Chapter 7 Advances in Caulifower (*Brassica oleracea* **var.** *botrytis* **L.) Breeding, with Emphasis on India**

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Abstract Among the cole vegetables, caulifower is a widely grown crop worldwide for its nutrients and favor. It is a thermosensitive crop for its curd formation and development. Different cultivar groups in caulifower are known such as Italian or Original, Cornish, Northerns, Roscoff, Angers, Erfurt, Snowball and Indian, based on phylogeny and plant traits. The Indian caulifower group evolved from European caulifower and later classifed as early, mid-early, mid-late and late, depending upon temperature requirements related to curd initiation and development. A large number of varieties and hybrids have been developed in tropical caulifower, for different maturity groups and established using a cytoplasmic male sterility (CMS) system for hybrid breeding. Recently, biotechnological tools such as DNA markers, genomics and tissue culture for doubled haploid development, prebreeding for introgressing genes/QTLs from alien brassicas were deployed in caulifower breeding. Resistant sources identifed in cole vegetables for black rot and downy mildew by genetic investigations revealed single dominant gene governance of resistance for both diseases. Caulifower is one of the best candidate crops for β-carotene biofortifcation, hence a natural mutant native *Or* gene was introgressed into Indian caulifower. Besides, transgenesis is underway to develop diamondback moth resistant varieties by stacking *cry 1b* and *1c bt* genes in caulifower. This chapter highlights recent developments in caulifower breeding particularly in tropical types.

Keywords Glucosinolates · Hybrid · Indian caulifower · Male sterility · Molecular markers · Orange caulifower · Resistance · Self-incompatibility

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7.1 Introduction

Caulifower (*Brassica oleracea* var. *botrytis* L.; 2n = 2x = 18) is an important cole vegetable belonging to Brassicaceae family. It grows at latitudes varying from 45° S in New Zealand to 65° N in Scandinavian countries. Asia is the leading producer followed by Europe and covers small hectarage in North America, South America, Africa, Australia and New Zealand. Globally, caulifower is cultivated on 1.38 million ha; India and China together account for about 69% area, of that, India alone contributes nearly 34%. Similarly, both countries share almost equally 75% of global caulifower production (24.18 million mt) (FAOSTAT [2017](#page-47-0)). India is the second largest producer of caulifower in the world after China. Presently, caulifower occupies 0.45 million ha in India with a production quantity of 8.9 million mt (NHB Database [2017\)](#page-50-0).

In 1822, caulifower was frst cultivated in United Province (now Uttar Pradesh) India during British rule as a choice food by the British. Later, introduced genotypes became adapted to local environments to evolve an entirely new ecotype, grouped as Indian caulifower or Tropical caulifower. It has a tolerance to high temperature for vegetative growth $(>35 \text{ °C})$ and for curd initiation and development stages (>27 °C) (Gill and Sharma [1996\)](#page-47-1). Caulifower growing areas expanded into tropical regions and seasons (as extra-early and early crops) in India (Kalia et al. [2016](#page-48-0)). The earliest varieties of this type were Early and Main Crop Patna and Early and Main Crop Benaras (Gill and Sharma [1996](#page-47-1)). Since then, major developments have occurred to breed improved varieties/hybrids through conventional and nonconventional breeding methods i.e., selection for simple traits, backcrossing for introgression of resistance or male sterility related genetic mechanisms, pre-breeding for development of genetic stock for novel or complex traits, and use of recent molecular and gene-editing tools. Consequently, caulifower cultivation area has expanded both temporally and spatially even in non-traditional areas and became established as an important vegetable crop in India as a popular cool season vegetable crop. However, development of tropical types extended the growing period to both extremes of winter season in India and other countries. Caulifower is popular among growers due to its short crop duration (60–80 days), high crop yield (15–35 mt/ha), low level of disease and insect pest incidence (mainly in the winter season crop) and better returns per unit area and time expended (Kalia et al. [2016\)](#page-48-0). The curd is the edible part of caulifower, which is made of pre-foral apical meristematic tissues. It is a dome of tissues made up of a mass of proliferated foral meristems at harvest. In some regions, tender leaves are eaten as a leafy vegetable, after boiling, frying or mixing with other vegetables. Consumers prefer caulifower for its unique taste, diverse delicacies, purported anti-cancer glucosinolates and other essential minerals and vitamins. Nutritionally, caulifower is a good source of dietary fibers (2%) , protein (1.9%) and potassium $(299 \text{ mg}/100 \text{ g})$. It is an ideal candidate crop for biofortifcation of β-carotene; hence, Kalia et al. [\(2018](#page-49-0)) developed the first β-carotene fortified tropical cauliflower, Pusa KesariVitA-1, that contains β-carotene in a range of 8–10 ppm in the edible portion of the curd (Anonymous [2016\)](#page-45-0). It has great potential to challenge widespread defciency in human populations in developing countries. Consumed singly or in combination with other vegetables, caulifower is also processed by blanching, pickling or freezing.

For systematic analysis and compilation of information on caulifower, the present chapter details a holistic presentation of information on important components such as an understanding of evolution using modern tools, development for hybrid breeding, use of molecular markers, developments in breeding for quality traits like glucosinolates and biofortifcation for β-carotene and anthocyanin content. It also describes resistance breeding for biotic and abiotic stresses along with the use of recent techniques such as transgenics for insect resistance.

7.1.1 History and Evolution of Indian Caulifower

The origin of caulifower is the island of Cyprus and the Eastern Mediterranean (Gustafson [1994\)](#page-47-2); it has been cultivated in Europe since the ffteenth century (Grout [1988\)](#page-47-3). It was dispersed to other areas like Syria, Turkey, Egypt, Italy, Spain and northwestern Europe (Boswell [1949](#page-45-1)), and is now grown worldwide including in parts of tropical regions during cooler months (Fig. [7.1\)](#page-3-0). In India, caulifower was frst introduced in Saharanpur, Uttar Pradesh (then called United Provinces) in 1822 (Swarup and Chatterjee [1972\)](#page-53-0). Afterwards, the local growers initiated production of caulifower seeds locally, which helped in its early adaptation to Indian climatic condition. Over the period of 1822–2019, introduced caulifower underwent remarkable adaptation to heat and humidity tolerance as well as other plant traits. The selection for good horticultural traits along with heat tolerance was a major attempt toward the development of Indian caulifower. It has an early maturing type, satisfactory seed yield (300–400 kg/ha) in north Indian conditions and has wide adaptability to hot and humid weather. The earliest varieties of caulifower in India were Early and Main crop Patna and Early and Main crop Banaras, developed by Sutton and Sons (Gill and Sharma [1996](#page-47-1)).

Swarup and Chatterjee ([1972\)](#page-53-0) demonstrated close morphological affnity between Indian caulifower and different western European types like Cornish, Roscoff, Italian, Northern, Angiers and Snowball or Erfurt, though not exactly the same. Giles [\(1941](#page-47-4)) opined that Indian caulifower is a dwarf selection of Erfurt or Snowball types, a view supported by Nieuwhof ([1969\)](#page-50-1). He reported that early varieties were selections from Erfurt-Alpha types, which performed better in warmer regions (20 $>$ °C). As per the climatic conditions of north India, the typical Indian caulifowers are categorized in two groups (I, II), and mature on an average daily temperature $20 > C$. They have a long stalk, open growth habit, exposed yellowish to creamy and uneven cruds, which loosen easily and with strong favor. Some of their characters are typical of the Cornish cultivar while some leaf and curd characters resemble Roscoff and Italian cultivars. Indian caulifower genotypes mature during December–January and show some phenotypic affnity with Snowball or Erfurt types (European Summer Group) and Italian autumn caulifower (Gill and

Fig. 7.1 Caulifower distribution and production data. (**a**) Distribution of caulifower types, (**b**) Production in the world. (Source: FAOSTAT [2018](#page-47-5))

Sharma [1996\)](#page-47-1). Group-I of Indian caulifower has a high degree of self-incompatibility, high heterosis and resistance to black rot disease (Swarup and Chatterjee [1972](#page-53-0)). The Cornish type was the earliest introduction to India, which contributed numerous genes to present-day Indian caulifowers. Indian seedsmen and growers have contributed signifcantly to the development of Indian caulifower varieties. The seedsmen of Hajipur (Bihar) specialize in the varieties of group I and II. Foreign seedsmen like Sutton and Sons (which later became Indian) played a signifcant role in this venture. The *aristocratic* character of European caulifower has undergone transformation to a *cosmopolitan* status showing fexibility of adaptation to regions from New Zealand to the Scandinavian countries. It is also signifcant to note that of the total area under caulifower in India about 40% is represented by hot weather tropical caulifowers, which includes Groups 1 and II (Seshadhri and Chatterjee [1996\)](#page-51-0). Crisp and Tapsell [\(1993](#page-46-0)) proposed the evolutionary development for caulifower as follows:

- (a) A wild, annual, eastern Mediterranean subspecies of *Brassica oleracea* (or *B. nivea*; white fower in cyme, a primitive form of broccoli, with terminal and perhaps lateral shoots of dense buds as the edible portion) was domesticated several years ago.
- (b) The introduction of this species took place in the east, and southern China where adaptive changes developed into the only Chinese *endemic* crop of *B. oleracea*, the Chinese broccoli or kale (*B. alboglabra*). It is a branched annual, usually with white fowers (yellow fowers also occur because of introduction of other *B. oleracea* crops and their possible part in natural crossing).
- (c) The ancestral broccoli dispersed to the west, where natural hybridization with other wild and cultivated *B. oleracea* group types resulting in many forms around the Mediterranean Region. Notably, hybridization with the yellowfowered, racemose, biennial western European *wild cabbage* gave rise to biennial types.
- (d) Around 500 years ago, selection for increased terminal head size and probably major gene mutation for greatly enlarged, immature foral buttons (i.e. curd) associated with decreased lateral branching from the stem below the curd. This phenotype may have arisen repeatedly within the diverse broccoli gene pool or may have spread by intentional or accidental introgression.

Over the years, many local types of caulifower evolved (large terminal curd) and broccoli (large terminal heads of tightly packed fower buds, or with many side branches) became established around the Mediterranean and in Europe. Annual caulifowers became an important crop in several inland regions, and biennial caulifower varieties (giving curds from late autumn until early summer) were developed in coastal regions where winter temperatures were buffered by the marine infuence.

(e) During British colonization, diverse types of caulifowers were introduced to India and Australia, where genetic recombination gave rise to distinct types, some adapted to tropical conditions. At least some of the adaptation shown by tropical caulifowers may have arisen by mutation. It was the single dominant

Early group white Indian cauliflower

Mid-Early group white Indian cauliflower

Indian cauliflower

Snowball group white cauliflower

Green cauliflower

Mid group orange cauliflower

Fig. 7.2 Diversity in curd colors in caulifower. (Photo is credited to Dr. Shrawan Singh)

gene and cytoplasmic effect, which confers *tropical* characteristics to an Indian cultivar in comparison with a European annual cultivar.

(f) The direction of breeding played a key role in evolution of modern-day caulifower. In recent years, breeding has concentrated on annual, white-curded types of caulifower with large, worldwide sales of seed.

In recent years, new colors also gained attention particularly orange (due to β-carotene), purple (anthocyanin) and green (chlorophyll) curding caulifower (Fig. [7.2\)](#page-5-0). These are showing uniqueness over the traditional cream white to persistent white curding caulifowers in terms of nutritional and specialty traits.

7.1.2 Curding and Flowering Trait Genetics

In caulifower, curd consists of a dense mass of arrested inforescence meristem, only ~10% of which develop into foral primordia and normal fowers. The caulifower *curd* phenotype in mutants of *Arabidopsis thaliana* is due to a class of fower developmental regulatory genes viz., *APETALA 1* (*AP1*, Mandel et al. [1992](#page-50-2)) and *CAULIFLOWER* (*CAL*; Kempin et al. [1995\)](#page-49-1) that specify the foral meristem identity (as opposed to the inforescence meristem) developing reproductive primordia. *Arabidopsis* mutants (*AP1* and *CAL*) are arrested in inforescence development at the meristem stage and develop into a dense mass similar to caulifower curd. Orthologous genes *BoCAL* are involved to alter inforescence in caulifower (Kempin et al. [1995\)](#page-49-1). The *BoCAL* allele has a premature termination codon at position 151 $(E \rightarrow stop)$ which appears to be of recent origin. Alleles carrying this nonsense mutation in exon 5 of *BoCAL* are fxed in caulifower and broccoli, both of which show evolutionary modifcations of inforescence structures (Purugganan et al. [2000\)](#page-50-3). Hence, specifc alleles of *BoCAL* were selected at an early stage of evolutionary domestication of subspecies within the vegetable crops of *Brassica oleracea*.

Based on molecular allelic variation, Smith and King [\(2000](#page-53-1)) suggested that heading Calabrese broccoli was the source of modern caulifower (compact curds) via an intermediate Sicilian crop type which has heads of an intermediate type. Close association of *BoAP1-a* and *BoAP1-c* with the self-incompatibility locus S may have reduced the number of S-alleles within the gene pool. Duclos and Bjorkman [\(2008](#page-47-6)) investigated the transcript abundance of *BoFUL* paralogues and *BoLFY*, fnding it was highest at inforescence meristem arrest and maintenance of this arrest is a consequence of suppression of *BoCAL*, *BoAP1-a*, or *BoLFY*, or failure to suppress *BoTFL1* (a strong repressor of fowering in *Arabidopsis*). Li et al. [\(2017](#page-49-2)) identifed a novel homologous gene containing the Organ Size Related (OSR) domain *CDAG1* (Curd Development Associated Gene 1) in caulifower. It has higher transcript levels in young tissue and promotes organ growth by increasing cell numbers, which results in a larger organ size and increased biomass. This gene inhibits transcriptional expression of endogenous OSR genes, *ARGOS* and ARL. Rosan et al. [\(2018](#page-51-1)) studied a genome-based model simulating the development of doubled haploid (DH) lines to time to curd induction and observed $R^2 = 0.40$ for the quantitative traits and $R^2 = 0.48$ for the GS model. Duclos and Bjorkman [\(2008](#page-47-6)) reported increased *BoAP1-a* and *BoAP1-c* transcript levels in caulifower just before foral-primordium initiation. Application of GAs during reproductive development stage does not activate meristem identity genes or A-function genes (Yu et al. [2004\)](#page-54-0). Hence, GAs $(GA_3 \text{ and } GA_{4+7})$ can trigger the vegetative-toreproductive transition in both caulifower and broccoli resulting in early curd formation (Duclos and Bjorkman [2015](#page-47-7)). Recently, Singh et al. ([2020\)](#page-53-2) studied genetics and expression analysis of anthocyanin accumulation in the curd portion of Sicilian purple to facilitate biofortifcation of Indian caulifower.

7.1.3 Caulifower Groups

Caulifower evolution continued in different regions depending upon prevalent climatic situations. The evolved groups remained geographically isolated for a long period (except for the Italians or Originals) within insulated populations and restricted breeding. Based on morphological characters, Swarup and Chatterjee [\(1972](#page-53-0)) classifed present-day cultivars of caulifower into seven broad groups (Table [7.1\)](#page-7-0) so that a proper understanding and relationship of them is possible. Further, Crisp ([1982\)](#page-46-1) also classifed caulifowers according to their phylogeny (Table [7.2](#page-7-1)). However, further studies were made to separate grouping of the North European annual and Australian types (Chatterjee [1993\)](#page-46-2).

		Probable period	
Cauliflower	Area/country of	of first	
types	origin	cultivation	Characters
Italians or Original	Mediterranean	Sixteenth century	Plants short, leaves erect broad with rounded tips, bluish green, curds good not protected by leaves
Cornish	England	Early nineteenth century	Plants vigorous, long stalked, leaves loosely arranged, broadly wavy, curds flat, irregular, loose, not protected, yellow, highly flavored
Northerns	England	Nineteenth century	Leaves petiolate, broad, very wavy, serrated, curds good, well protected
Roscoff	France	Nineteenth century	Plants short, leaves long erect, slightly wavy with pointed tip, midrib prominent, bluish green, curds white or creamy, hemispherical, well protected
Angers	France	Nineteenth century	Leaves very wavy, serrated, greyish green; curds solid, white, well protected
Erfurt and Snowball	Germany and Netherlands	Eighteenth century	Plants dwarf; leaves short, erect, glaucous green, curds solid, well protected
Indian cauliflower	India	Late nineteenth century	Plants short, long stalked, leaves loosely arranged, broadly wavy, curds flat, somewhat loose, yellow to creamy, not protected and highly flavored

Table 7.1 Broad groups of cauliflower based on origin and morphological characters

Source: Adapted from Sharma et al. ([2004\)](#page-52-0)

Group	Chief Characteristics	Common types
Italian	Very diverse, include both annuals and biennials and curds with peculiar conformations and colors	Jezi, Naples (Autumn Giant), Romanesco, Flora Blanca
North-West European biennials	Derived within the last 300 years from Italian material	Old English, Walcheran, Roscoff, Angers, St. Malo
North European annuals	Developed in northern Europe for at least 400 years. Origin unknown, perhaps Italian or Eastern Mediterranean	Lecerf, Alpha, Mechelse, Erfurt, Danish
Asian	Recombinants of European annuals and biennials developed within 250 years, adapted to tropics	Four maturity groups are recognized by Swarup and Chatteriee (1972)
Australian	Recombinants of European annuals and biennials and perhaps Italian stock, developed during the last 200 years	Not yet categorized

Table 7.2 Grouping of cauliflower according to phylogeny

Source: Adapted from Sharma et al. ([2004\)](#page-52-0)

7.1.4 Indian Caulifower Classifcation

Indian (or Asian) caulifower is classifed into four groups viz. early, mid-early, mid-late and late or snowball types, based on thermosensibility (Table [7.3\)](#page-8-0). The frst three types include Indian or tropical types and the selections made for this purpose perform well, producing quality curds even during May–June, making it possible to grow caulifower almost year around (Chatterjee [1993](#page-46-2); Singh and Sharma [2001\)](#page-52-1). There are several local cultivars in India of varying maturity, commonly named after the season of curd maturity, such as, Kunwari (September–October), Katki (October–November), Aghani (November), Poosi (December) and Maghi (January). These cultivars are highly heterozygous with respect to all characters, whether vegetative, curd or maturity. These cultivars have short a short stature, bluish green leaves with a waxy bloom and with a very small meristem curd tending to grow loose faster. They are also sensitive to buttoning earlier. Mainly private seed companies of Hajipur (Bihar) and Ayodhya the then Faizabad (Uttar Pradesh) regions market seeds of these local types. Many of these local cultivars are cultivated in Bihar, Uttar Pradesh, Punjab, Haryana, Rajasthan, Madhya Pradesh, Maharashtra and

Maturity group	Traditional groups	Sowing time	Mean temperature for curd initiation $\&$ development	Harvest or period	Cultivar
Early	Kartiki	June	$20 - 27$ °C	September- November	Pusa Meghna Pusa Ashwini Pusa Kartiki Pusa kartik Sankar Pusa Deepali
Mid-early	Aghani	End of July-August	$16 - 20$ °C	November- December	Pusa Sharad. Pusa Hybrid-2, Improve Japanese Pusa synthetic
Mid-late	Poosi	End of August- September	$12 - 16$ °C	December- January	Pusa Himjyoti Pusa Paushja Pusa Shukti
Late	Maghi	September- November	$10-16$ °C	January- March	Pusa Snowball $K-1$, Pusa Snowball 1. Pusa Snowball $K-25$ Pusa Snowball Hybrid-1

Table 7.3 Grouping of caulifower based on temperature requirement for curd initiation and development

Source: Singh et al. [\(2018](#page-52-2)) a Under northern Indian plains Gujarat. The earliest selected local caulifower varieties, Early and Main crop Patna, Early, and Main Crop Banaras were from M/s Sutton and Sons, India, in 1929. However, the systematic breeding program in Indian caulifower started around fve decades ago and helped in the genetic shift towards desirable traits and germplasm diversifcation, which, later on, acted as source for breeding programmes across the countries. Over the years, the work of breeding for heat tolerant varieties and temporal shift for earliness in Indian caulifower has resulted in the development of a range of varieties (Table [7.3\)](#page-8-0). These are open-pollinated varieties, which further served as a source breeding materials for developing varieties for local climatic condition in different regions of the country. Today, a number of varieties and hybrids in the public and private sector are available for different maturity groups. Table [7.3](#page-8-0) shows important public sector varieties/hybrids.

7.2 Genetic Diversity and Exploration of Wild Relatives

7.2.1 Genetic Diversity

The greatest genetic diversity of caulifower is in the Mediterranean gene center including Greece, Syria, Cyprus, Sicily, Italy, Spain and Portugal. However, wild species or subspecies and improved heterogeneous cultivars have been primary genetic resources for various characters available to the breeders. Introgression from wild species or primitive forms of *Brassica oleracea* in the Mediterranean area and northwestern Europe has resulted in the great genetic diversity found in caulifower. The annual types of caulifower occur in Italy and surrounding areas. The introduction of improved cultivars and hybrids into Europe has replaced many of the landraces, primitive cultivars and traditional varieties or types. There is extensive genetic erosion but fortunately, many genetic resources are still available in the Mediterranean gene centers. In addition to landraces in Italy, caulifower genetic diversity also exits in France, UK, Sweden, Denmark and the Netherlands. In Europe, researchers developed caulifower varieties in the sixteenth century, such as, Originals or Italians (Jezi, Naples, Romanesco, Flora Blanca), Erfurt, Alpha and Snowball in Germany and the Netherlands, Cornish and Northerns in England and Roscoff and Angers in France. The European biennials include Old English, Walcheran, Roscoff, Angers and St. Malo and the annuals like Alpha, Erfurt, Danish, Lecerf and Mechelse. The two other groups of caulifower suggested are the Indian (or Asian) and Australian, due to recombination of European annuals and biennials.

Genetic diversity is an important factor and pre-requisite for heterosis breeding. Hybrids between genetically-diverse parents manifest greater heterosis than those of closely-related parents. However, Tonguc and Griffths [\(2004a\)](#page-53-3) reported a very low extent of genetic diversity, which hinders modern breeders from producing new caulifower varieties with high yield and specifc qualities. Researchers have employed different molecular markers to quantify the genetic diversity level in cauliflower. Dey et al. (2011) (2011) did line \times tester analysis in Snowball group of caulifower using three CMS lines (Ogu1A, Ogu2A and Ogu3A) and nine diverse lines. The number of heterotic hybrids for yield and earliness was low, indicating the narrow genetic base of the Snowball caulifower. A great extent of variability evolved in tropical early caulifower (Santhosha et al. [2011\)](#page-51-2). The authors studied 51 genotypes using 16 quantitative characters and reported 14 clusters, of them genotypes of Cluster 8 (IIHR-323-13, IIHR-214-5, IIHR-277-14) and cluster 10 (IIHR-263, IIHR-272) as the best choice for hybridization. Similarly, Astarini et al. ([2006\)](#page-45-2) also reported genetic variation and relationships among 8 Indonesia-, Australian- and European-based cultivars and within Indonesia open-pollinated cultivars using RAPD and ISSR markers. The comparison between the two groups showed that Indonesian cultivars evolved to unique genotypes and would be promising sources of genes for future crop improvement. El-Esawi et al. [\(2016](#page-47-8)) reported 27.1% genetic variation among accessions while 72.9% within the accessions in cabbage, caulifower, Brussels sprouts and kale using SSR markers from Ireland. Yousef et al. [\(2018](#page-54-1)) characterized 192 caulifower accessions from the USDA and IPK gene banks with genotyping by sequencing (GBS) which formed two major groups representing the two gene banks. They indicated that the composition and type of accessions have a strong effect on the germplasm structure, although regeneration procedures and local adaptation to regeneration conditions also exert infuence. Meanwhile, primary and secondary centers of diversity still have wild relatives and/ or original types of caulifower. However, local landraces are being replaced rapidly by improved cultivars and hybrids due to yield advantages. Hence, in situ conservation of caulifower genetic resources is currently diffcult for caulifower. However, ex situ conservation is a common approach. The Horticultural Research International, Wellsbourne, Warwick, UK; Instituut voor de Veredeling van Tuinbouwgewassen, Wageningen, Netherlands; Instituto del Germoplasm, Bari, Italy; Indian Agricultural Research Institute, New Delhi; Indian Agricultural Research Institute Regional Station, Katrain, Himachal Pradesh and the National Bureau of Plant Genetic Resources, New Delhi are managing signifcant collections of caulifower germplasm. Germplasm conservation through in vitro propagation of caulifower is feasible using seedling explants (Arora et al. [1997\)](#page-45-3), protoplast culture (Yang et al. [1994\)](#page-54-2) and anther culture (Yang et al. [1992](#page-54-3)). Culture of curd explants on MS medium with 6-benzyladenine (cytokinin) and gibberellic acid is an effective way to regenerate caulifower plants (Bhalla and De Weerd [1999\)](#page-45-4).

7.2.2 Pre-breeding for Caulifower Improvement

Pre-breeding is an important activity in crop improvement and covers all activities designed to (i) identify desirable characteristics and/or genes from nonadapted (exotic or semi-exotic) materials and (ii) transfer these traits into an intermediate set of materials. In case of the diverse cole vegetables, a large number of species have been exploited for CMS or other important traits. The wild or related species for

B. oleracea are canola (*Brassica napus* L. and its hybrids with *B. campestris*), *B. macrocarpa*, *B. villosa*, *B. rupestris* and *B. incana* in addition to turnip (*B. rapa* ssp. *rapa* L.), *B. campestris* L., *B. napobrassica* L., *B. nigra* Koch, *B. juncea* (L.) Czern. and *B. carinata*. Sharma et al. [\(2016](#page-52-3)) initiated introgression of black rot resistance *Xca1bc* locus (on B7 chromosome) from *B. carinata* to *B. oleracea* var. *botrytis* using ILPAt1g70610 marker and embryo rescue. Dey et al. ([2015\)](#page-46-4) attempted introgression of black rot resistance (for both race 1 and 4) from *B. carinata* into Snowball caulifower using embryo rescue.

7.2.3 Embryo Rescue

Embryo rescue can help overcome natural reproductive barriers in the development of interspecifc hybrids in *Brassica* (Ayotte et al. [1987;](#page-45-5) Hansen and Earle [1995;](#page-48-1) Momotaz et al. [1998](#page-50-4); Niemann et al. [2013;](#page-50-5) Weerakoon et al. [2009\)](#page-54-4). Wide crosses between crop plants and their wild relatives have become routinely, possible by the embryo rescue technique. Different techniques of plant cell and tissue culture, such as ovary, ovule and embryo culture as well as protoplast fusion, have proved useful for production of interspecifc hybrids. Rescue of hybrid embryos and their culture in vitro helps to overcome post-fertilization barriers in interspecifc crosses. Since its frst use in *Brassica* by Nishi et al. [\(1959](#page-50-6)), extensive investigations to improve the techniques for obtaining higher seed set have been carried out by Inomata [\(1993](#page-48-2), [2002\)](#page-48-3) and Zhang et al. ([2003,](#page-54-5) [2004\)](#page-54-6). The successful application of this technique depends on the stage of the rescued embryo cultured in vitro*.* Several attempts have been made to transfer desirable gene(s) from alien *Brassica* spp. to *B. oleracea*, such as powdery mildew resistance (Tonguc and Griffths [2004b\)](#page-53-4), downy mildew (Chiang et al. [1977](#page-46-5)), male sterility (Chiang and Crete [1987](#page-46-6)) and atrazine resistance (Jourdan et al. [1989\)](#page-48-4). Progress toward marker-assisted *Xcc* resistance gene transfer from *B. carinata* to caulifower has been very slow (Tonguc and Griffths [2004c;](#page-53-5) Tonguc et al. [2003](#page-53-6)).

7.2.4 Conservation Strategies

Sporophytic self-incompatibility (SI) in caulifower prevents pollination of fowers on the same plant. In caulifower, it is active only after anthesis; hence, the germplasm having SI needs to be maintained at bud stage by bud-pollination at 2–4 days prior to anthesis (Kalia [2009\)](#page-48-5). Singh and Vidyasagar ([2012\)](#page-52-4) reported that NaCl sprays (3–5%) are effective in temporarily breaking down self-incompatibility in cabbage. However, strong SI lines and male sterile lines can also be maintained by tissue culture (Bhalla and De Weerd [1999](#page-45-4); Bhatia et al. [2014](#page-45-6)).

7.3 Breeding Objectives

In caulifower, the most important objectives of breeding new varieties/hybrids are high commercial quality, including adequate curd size and shape, good and attractive color, compact and frm curds and uniformity of feld appearance of plant type (Kalia [1994\)](#page-48-6). A high level of uniformity is diffcult to achieve in the case of openpollinated varieties and in F_1 hybrids using conventionally developed inbred lines due to the cross-pollinating nature of caulifower. Hence, use of doubled haploid (DH) lines is needed to produce F_1 hybrids with a great extent of uniformity and in reduced time span. The different kinds of CMS system established in caulifower genotypes for use in hybrid breeding can be used to tap the heterotic potential of caulifower groups. Development of varieties/hybrids resistant to diseases and insect pests is an important objective both to reduce pesticide load on food and in the environment. Quality traits such as curd favor, persistent white color, curd texture and compactness and shape need adequate attention. In caulifower, selective approach for glucosinolates is required because some have harmful health effects while there are glucosinolates, which possibly counter cancerous agents. Novel or *specialty traits* desired by certain consumers such as orange, green and purple curds in caulifower could be tackled for better consumer health and premium price for farmers. Further, in a changing climatic scenario, development of resilient open pollinated/hybrids varieties enabled with traits such as reduced crop period, extended reproductive phase, elongated root length and better blanching habit are desirable. On the basis of genetic stocks studied in Indian caulifowers, the ideotype of caulifower should possess: (i) stem length: 12–25 cm, (ii) plant type: No. 3, (iii) frame (spread): 35–45 cm, (iv) leaf number per plant: 18–22, (v) leaf length: 50–55 cm, (vi) curd shape: hemispherical, (vii) curd diameter: 15–18 cm, curd weight: 750–1000 g, (viii) curd color: retentive white, (ix) resistance to: black rot, curd and inforescence blight and (x) curding period: better plasticity for extended growing period.

7.4 Divergence in Important Characters

7.4.1 Maturity of Curd

Chaterjee and Swarup [\(1972](#page-46-7)) classifed Indian caulifowers into three maturity groups: (i) Maturity group I – Curds harvested from September to early November, curds loose, cream to yellow and strong flavored; (ii) Maturity group $II -$ Curds harvested from mid-November to early December, curds are somewhat loose, cream white and have strong flavor and (iii) Maturity group III – Curds harvested from mid-December to mid-January, curds are more compact and somewhat whiter curds not so strongly favored and has Plant type 3 features.

Group I and II have Plant Type 2 and characteristic typical features of the Cornish type. However, intensive development in the past three decades transformed Indian type with development of varieties having short-to-medium stalk length, white, compact and partial dome shape with curds covered partially. The varieties of Maturity group I with partial covering and good curds are Pusa Deepali, Pusa Ashwini and Pusa Kartiki.

7.4.2 Plant Type

On the basis of growth habit, three plant types were identifed: (i) Plant type No. 1 – long stalk, curds completely exposed with fat leaves; (ii) Plant type No. 4 – completely erect habit of leaves with covered curds and (iii) Plant types No. 2 and 3 are intermediates, the former being close to No. 1 and the latter approaching No. 4 (Swarup and Chatterjee [1972](#page-53-0)). Among them, Plant type No. 3 was considered best as it had long, erect leaves with or without blanching habit and medium-sized curds. It corresponds to the plant type of Snowball. Plant type No. 2 is the most common among Indian caulifowers.

7.4.3 Stem Length

There is large variation in stem length of different caulifower germplasm. Chatterjee and Swarup [\(1972](#page-46-7)) classifed Indian caulifower into three groups based on stem length: (i) shoot (215 cm) ; (ii) medium $(16-20 \text{ cm})$ and (iii) long $(21 > \text{ cm})$. Stalk duration was longer in Maturity Groups I (September to Early November) and Group II (Mid November to Early December) than Group III (Mid December to Mid January). Long stem was only present in Group I. Medium stem length was predominant in Maturity Group II. Maturity group III mostly had a short-stalk (54.5%) and medium-length stalk (45.5%).

7.4.4 Stem Pigmentation

The stem may be green or pigmented as in the Snowball group. However, the intensity of pigmentation may vary in different types of caulifower. Stem pigmentation is important as a dominant marker gene character. Purple pigmentation appears in the apical region of some genotypes but that does not persist in later growth and curding stages.

7.4.5 Leaf Characters

The leaves may be long and narrow, long and broad, and short and broad. The leaf margins are straight or broadly wavy. Leaf color varies from bluish green and wavy green to glossy green. A recessive gene governs the glossy leaf. The number of leaves ranges from 18 to 50 during the curding stage. Generally, there are more leaves in early-maturing types than in the late-maturing types.

7.4.6 Self-Blanching Character

When the inner leaf whorls are joined at the top of the curd, the plant is known as a self-blanching type. Caulifower types vary greatly in this character i.e*.*, not covered (early group), partly covered (mid group) and covered (Snowball group). However, partial covering also has been introgressed in some early varieties.

7.4.7 Curd Shape

The shape of caulifower curd may be circular (Pusa Himjyoti), broad elliptic (mid and Snowball groups) and narrow elliptic (early group). Further, curd doming is another trait which appears to be weak (early group), medium (mid and Snowball groups) and strong (Pusa Paushja).

7.4.8 Curd Size and Weight

Caulifower germplasm has broad genetic variation concerning size and weight of the curds. Polar diameter is defned as small (<15 cm), medium (15–20 cm) and large (20 > cm) curds. Similarly, equatorial diameters are also categorized as small $\left($ <15 cm), medium (15–20 cm) and large (20 > cm) curds. Most early varieties are grouped in the small category, while Snowball types have large curds.

7.4.9 Curd Compactness and Texture

Loose, medium and compact are common categories of curd compactness while fne and coarse are two categories of curd texture. Loose curds are defned as having a surface which feels spongy to the touch, sometimes caused by wilting and also because the curd has thin interstitial branches or the segments of curd have

elongated due to its maturity before the subtending leaves have folded back to expose the curd. Early type varieties have loose to medium compact curds with fne texture while the Snowball type produces compact curds with fne texture. Bracting in curd ranges from being barely visible to several centimeters in length, from being few in numbers to several thousand, from white to green and with somewhat purple tips. The appearance of bracts in curds is under genetic control but their color (white or green) and size (small to large) are infuenced by temperature factors. Riciness, ricyness, or wooliness is due to the appearance of miniature foral buds as outgrowths about 1 mm in diameter above the curd surface, which is clearly visible under a microscope.

7.4.10 Curd Color

Curd colors in Indian caulifowers have different shades, which range from yellow to bright white. The bright white curds, as in Snowball, have wide market preference. The early-maturing hot weather caulifowers are mostly yellowish to somewhat creamy white. Curd color is infuenced by the blanching habit of the variety and growing temperature. Additionally, orange (β-carotene), green (chlorophyll) and purple (anthocyanin) colors are not uncommon in caulifower. One semidominant gene *Or* determines carotenoid accumulation in the curd portion, giving an orange color. Green curd color curd is governed by high chlorophyll content and controlled by two genes and bleaching (white) genes perhaps three in number causing curds to remain white in the presence of sunlight, probably due to lower peroxidase activity. Yellow or pink discoloration may transiently appear in curds when unexposed to sun and may persist after maturity. They are probably due to favonoids; however, genotypes with a waxy coating to the leaf consistently show pink curds.

7.4.11 Curd Maturity

Based on curd initiation (i.e. days to 50% of the plants with curd initiation from sowing of seed), caulifower varieties are grouped into three categories Early $\left($ <75 days), Medium (75–100 days) and Late (100 > days). Most of the early types are early curd-forming varieties while Snowball group varieties are late curdforming varieties.

7.5 Gene Action

Caulifower was frst morphologically distinguished based on a few gene differ-ences (Giles [1941](#page-47-4)). These crops have same chromosome number $(n = 9)$ and there are almost no differences in chromosome morphology. *Brassica oleracea* is triple tetrasomic with the genomic formula A BB CC D EE F with six basic chromosomes, which show some secondary pairing (Robbelen [1960](#page-51-3)). The caulifower genome size is 584.60 Mb, contained 47,772 genes and 56.65% of the genome is composed of repetitive sequences (Sun et al. [2019\)](#page-53-7).

In caulifower, the transformation from vegetative phase to curding is governed by temperature; varieties differ in optimum temperature requirement for curd formation (Haine [1959](#page-48-7); Kato [1964;](#page-49-3) Sadik [1967\)](#page-51-4). Hence, any study on this character must take into consideration the suitability of prevailing temperatures for curding of the varieties. Watts ([1964\)](#page-54-7) made two series of diallel crosses between (a) eight varieties of autumn caulifower (intervarietal) and (b) six inbreds of a single variety of early-summer type. He found no F_1 (in either series), which was earlier than the early parent was. Further, in autumn types, he noted additive effects and those varieties with early mid-maturity possessed dominant polygenes while those with late maturity possessed recessive polygenes. In the early-summer type (intravarietal diallel), some interaction was noted. There was an association between early curding and low leaf number and between later curding and high leaf number. Swarup and Pal [\(1966](#page-53-8)), in a similar study of late-maturing caulifower, found that dominance and epistasis contributed most towards the inheritance of curd maturity. Heterosis was manifested in earliness.

Nieuwhof and Garetsen [\(1961](#page-50-7)) reported that curd compactness or solidity is controlled by polygenic factors. They observed a negative correlation between the frmness of curd and seed yield. Combining ability analysis of seven inbred lines of curd maturity group III (mid-December to mid-January) indicated that nonadditive effects were more important in the expression of plant height, plant spread, curd maturity, curd weight and curd size index (Lal et al. [1978\)](#page-49-4). The nature of gene effects was studied in 36 cross combinations obtained by crossing 6 inbred lines of maturity group II and 6 of maturity group III. Lal et al. ([1979\)](#page-49-5) concluded that dominance and epistasis were quite high in the expression of curd weight and curd size indices. The crosses showing high performance for these characters may be utilized for heterosis breeding. Some crosses also revealed a signifcant additive component of variation indicating the possibility of improvement in these characters by selection. High heritability and genetic advance were observed for traits such as net curd weight, total plant weight, harvest index, curd size index, curd diameter, stalk length and leaf length, respectively, in Indian caulifower (Dubey et al. [2003](#page-47-9)).

Several caulifower researchers have reported the genetics of qualitative and quantitative traits (Table [7.4](#page-17-0)), genetic advance, heritability and combining ability. Ahluwalia et al. [\(1977](#page-45-7)) described the inheritance of qualitative characters in Indian caulifower in detail.

Character	Nature of gene action	References
Curd weight	Dominance and epistasis Pronounced overdominance and epistasis Additive and dominance gene action	Gangopadhayay et al. (1997), Jyoti and Vashistha (1986), Sharma et al. (1988), Singh et al. (1975, 1976a) and Swarup and Pal (1966)
Curd: Plant ratio	Partial dominance	Kale et al. (1979)
Curd diameter	Predominance of dominance gene action	Lal et al. (1979)
Curd size index	Pronounced overdominance and epistasis Dominance and epistasis Additive dominant gene action Partial dominance	Kale et al. (1979), Lal et al. (1979), Sharma et al. (1988), Singh et al. (1975, 1976a) and Swarup and Pal (1966)
Curd angle	Pronounced additive gene action Additive and dominant gene action	Chand (1980), Dadlani (1977) and Lal et al. (1979)
Curd compactness	Polygenic Dominance and additive gene action Additive	Lal et al. (1979), Nieuwhof and Garretsen (1961) and Vashistha et al. (1985)
Maturity earliness	Partially dominant gene action Dominance and epistasis Predominance of additive gene action Additive gene action Additive and dominant gene action	Gangopadhayay et al. (1997), Kale et al. (1979), Lal et al. (1979), Mahajan et al. (1996), Sandhu and Singh (1977), Sharma et al. (1988), Singh et al. (1975, 1976b), Swarup and Pal (1966) and Watts (1964)
Maturity lateness	Recessive polygenes	Watts (1963)

Table 7.4 Genetics of quantitative characters in cauliflower

Source: Sharma et al. ([2004\)](#page-52-0)

The information on genetics of traits is a prerequisite for their improvement. Genes for other traits such as downy mildew and black rot resistance were investigated and symbols given were *Ppa3* and *Xca1bo,* respectively. In the selection of parental/inbred lines for improvement of traits of signifcance, it is essential to know the gene action for the particular trait. Classical studies of caulifower genetics reviewed by Bose et al. [\(2003](#page-45-9)) found that the gene action of curd weight is dominant and due to epistatic or partial dominance, additive gene action or additive and dominant. For curd to plant ratio, gene action was reported as partially dominant while for curd depth it is additive and dominant gene action. Curd size index is governed by epistasis or overdominance and epistasis, additive gene action or partial dominance. The gene action for curd angle is due to additive gene action, and additive and dominance gene action. Polygenic or two genes *Co1* and *Co2* or additive gene action was reported for curd compactness while the earliness (maturity) trait is controlled by recessive polygenes while traits such as plant height, plant expansion, number of leaf blades and heading stage are governed by nonadditive gene action.

Several plant characters in caulifower have simple inheritance, such as plant type, leaf characters (petiolate, leaf apex, margin, arrangement, glossiness), stalk length, curd color and fower color (Table [7.5\)](#page-18-0). The important quantitative traits governed by polygenes in caulifower include curd diameter, compactness, maturity, weight, depth, size-index, shape and yield (Table [7.6\)](#page-19-0). The loose, bracted (small light to dark green or slightly purple leafness in curd portion) and ricey defects (uneven lengthening of peduncles of preforal buds on curd surface leading to a condition known as *ricey* or *riceyness*) in caulifower curds are perhaps polygenic characters greatly infuenced by environment. Possibly looseness and riceyness are highly heritable, hence they need proper tracking during breeding.

Plant characters	Inheritance pattern		
Plant type	Single gene, erect dominant (EE)		
	Three major genes-additive, dominant and epistasis		
Leaf characters			
Petiolate	Single gene, dominant (PET)		
Leaf apex	Single gene, dominant for round apex (RO)		
Leaf margin	Single gene, dominant for wavy (WY)		
Leaf arrangement around curd	Two genes, dominant for semi-blanched (Bl_1, Bl_2)		
Glossy leaf Single gene, recessive (gl)			
	Two genes, inhibitory (IG)		
Stalk length	Single gene, long stalk dominant		
Curd color			
Orange	Single semidominant gene Or		
Green	Two genes		
Purple	Single semi-dominant gene Pr		
Retentive white	Three genes for bleaching, controlling peroxidase activity		
Flower color (white, yellow,	Two independent genes, dominant, epistatic interaction with		
cream)	few modifiers		

Table 7.5 Important simple inherited characters in caulifower

Source: Swarup ([2006\)](#page-53-9)

Character	Inheritance
Curd maturity	Polygenic, predominance of additive gene action; earliness partially dominant gene action additive and dominant gene action; lateness controlled by recessive polygenes, dominance towards earliness
Curd diameter	Polygenic, predominance of dominant gene action; additive and dominant gene action; low heritability
Curd depth	Polygenic, additive genetic variance; dominant and additive gene action
Curd-size index (diameter/depth)	Polygenic, epistasis; overdominant and epistasis; dominance and epistasis; partial dominance and additive gene action; partial dominance; highly complex inheritance
Curd compactness	Polygenic, dominant and additive gene action; two major genes (Co_1, Co_2) in which $Co1$ is epistatic to $Co2$; semi-compactness controlled by both recessive genes
Curd weight	Polygenic, dominant and epistasis in Snowball group epistasis; partial dominance and additive gene action; overdominance and epistasis; additive and dominant gene action, in tropical cauliflower
Curd shape	Polygenic, partial dominance for smooth curds in Italian types

Table 7.6 Inheritance of important quantitative traits in caulifower

Source: Swarup ([2006\)](#page-53-9)

7.6 Genetic Mechanisms for Hybrid Breeding

In caulifower, the extent of heterosis in terms of yield has a range of 15–50% depending upon the crop. In caulifower, heterosis of hybrids over open-pollinated cultivars may be only 10%, but a high degree of natural outcrossing and greater uniformity in yield and quality relative to open-pollinated varieties make hybrids the preferred choice for cultivation. The superiority of hybrids over the mean parental value depends directly on the existence of dominance and indirectly through interactions involving the dominance effect at different loci. There are several helpful biometrical procedures available to understand the heterosis in terms of actions and interactions at a variable number of loci. This procedure allows the partitioning of heterosis based on the relative roles of additive, dominance, epistasis, linkages, maternal effect and genotype x environmental interactions. It is also observed that the heterosis in caulifower fnds support for its physiological basis of faster growth rate, higher leaf area index, stout stem and root portions and greater biomass production (Sharma et al. [2004\)](#page-52-0).

Caulifower has a good amount of diversity with an adequate level of heterosis. However, hybrid breeding has constraints because of (i) lack of stable selfincompatible lines/cytoplasmic male sterile lines which results in sib-mating within the parental lines; (ii) nonsynchronous of fowering time between male and female genotypes; (iii) shorter period of fowering fush in caulifower due to cymose inforescence which leads to nonsynchrony of fowering of parent inbreds and (iv) minor heterosis for curd size in some combinations in comparison to other brassicas. Heterosis was exploited in caulifower in the development of Pusa Hybrid-2 (November–December maturity group) for earliness, high yield, bigger curd size, better curd quality, uniform maturity and disease resistance (Singh et al. [1994\)](#page-52-8).

Investigations on degree of heterosis in caulifower revealed variation for adaptive trait such as for days to curd maturity $(-3.92-16.3\%)$, plant height (−10.40–31.33%), plant spread (−10.68% to −29.52%) and number of leaves/plant (−10.44% to −39.27%) (Garg and Lal [2005](#page-47-11)). The heterotic combinations have better performance for quality traits such as curd compactness (−36.37–0.58%) and color. High heterosis was recorded for yield traits, which ranged from −51.77% to 24.25%. Better hybrid performance against abiotic stresses like heat and humidity could be due to changes in the salicylic acid- and auxin-regulated pathways (Groszmann et al. [2015\)](#page-47-12). These authors indicated that hybrids with larger leaves have greater capacity for energy production to support increased growth vigor and seed yields of the hybrids. Sheemar et al. [\(2012](#page-52-9)) observed that net curd weight had signifcant positive correlation with total plant weight and leaf width. Yield attributes such as size and weight of curds, harvest index and yield per hectare are considered when evaluating heterotic combinations. The heterobeltiosis for harvest index ranged from −47.59% to 15.0%; for curd diameter from −22.22% to 35.63% and for net curd weight, it ranged from 11.19% to 45.38% (Singh et al. [2009](#page-52-10)). For days to harvest, negative heterosis was reported in all heterotic combination in the range of −4.59% to −1.46%. However, information on the extent of heterosis on fowering behavior, seed production traits, growth attributes such as leaf area, leaf number, canopy parameters, erectness, blanching habit, leaf shape and orientation and plant spread is still not clear. Dey et al. ([2014\)](#page-46-9) reported heterosis for important vitamins and antioxidant plant pigments in Snowball caulifower. They observed high a SCA effect and a predominant role of nonadditive gene action for most of the quality traits in heterotic hybrid combinations. Kumar ([1983\)](#page-49-6) reported maximum heterosis for survival percentage of seedlings and total minerals.

7.6.1 Self-Incompatibility

Self-incompatibility (SI) is the inability of a plant to set seed when self-pollinated, even though it can form normal zygotes when cross-pollinated and its pollen can fertilize other plants. The SI system is a genetically controlled mechanism, which favors cross-pollination and is commonly used in hybrid seed production of cole crops. All *Brassica* vegetables have sporophytic SI systems, being strongest in kale and weakest in (European) summer caulifower. Caulifower has homomorphic sporophytic SI with trinucleate pollen and pollen germination inhibition occurs at the stigmatic surface. In this system, inhibition of self-incompatible pollen takes place on the surface of the papilla and deposition of callose takes place inside the papillae. A detailed investigation of Indian caulifower self-incompatibility revealed that inbred lines of maturity group I have the strongest self-incompatibility followed by maturity group II; group III showed weak self-incompatibility (Sharma et al. [2003\)](#page-52-11). However, some reports indicated a strong self-incompatibility in all the maturity

groups of Indian caulifower while maturity Group II exhibited an intermediate position for self-incompatibility (Chatterjee and Swarup [1984](#page-46-10)). Singh et al. [\(2002](#page-52-12)) reported a high level of self-incompatibility in 13 genotypes from different groups such as in Group I, Group-II, Group III and Group-IV. Sharma et al. [\(2003](#page-52-11)) investigated SI level in early group genotypes in Punjab and found that Early Kumari and NDC-1 were strongly self- incompatible. Being a natural mechanism, selfincompatibility has no adverse side effects, such as those often found with cytoplasmic or chemically-induced sterility.

Bud pollination and spraying with 3% NaCl solution (Kucera et al. [2006](#page-49-7)) are used to break SI to maintain SI lines. In caulifower, cvs. Pusa Kartik Sankar and Pusa Hybrid-2 and in cabbage cv. Pusa Cabbage Hybrid-1 have been developed using SI lines and released for commercial cultivation in India (Sharma et al. [2004\)](#page-52-0). Hadj-Arab et al. ([2010\)](#page-47-13) studied variability of the SI response in homozygous plants in caulifower and reported continuous phenotypic variation for SI response in offspring plants. They observed that SI levels decreased during the life of the fower. This is mainly due to two key genes S-locus receptor kinase (SRK) and S-locus cysteine-rich (SCR/SP11) genes. Zeng and Cheng ([2014\)](#page-54-10) cloned yellow mustard *S*-locus genes of SI lines using the *S*-locus gene-specifc primers from *Brassica rapa* and *B. oleracea*. The study indicated that self-incompatibility was dominant over self-compatibility and controlled by a one-gene locus. The authors developed dominant and codominant markers in yellow mustard which may be useful in caulifower. Verma et al. ([2017\)](#page-54-11) characterized SI lines of early and mid-maturity Indian caulifowers using quantitative and molecular analyses and reported higher diversity in the mid-maturity group.

7.6.2 Cytoplasmic Male Sterility (CMS)

The *Ogura* cytoplasm of the radish genus *Raphanus* is the most important source of sterile cytoplasm used in caulifower. Cybrids were utilized to transfer CMS to caulifower from *Ogura*. However, there were problems of temperature sensitivity and chlorosis in hybrid plants, which were overcome by protoplast fusion. CMS male sterility, especially *Ogura*, has been established in different groups of caulifower and is being explored for F_1 hybrid development at the Indian Agricultural Research Institute (IARI), New Delhi. The transfer of sterile *Anand* cytoplasm from *Brassica rapa,* originally derived from the wild species, *B. tournefortii* via *B. napus* into cauliflower is also being explored as a new source to facilitate F_1 hybrid breeding. A recessive *ms* gene in caulifower has been tagged by using RAPD and RFLP markers to accelerate hybrid breeding.

The transgenes, *Barnase, Bar* and *Barstar* are being utilized to develop transgenic caulifower hybrids. The male sterility transgene *Barnase* is also in *Bacillus amyloliquifaciens,* in which ribonucleases destroy the tapetum layer in the pollen to produce stable male sterile plants. The male sterility *ms* gene is linked to the herbicide resistance gene, *bar.* The restorer line for the *barnase* sterile lines can be obtained by expressing the gene coding for *barstar.*

Caulifower has a SI system, which favors outcrossing but has limitations of breakdown and maintaining SI inbreds. Hence, the search for other mechanism such as cytoplasmic male sterility (CMS) in its own germplasm or related species was done. The CMS system is the most reliable for hybrid seed production and various types of CMS have been developed to breed vegetable crops. CMS is a maternallyinherited trait encoded in the mitochondrial genome. No CMS system is yet reported in *Brassica oleracea*, however, *Ogura* sterile cytoplasm was frst introduced into caulifower (Ogura [1968](#page-50-9)). Later, it was transferred into heat-tolerant Indian caulifowers from kale and broccoli. Four lines (MS-91, MS-51, MS-11, MS-110) were used to transfer *Ogura* CMS via kale into fve lines (MS-01, MS-04, MS-05, MS-09, MS-10); this CMS system was transferred from broccoli for use in heterosis (Sharma and Vinod [2002](#page-51-7)). Use of male sterile lines not only extended the range of heterosis but also improved the quality and effciency of hybrid seed production. Ruffo-Chable et al. ([1993\)](#page-51-8) reported the infuence of temperature on the male sterile phenotype, while Kaminski et al. [\(2012](#page-49-8)) observed the presence of atypically developed plants with chimeral generative stacks or partially-fertile fowers among segregating test cross progeny. In brassicas, several other CMS systems (*oxyrrhina, polima, tournefortii*, *erucastrum, moricandia*) are being investigated, but so far, these could not be successfully used for hybrid seed production due to various limitations viz., breakdown of male sterility, chlorosis and abnormalities in petals, poor nectarie function and lack of appropriate restorer lines, all of which need more attention. However, the cytoplasmic male sterility (CMS) system has been introgressed into tropical types of caulifower genotypes for use in hybrid breeding. The CMS system is much more effective than the SI system due to its stable genetic mechanism, because the SI system is comparatively weak within the mid-group of caulifower. Besides, the open-pollinated varieties are a better choice for nonconventional areas and improvement of land races by appropriate selection methods. Some of the traits like black rot, yellowish and loose curds, advanced earliness and stability in performance need more attention for further improvement of tropical caulifowers. Jourdan et al. [\(1985](#page-48-10)) reported high regeneration capacity from cultured mesophyll cells in a caulifower line carrying *Ogura* CMS. Further, the cell fusion technique is also used to produce male sterile lines from wild species not used in interspecifc and intergeneric sexual hybridization. Liu et al. ([2006,](#page-49-9) [2007\)](#page-49-10) reported use of antisense RNA or RNAi to silence relevant gene expression of pollen development related gene *BcMF3* and *BcMF4* from Chinese cabbage pakchoi to inhibit development of pollen.

Dey et al. ([2011\)](#page-46-3) reported development of *Ogura*-ddbased improved CMS lines of snowball caulifower viz., Ogu1A, Ogu2A and Ogu3A through conventional backcrossing. Chamola et al. [\(2013](#page-45-10)) transferred cytoplasmic male sterility from alloplasmic *Brassica juncea* and *B. napus* to caulifower through interspecifc hybridization and embryo culture. The CMS system has been used in commercial F_1 hybrid production in *B. oleracea* using an improved *Ogura* cytoplasm (Pelletier et al. [1989](#page-50-10)). Introgression of *Ogura* cytoplasm also altered important quality traits in *Ogura* cybrid cytoplasm-based caulifower CMS lines (Dey et al. [2017a\)](#page-46-11). Dey

et al. [\(2014](#page-46-9)) did not observe signifcant differences among A and B lines for most of the vegetative traits but they varied in curd maturity, leaf number, leaf size and plant height. They also investigated 25 CMS lines for different agronomic and foral traits along with combining ability and SSR marker analyses (Dey et al. [2017b\)](#page-47-14). Bhatia et al. ([2014\)](#page-45-6) developed a protocol for in vitro maintenance of *Ogura* CMS lines of caulifower using hypocotyls and curds as explants by using MS medium supplemented with 2.5 mg/l kinetin, 0.2 mg/l NAA and 0.2 mg/l GA₃.

7.6.3 Combining Ability for Exploiting Heterosis

The selection of inbreds of varieties for use in heterosis breeding should be based on their combining-ability performance. Combining ability is effective for the selection of excellent parents in early generations, because evaluation of all possible crosses is time consuming and laborious in a breeding program. Generally, SCA values of the cross give better predictive information than GCA of the parents. Single or three-way crosses can provide the SCA analysis while polycrossing is used for GCA analysis, and top and diallel crosses for analysis of both SCA and GCA. The lines with high GCA values are useful in a hybridization program to develop improved lines and those with better SCA for hybrid breeding.

A number of investigations have been carried out on identifcation of inbreds or varieties for use in heterosis breeding through SCA in caulifower. Dixit et al. [\(2004](#page-47-15)) reported suffcient heterosis for early maturity, net curd weight, curd size index and curd yield. Earliness is an important trait of tropical caulifower, which has suffcient heterosis (Gangopadhyay et al. [1997;](#page-47-10) Sharma et al. [1983](#page-51-9)). Dey et al. [\(2014](#page-46-9)) reported that the CMS line Ogu12A of caulifower was a good general combiner (GCA effect) for most of the important vitamins and antioxidant pigments. The proportions of gca/*σ*2sca were <1 in 40 hybrids indicated for the presence of nonadditive gene action for the traits. The study suggested that high heterosis for ascorbic acid, anthocyanin and carotenoids in caulifower indicated the scope for development of F_1 hybrids rich in phytonutrients. Thakur et al. [\(2004](#page-53-10)) investigated the extent of heterosis for curd compactness and revealed appreciable heterosis over the better parent. Saha et al. ([2015\)](#page-51-10) reported that overdominance had a predominant role for marketable curd weight, curd diameter and curd depth. For marketable curd weight, dominance (h) and dominance \times dominance (l) components with duplicate type of epistasis were present. Lines IHR3, IHR4, IHR9 and IHR36 were good combiners for most of the characters. Sheemar et al. ([2012\)](#page-52-9) reported that the net curd weight correlated signifcantly and positively with total plant weight, and total plant weight had the highest positive direct effect on net curd weight, harvest index and curd depth. They also reported that the net curd weight, curd depth and curd diameter were signifcantly correlated with days to 50% curd maturity, and the net curd weight with total plant weight and leaf width.

Varalakshmi (2009) (2009) performed line \times tester analysis involving four lines and five testers in early caulifower and reported predominance of nonadditive gene action

for days to 50% curd initiation, 50% curd maturity, leaf number, leaf weight, stalk weight, curd size and curd weight. In Snowball cauliflower, Ram et al. ([2017\)](#page-51-11) found a wide range of heterosis for important dietary minerals and identifed CMS lines with good combining ability. Verma and Kalia [\(2017](#page-54-13)) analyzed genetic component of variance and reported the preponderance of dominant variance and nonadditive gene action for leaf, plant and curd traits. In hybrids, the contribution of lines was higher over the testers for all traits. They also analyzed genetic diversity and its relation to heterosis in early- and mid-maturity groups of Indian caulifower (Verma and Kalia [2017\)](#page-54-13). Additive genetic effect is more important than nonadditive effects in the expression of resistance to diseases, average curd mass, curd color and hollow stalk incidence in Brazilian caulifower materials (Arashida et al. [2017\)](#page-45-11).

7.6.4 DNA Markers for Heterosis Breeding

Research is heading towards detection and mapping of heterosis quantitative trait loci (Heterosis QTLs; hQTLs). For this, identifed heterotic groups based on marker data and complementary groups are crossed to produce hybrids. After this, genomic regions involved in heterosis are identifed and the target regions introgressed into appropriate inbreds to enhance hybrid performance. Marker-based estimates of genetic diversity between the parents would predict heterosis more precisely than that of phenotypic diversity, but this expectation is not yet realized in caulifower. Molecular markers assign the inbred lines to appropriate heterotic groups and identify the heterotic loci. A detailed analysis of these loci may provide a better insight into the genetic basis of heterosis and afford a more reliable heterosis prediction.

For heterosis breeding, identifcation of the useful hybrid combinations based purely on feld evaluation is expensive and quite time demanding. The use of robust DNA markers linked to the hQTLs is quite interesting. These markers can be identifed with standard protocols of marker development or identifcation. To search for hQTLs, the F_2 population is ideal because it provides estimates of different components of genetic variance. The doubled haploid (DH) population is very suitable for mapping of economic traits but not for identifcation of hQTLs because it consists of only homozygous plants, which carry only additive and additive x additive interaction genetic variances.

Heterotic genes are now also being sought using genomics, however, there is no report on the use of molecular markers or transcriptomics to understand the hQTLs. Chétritl et al. [\(1984](#page-46-12)) constructed a physical map of the caulifower mitochondrial DNA with the restriction endonucleases Sall, Kpnl and Bgll. The 26S and 18S – 5S ribosomal RNA genes appeared to be separated by about 75 kb in this map. However, further use of such information in male sterility is not clear. Gu et al. [\(2008](#page-47-16)) constructed a genetic linkage map (668.4 cM) of caulifower using 234 AFLP and 21 nucleotide binding site (NBS) markers with an average distance of 2.9 cM between adjacent mapped markers, in order to identify potential molecular markers linked to important agronomic traits that could be useful in crop improvement. Li and Garvin [\(2003](#page-49-11)) mapped the *Or* gene in caulifower by using AFLP markers which later converted to RFLP and SCAR markers. The *Or* gene is semi-dominant in nature which induces accumulation of β-carotene in plant tissue and turns it orange. This has triggered interest in caulifower breeders to use the technique for rapid introgression of the *Or* gene in commercial caulifower varieties or developed hybrids to counter human vitamin A deficiency. Saxena et al. ([2009\)](#page-51-12) identified two RAPD markers $D-3_{450}$ (5' GGACCCAACC 3') and C-20₃₅₀ (5' ACTTCGCCAC 3') flanking the stalk rot (*Sclerotinia sclerotiorum*) resistance gene in caulifower with a distance of 2.7 cM, and 4.2 cM, respectively, in a F_2 mapping population of Olympus (R) \times Pusa Snowball (S). *Purple* (*Pr*) gene mutation in caulifower confers anthocyanin accumulation and intense purple color in the curds. Chiu et al. ([2010\)](#page-46-13) isolated the *Pr* gene via a combination of candidate gene analysis and fne mapping which offers a genetic resource for development of new varieties in caulifower with enhanced health-promoting properties and visual appeal. These reports suggest systematic efforts on the development of DNA markers to understand the heterotic genes for better yield and superior quality. However, due to limitations in marker-based genotyping approaches and high similarity among caulifower genotypes, the development of high polymorphic marker systems such as new sequence based markers linked to CMS locus could be useful tool for hybrid breeding.

Morphological traits and isozyme markers have been used for analysis of genetic diversity and relatedness in caulifower germplasm, but they have several disadvantages, such as their limited number, environmental dependence and temporal and spatial expression; hence, DNA markers could be a useful tool to predict the genetic divergence in the parents in testing for heterosis. DNA markers are more efficient tools for rapid detection of genetic purity of commercial hybrids than the conventional grow-out test (GOT) method, due to environmental independence and a lesser time requirement (Nicholas et al. [2012](#page-50-11)). Pattanaik et al. [\(2018](#page-50-12)) reported promises of simple sequence repeat (SSR) markers in caulifower hybrid purity.

DNA marker use in cultivar identifcation, diversity analysis, construction of genetic maps and tagging agronomically-important genes is reliable. The markers are used to correlate genetic diversity and heterosis in several crops such as maize (Kiula et al. [2008](#page-49-12)), pearl millet (Singh and Gupta [2019](#page-52-13)), rice (Zhang et al. [1996\)](#page-54-14) and wheat (El-Maghraby et al. [2005](#page-47-17)). Hence, this approach can predict heterosis in hybrids, reducing labor and the time needed to evaluate hybrids for heterosis or combining ability in the feld.

7.6.5 Heterosis Fixation

Heterosis declines in successive generations because of meiotic recombination during gamete formation and genetic segregation. This requires constant renewal of hybrid seeds and proper maintenance of parental stocks. It avoids unwanted seed progeny and minimizes the cost of seed production. Heterosis fxation can employ doubled haploids, apomixes and mass propagation of hybrids. The DH technology

is more useful in caulifower but apomixes is not yet reported in this crop. Mass propagation is also feasible but has limited application. In mass production of hybrids, plants can be propagated asexually on a large scale under in vitro conditions either directly from apical, axially adventitious buds, or indirectly through somatic embryogenesis.

7.7 Open Pollinated and Hybrid Variety Development in India

Breeders the world over serve and need to satisfy two constituencies: growers and consumers. From the growers' viewpoint, the breeding aims for caulifower improvement are: (a) increased crop productivity; (b) reduced losses due to diseases, insect pests and physiological disorders; (c) heat tolerance; (d) improved curd/plant weight ratio and (e) uniformity in appearance and maturity. From the consumers' perspective, quality is the main concern, in terms of curd whiteness, structure (density, surface texture, suitability for foreting), freedom from diseases, nutrient rich and good sensory traits.

Previously, there was a lack of useful SI alleles limiting hybrid breeding in caulifower. However, the advent of cytoplasmic male sterility has stimulated hybrid development in caulifower in both the public and private sectors.

In India, the main emphasis in caulifower improvement, of late, is on development of cultivars and hybrids (SI and CMS system-based) with heat tolerance and resistance to diseases (black rot, downy mildew, *Alternaria* leaf spot, *Sclerotinia* rot) and insect pests (diamondback moth, cabbage butterfy). Earlier emphases were on breeding heat-tolerant early-maturing varieties and hybrids. As a result, improved varieties namely Pusa Meghna, OPusa Early synthetic and F1 hybrid Pusa Kartik Sankar ($CC \times DC$ 41-5) were developed and released for cultivation. Earlier, Pusa hybrid 2 ($CC \times$ Sel 1-3-18-19) was the first hybrid developed in cauliflower in India using the SI mechanism in the November–December maturity group (Singh et al. [1994\)](#page-52-8).

An emphasis on resistance breeding led to the isolation of multiple resistance sources viz., Kn-81 (DM, SR, Alt), BR-2 (DM, BR, DMB), Lawyana (BR, SR, DBM) and Armel (BR, SR, DM). These were involved in hybridization with commercial consumer-acceptable land varieties/lines of each maturity group to transfer resistance. The improved elite material was achieved in advanced F_5/F_6 generation stages, which are being evaluated for resistance to diseases/insect pests, yield potential and other horticultural traits.

In India, caulifower breeding is being carried out at the ICAR institutes namely IARI, New Delhi (tropical caulifower) IARI Regional Station, Katrain (Snowball group), Indian Institute of Vegetable Research, (IIVR), Varanasi and Indian Institute of Horticultural Research (IIHR), Bangalore. The State Agricultural Universities (SAUs) working on caulifower are located at Ludhiana, Pantnagar, Hisar, Solan, Sabour and Palampur. Table [7.7](#page-27-0) provides details of the caulifower varieties developed for different maturity groups.

Variety	Pedigree	Source	Year	Maturity	Yield (mt/ ha)	Remarks
September maturity group (Sowing: end of May; transplanting: mid July; temperature for						
curding: $20-30$ °C)						
Pusa Meghna	Selection	IARI, New	2004	End of	12	Compact, cream
		Delhi		September		white curds
October maturity group (Sowing: mid June; transplanting: mid July onwards; temperature for						
curding: $20-25$ °C)						
Pusa Kartiki	Selection	IARI, New Delhi	2015	October 2nd fortnight	22	Compact, white curds
Pusa Ashwini	Selection	IARI, New Delhi	2015	October 1st fortnight	18	Compact, white curds
Sabour Agrim	Selection	BAU, Sabour	2013	Mid October	15	Compact white curds
Kashi Kunwari	Selection	IIVR, Varanasi	2005	Mid October	16	White, compact
Pusa Kartik	F_1 hybrid (CC	IARI . New	2004	Mid	16	Compact, white
Sankar (F_1)	x DC 41-5)	Delhi		October		curd
Pant Gobhi 3		GBPAUT, Pantnagar	1993	Mid October	10	Cream, compact curds
Pusa Deepali	Selection from local cultivar	IARI, New Delhi	1975	End of October	12	White, self blanching
November-December maturity (Sowing: end of July; transplanting: end of August; temperature for curding: $15-20$ °C)						
Kashi Agahani	Selection	IIVR. Varanasi	2008	End of December	22	White, compact curd
Pusa Sharad	Selection	IARI, New Delhi	2004	Mid November	24	White, compact curds
Pusa Hybrid 2	CC 32 \times DC	IARI, New	1994	Mid	23	White, compact
(F_1)	18-19	Delhi		December		
Pant Gobhi 4	Recurrent selection in local collection	GBPAUT, Pantnagar	1993	November	12	Creamy white compact
Hisar 1	Selection	HAU, Hisar	NA	End of November	12	Cream, compact curds

Table 7.7 Varieties of Indian cauliflower developed and released in different maturity groups

December-January maturity group (Sowing: end of August; transplanting: end of September to mid-October; temperature for curding: 12–15 °C)

(continued)

Table 7.7 (continued)

7.7.1 Breeding Open-Pollinated Varieties

IARI frst released a tropical caulifower variety, Pusa Katki, in 1954, which was suitable for October maturity. Later on, Pusa Meghna was released for September– October maturity and in recent years, Pusa Ashwini and Pusa Kartiki were also added to the early group varieties (Kalia et al. [2016\)](#page-48-0) These two varieties mature in the second fortnight of October and the end of October, respectively, in sequence, even on the same date of transplanting. Pusa Sharad is the only mid-season variety developed by IARI, New Delhi (Sharma et al. [1999](#page-51-13)) while Pusa Paushja and Pusa Shukti are in the mid-late group which maturing in December–January (Kalia et al. [2016\)](#page-48-0).

7.7.2 Heterosis Breeding

Although, Jones ([1932\)](#page-48-11) frst reported heterosis in caulifower it took a long time to tap its potential at a commercial scale. In India, Swarup and Pal [\(1966](#page-53-8)) and Pal and Swarup [\(1966](#page-50-13)) found appreciable heterosis in Snowball caulifower for earliness (5–7 days), curd weight (24.5–28.2%), curd size index (22.54–34.85%) over the better parent. Later, a number of reports indicated an appreciable amount of heterosis in different maturity groups of Indian caulifowers (Swarup and Chatterjee [1972,](#page-53-0)

[1974;](#page-53-11) Deshpande [1975;](#page-46-14) Gangopadhayay et al. [1997](#page-47-10); Kumaran [1971;](#page-49-13) Sandhu et al. [1977;](#page-51-14) Singh et al. [1975](#page-52-5); Swarup and Chatterjee [1972\)](#page-53-0). Hoser-Krauze et al. [\(1982](#page-48-12)) used three SI lines of Indian caulifower and three SI lines of temperate caulifower and reported heterosis for earliness, curd diameter, curd weight and quality.

7.7.3 Synthetic Varieties

About four to six inbred lines were used to develop synthetics. The inbred lines after testing their general combining ability in a diallel cross, polycross or in a top cross, can be synthesized to form a synthetic variety. Exploiting the pronounced additive genetic variance can develop a synthetic variety. A variety produced in this manner has a threefold advantage: (1) its seeds can be easily produced from open pollination and maintained by the farmer; (2) it is useful particularly in locations no commercial seed industry exists and (3) it is broad-based and, therefore, better adapted to changing growing environments. Bhatia et al. [\(1978](#page-45-12)) reported that S_1 , S_2 and S_3 generations of early maturing synthetic caulifower showed 72.0%, 37.0% and 20.8% increase in curd weight, respectively, over that of the standard variety, Improved Japanese. This indicated a high-yield potential of synthetics evolved from Indian caulifowers. In India, Pusa Early Synthetic and Pusa Synthetic caulifower varieties were developed by synthesizing 6 and 7 parents, respectively (Gill [1993;](#page-47-18) Singh et al. [1997\)](#page-52-14).

7.7.4 Intervarietal Crosses for Yield Improvement

Selection in F_2 generation of intervarietal crosses can recombine and fix favorable yield genes. A well-pronounced additive variance for yield or other characters in the elite F_2 generation offers great potential for improvement in such characters through appropriate selection procedures. The occurrence of transgressive segregants (i.e., individual plants, the performance of which is better than that of both the parents of the cross) towards favorable directions for many desirable economic characters in the $F₂$ generation of intervarietal crosses of cauliflower was reported by Swarup and Pal [\(1966](#page-53-8)). Such segregants can serve to develop superior lines/varieties.

7.7.5 Mutation Breeding

Mutation is a useful technique to generate variability for rare or unavailable traits in usable germplasm. Caulifower, as a member of the Brassicaceae family, has vast diversity. However, the search for traits such as resistance to black rot, *Alternaria* leaf spot, insect resistance and quality traits needs to explore mutations to create favorable alleles. Narayanaswamy [\(1988](#page-50-14)) treated Pusa Deepali and Early Kunwari seeds with ethyl methanesulfonate (EMS) and identified LD_{50} 0.77% and 0.70% for black rot resistance; however, none of the M_1 plant were found to be resistant. While observing the mutation in seeds taken into space by satellite, there was reportedly signifcant phenotypical changes in both the size of the plant and the weight of the fower head in caulifower, whereas no major change was noted in broccoli except for a single plant. However, they did report black rot resistance in caulifower.

7.7.6 Doubled Haploids

The DH technique is very useful for the development of homozygous and homogenous inbred lines within a short time period. These lines can be used as parents in hybrid breeding. Wijnker et al. [\(2007](#page-54-15)) used *reverse breeding* by which they identifed superior hybrid genotypes in segregating populations and introduced a gene through genetic transformation for induced suppression of meiotic recombination and developed several DH lines. Further, Dey et al. ([2014\)](#page-46-9) suggested that in vitro maintenance of CMS lines of Indian caulifowers can be used as an alternative method for conventional CMS-based hybrid seed production.

7.8 Breeding for Improved Quality

The extrinsic quality characters in caulifower are curd color, solidity, free of bracting, pinking and riceyness. The caulifower curd consists of a mass of short peduncles bearing many thousands of apical meristems. These meristems normally develop to bear fowers, but a large proportion of them (usually over 90%) abort before or occasionally during the foral phase. The curd is, thus, a precociously developed foral button, and its appearance at the marketable stage is affected by the normal ontogeny of fowering and by the death of excess foral material.

The quality of the curd, in terms of cosmetic appeal to consumers is largely determined by the timing of the morphological changes associated with fowering in relation to the curd reaching a marketable size. The distinct features characteristic of poor cosmetic quality are disfguring defects like elongation of the peduncles, precocious development of apical meristems into fower buds, growth of bracts and leaves from the peduncles through the surface of the curd and development of pink or purple colorations in the curd. The frst two of these defects, known respectively as looseness and riceyness, are essential parts of the fowering process, and selection against them is largely a matter of ensuring that they appear as late as possible after the curd has reached a marketable size. Bracting and pinking, do not appear to be essential for fowering and, therefore, selection against them is for their complete elimination.

7.8.1 Curd Appearance

The environment affects the expression of bracting, pinking and riceyness, but these may be completely absent in some seasons while a serious problem in others. Comparison of the morphology of curds when aseptically cultured in nutrient solution, with the effects of their genotype on bracting and pinking as shown by a progeny test, Crisp et al. ([1975a](#page-46-15), [b\)](#page-46-16) found that the formation of bract-like structures in culture was a refection of the genotypic tendency to do this in the feld. Thus, assessment in culture as well as in the feld increased the selection pressure against bracting. The results with pinking are even more useful because while the assessments of purple colorations in the feld and in culture were phenotypically independent, both were genotypically related to the appearance of the defect in the feld, possibly owing to different genes governing the same phenotype. With respect to riceyness, which has been found to have inherent association with endogenous synthesis of auxins, the screening of genotypes can be done at an early stage by growing young cotyledons aseptically on auxin-free nutrient medium where ricey susceptible genotypes will strike roots within a week (Kalia [1994](#page-48-6)).

This suggests that a two-tier system can be applied to selecting against bracting, pinking and riceyness in caulifower to produce potential new cultivars with bractfree, ricey-free non-pink curds. In addition, the assessment of bracts in artifcial culture media may be carried out for screening of anthocyanin production (Crisp et al. [1975a](#page-46-15), [b](#page-46-16)) and for vegetative propagation of selected plants (Crisp and Walkey [1974\)](#page-46-17) which may be necessary in a breeding program. Teakle [\(2004](#page-53-12)) reported two MADS-box regulatory genes (*BoAP1-a*, *BoCAL-a*) that are present at loci having key roles in determining the formation of caulifowers. A number of cDNA for different MADS-box genes have been isolated from caulifowers, which include both foral promoters and repressors. The expression pattern and genetic map position of these genes will help predict their potential relationship with caulifower quality i.e. bracting and riceyness.

In caulifower, major emphasis is given to curd quality because traits like higher yield, disease resistance and wider adaptability become meaningless unless the curd of the variety has good marketability. Hence, the efforts to breed for correction of physiological abnormalities of the curd are fully justifable. Proper use of nutrients and moisture along with the correct selection of a variety for specifc season prevents these abnormalities, but they can be corrected genetically as well. This is because the expression of most of these defects is under genetic control. Loose curd, curd bracting and precocious fower bud formation appear to be due to high apparent genotype \times environment interactions. Crisp et al. [\(1975a,](#page-46-15) [b\)](#page-46-16) estimated and recorded heritability of the appearance of bracts through the surface of the cauliflower curd to be 0.73 ± 0.10 under field conditions.

7.8.2 Dietary Nutrients

Caulifower curd is a good source of dietary fber and microelements. The intrinsic quality traits include nutrition-related parameters like vitamins, minerals, protein, carbohydrates, fats and favor. Inherently, caulifower is not very rich in nutritional traits, but since the Indian caulifowers represent a new group with wide variability for horticultural traits, which has been exploited for varietal improvement, the variability with respect to nutritional traits needs to be investigated and exploited in the present scenario of nutritional security. Caulifower favor is very delicate which increases its popularity, therefore breeding for favor will also draw the attention of breeders in future programs.

7.8.3 Curd Flavor

Caulifower contains glucosinolates which on hydrolysis (by myrosinase) gives characteristics volatile favor products i.e., nitriles and isothiocyanates. Broccoli and Brussels sprout have strong favor due to glucosinolates. Breeding for glucosinolates in caulifower has diffculties of poor repeatability of their estimations and varies with individual plant, which may be fxed at an early life stage, perhaps by environmental factors (Sones et al. [1984](#page-53-13)). Varietal difference in both individual and total glucosinolate content is found in caulifower (Sones et al. [1984\)](#page-53-13). Hill et al. [\(1984](#page-48-13)) estimated appreciable additive heritability of 0.32 for the total glucosinolate content. Breeding for different glucosinolate contents may, therefore, be possible.

7.8.4 Curd Color

Ahluwalia et al. ([1977\)](#page-45-7) studied inheritance of various traits including curd color in Indian caulifowers and gene symbols were assigned for curd color-yellow Y and white- y. The first ever beta-carotene rich variety Pusa KesariVitA-1 in the mid maturity group of Indian caulifowers using marker-assisted selection was developed by IARI, New Delhi (Fig. [7.3](#page-33-0)) (Anonymous [2016\)](#page-45-0). This variety has great prospect in programs to mitigate human vitamin A defciency in tropical regions, particularly India. The commonly-used breeding methods for quality traits are selection, backcrossing, hybridization and hybrid breeding. The successful examples of transfer of quality-enhancing genes in prominent varieties using backcrossing is introgression of the *Or* gene in Indian caulifower (Kalia et al. [2018;](#page-49-0) Muthukumar et al. [2017\)](#page-50-15). The β-carotene content in promising lines showing more than 10 μ g g⁻¹ β-carotene content in the curd portion were identified by Kalia et al. [\(2018](#page-49-0)).

Identified molecular markers for foreground selection of Or gene

M- Marker ladder 50bp, P_1 - EC625883 homozygous Or inbred line, P_2 –DC 309 homozygous white, F_1 -1-4 Dark orange,, 5-9 BC₂F₁ Dark orange individuals, 10-14 BC_2F_1 white individual. The fragments were seperated on 3.0% metaphor agaraose gel.

Fig. 7.3 (**a**) Pusa KesariVitA1 harvested marketable caulifower curds, (**b**) Marker assisted foreground selection of *Or* gene, (**c**) HPLC peak of betacarotene. (Photos are credited to Dr. P. Muthukumar)

7.8.5 Glucosinolates

Natural variability in caulifower (European) for total glucosinolate content in leaves was reported to be 46–87 μmol/g dry weight (Menard et al. [1999](#page-50-16)) and 19.5–42.6 mg/100 g fw (Ciska et al. [2000\)](#page-46-18). They also reported wide variation in

b

individual glucosinolates such as sinigrin (5.7–12.9 μmol/g dw), glucoiberin $(0.5-6.6 \text{ mg}/100 \text{ g} \text{fw})$, glucoibervirin $(0.6-2.9 \text{ mg}/100 \text{ g} \text{fw})$ and indole (15.2–24.9 mg/100 g fw) which are comparable with total glucosinolate content $(0.6-35.6 \text{ mg}/100 \text{ g}$ fw) and glucoraphanin $(0.8-21.7 \text{ \textmu})$ mol/g dw) and indole $(0.4–6.2 \text{ \mu mol/g dw})$ in green broccoli (Kushad et al. [1999\)](#page-49-14). The genetics of the glucosinolate content in caulifower is governed by quantitative factors with environmental infuence (Hirani et al. [2012](#page-48-14)). The glucosinolate content also varies considerably among plant ontogenetic stages and plant organs (Van Leur et al. [2006\)](#page-53-14). Variation in cauliflower genotypes for total glucosinolates (19.5–42.6 mg 100 g⁻¹ fw) (Verkerk et al. [2009](#page-54-16)) indicate great scope for its improvement through breeding. Vanlalneihi [\(2016](#page-53-15)) analyzed sinigrin in curd and leaf parts of 48 inbred lines of caulifower comprising early (16), mid-to-early (15) and mid-to-late (17) at IARI, New Delhi. The author reported the highest sinigrin content in curds of DC 41–5 (16.37 µmol 100 g^{-1} fw) and leaves of CC 13 (15.43 µmol 100 g^{-1} fw) in the early group. The highest GCV (57.22%) and PCV (57.25%) were recorded for curd sinigrin. In the mid-maturity group, DC 326 had the highest curd sinigrin (36.93 μmol 100 g−¹ fw) whereas leaf sinigrin was highest in DC 306 (39.50 μmol 100 g−¹ fw). Vanlalneihi et al. [\(2019a,](#page-53-16) [b](#page-53-17)) analyzed curd and leaf sinigrin which were estimated to be highest for Pant Gobhi 2 and Selection 1-2 with 16.45, 17.56 μmol 100 g⁻¹ fw, respectively. This study concluded that the mid-early maturity group genotypes had maximum sinigrin content. Neelavathi et al. [\(2014](#page-50-17)) analyzed glucosinolates in 2 caulifower varieties Pusa Sharad (149.27 μmol/100 g), Pusa Himjyoti $(85.44 \mu mol/100 \text{ g})$ and a hybrid Pusa Hybrid-2 $(63.74 \mu mol/100 \text{ g})$. Pusa Sharad was harvested in November while Pusa Hybrid-2 and Pusa Himjyoti in December were subjected to variation in temperature to affect the glucosinolate content in these varieties but there is no confrmation of evidence to support the role of environment or genotype for this great extent of variation. This kind of information is scare in Indian caulifowers particularly under Indian growing condition. Therefore, to initiate a breeding program for development of varieties, it is essential to know the variation in glucosinolate content in available germplasm, its genetic control and to develop closely-linked markers.

7.9 Breeding for Climate Resilience

Caulifower is thermosensitive and temperature plays a key role in curd initiation and development. The ideal temperature for seedling growth is around 23 °C, which can be 10–20 °C at later stages. The seedlings of early Indian caulifowers grow well even at higher temperature during May–June in north India but need partial shade (50–70%). Caulifower seedlings cease to grow at temperature slightly above 0 °C. The Indian cauliflowers can grow under high temperature $(>35$ °C) during vegetative stage but $15-20$ °C is favorable for plant growth. The plants remain in a vegetative stage, if temperature remains higher than required for curding in specifc cultivars. In contrast, early-group varieties form small size curd *buttons*, if temperatures remain lower than required for curding. Any fuctuation in temperature at the time of the development stage adversely affects curd quality. Curd disorders such as riceyness, bracting or leafyness occur due to lower and higher temperatures than required for curding, respectively. Hence, Indian caulifowers form curds in the range of $12-27$ °C while Snowball type form curds at 10–16 °C. Based on the temperature requirement for curd initiation and development, caulifower cultivars are divided into four maturity groups (Table [7.3](#page-8-0)). These maturity groups are also known by the name of Hindu calendar months for their maturity and arrival in market *viz*., Kunwari (September–October), Kataki (October– November), Agahani (November) and Poosi (December) and/or Maghi (January). Snowball types belong to the late group. Earlier, caulifower formerly grown only during winter season but now cultivated from May to March and curds are available from the end of August to March under North Indian conditions. Specifc cultivars are available with the ability to form curd at a temperature range of 10–27 °C. If the early cultivars are planted late then instead of normal curd, they form small buttons and ricey curds. Similarly, if late types are planted early in the season, they would continue to grow vegetatively-forming curds only when the required temperature range is reached.

7.10 Breeding for Biotic Stress Resistance

7.10.1 Common Diseases and Insect Pests

Downy mildew [*Hyaloperonospora parasitica* Constant (Pers.:Fr) Fr.], black spot (*Alternaria brassicae*, *A. brassicicola* and *A. alternate*), *Sclerotinia* rot [*Sclerotinia sclerotiorum* (Lib) deBarry] and black rot [*Xanthomonas campestris* pv. *campestris* (Pam.) Dowson] and bacterial soft rot (*Erwinia carotovora*) are common diseases infecting caulifower. Inheritance of downy mildew resistance in caulifower is governed by a single dominant gene *Ppa3* (Singh et al. [2013](#page-52-15)) and black rot resistance by a single dominant gene (Saha et al. [2015\)](#page-51-10). Stalk rot or white mould in Snowball caulifower is polygenically inherited (Thakur [2013\)](#page-53-18).

Diamondback moth (*Plutella xylostella* L.), tobacco caterpillar [*Spodoptera litura* (F.)], cabbage butterfywhite (*Pieris rapae* L.), cabbage head borer [*Hellula undalis* (F.)], Bihar hairy caterpillar (*Spilosoma oblique* Walker, cabbage aphids [*Brevicoryne brassicae* (L.)] and painted bug [*Bagrada hilaris* (Burmeister) (cruciferarum)] are important insect pests of caulifower. Pesticide residues pose a major health problem; therefore, placing emphasis on host plant resistance is important. Identifed resistant sources and an understanding of genetics of resistance for a particular disease and insect pest is essential.

7.10.2 Disease Resistance Breeding

Resistance breeding has resulted in various successful resistant varieties. The development of resistant varieties requires thorough understanding of evolutionary interrelationship of host and pathogen. The success of resistance breeding depends on selection of right genetic sources of resistance, racial composition of pathogen and genetic basis of host-pathogen interaction. It is also essential to have the knowledge and scope of manipulation of host-pathogen interaction. Resistance is a relative term refecting hereditary capability of the host to reduce the development of pathogen after its infection so that the severity of disease is minimized (Chahal and Ghosal [2002\)](#page-45-13). The strategy for resistance breeding depends on knowledge of genefor-gene relationship and host-pathogen interaction for effcient deployment of resistance genes in alternate forms. Fehr [\(1984](#page-47-19)) categorized three alternate strategies such as: (i) development of cultivars with single major gene against the prevalent pest; (ii) combining genes controlling prevalent and minor races of pests in the form of mixture of different genotypes especially as multiline varieties and (iii) stacking genes controlling prevalent and minor races into a single cultivar i.e., pyramiding of resistance genes. Investigations indicate that a single dominant gene governs the resistance in caulifower for downy mildew and *Sclerotinia* rot, hence their manipulation is easy. Hybrid breeding, backcross breeding and recurrent selection are common methods employed in caulifower resistance breeding. In the case of black rot, four races have been reported and deployment of resistance genes for each race in a cultivated variety can be done through gene pyramiding. The steps in resistance breeding are: (i) Collection and maintenance of resistance genes for use in breeding programme. The sources of R gene may be in advance breeding lines, or new genetic stocks developed through pre-breeding, commercial varieties, landraces or primitive cultivars and wild relatives in the form of original progenitors or related species; (ii) Incorporation of one of the resistance gene by incorporation of a resistance parent in hybridization program. This method does not disturb the overall genetic constitution of the recipient commercial variety. The monogenic dominant resistance to downy mildew and black rot can be transferred into cultivated varieties by backcrossing. Further, use of one resistant parent having desirable horticultural traits in hybrid breeding can result in resistant hybrids against these pathogens. The gene pyramiding approach can also be employed to develop varieties having resistance to both the diseases. The breeding efforts in caulifower are briefy summarized by disease below.

7.10.2.1 Downy Mildew

Downy mildew [*Hyaloperonospora parasitica* (Pers.) Constant. 2002] is a devastating disease of mid-maturity Indian caulifowers. It is an obligate fungal parasite and systemic in nature. Its infection occurs at seedling stage to seed stage but is most devastating during curd stage (Crute and Gordon [1987\)](#page-46-19). Among Indian caulifowers, indigenous genotypes BR-2, CC and 3-5-1-1; and exotic genotypes EC177283, EC191150, EC191157, Kibigiant, Merogiant, EC191140, EC191190, EC191179 and Noveimbrina have been found to be resistant (Mahajan et al. [1991;](#page-50-18) Singh et al. [1987](#page-52-16)). MGS2-3, 1-6-1-4,1-6-1-2 and 12C (Chatterjee [1993](#page-46-2)); KT-9 (Sharma et al. [1991\)](#page-51-15) Early Winter Adam's White Head (Sharma et al. [1995](#page-51-16)); CC-13, KT-8, xx, 3-5-1-1,CC (Trivedi et al. [2000](#page-53-19)); Perfection, K1079, K102, 9311 F1 and 9306 F1 (Jensen et al. [1999](#page-48-15)); Kunwari-7, Kunwari-8, Kunwari-4 and First Early Luxmi (Pandey et al. [2001\)](#page-50-19) are reportedly resistant to moderately-resistant. Pusa Hybrid-2 (Singh et al. [1994\)](#page-52-8), Indian caulifower, and Pusa snowball K-25, Snowball type, with resistance to downy mildew, were released for commercial cultivation in India. Resistance to downy mildew has been ascribed to a single dominant gene (Jensen et al. [1999](#page-48-15); Mahajan et al. [1995](#page-50-20); Sharma et al. [1991\)](#page-51-15), single gene with recessive effects (Mahajan et al. [1995](#page-50-20)) or several genes (Hoser-Krauze et al. [1995\)](#page-48-16). Singh et al. ([2013\)](#page-52-15) identifed the seven most resistant genotypes: BR-2, CCm, 3-5-1-1, CCm-6, CCm-5, MGS-2-3 and cc-12 among Indian caulifowers.

7.10.2.2 *Sclerotinia* **Rot**

The causal organism of this disease is *Sclerotinia sclerotiorum*. This disease has a wide host range infecting most dicot crops, but is more severe in the seed crop of caulifower, although it may attack the crop at an early growth stage as well. Moderate resistance to this pathogen is reported in EC131592, Janavon, EC103576, Kn-81, Early Winter Adam's White Head, EC162587, EC177283 (Baswana et al. [1991;](#page-45-14) Kapoor [1986](#page-49-15); Sharma et al. [1995](#page-51-16), [1997;](#page-51-17) Singh and Kalda [1995](#page-52-17)). Resistance is polygenically controled and recessive in nature (Baswana et al. [1993;](#page-45-15) Sharma et al. [1997](#page-51-17)). Pusa Snowball K-25 developed by using EC103576 as a resistant source with Pusa Snowball-1 possessing feld resistance to *Sclerotinia* rot. Pandey et al. [\(2003](#page-50-21)) reported moderately-resistant lines of early caulifower to *Sclerotinia* rot, namely Kataki-6, Kataki-13, Patna Kataki, Deep Malika, Suryamukhi, Pusa Himkaran, Early Laxmi and PDVR early. However, Kataki-13 and Kataki-6 showed a high degree of tolerance. Saxena et al. ([2009\)](#page-51-12) reported the polygenic nature of *Sclerotinia* resistance and identifed two RAPD markers D-3450 (5' GGACCCAACC 3') and C-20350 (5' ACTTCGCCAC 3') fanking the stalk rot resistance gene at a distance of 2.7 cM and 4.2 cM, respectively, in the resistant genotype Olympus.

7.10.2.3 Black Rot

Xanthomonas campestris (Pam) Dawson bacterium is the causal organism of this disease. Symptoms begin as yellowing of leaves from leaf margin and extending in the direction of the midrib, followed by blackening of veins (vascular bundles). Caulifower lines reported as resistant sources are Sn 445, Pua kea and MGS2-3 (Sharma et al. [1972\)](#page-51-18); RBS-1, EC162587 and Lawyana (Sharma et al. [1995\)](#page-51-16); Sel-12 (Gill et al. [1983](#page-47-20)); Sel-6-1-2-1 and Sel-1-6-1-4 (Chatterjee [1993\)](#page-46-2) and Avans and

Igloory (Dua et al. [1978\)](#page-47-21). Some of the above sources have been used in the development of resistant varieties. Pusa Shubhra was developed, using Pua Kea and MGS2-3 lines and recommended for commercial cultivation (Singh et al. [1993\)](#page-52-18). Pusa Snowball K-1 was also reported to be feld resistant to black rot (Gill et al. [1983\)](#page-47-20). The resistance was dominant and governed by polygenes and the dominance components of variation were more pronounced than additive (Sharma et al. [1972\)](#page-51-18). However, Jamwal and Sharma [\(1986](#page-48-17)) reported that a single gene governs dominant resistance. Of the 54 accessions wound-inoculated with 4 isolates of Xcc race 4 at the juvenile stage, A 19182 and A 19183 exhibited no symptoms, and the accessions including PI 199947, PI 199949 and PI 194256 segregated for resistance to Xcc race 4 (Tunguc and Griffiths [2004a](#page-53-3), [b](#page-53-4), [c](#page-53-5)). Tonguc et al. [\(2003](#page-53-6)) analyzed 3 segregating F_2 populations for black rot resistance along with 8 polymorphic RAPD markers. Segregation of markers with black rot resistance indicates that a single, dominant major gene controls black rot resistance in these plants. Stability of this black rot resistance gene in populations derived from 11B-1-12 may complicate introgression into *Brassica oleracea* genotypes for hybrid production. Recently, Saha et al. [\(2015](#page-51-10)) identifed new resistance sources for black rot pathogen Xcc race 1 in Indian caulifower namely BR-207, BR-1, BR-202-2 and AL-15.

7.10.2.4 Black Leaf Spot

In cole vegetables, the black leaf spot disease is caused by *Alternaria brassicae* or *A. brassicicola*. Brown to black, small to elongated spots appear on leaves and stem. In younger plants, it may cause symptoms like that of *Rhizoctonia solani*. When the fungus infects the curd, especially in the case of seed crop, the disease is referred to as inforescence blight. Resistance was found in Indian caulifower lines, MGS2-3, Pua Kea and 246-4 (Sharma et al. [1972\)](#page-51-18), 23-7, 466, MS98, 210-21, Sel-9, 443-7 (Trivedi et al. [2000](#page-53-19)) and Snowball KT-9 (Sharma et al. [1991\)](#page-51-15). Resistance to curd blight is dominant in nature, polygenically inherited, and in general additive effects were found more pronounced than dominant ones (Sharma et al. [1975](#page-51-19)). Pusa Shubhra having resistance to curd blight has been released for commercial cultivation (Singh et al. [1993\)](#page-52-18). Both additive and dominant gene action played a role in resistance but partial dominance is more important (King and Dickson [1994\)](#page-49-16).

7.10.3 Breeding for Insect Pest Resistance

Dickson et al. ([1986\)](#page-47-22) identifed a glossy-leaved caulifower which exhibited high resistance to diamondback moth. Resistance to cabbage head borer (*Hellula undalis* L. *fabricius*) is reported in caulifower genotypes ES-97, ES-96, Katiki (J.B), KW-5, KW-8, KW-10, Kunwari (RB), Kathmandu Local, Early Patna, EMS-30 and PSK-16 (Lal et al. [1991](#page-49-17)). Lal et al. [\(1994](#page-49-18)) also found resistance under feld conditions in Indian cauliflower F_1 hybrids like aa X ES102, aa \times Kataki (JB), aa \times First Early, aa \times First Crop, aa \times Sel.100, aa \times Sel.41 and aa \times 824 to Bihar hairy caterpillar (S*pilosoma oblique*). Aphids cause major losses to cole crops. The aphid species responsible for economic losses in caulifower and other cole crops are cabbage aphid (*Brevicoryne brassicae*), green peach aphid (*Myzus persicae*) and turnip aphid (*Lipaphis erysimi*). Resistance to cabbage aphid is reported in NY 13816, NY101181, NYIr 9602 and NYIR 9605, but work on caulifower is very scanty. Naturally occurring compounds like glucosinolates, pipecolic acid and β-nitroprionic acid in the tissue of *Brassica* plants are responsible for resistance to cabbage looper and the imported cabbageworm. Breeding resistant varieties in caulifower and other cole crops for most insect pests and some diseases remains elusive because hardly any germplasm source with a desirable degree of resistance is available.

7.11 Molecular Markers

Marker-assisted selection (MAS) is an indirect process where selection is based on a marker instead of the trait itself. The successful application of MAS relies on the tight association between the marker and the major gene or QTL responsible for the trait (Singh and Singh [2015\)](#page-52-19). They ensure a reasonable likelihood that the genotype combining favorable alleles is present in the population (Ishii and Yonezawa [2007\)](#page-48-18). Kalia et al. ([2017\)](#page-48-19) identified two closely linked (1.6 cM) markers (RAPD-OPO-04833 and ISSR-11635) to black rot resistance locus (*Xca1bo)* and converted them into sequence characterized amplifed region (SCAR) markers. These two SCAR markers, ScOPO-04833 and ScPKPS-11635, were linked with black rot resistance locus *Xca1bo*. Singh et al. ([2012\)](#page-52-20) mapped the RAPD and ISSR markers with downy mildew resistance gene (*Ppa3*) in caulifower. They also investigated 20 polymorphic primers in bulked segregant analysis and identifed seven as putatively linked markers between resistant and susceptible bulks generated from F_2 population developed for downy mildew in Indian caulifowers (Singh et al. [2015\)](#page-52-21). However, only three (OPC141186, OPE141881, and ISSR231103) of distinguished resistant and susceptible individuals of respective bulbs, hence used for genotyping of mapping population and development of linkage map with *PPa3* gene.

To fnd markers, the SNPs discovery was performed by genotyping by sequencing (GBS) in caulifower and broccoli by Stansell et al. [\(2018](#page-53-20)) while investigating phylogenetic patterns, population structure and domestication footprints, with and without reliance upon a reference genome, and produced $141,317$ and $20,815$ filtered SNPs, respectively. Further, Sun et al. [\(2019](#page-53-7)) sequenced and assembled cauliflower genome (584.60 Mb) with a contig N50 of 2.11 Mb, and contained 47,772 genes. According to them, chromosome number 03 of caulifower shared the most syntenic blocks with the A, B (*Brassica* species) and C (*B. oleracea*) genomes indicating that this is the most ancient one in the caulifower genome.

7.11.1 Marker-Assisted Improvement of Quantitative Traits

Early inheritance studies focused on morphological traits, although complex inheritance was often observed, suggesting that many genes controlled the traits. Lan and Paterson [\(2000](#page-49-19)) attempted to resolve these complex inheritance patterns i.e., a series of QTLs associated with cauliflower curd traits using $3 F₂$ populations. Segregating populations of F_1 derived doubled haploid (DH) lines from divergent parents had been used to for construction of linkage map of the *Brassica oleracea* genome and identifcation of QTLs controlling developmental characteristic (Sebastian et al. [1991,](#page-51-20) [2002\)](#page-51-21). Genetic linkage maps of *B. oleracea* created at the then HRI, Wellesbourne (UK), comprise over 600 molecular markers (King [2004\)](#page-49-20).

7.11.2 Genomic Selection

Genomic selection is a recent approach that also relies on marker-assisted selection. It enables the simultaneous selection for tens or hundreds of thousands of markers, which cover the entire genome. It is thought to provide the key in maximizing the full potential of MAS, especially for breeding complex traits. Genomic selection requires the availability of phenotypic and genotypic data for the reference population. The objective of genomic selection is to predict the breeding values of each individual instead of identifying QTLs for use in a traditional marker-assisted selection. GWS makes use of genomic estimated breeding values (GEBVs) as a selection parameter, rather than the estimated breeding values.

In caulifower, Thorwarth et al. ([2017\)](#page-53-21) found genomic selection effective in predicting the QTLs for improving curd-related traits.

7.11.3 Association Studies

Association mapping uses natural genome-wide distribution of various genes together with other detectable loci/markers in predicting the marker-trait associations (Singh and Singh [2015](#page-52-19)). However, such studies were not explored in Indian caulifowers, although Thorwarth et al. [\(2017](#page-53-21)) performed genome-wide association studies (GWAS) for genomic prediction to improve curd-related traits in caulifower and identifed a total of 24 signifcant associations for curd-related traits with prediction abilities ranged from 0.10 to 0.66 for different traits and did not differ between prediction methods. Matschegewski et al. [\(2015](#page-50-22)) also performed GWAS for genetic dissection of temperature-dependent curd induction in caulifower using a panel of 111 caulifower commercial parent lines and identifed 18 QTLs for curding time localized on 7 different chromosomes. They also done transcriptional profling of fowering genes *FLOWERING LOCUS C* (*BoFLC*) and *VERNALIZATION* *2* (*BoVRN2*) and observed the increased expression levels of *BoVRN2* in genotypes with faster curding. Rosan et al. [\(2018](#page-51-1)) tried QTL and GS models to predict time to curd induction, and one of them generated slightly better results $(R^2 = 0.52 - 0.61)$.

7.12 Transgenesis and Gene Editing

The transgenic approach was performed by Lu et al. [\(2006](#page-50-23)) in cauliflower and transformed plants with *Or* transgenesis associated with a cellular process that triggers differentiation of proplastids or other non-colored plastids into chromoplasts for carotenoids accumulation. A successful protocol for genetic transformation of caulifower employing the process of agroinfection was proposed by Kowalczyk et al. [\(2018](#page-49-21)) with variety Pioneer transformation via *Rhizobium rhizogenes* (ATCC 18534, A4) with higher (72%) transformation effciency GUS assay (55%). In the absence of availability of resistance in the caulifower germplasm, especially for diamondback moth, genetic engineering offers the same and a lasting solution. Kalia et al. [\(2020](#page-49-22)) investigated insecticidal effcacy of *Cry1B/Cry1C* genes in transgenic caulifower, assessed by feeding neonates of diamondback moth on detached leaves. From a large number of transformed lines analyzed, it is obvious that the *Cry1B/Cry1C* genes potentially exhibited insecticidal activity. During this, they developed a regeneration protocol for Indian caulifower variety Pusa Meghna (Fig. [7.4\)](#page-42-0). Chakrabarty et al. [\(2002](#page-45-16)) evaluated a number of factors that infuence genetic transformation to optimize *Agrobacterium*-mediated transformation of hypocotyl explants of caulifower variety Pusa Snowball K-1. They mobilized synthetic *cryIA(b)* gene into cauliflower and observed effectiveness of the transgene against infestation by diamondback moth (*Plutella xylostella*) larvae. Chikkala et al. [\(2013](#page-46-20)) reported production of transgenic caulifower with plastid division gene *BoMinD* by using PEG mediated transformation of mesophyll protoplasts which has had abnormally-shaped chloroplasts but devoid of true macrochloroplast or minichloroplast phenotype. Ding et al. [\(1998](#page-47-23)) performed *Agrobacterium*mediated transformation of caulifower with a trypsin inhibitor gene TI gene from a sweet potato cultivar and reported in planta resistance to local insects to which the control plants were vulnerable. Chen et al. ([2008\)](#page-46-21) cloned three putative chlorophyllase genes; of them, only *BoCLH1* transcribed during postharvest senescence and antisense *BoCLH1* transcripts showed positive correlations with slower postharvest yellowing. Russell et al. [\(2017](#page-51-22)) reviewed progress on deployment of pyramided *Bt* genes *cry1B* and *cry1C* for the control of *Plutella xylostella*, *Crocidolomia pavonana*, *Hellula undalis* and *Pieris* spp. in caulifower. Recently, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) were reported as an effcient and recent tool for genome editing (Cong et al. [2013\)](#page-46-22). It consists of a nuclease (Cas9) and two short single-strand RNAs (crRNA and tracrRNA) which are fused to form single-guide RNA (sgRNA), for genome editing. Cas9 and a gRNA form a ribonucleoprotein complex and bind to genomic DNA. In cole vegetables, Jansson ([2018\)](#page-48-20) was the first to describe the gene editing

Fig. 7.4 Standardization of protocol for tissue culture of Pusa Meghna caulifower. (**a–b**) Callusing and shoot emergence, (**c**) Shooting, (**d–e**) Shoot growth, (**f**) Rooting, (**g**) Ex vitro plant. (Photos are credited to late Ms. P. Choudhary)

using CRISPR-Cas9 (a *Brassica* deletion mutant) in cabbage as model plant and PsbS as target gene.

7.13 Conclusion and Prospects

Caulifower originated in Cyprus and around the Mediterranean coast and evolved into different phylogenic groups in the European region; however the highest share (75%) in current global production is from China and India. This could have happened due to development of tropical types that expanded the growing regions and seasons. In India, caulifower has four maturity groups which expanded the harvest season from September to March. Caulifower has diverse germplasm due to diverse groups worldwide but the use of exotic brassicas is also possible. The wild/related species have effectively been exploited to transfer different types of cytoplasmic male sterility systems in caulifower. Similar attempts are under way for introgression of resistance to diseases such as black rot using *Brassica caritana* and *B. juncea*. The use of novel tools and techniques such as molecular breeding, genomics, association mapping, genomic selection, TILLING, transgenic and CRISPR/Cas9 have great prospect in caulifower breeding for handling complex traits for yield, stress tolerance and climate change.

Appendices

Appendix I: Research Institutes Relevant to Caulifower

Appendix II: Genetic Resources of Caulifower

(continued)

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