in the arid and semiarid tropics of southern parts of Haryana, Rajasthan, Madhya Pradesh, Gujarat, and Uttar Pradesh. The growth period could be 70–150 days based on the variety and the environmental conditions (Ashri [1998\)](#page-28-1). Sesame seed requires temperature around 20 °C for germination, and more than 23 °C favors good growth and high yields. The crop rarely requires redundant irrigation factually due to its high susceptibility toward the moisture stress. Sesame production is progressively endorsed because of relatively simple cultivation as it can be grown on various kinds of soil, tolerance to high temperatures, less labour-intensives and fexibility to fts in crop rotation schemes (Langham [2007;](#page-32-2) Dossa et al. [2017a](#page-29-1), [b\)](#page-29-2). The overall sesame production is below the expectation worldwide mostly due to absence of effective pest control methods, occurrence of biotic and abiotic stress, and notably lack of a pertinent breeding program (Duhoon [2004;](#page-30-2) Ram et al. [2006\)](#page-34-2). The statistics have shown a decline in production of sesame despite the fact that it is a highly self-suffcient crop (Anthony et al. [2015;](#page-27-1) FAOSTAT [2015\)](#page-30-3). Declining production and low yield are also of great concern because of its economic importance and high medicinal value. The commercial varieties of sesame are susceptible to biotic and abiotic stress factors including photosensitivity and experience early senescence (Rao et al. [2002](#page-34-3)). Various studies have elucidated the presence of biotic and abiotic stress-resistant genes in wild species of sesame (Joshi [1961;](#page-31-2) Weiss [1971;](#page-37-2) Brar and Ahuja [1979;](#page-29-3) Kolte [1985](#page-31-3)).

Late maturing cultivars are proclaimed to have high oil contents than the early maturing counterparts. Amount of oil content also varies according to the location of capsules on the same plant such as the seeds on the main stem from the basal capsules carry more oil than those placed toward the apex and on side branches (Mosjidis and Yermanos [1985\)](#page-33-3). Similarly, brown and white seeded cultivars often have high oil content than black ones, indicating a probable genetic linkage between seed coat color and oil content. Black seed coats are usually thicker than brown- and white-colored coats.

Global sesame production and area coverage were 5,531,948 tons and 9,983,165 hectare, respectively, in 2017 (FAOSTAT [2019\)](#page-30-4). Prime sesame producing countries are Tanzania, India, Nigeria, China, and Ethiopia (FAOSTAT [2019\)](#page-30-4). Worldwide sesame seed consumption in 2018 was reported to be USD 6559.0 million and is approximated to extend up to USD 7244.9 million by 2024, at 1.7% CAGR (compound annual growth rate). India and China are among the top producers of sesame globally, and average yield is highest in China (1223 kg/ha) trailed by Nigeria (729 kg/ha) and Tanzania (720 kg/ha) (FAOSTAT [2020](#page-30-5)). India was the largest exporter of sesame seeds with annual production recorded 850,000 tons, and total area under cultivation was 1951,000 hectares in 2015–2016. West Bengal was top producer trailed by Madhya Pradesh, Rajasthan, and Uttar Pradesh (Anonymous 2016).

Sesame seeds are used to produce high-quality edible oil also used in fsh canning and the production of butter substitutes like margarine (Uzo [1998](#page-36-2)). Varieties of steroids present in sesame oil increase the insecticide potency of pyrethroids which are used as commercial and household insecticide. Seeds as well as the leaves of sesame are used to prepare food delicacies such as variety of porridges and are also consumed with fried groundnut. Consumption of sesame in the form of porridge is well known from the tribal populations which are eaten by the Fulani tribe mixed with the millet and by Tiv people with the boiled yam (Uzo [1998\)](#page-36-2). Dried sesame leaves after pulverization are used in soups as it is a rich source of minerals and

proteins. The seeds are often consumed as a candy and as snacks mixed with sweetener and groundnuts or with partially cooked cowpea. The seeds are used in bread and confectionery industry as well. Sesame four is more compatible with wheat four than other oilseeds for producing bread with good loaf volume and crumb texture. The brown and black varieties are fermented to produce local brew beer (Burukutu). Sesame oil is used as a substitute of other solvents in paints, liniments, ointments, and cosmetics (Mbaebie et al. [2010\)](#page-32-3).

Commercially sesame semidrying oil is used as an alternate of olive oil, corn oil, and cotton seed oil for restraining bacterial infection on umbilical cord of infants. Studies have also revealed the anticancer and neuroprotective properties of sesame oil to cure hypoxia or brain damage (Hibasami et al. [2000;](#page-31-4) Miyahara et al. [2001;](#page-33-4) Cheng et al. [2006](#page-29-4)). Antioxidants have been proclaimed to possess health promoting effects like reducing hypertension and cholesterol levels in humans (Noguchi et al. [2001;](#page-33-5) Sankar et al. [2005\)](#page-34-4). The cake left after oil extraction is rich in phosphorus and calcium and thus used as animal feed. Other uses of sesame seed and oil for medical purposes include the treatment of sores, ulcers, diarrhea, and dysentery. Historically, it is used for customary marriage rites of some tribes in Nigeria. The dried stems of plants are tied together to be made broom, while the ashes of dried shrub are used for the production of black soap. The plant residue is also plowed into the soil to enrich it. The dehulled press cake of sesame is used for the treatment of malnutrition in children because of the presence of globulin as a principal protein in it (Abdullahi [1998](#page-27-2)).

#### **15.2 Classical Breeding in Sesame**

Sesame is often described as the oldest oilseed crop cultivated in ancient times both for its edible seed and mainly for its oil. Sesame oil is esteemed as a best vegetable oil because of its high nutritional quality and stability to oxidative rancidity (Biswas et al. [2018\)](#page-28-3). Despite this, sesame is mainly grown under marginal and submarginal land and also known as poor's farmer crop. Sesame is an underexploited oilseed crop, yet still it holds tremendous potential for enhanced food value (Manjeet et al. [2020\)](#page-32-4). Sesame is one such crop that justifes imperative and instant consideration of the scientifc communal. The sesame plant type is not well adapted to current farming schemes since of its unstipulated growth habit causing varying ripening of capsules, their varying susceptibility to different stresses, and unavailability of non-shattering varieties suited for mechanical harvest (Ashri [1998\)](#page-28-1). The important breeding objectives of sesame are high yield potential along with high oil content; yield stability through tolerance against different abiotic stresses, viz., drought, salinity, heavy metal stress, water logging, and temperature extremities; resistance to biotic stresses like phyllody and charcoal rot, *Alternaria* blight, leaf curl virus, and several insect pests; and good confectionary quality to meet industrial demands (Ashri [1998;](#page-28-1) Islam et al. [2016;](#page-31-5) Sinha et al. [2020\)](#page-35-1). India is a center of origin and diversity for sesame, and several of important germplasm contain economically important traits that are largely underexplored for use in sesame improvement

programs (Bisht et al. [1998](#page-28-4); Bedigian [2003\)](#page-28-5). However, a systematic screening of these germplasm or their characterizations is still totally lacking. Hence, germplasm evaluation and pre-breeding are still a key approach for sesame improvement. The important breeding objectives in sesame will be discussed in the following heads.

#### *15.2.1 High Seed Yield*

In spite of being a great source of very healthy edible oil in terms of presence of huge amounts of polyunsaturated fatty acids and several antioxidants, sesame is cultivated on very small acreage due to availability of poor yielding dehiscent varieties with low harvest index. Lack of improved varieties is the major reasons behind low seed yield in sesame (Pathak et al. [2014a](#page-33-1), [b](#page-33-2)). So, productivity enhancement by accumulating desirable alleles into single genetic background is the prime objective for sesame breeders. Sesame seed yield is depending upon its several component traits like number of primary and secondary branches per plant, number of capsules per plant and capsule length, seed weight, and number of seeds per capsule (Teklu et al. [2014;](#page-36-3) Mustafa et al. [2015;](#page-33-6) Ramazani [2016;](#page-34-5) Shakeri et al. [2016](#page-35-2);). As yield is a complex trait with low heritability, therefore, indirect selection for yield through its abovementioned attributing traits may enhance productivity in sesame. Another very important yield attributing trait in sesame is harvest index, which directly related with high yield in sesame (Day et al. [2002](#page-29-5)). However, to improve harvest index in sesame, plant type should be of medium plant height with high density capsule bearing starting from 15 to 20 cm above the ground (Tripathy et al. [2019\)](#page-36-4).

#### *15.2.2 Early Maturity and Short Plant Stature*

Early maturity and short plant stature are the two important agronomic traits in sesame which makes it ft for cultivation for farmers (Uzun and Çagırgan [2006\)](#page-36-5). Early maturity not only helps in reducing crop cultivation cost but also provides enough times for succeeding crop. Short plant stature is very helpful to breed lodging-tolerant sesame and an important component toward mechanical harvesting in sesame (Ashri [1998\)](#page-28-1).

## *15.2.3 High Oil Content*

High oil content is another important breeding objective as oil is chief produce for oilseed crops. Among different oilseed crops, sesame has high oil content (∼55%) which makes it suitable as a key oilseed crop (Yadava et al. [2012](#page-38-2)). Sesame oil content and its quality are varying with the genotype, color (black to white), and size of the seed. Oil content in sesame gene pool varies from 35% to 63% indicating the

presence of suffcient genetic variation for oil content in sesame which is a prerequisite for breeding cultivar with high oil content. Also, genotypes with white seed coat color possess higher oil content than the dark seed coat-colored genotypes. Oil content in sesame is oligogenic to polygenic; therefore, breeders should pay attention on recurrent selection schemes to develop high oil content genotypes (Velasco and Fernández-Martínez [2002](#page-36-6); Islam et al. [2016](#page-31-5)).

## *15.2.4 Fatty Acid Compositions of Oil*

The worth and usefulness of an oilseed crop for both dietary and manufacturing purposes mainly depend upon the fatty acid composition of its oil (Dyer et al. [2008\)](#page-30-6). Varietal development with desirable fatty acid compositions could augment the usefulness of the oil for defnite comestible purposes. Sesame geneticists and breeders prefer to select those lines which exhibit high oil content with high polyunsaturated fatty acids like oleic, linoleic, and linoleic acid. Beside this, the presence of several antioxidant compounds like minerals, vitamins, phytosterols, tocopherols, and unique class of lignans such as sesamin and sesamolin adds further nutritional value in sesame. These components mainly help in scavenging of reactive oxygen species and are very helpful for recoverable patients. Hence, breeding for these quality components traits also becomes important for sesame breeders. The high PUFA compositions along with high antioxidant components like tocopherol, sesamin, and sesamolin are desirable for high-quality export value in sesame (Hwang et al. [2005;](#page-31-6) Gupta [2015\)](#page-30-7). For confectionary purposes, cultivars should have white seed color, bold size, and appealing shape. Beside this, germplasm should be screened for required texture and seed coat thickness and oil flavor using specific descriptors (Tripathy et al. [2019](#page-36-4)).

#### *15.2.5 Shattering Resistance*

Capsule shattering often leads to heavy yield losses in sesame. Most of the sesame varieties are of shattering type, and almost all of the felds are harvested by hand which leads to approximately 60% yields loss (Langham [2007](#page-32-2)). There is a prerequisite to reorient breeding approach to reduce the high cost of manual harvesting and yield loss due to shattering. Development of new high-yielding cultivars with semi-indehiscent capsules is a possible option to ft mechanized farming.

#### *15.2.6 Abiotic Stress Tolerance*

Sesame is usually grown under marginal to submarginal land and faces several types of environmental extremities. However, only limited efforts have been made to develop genotype with high yield potential and improved tolerance to abiotic

stresses. Sesame withstands water scarcity to some extent because of its extensive root system but may experience huge yield losses under different environmental stresses such as fooding, salinity, heavy metals stresses, and temperature extremities. This crop is considered moderately salt tolerant and can give gainful output on saline soils. Salinity affects water potential and causes ion imbalance and toxicity in living cells; this altered water status leads to initial growth reduction and reduction in productivity and may lead to death of plant. Stress affects all the major metabolic processes such as germination, seedling growth and survival, accumulation of photosynthetic pigments, photosynthesis, and respiration processes which leads to water scarcity, nutrient imbalance, and oxidative stress in salt affected plant. Abbasdokht et al. [\(2012](#page-27-3)) revealed that germination percentage, shoot length, shoot dry weight, root length, and germination rate decreased as the salinity concentration increased in sesame. There was no signifcant difference between the cultivars up to 0.16 ds.m−<sup>1</sup> salinity levels; however, there were signifcant differences between the cultivars beyond 0.16 ds.m−<sup>1</sup> , and they also conclude that selection within cultivars for salt tolerance could be possible at germination stage. Bahrami and Razmjoo [\(2012](#page-28-6)) concluded that germination and seedling growth were strongly inhibited by 12.05 dSm−<sup>1</sup> among the ten cultivars they studied.

Sesame is usually cultivated under rainfed conditions where precipitation is irregular. It is regularly subjected to mild to severe water defcit stress. Vegetative stage is most sensitive to drought stress (Boureima et al. [2011\)](#page-28-7). Drought stress is the main constraint in production potential of the crop in the semiarid regions (Boureima et al. [2012](#page-28-8)). Drought affects the plant metabolism, growth development, and yield. Different cultivars respond differently to drought stress with some cultivars being highly resistant and others more susceptible (Boureima et al. [2011\)](#page-28-7). Due to extensive rooting system, sesame can overcome drought although it experiences substantial yield losses if drought occurs when it is cultivated on marginal and rainfed areas. Sesame seed yield is more affected by drought than any other morphological characters. Kim et al. [\(2007](#page-31-7)) investigated the drought effect on yield, and its component traits in sesame found that water stress signifcantly decreased sesame yield by decreasing the number of seeds per capsule. Dossa et al. [\(2019](#page-29-6)) identifed 543 sesame core abiotic stress-responsive genes using meta-analysis of 72 RNA-Seq datasets from drought, water logging, salt, and osmotic stresses using contrasting sesame genotypes. You et al. ([2018\)](#page-38-3) performed transcriptomic analysis to study the expression profling of stress-responsive genes in different tissue and development stage under various abiotic stresses. They found that the genes, namely, *SiGolS* and *SiRS*, were signifcantly regulated by drought, salt, osmotic, and water logging stresses but slightly affected by cold stress. Wang et al. [\(2018](#page-37-3)) studied the transcriptomic profling of *SibZIPs* gene. Their results indicated that this gene exhibited considerable changes against abiotic stresses, including salt, drought, water logging, osmotic, and cold. Li et al. [\(2017](#page-32-5)) studied *SiWRKY* gene expression patterns and revealed that 33 and 26 *SiWRKYs* gene expression was strongly responded to water logging and drought stress, respectively. Drought tolerance in sesame is associated with wax depositions, root length, transpiration rate (Sun et al. [2010\)](#page-35-3), and higher activities of few antioxidant enzymes like superoxide dismutase, catalase,

polyphenol oxidase, and peroxidase (Fazeli et al. [2007](#page-30-8)) as well as antioxidant metabolites content, viz., carotenoids (Kadkhodaie et al. [2014](#page-31-8)), and higher levels of ABA, proline, arginine, lysine, aromatic and branched chain amino acids, GABA, saccharopine, 2-aminoadipate, and allantoin under drought stress (You et al. [2019\)](#page-38-4). Water logging is another important stress, and sesame crop is highly susceptible to fooding, as the crop undergoes immediate senescence and declines within 2–3 days of exposure to waterlogging stress (Anee et al. [2019](#page-27-4)). High rainfall during monsoon often leads to yield losses in sesame, and there is a need to develop improved cultivars that could survive the waterlogging stress. Anee et al. ([2019\)](#page-27-4) observed that lipid peroxidation as well as hydrogen peroxide  $(H<sub>2</sub>O<sub>2</sub>)$  and methylglyoxal contents increased while leaf relative water content, proline content, and chlorophyll and carotenoid contents decreased under prolonged water logging stress in sesame. Beside this, glutathione and oxidized glutathione contents increased under waterlogging, while the GSH/GSSG ratio and ascorbate content decreased. Ascorbate peroxidase, monodehydroascorbate reductase, glutathione peroxidase, and glyoxalase I activity increased under water logging, while dehydroascorbate reductase, glutathione reductase, and catalase activity showed decreasing trend. Wang et al. [\(2012](#page-37-4)) found strong association between cell wall modifcation and growth pathways, glycolysis, fermentation, mitochondrial electron transport, and nitrogen metabolism with waterlogging tolerance in sesame. These traits should be under consideration when breeding for fooding tolerance in sesame. Salinity often limits sesame cultivation especially in arid and semiarid regions. Sesame cultivars show a considerable variation in the degree of salt tolerance (Bekele et al. [2017\)](#page-28-9). Zhang et al. ([2019\)](#page-38-5) compared salt tolerant and sensitive genotype of sesame and revealed that tolerant genotype has higher seed germination percentage, more plant survival rate, as well as better growth rate than susceptible one. Their transcriptome study revealed strongly induced salt-responsive genes in sesame mainly related to amino acid metabolism, carbohydrate metabolism, biosynthesis of secondary metabolites, plant hormone signal transduction, and oxidation-reduction process, while metabolomics investigation revealed amino acid metabolism and sucrose and raffnose family oligosaccharide metabolism impart salt tolerance in sesame. Several antioxidant enzymes, viz., superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase as well as malondialdehyde and proline content, have been found closely related to salt tolerance in sesame (Koca et al. [2007](#page-31-9)). In addition to this, seed germination percentage, root and shoot length, root to shoot length ratio, and seedling fresh weight are also associated with salinity tolerance particularly at seedling stage (El Harfi et al. [2016](#page-30-9)). Heavy metal stresses also adversely affect sesame yield considerably. Heavy metal stress tolerance in sesame was associated with accumulation of more dry mass during early growth phase and nitrate reductase activity. Considerable genetic diversity exists in collected sesame germplasm which could exploit to breed cultivar tolerance to abiotic stresses. In addition to this, sesame crop wild relatives have been reported to have agronomically desirable alleles for stress tolerance including both biotic and abiotic stresses.

## *15.2.7 Biotic Stress Tolerance*

Biotic stresses such as pathogens, insects, and weeds adversely affect sesame crop which cause unpredicted losses in productivity and production due to lack of proper management practices including unavailability of resistant varieties (Girmay [2018\)](#page-30-10). Hence, development of resistant cultivars helps in sustainability and in improving yield stability in sesame. The important diseases, their causal organism, and estimated yield losses are presented in Table [15.2](#page-9-0). Plants face a combination of different biotic stresses, and to mitigate the effects, they evolved complex signaling pathways. In general, disease resistance in crop plants is two types: whether a hypersensitive or incompatible type is governed by few genes and which is often known as oligogenic or vertical resistance. Second one is partial resistance which is governed by many genes and popularly known as quantitative or horizontal resistance (Singh et al. [2020](#page-35-4); Beebe and Corrales [1991](#page-28-10); Vale et al. [2001](#page-36-7)). In sesame, genetics of disease resistance varied as it is was monogenic to oligogenic against phyllody and *Alternaria* blight, while it was polygenic against charcoal rot (El-Bramawy and Shaban [2007;](#page-30-11) Eswarappa et al. [2011](#page-30-12); Shindhe et al. [2011;](#page-35-5) Lokesha et al. [2013](#page-32-6); Renuka and Lokesha [2013\)](#page-34-6). However, breeding for single disease resistance is often not much effective; hence there is a need to develop cultivars with multiple resistances to the above biotic stresses including both disease and

Major diseases	Causal organism	<b>Yield losses</b>	References
Phyllody	Phytoplasma like organism	Up to $80\%$	Ganem Junior et al. (2019)
Dry root rot	Rhizoctonia bataticola Taubenh	$80 - 100\%$	Renganathan (2020)
<i>Phytophthora</i> blight	Phytophthora parasitica var. sesame Dastur	Up to $100\%$ loss when infection occurs severely at seedling stage	Kumari et al. (2019)
Alternaria leaf blight	Alternaria sesame Kawamura (Mohanty and Behera)	20–40% yield losses	Pawar et al. (2019)
Charcoal rot	Macrophomina phaseolina	$5 - 100\%$	Deepthi et al. (2014a, b)
Leaf curl virus disease	Gemini virus		Manjeet et al. (2020)
Insect pests			
Common name	Scientific name	<b>Yield losses</b>	References
Leaf Webber or roller and capsule borer	Antigastra catalaunalis Duponchel (Lepidoptera: Crambidae)	Up to $90\%$ yield losses	Pandey et al. (2018)
Gall fly	Asphondylia sesami Felt (Diptera: Cecidomyiidae)	Up to 100% in susceptible genotypes and under favorable conditions	Adam et al. (2020)

<span id="page-9-0"></span>**Table 15.2** Major disease and insect pests and their estimated yield in sesame

<span id="page-10-0"></span>

**Fig. 15.1** Symptoms of sesame phyllody in the feld. (**a**) The entire sesame inforescences are replaced by short twisted leaves closely arranged on top of the stem with very short internodes, but leaves on the lower part of infected plant did not exhibit any visible symptoms. (**b**) Floral virescence and dark exudates appear on foliage foral parts. (**c**) Floral virescence

insect pest. The symptoms of various diseases, viz., phyllody, charcoal rot, and leaf curl virus disease, are shown in Figs. [15.1](#page-10-0), [15.2,](#page-11-0) and [15.3](#page-11-1).

# **15.3 Sesame Classical Breeding Methods**

Sesame is known as a predominantly self-pollinated crop. Hence, the selection methods employed for breeding self-pollinated plants are equally effective in sesame. Existence of genetic variation is a precondition for hybridization and accumulation of desirable alleles into single genetic background through selection. Domestication, plant introduction, mass, and pure line selection are the important breeding method applicable for existing genetic variability. Meanwhile, creation of genetic variation through crossing contrasting genotypes followed by pedigree, bulk, and single seed descent selection method is another important breeding scheme for sesame breeders. Recurrent selection and diallel mating selective schemes are the two most effective although time-consuming breeding methods in sesame. However, transferring one or two genes of agronomically important traits

<span id="page-11-0"></span>

**Fig. 15.2** Field symptoms of sesame charcoal rot. (**a**) Infected sesame plant showing charcoal rot symptoms on lower portion of the stem. (**b**) Severe charcoal rot infection leads to stem breakage. (**c**) Root portion showing typical charcoal rot symptoms and devoid of lateral and fner roots

<span id="page-11-1"></span>

**Fig. 15.3** Symptoms of sesame leaf curl virus disease. (**a**) Severely infected sesame plant showing leaf curling and plant stunting. (**b**) Close look of sesame twin with severe curling along with thickening of leaves. (**c**) Underside of an infected sesame leaf showing vein swelling and upward curling along with leaf thickening

from known source to high-yielding, well-adapted genotype is possible through backcrossbreeding schemes. Mutation breeding is another key approach for creating new desirable allele which is totally lacking in germplasm. Mutation breeding is generally employed for quality traits, while backcross method is often used for transferring disease resistance alleles and for transferring genes responsible for male sterility and fertility restoration. Baydar [\(2005](#page-28-11)) applied pedigree selection method to improve the ideal type of sesame and revealed that the bicarpels, monoto tricapsule, with a greater number of branches were considered as ideal plant types in breeding for high-yielding varieties. Ismail et al. ([2013\)](#page-31-10) performed pedigree selection in sesame for seed yield per plant and compared yield after two selection cycle. The average yield after two selection cycle of selected families surpassed as compared to their parents. The plant geneticists and breeders are always interested in identifying gene/allele source responsible for desirable agronomic traits and determining the genetic basis of agronomic traits in order to design proper breeding approaches for development of new cultivars. Information about inheritance pattern of any particular trait is indispensable in deciding most appropriate breeding method for the development of elite genotype/variety. The information about nature of gene action for different agronomic traits in sesame is given in Table [15.3,](#page-13-0) while the gene/allele source for different agronomic traits is given in Table [15.4](#page-15-0).

We at CCS Haryana Agricultural University, Hisar, India, have been maintaining about 550 germplasm lines, and these were evaluated for various agronomic traits during *Kharif*, 2014. The range of variation for different agronomic traits revealed substantial and potential variability in different genotypes. Among these germplasm lines, four lines, viz., TC 180, TC 26, TC 104, and TC 174, were promising for seed yield per plant, while the genotypes, viz., TC 302, TC 354, TC 301, TC 183, F 45, and TC 353, were promising for early fowering and maturity. For the development of short stature cultivars, the genotypes, viz., TC 302, TC 24, TC 325, TC 327, TC 167, TC 342, TC 27, and TC 45, were having desirable short plant height. These genotypes might be useful for development of high-yielding variety in sesame. Two high-yielding sesame varieties, viz., HT 1 and HT 2 (HT 9713), were developed at CCS HAU for farmer's cultivation. (Fig. [15.4\)](#page-16-0). The variety HT 1 possesses medium to long height and dark green-colored leaves, resistant to phyllody and leaf curl virus with an average yield of 2.4 q/acre. The variety HT 2 is a short stature, early maturing, high-yielding variety developed from pedigree selection of population developed from the cross HT  $1 \times$  HT 15, and it possessed both high yield and disease resistance as it showed resistant reaction to all the major diseases (phyllody and leaf curl virus) as well as to leaf roller/capsule borer.

#### *15.3.1 Heterosis Breeding in Sesame*

In spite of many efforts through classical breeding methods, viz., mass, pedigree, backcross and recurrent selection, still there have not been accomplished a foremost yield revolution in sesame productivity. Therefore, heterosis breeding could have potential to break yield plateau in sesame by exploiting the advantage of heterosis

Trait	Gene action	References
Hairiness	Single dominant gene	Yol and Uzun $(2011)$
Number of capsules per leaf axil	Monogenic (with one capsule per leaf axil character was dominant to three capsules)	
Seed coat color	Oligogenic (two major genes V and $B)$	Pandey et al. (2013)
Seed yield per plant	Polygenic with the importance of both additive and nonadditive gene action along with duplicate epistasis and complementary epistasis	Gaikwad et al. (2010, 2009), Sharmila et al. $(2007)$ , Raikwar $(2018)$ , Rajput et al. $(2017)$ , Dasgupta et al. $(2018)$ , Elaziz and Ghareeb (2018), Anyanga et al. (2016), Solanki and Gupta (2003), and Tripathy et al. $(2016a, b)$
Number of seeds per capsule	Polygenic along with duplicate epistasis; both additive and nonadditive gene action were important	Gaikwad et al. (2010), Gaikwad et al. (2009), Aladji et al. (2014), Dasgupta et al. (2018)
1000-seed weight	Polygenic with both additive and nonadditive gene effects along with duplicate epistasis	Gaikwad et al. (2010), Dasgupta et al. (2018) and Elaziz and Ghareeb (2018)
	Additive gene effects	Rajput et al. (2017)
	Polygenic with both additive and nonadditive gene effects along with both duplicate and complementary epistasis	Sharmila et al. (2007)
Capsule length	Polygenic with the preponderance of both additive and nonadditive gene effects; both duplicate and complementary epistasis present	Gaikwad et al. (2010), Aladji-Abatchoua et al. (2014), Sharmila et al. (2007), Rajput et al. $(2017)$ and Dasgupta et al. $(2018)$
	Polygenic with preponderance of additive gene effects	Gaikwad et al. (2009)
Number of primary branches	Polygenic with both additive and nonadditive gene action along with complementary and duplicate epistasis	Gaikwad et al. (2010) and Aladji- Abatchoua et al. (2014)
Number of capsules on main axis	Both additive and nonadditive gene action along with duplicate and complementary epistasis	Gaikwad et al. (2010, 2009)
Number of capsules per plant	Mostly polygenic, both additive and nonadditive gene actions important, mostly governed by duplicate epistasis	Gaikwad et al. (2010, 2009), Aladji et al. (2014), and Elaziz and Ghareeb (2018)
Days to $50\%$ flowering	Mostly governed by additive gene action	Gaikwad et al. (2010, 2009)

<span id="page-13-0"></span>Table 15.3 Gene action responsible for seed yield and its component traits as well as other important agronomic traits in sesame

(continued)

Trait	Gene action	References
	Both additive and nonadditive gene effects	Aladji et al. (2014), Rajput et al. (2017) and Sumathi and Muralidharan (2009)
Days to maturity	Both additive and nonadditive gene actions, duplicate epistasis	Gaikwad et al. (2010, 2009), Aladji- Abatchoua et al. (2014), Rajput et al. (2017), and Sumathi and Muralidharan (2009)
Days from flowering to capsule maturity	Both additive and nonadditive gene actions	Aladji-Abatchoua et al. (2014)
Plant height	Polygenic inheritance with both additive and nonadditive gene effects	Gaikwad et al. (2010, 2009), Aladji et al. (2014), Raikwar (2018), Rajput et al. $(2017)$ and Dasgupta et al. $(2018)$
Harvest index	Additive gene effects	Tripathy et al. (2019)
Leaf chlorophyll content	Nonadditive	
Capsules bearing nodes	Additive	
Photoperiod response	Polygenic inheritance	
Photosynthetic rates	Additive gene action	
Oil content	Polygenic inheritance with both additive and nonadditive gene effects	Rajput et al. $(2017)$
	Preponderance of nonadditive gene effect	Tripathy et al. $(2016a, b)$
Alternaria leaf spot resistance	Additive gene effects	El-Bramawy and Shaban (2007)
Fusarium wilt	Both additive and nonadditive	
Charcoal rot	Both additive and nonadditive	
Monostem/shy branching	Monogenic to oligogenic with complementary epistasis	Sumathi and Muralidharan (2009)
Capsule shape	Governed by dominant epistasis; long capsule is dominant over dense capsule	Yol $(2017)$
Shattering resistance	Governed by two pairs of genes with duplicate dominant epistasis and duplicate recessive epistasis	Kotcha et al. (2012)
Genetic male sterility	Governed by single recessive genes	Liu et al. (2013)
Sesame gall midge	Nonadditive gene effects	Ubor et al. $(2015)$
Powdery mildew tolerance	Governed by two independent recessive genes with complementary epistasis	Rao et al. (2012)
Sesamolin content	Additive and nonadditive	Khuimphukhieo et al. (2020)

**Table 15.3** (continued)

Genetic sources	Trait	References
$C_{3.8}$	Drought tolerance	Abdelraouf and Anter (2020)
Darab 14, Shaban and Yekehsaud		Asadi et al. (2020)
TEX-1		Song et al. (2020)
shi165, lc162, mc112, lc164, icn115, icn141, mt169, dwf172, cc102, 38–1-7, and <b>Birkans</b>		Boureima et al. (2012)
Sistan and TN238		Khammari et al. (2013)
LC 164, LC 162 and BC 167 and 32-15		Boureima et al. (2016)
KC50658 and Oltan		Abbasali et al. (2017)
$C8.4$ and $C8.8$	Salinity tolerance	Anter and El-Sayed (2020)
BD 6980 and BD 6985	Water logging tolerance	Saha et al. (2016)
ZZM1501, ZZM2113, ZZM2147, ZZM2208, ZZM3342, ZZM3379, ZZM3410, ZZM4780, ZZM4781, 09-P65, Liaopinzhi 3, and Luozhi 15		Wang et al. (2011)
P5(NM59), C6.3, C1.10, and C3.8	Charcoal rot resistance	Shabana et al. (2014)
1.6, C1.10, C3.8, C6.3, C6.5, and C9.15	<i>Fusarium</i> oxysporum resistance	
Zhongzhi No. 13	Charcoal rot resistance/	Wang et al. (2017)
PKDS-91	tolerance	Deepthi et al. (2014a, $\mathbf{b}$
87,008		Farooq et al. (2019)
Sesame lines No., 33, 3, 15, 64, 40, 63, 14, 39, 4, 16, 13, 80, 58, and 79		Bedawy and Moharm (2019)
Potak-e-Mousian, MahalliIranshahr and Safiabad line 3		Garmaroodi and Mansouri (2014)
ORM 7, ORM 10, and ORM 17		Thiyagu et al. $(2007)$
T6, Dashtestan 2, Darab 1, AT1, and AT2		Zaker et al. (2020)
Chinese, Varamin 2822, and PotkeMusian		Sadeghiy Garmaroudi et al. (2003)
JLS 110-12, HT 9913, T 78, and KMR 60	Multiple disease resistance (phyllody, charcoal ro, and sesame leaf curl virus)	Manjeet et al. (2020)
HuRC-4 and HuRC3	Multiple disease resistance (resistant to bacterial blight, Fusarium wilt, and phyllody)	Belay (2018)

<span id="page-15-0"></span>Table 15.4 Agronomically important gene/allele source within cultivated gene pool in sesame

(continued)

Genetic sources	Trait	References
RJS78, RJS147, KMR14, KMR79, Pragati, IC43063, and IC43236 and two wild spp., i.e., Sesamum alatum and Sesamum mulayanum	Phyllody resistance	Singh et al. $(2007)$
KAU-05-2-12, PC-14-2 and Kanakapura		Mahadevaprasad et al. (2017)
NS98002-04, NS98003-04, NS99005-01 and NS01004-04		Akhtar et al. (2013)
RT-273	Alternaria blight resistance	Lokesha and Naik (2011)
NS 11204	Sesame leaf curl virus resistance	Sarwar et al. (2006)
TSP 933229 and TR 3821512	High oleic acid	Baydar et al. (1999);
Majengo, Stewa,		Were et al. $(2006a, b)$
TSP 932410 and TSP 932,403	High linoleic acid	
Webuye, Kisumu301, ug1, Koyonzo		

**Table 15.4** (continued)

<span id="page-16-0"></span>

**Fig. 15.4** Sesamum varieties developed by CCS, HAU, Hisar. A. Variety HT I possessing resistance to phyllody and leaf curl virus with an average seed yield of 2.4 q/acre. B. HT 2, a dwarf, early maturing and high-yielding variety. C. White-colored bold seeds of variety HT1. D. Whitecolored bold seeds of variety HT2

or hybrid vigor phenomenon (Mothilal and Ganesan [2005;](#page-33-10) Monpara and Pawar [2016\)](#page-33-11). Heterosis is the term used for superiority of  $F_1$  hybrids as compared to their parents. Heterosis was already exploited in many important oilseed's crops including rapeseed-mustard, groundnut, and soybean to some extent. Recently, several workers reported the existence of noteworthy heterosis in certain cross combinations of sesame (Murty [1975](#page-33-12); Sasikumar and Sardana [1990;](#page-34-14) Jiarong [1991](#page-31-14); Quoada and Layrisse [1995](#page-34-15); Uzun et al. [2004;](#page-36-12) Sumathi and Muralidharan [2008;](#page-35-12) Banerjee and Kole [2010](#page-28-18); Jadhav and Mohrir [2013](#page-31-15); Parimala et al. [2013;](#page-33-13) Saravanan and Nadarajan [2002;](#page-34-16) Lal Jatothu et al. [2013;](#page-32-11) Vavdiya et al. [2013](#page-36-13); Hassan and Sedeck [2015;](#page-31-16)). Hence, there is urgent need to exploit this heterosis to capitalize on seed yield and oil content in sesame. Effective male sterility-fertility restoration system might provide opportunity to develop effcient system for commercial hybrid seed production in sesame. Several workers reported genetic male sterility in sesame like corolla recessive genic male sterile mutant (Langham 1947), greenish anther color at pollen dehiscence-associated male sterility (Osman and Yermanos [1982](#page-33-14)), short anther's flaments and cold night temperature-based recessive genetic male sterility (Brar [1982\)](#page-29-10), and mutagen-induced monogenic recessive genetic male sterility (Rangaswamy and Rathinam [1982\)](#page-34-17). Cytoplasmic male sterility was also identifed in sesame wild relative *Sesamum malabaricum* (Prabakaran et al. [1995](#page-33-15); Bhuyan and Sarma, [2003](#page-28-19); Prabakaran [1998\)](#page-33-16). This CMS system was used to develop 36 cross combinations with high heterotic effects (77–540%) for many seed and oil yield (Tripathy et al.  $2019$ ). Several experimental  $F_1$  cross combinations have been developed in India which exhibited heterotic effect of 31.0–44.3% in seed yield and 13–48% in oil yield over commercial pure line variety TKG 22 (Gangaiah [2008](#page-30-18)).

We at CCS HAU evaluates several lines in different cross-combinations for exploiting heterosis in sesame through cytoplasmic genetic male sterility system (CMS) and also to develop resistant lines against insect-pests and diseases. Also, the possibility for exploitation of inter-specifc hybridization was explored at CCS HAU by exploiting *Sesamum malabaricum*. The genotypes, viz., IC 043144–1 and JJK/MIS10–67 of *S. malabaricum*, were crossed with HT 1, HT 2, HT 9316, HT 9907, HT 9913, TKG 22, MT 11–8-2, LT 210, HTC 1, and KMR 60. The 49 newly developed F1 hybrids were evaluated for seed yield and its component traits. Out of these cross combinations, eight showed higher seed yield over local check HT 2. Highest seed yield per plant was observed in hybrid HT  $20 \times$  HT  $2 (7.3 g)$ , followed by HT  $45 \times RT$  125 (6.7 g), CST 2001–9  $\times$  HT 2000 (6.5 g), OC 201  $\times$  HT 9316  $(6.0 \text{ g})$ , OC 251  $\times$  HT 2000  $(6.0 \text{ g})$ , T 78  $\times$  HT 2 (4.5 g), KMR 41  $\times$  RT 125 (4.4 g), RH 54 × HT 9316 (4.0 g), and local check HT 2 (3.6 g) during *Kharif*, 2015. This indicates the potential of  $F_1$  hybrids for breaking yield plateau in sesame. For charcoal rot resistance breeding at CCS HAU, 24 germplasm lines, viz., NIC 7837, NIC 7875, HT 1, NIC 17274, HT 2, NIC 17849, HT 15, SI 2174–1, SI 3296, IS 92–2, and HT 9913, were found moderately resistant.

## **15.4 Molecular Breeding in Sesame**

Crop breeding together with improved agronomic practices resulted in the Green Revolution in the 1960s with spectacular yield gains, particularly for staple crops like wheat and paddy in developing countries (Lenaerts et al. [2019\)](#page-32-12). However, crop yield augmentation has been slowing down more in recent times. Changes in climatic patterns, arable land, and water accessibility now endow with further challenges for ensuring yield steadiness across varied environment. Changing climatic conditions affect farming and foodstuff formation in multifaceted ways. It infuences food production straightforwardly by altering in agroclimatic environment and on another way by distressing development and allocation of income and thus demands for agricultural outcomes (Shetty et al. [2013](#page-35-13)). Advanced biological techniques in plant breeding like genomics, proteomics, bioinformatics tools, molecular breeding, and plant tissue culture and genetic engineering have already led to signifcant impacts on several important crops including rice, wheat, rapeseed-mustard, soybean, maize, potato, sorghum, and pearl millet (Varshney et al. [2005\)](#page-36-14). Sesame is an underexploited crop of tropical and subtropical region of the world. As sesame is the crop of developing countries, major efforts for sesame improvement were made only through classical plant breeding methods (Gupta [2015\)](#page-30-7). However, under changing climatic conditions and evergreen increasing human populations, efforts should be directed toward the use of recent biotechnological techniques to boost up the sesame production and productivity. The molecular breeding work in sesame began very late with only one genetic map published and no information on QTL mapping before 2013 (Dossa et al. [2017a,](#page-29-1) [b](#page-29-2)). However, over the last decade, some noteworthy advancement has been made in sesame breeding programs to use advanced molecular biology techniques including plant tissue culture techniques; highly informative molecular marker techniques like SNPs; high density linkage and genetic maps; omics studies including genomics, proteomics, transcriptomics, and metabolomics; and advanced bioinformatics tools (Tripathy et al. [2019](#page-36-4)). In addition, the draft of sesame genome triggered functional analyses of candidate genes related to important agronomic traits (Wei et al. [2017a](#page-37-9), [b](#page-37-10); Zhang et al. [2013a](#page-38-9), [b\)](#page-38-10). With these invaluable efforts, sesame has some important genomic resources and platforms for improvement, and presently, sesame has shifted from an "orphan crop" to a "resource-rich crop." Among different advanced techniques, molecular marker technologies have considerably accelerated the classical sesame breeding programs in enhancing the genetic gain and minimizing the breeding cycles in many crop species (Dossa et al. [2017a,](#page-29-1) [b](#page-29-2)). Molecular marker technologies in sesame are witnessing signifcant progress, and it is clear that sesame is no longer far behind large crops in this feld. Various kinds of molecular markers have been developed and used to sesame genotyping including randomly amplifed polymorphic DNA (RAPD), amplifed fragment length polymorphism (AFLP), microsatellites or simple sequence repeat (SSR), and inter-simple sequence repeats (ISSR) employed mainly for genetic diversity analysis at DNA level. The next class of markers concerned mostly of expressed sequence tags-SSR (EST-SSR), cDNA-SSR, genome

sequence-SSR (gSSR), and chloroplast SSR (cpSSR) which were mainly employed for association mapping, germplasm characterizations, and molecular breeding in sesame (Dar et al. [2017;](#page-29-11) Dixit et al. [2005](#page-29-12); Wei et al. [2014a,](#page-37-11) [b;](#page-37-12) Kizil et al. [2020;](#page-31-17) Cui et al. [2017](#page-29-13); Li-Bin et al. [2008](#page-32-13);Wei et al. [2011](#page-37-13); Zhang et al. [2013a,](#page-38-9) [b](#page-38-10); Kumar and Sharma [2011\)](#page-32-14). Recently, with the discovery of next-generation sequencing technology (NGS), another class of molecular markers emerged. SNPs are more useful as genetic markers than many other simple markers because they are the most abundant and stable form of hereditary difference in most genomes (Uncu et al. [2016;](#page-36-15) Wei et al. [2014a](#page-37-11), [b\)](#page-37-12). Therefore, high-throughput methods available for SNP detection and genotyping have been used in sesame research including restriction siteassociated DNA sequencing (RAD-seq), specifc length amplifed fragment sequencing (SLAF-seq), RNA-Seq, whole-genome sequencing (WGS), genotyping by sequencing (GBS), and insertion/deletions (Indels) (Uncu et al. [2016](#page-36-15)). Using these marker techniques, several important genes and QTLs were mapped and validated in sesame till now (Table [15.5](#page-20-0)). Different types of molecular markers have been developed and used successfully for genetics and breeding activities in *Sesamum indicum.* The following sections provide brief information related to different types of molecular markers used in sesame based on their detection method.

## *15.4.1 RFLP (Restriction Fragment Length Polymorphism)*

RFLP was the frst molecular marker and the barely marker system based on hybridization. Polymorphism occurs among individuals of same species as a result of insertion/deletion (InDels), translocation, duplications, inversions, and point mutations. RFLP begins with the isolation of pure genomic DNA, after which isolated DNA is treated with restriction enzymes resulting in a large number of fragments varying in length. Agarose or polyacrylamide gel electrophoresis (PAGE) is used to study the polymorphism among genomic DNA (Kundan et al. [2014](#page-32-15)).

#### *15.4.2 RAPD (Randomly Amplifed Polymorphic DNA)*

Williams et al. and Welsh and McClelland independently developed RAPD technique (Welsh and Mcclelland [1990](#page-37-14); Williams et al. [1990\)](#page-37-15). Simple, short (ten nucleotides), and random primers were used for PCR amplifcation of genomic DNA. When two hybridization sites are similar and in the opposite direction, PCR amplifcation takes place. Sharma et al. studied the characterization and analysis of genetic variance in Indian sesame (*Sesamum indicum* L.) genotypes. To fnd out the extent of genetic diversity between 60 sesame varieties in diverse geographical regions of India, 20 phenotypic (qualitative and quantitative) traits and 200 RAPD markers were used. In accessing the diversity, 14 RAPD markers were found to be useful. Among the population, high level of genetic variability  $(HT = 0.1991)$  and

Traits	Genes/QTLs	References	
Flowering time	SiDOG1 (SIN_1022538) and SiIAA14 (SIN_1021838)	Wei et al. (2015)	
Seed yield	$Qgn-1$ , $Qgn-6$	Mei et al.	
		(2017)	
Seed coat color	QTL-1, QTL11-1, QTL11-2, QTL13-1	Zhang et al. (2013a, b)	
	qSCa-8.2, qSCb-4.1, qSCb-8.1, qSCb-11.1, qSCl-4.1, qSCl-8.1, qSCl-11.1, qSCa-4.1, and qSCa-8.1	Wang et al. (2016)	
1000-seed weight	$Qtgw-11$	Wu et al. 2014	
Flowering times	SiDOG1 (SIN_1022538) and SiIAA14 (SIN_1021838)	Wei et al. (2015)	
Capsule length	Qcl-3, Qcl-4, Qcl-7, Qcl-8, and Qcl-12	Wu et al.	
First capsule height	Ofch-4 and Ofch-12	(2014)	
Plant height	Qph-6 and Qph-12	Wei et al.	
	SiDFL1 (SIN_1014512) and SiILR1 (SIN_1018135)	(2015)	
	Qph-8.2, Qph-3.3	Wang et al. (2016)	
Semidwarf sesame plant phenotype	QTL (qPH-3.3), gene[SiGA20ox1(SIN_1002659)]	Wang et al. $(2016)$ and Wei et al. (2016)	
Capsule length and capsule	Qcl-3, Qcl-4, Qcl-7, Qcl-8, and Qcl-12	Wu et al. (2014)	
number	SiLPT3 and SiACS8	Zhou et al. (2018)	
Number of capsules per axil	SiACS (SIN_1006338)	Wei et al. (2015)	
Mono flower vs. triple flower	<b>SiFA</b>	Mei et al. (2017)	
Determinate trait in sesame	geneSiDt (DS899s00170.023)	Zhang et al. (2016)	
Oil content	SIN_1003248, SIN_1013005, SIN_1019167, SIN_1009923SiPPO (SIN_1016759), SiNST1 $(SIN_1005755)$	Wei et al. (2015)	
Biotic and abiotic stresses			
Drought tolerance	TF (transcription factor) families (AP2/ERF and HSF)	Komivi et al. $(2016)$ , Dossa et al. (2016)	
Water logging tolerance	qEZ09ZCL13, qWH09CHL15, qEZ10ZCL07, qWH10ZCL09, qEZ10CHL07, and qWH10CHL09	Wang et al. (2016)	
Drought, salinity, oxidative stresses, charcoal rot	Osmotin-like gene (SindOLP)	Chowdhury et al. (2017)	

<span id="page-20-0"></span>Table 15.5 Important gene(s)/QTLs responsible for different agronomic traits mapped in sesame

(continued)



**Table 15.5** (continued)

(continued)

<b>Traits</b>	Genes/OTLs	References
Seed potassium concentration	$OTL-qK-1$	Teboul et al. (2020)
Seed zinc concentration	$QTL-qZn-5$ ; $qZn-6$	
Seed iron concentration	OTL-qFe-6	
Seed magnesium concentration	$OTL-qMg-2$	
Black seed coat development	SIN 1018961 and SIN 101895; SIN 1006242 and SIN 1016759/PPO, SIN 1026689 and SIN 1006025, SIN 1025056	Dossou et al. (2020)
Internode length and plant height	SiDWF1	Miao et al. (2020)

**Table 15.5** (continued)

within population less variability  $(HS = 0.0749)$  were observed. Among the sesame population, mean coefficient of gene differentiation (GST =  $0.6238$ ) was  $62.38\%$ and 37.62% within the population. The above information suggests that the Indian sesame lines are genetically different, which should be used to improve the sesame crop (Sharma et al. [2014\)](#page-35-15). Dar et al. reported the assessment of genetic variance in sesame using 22 RAPD, and 18 SSR primers were used for the study of 47 diverse sesame accessions cultivated in different agroclimatic regions of India. One hundred ninety-one polymorphic bands were observed with RAPD primers while SSR gives 64 bands. Maximum PIC was reported with SSRs (0.194) compared to RAPD (0.186). In describing genetic variation between the varieties studied, RAPD primer RPI-B11 and SSR primer S16 were the most informative (Dar et al. [2017](#page-29-11)).

# *15.4.3 AFLP (Amplifed Fragment Length Polymorphism)*

The combination RFLP and RAPD markers results in the development of AFLP markers, in which digestion of genomic DNA is followed by PCR amplifcation. AFLP is a cost-effective technique, in which there's no need of former sequence information. In AFLP, two restriction enzymes (a frequent cutter and a rare cutter) are used. After restriction digestion, oligonucleotide fragments were used for PCR amplifcation (Vos et al. [1995](#page-36-16)). Laurentin and Karlovsky studied the genetic variance in a sesame germplasm set using AFLP. Great genetic variability was studied within the 32 sesame associations from the Venezuelan Germplasm Collection which represents genotypes from 5 diversity centers (India, Africa, China-Korea-Japan, Central Asia, and Western Asia). Out of the 457 AFLP markers recorded, 93% were polymorphic. The Jaccard similarity coeffcient ranged from 0.38 to 0.85 between pairs of accessions. According to geographical origin, five groups of genetic diversity study discovered that only 20% of the total diversity was due to diversity among groups that used Nei's coefficient for population differentiation. Similarly, only 5% of the total diversity is accredited to differences between groups through analysis of molecular variance (AMOVA). This study showed that 32 sesame associations were genetically highly variable and did not show a link between geographical origin and AFLP patterns. This suggests that there was a large gene flow among diversity centers (Laurentin & Karlovsky [2006](#page-32-0)).

#### *15.4.4 SSR or Microsatellites (Simple Sequence Repeats)*

SSRs are short tandem repeats of one to six nucleotides having simple sequence length polymorphism, which are present profusely in the genome of different taxa. Microsatellites are distributed throughout the whole genome, viz., nuclear and mitochondrial as well as chloroplast genes. They are also present in the protein coding genes and expressed sequence tags (ESTs). Due to the presence of different numbers of repeats in microsatellite regions, high polymorphism is easily detected by PCR (Kalia et al. [2011](#page-31-20)). Zhang et al. studied the development and validation of genic-SSR markers in sesame by RNA-seq. In this study, 75 bp and 100 bp paired RNA seq were used to sequence 24 cDNA libraries, and 42,566 uni-transcripts were collected from more than 260 million fltered readings. The total length of unitranscript was 47.99 Mb, and 7324 SSRs (SSRs  $\geq$ 15 bp) and 4440 SSRs (SSRs ≥18 bp) were acknowledged. On a usual, there was one genic-SSR per 6.55 kb (SSRs≥15 bp) or 10.81 kb (SSRs≥18 bp). A total of 2164 genic-SSR markers have been developed in sesame using transcriptomic sequencing. Two hundred seventysix of 300 validated primer pairs successfully yielded PCR amplicons in 24 culti-vated sesame accessions (Zhang et al. [2012](#page-38-14)). Park et al. reported the genetic diversity, phylogenetic conditions, and population structure of 227 connections of sesame seed collections collected from 15 countries in 4 different continents. Among sesame accessions, a total of 158 alleles were detected, with an average of 11.3 alleles per locus. The average polymorphism content value was 0.568. It indicates a high genetic variance at 14 loci both among and within the population. UPGMA and the unweighted pair group method formed four robust clusters among the 277 core collection accessions of sesame. Similar patterns were obtained using country-based dendrograms and model-based analysis, as certain geographically distant connections were grouped in the same cluster (Park et al. [2014](#page-33-19)). Surapaneni et al. ([2014\)](#page-36-18) studied the genetic characterization of 68 Indian sesame cultivars and 3 related wild species using 102 SSR markers. By constructing the genomic libraries, 62 novel sesame-specifc microsatellites were isolated from the study. The content of polymorphic information in the markers of the markers ranged from 0.43 to 0.88 with an average of 0.66. All connections were grouped into two large clusters with a genetic similarity between 0.40 and 0.91 by UPGMA cluster analysis. A high percentage of variation (87.1%) was observed within the population by AMOVA analysis. An overall  $F_s$  of 0.11 among the populations indicated low population differentiation. The study reveals that the development of SSR markers will be

constructive for genetic analysis, linkage mapping, and selection of parents in future breeding programs. Uncu et al.  $(2015)$  $(2015)$  used a pyro-sequencing approach for the development of genomic SSR markers. They approached successfully in identifying 19,816 nonredundant SSRs, 5727 of which were identifed in a coting assembly that covers 19.29% of the sesame genome. Molecular genetic diversity and population structure in a collection of world affliations were analyzed using a subset of the newly identifed SSR markers. The results of two analyses almost overlapped and suggested a correlation between genetic similarity and geographical closeness. Iqbal et al. [\(2018](#page-31-21)) reported on the calculation of the genetic diversity of sesame genotypes using morphological traits and SSR gene markers. To access the molecular genetic diversity at the molecular level of 70 genotypes from ecogeographic regions of the world, 235 gene markers were developed by mining expression sequence tag data from the NCBI database. The PIC content ranged between 0.36 and 0.82 with an average of 0.61. Neighbor-joining (NJ) analysis discovered that the fve main groups and grouping were independent of geographic origin. Stavridou et al. ([2021\)](#page-35-16) studied the characterization of genetic diversity present in a varied sesame landrace set using seven expressed sequence tag-simple sequence repeat (EST-SSR) markers coupled with a high-resolution melting (HRM) analysis. The PIC value of 0.82 indicates that the selected markers were highly polymorphic. The sesame genotypes were classifed into four major clades based on the principal coordinate analysis and dendrogram reconstruction of molecular data.

## *15.4.5 ISSR (Inter-simple Sequence Repeat)*

Zietkiewicz et al. [\(1994](#page-38-15)) developed the ISSR marker technique. ISSR is based on the amplifcation of DNA segments situated in between two identical but oppositely oriented microsatellite repeat regions, at a distance which allows amplifcation. Parsaeian et al. [\(2011](#page-33-20)) conducted a research to study the genetic variations between 18 genotypes of sesame taken from diverse agroclimatic parts of Iran along with 6 exotic genotypes from the Asian countries by means of combined agro-morphological and ISSR marker traits. Total 13 ISSR primers were chosen for molecular analysis revealed 170 bands, of which 130 (76.47%) were polymorphic. On the basis of ISSR profles, the generated dendrogram divided the genotypes into seven groups. A nonsignifcant co-phenetic correlation was observed in the Mantel test by studying genetic variation in sesame using agro-morphological traits and ISSR markers.

## *15.4.6 SNP (Single Nucleotide Polymorphism)*

Single base pair changes present in the sequence of an individual's genome are known as SNPs. SNPs are results of transition or transversion, and in plants, SNP frequency ranges between 1 SNP in every 100–300 bp. On the basis of different molecular mechanism, diverse types of SNP genotyping assays have been developed, and among them, allele-specifc hybridization, invasive cleavage, primer extension, and oligonucleotide ligation are most important (Sobrino and Carracedo [2005\)](#page-35-17). Several recent high-throughput genotyping methods such as chip-based NGS, GBS, and NGS and allele-specifc PCR make SNPs the most attractive markers for genotyping (Agarwal et al. [2008\)](#page-27-12). Uncu et al. ([2016\)](#page-36-15) reported an identifcation and mapping of high-throughput SNPs in the sesame genome with genotyping by sequencing (GBS) analysis. SNPs preferred through a high stringency fltering protocol (770 SNPs) for better map precision were used in concurrence with SSR markers (50 SSRs) in linkage analysis. This results in 13 linkage groups spanning a total genetic distance of 914 cM with 432 markers (420 SNP, 12 SSR). Wei et al. studied the three kinds of markers (SNPs, InDels, and SSRs) used for DNA fngerprinting of 151 sesame cultivars released in China. The 140 polymorphic markers used (47 SNPs, 47 InDels, and 46 SSRs) bare a narrow range of genetic variations. Of the 151 cultivars, 3 cultivars (AH03, AH04, and AH05 from Anhui Province) were considered synonymous cultivars due to their high coefficients of genetic similarity (> 98%). To distinguish all sesame cultivars overall, 15 SNPs, 14 InDels, and 9 SSRs were sufficient (Wei et al. [2017a](#page-37-9), [b](#page-37-10)).

#### **15.5 Plant Tissue Culture in Sesame**

Sesame is prevalently self-pollinated; however, the hybrids by conventional crosses with wild types were diffcult to produce because of the sexual incompatibility (Tiwari et al. [2011](#page-36-20); Kulkarni et al. [2017](#page-32-17)). Protoplast fusion and somatic hybridization using tissue culture techniques are effective strategies to overcome sexual incompatibility. Interspecifc hybrids have been successfully developed between cultivated variety of sesame and its wild relatives *S. occidentale* and *S. radiatum* through ovule and ovary culture by Dasharath et al. ([2007\)](#page-29-16). Rajeswari et al. [\(2010](#page-34-19)) have standardized an efficient protocol to produce hybrids of a cross between *Sesamum indicum* and *S*. *alatum* using ovule culture. The developed hybrids were resistant against phyllody disease. In vitro culture has been largely investigated in sesame which may result in somaclonal variations. Somaclonal variations are induced during callus induction and callus proliferation (Hoffman et al. [1982\)](#page-31-22). Repeated and prolonged subculturing of calli enhances the frequency of gross chromosomal aberrations and gene mutations (Sanal and Mathur [2008\)](#page-34-20). Regenerants resulting from such cultures are liable to carry heritable variations for seedling vigor, growth, capsule dehiscence, placental thickness, seed dormancy, seed size, yield, oil content, and oil quality (Bairu et al. [2006;](#page-28-21) Ram et al. [1990\)](#page-34-21). Sesame is exceedingly recalcitrant for in vitro regeneration.

Attempts have been made for direct as well as indirect regeneration and callus induction of sesame in tissue cultures using various explants such as cotyledons and/or hypocotyl (Younghee [2001;](#page-38-16) Baskaran and Jayabalan [2006](#page-28-22); Were et al. [2006a](#page-37-7), [b;](#page-37-8) Chakraborti and Ghosh [2009](#page-29-17); Yadav et al. [2010;](#page-38-17) Al-Shafeay et al. [2011;](#page-27-13)

Shashidhara et al. [2011;](#page-35-18) Rao and Honnale [2011;](#page-34-22) Honnale and Rao [2013](#page-31-23); Pusadkar et al. [2015](#page-33-21)), shoot tips (Lee et al. [1985;](#page-32-18) George et al. [1987;](#page-30-20) Baskaran and Jayabalan [2006\)](#page-28-22), de-embryonated cotyledon (Seo et al. [2007;](#page-35-19) Lokesha et al. [2012](#page-32-19); Malaghan et al. [2013](#page-32-20); Chowdhury et al. [2014](#page-29-18); Pusadkar et al. [2016\)](#page-34-23), embryo (Saravanan and Nadarajan [2005\)](#page-34-24), and nodes (Gangopadhyay et al. [1998\)](#page-30-21). Cotyledon or hypocotyl has proven signifcantly successful as an explant for plant regeneration by somatic embryogenesis (Younghee [2001;](#page-38-16) Baskaran and Jayabalan [2006;](#page-28-22) Yadav et al. [2010;](#page-38-17) Honnale and Rao [2013\)](#page-31-23). All these studies achieved varying degree of success in terms of callus growth and regeneration. Callus induction and shoot regeneration frequency were signifcantly enhanced by supplementing cytokinins and auxins with nutrient media (Baskaran and Jayabalan [2006,](#page-28-22) Wadeyar and Lokesha [2011;](#page-37-20) Honnale and Rao [2013;](#page-31-23) Zamik et al. 2017; Gayatri and Basu [2020\)](#page-30-22). Auxins and cytokines alone as well were found capable of promoting regeneration in cultures (Baskaran and Jayabalan [2006](#page-28-22); Yadav et al. [2010](#page-38-17)). Genotypes, explant type, type of growth regulators, concentration of growth regulators, age of the explants, and the developmental stage of explants were revealed as crucial factors that governed in vitro shoot regeneration and somatic embryogenesis (Mary and Jayabalan [1997;](#page-32-21) Venkatachalam et al. [1999](#page-36-21); Seo et al. [2007;](#page-35-19) Malaghan et al. [2013;](#page-32-20) Zimik and Arumugam [2017\)](#page-38-18). However, some cotyledonary explants were also found to undergo necrosis after supplementing with cytokinins (Baskaran and Jayabalan [2006;](#page-28-22) Were et al. [2006a,](#page-37-7) [b\)](#page-37-8). Auxin and cytokinin treatment has been found to induce rooting in cotyledon culture of sesame (Were et al. [2006a,](#page-37-7) [b;](#page-37-8) Seo et al. [2007;](#page-35-19) Zimik et al. 2017). ABA and  $AgNO<sub>3</sub>$  were also reported to enhance shoot regeneration if supplemented with plant growth regulators (Seo et al. [2007,](#page-35-19) W deyar and Lokesha  $2011$ ). AgNO<sub>3</sub> inhibits ethylene which is produced during in vitro culture (Chi and Pua [1989](#page-29-19)) and responsible for recalcitrant behavior of tissues in culture (Chi and Pua [1989;](#page-29-19) Shashidhara  $2005$ ). AgNO<sub>3</sub> have also been reported to enhance conversion of somatic embryos to plants (Honnale and Rao [2013](#page-31-23); Xu et al. [1997\)](#page-38-19). Abscisic acid promotes seed maturation and inhibits seed germination (Zeevaart and Creelman [1988](#page-38-20)). The impact of ABA is broadly studied in somatic embryogenesis. ABA treatment prevents the precocious germination of embryos (somatic) and generation of secondary embryo (Choi and Jeong [2002](#page-29-20); García-Martín et al. [2005](#page-30-23)).

#### **15.6 Concluding Remarks**

Due to increasing health awareness, people are more concerned about nutrition quality of food. Sesame is a very promising crop of future prospects due to its highquality oil for nutritional and industrial purposes. Sesame is an underutilized and poor farmer's crop and is ft for sustainable agricultural development. Improvement of the sesame crop can be achieved by various methods such as conventional and molecular breeding methods to obtain agronomically elite lines. Breeding efforts so far made in the country have not resulted in any substantial and signifcant breakthroughs in terms of productivity and yield stability. Data so far available from yield trials carried out at CCS HAU clearly show that none of the high-yielding varieties are available till date with incorporating multiple stress tolerance. The future of sesame as a commercial crop totally now totally depends upon developing highyielding strains with inbuilt resistance to various biotic and abiotic stresses to overcoming the present yield barriers. Unluckily, the on hand germplasm materials have not yet been fully and thoroughly exploited in sesame crop improvement program. Recent years have witnessed a continuously increasing number of functional genes discovered for key agronomic traits in sesame, thanks to the availability of omics tools. In this regard, the future strategies in the sesame breeding are the functional validation of these gene resources through genetic engineering approaches and marker-assisted breeding.

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