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Double Haploidy and Marker Assisted Selection in Cauliflower Breeding: A Review

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Abstract

Cauliflower iscommonly grown vegetable of Cruciferae family and is grown throughout the world. Today, it is gaining popularity among consumers as it contains potent anti-cancerous compounds like Sulphorphane and helps in fighting cancer. Also, the growers are concentrating more on cauliflower than cabbage due to its high market demand. This indicates the strong need to focus on cauliflower improvement. Breedingstrategies related to cauliflower have been reviewed by many workers but information related tobiotechnological aspects particularly double haploidy and marker assisted breeding is lacking. For commercial hybrid seed production, exploitation of double haploidy (DH) became the need of the hour.Furthermore, for developing homozygous lines in cauliflower, anther and microspore cultures are considered more suitable. Marker-assisted selection (MAS) has its importance in accelerating cauliflower genetic improvement. Developing marker-assisted selection for new sources of resistance might be a valuable tool in breeding new cultivars with enhanced resistance. Details of primers used for cultivar identification and hybridity tests might help young scientists for designing primers for specific purposes. Therefore, an attempt has been made in this paper to review the role of double haploidy and marker assisted selection in cauliflower breeding.

Keywords: *Breeding, cauliflower, double haploids, marker assisted selection, primers*

Introduction

The plant family *Brassicaceae* comprises 338 genera and about 3709 species (Warwick et al. 2006; Al-Shehbaz et al. 2006) of which genus *Brassica* is considered to be the most important from economic point of view (Singh et al. 2018). In general, vegetable species of this genus fall under *Brassica oleracea* (2n=18), and their varieties shares a common C genome (n=9) often known by name “cole vegetables” (Maggioni et al. 2018). In cole group, cauliflower (*Brassica*

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oleracea L.var. *botrytis* L.) is considered to be an economically important vegetable crop (Verma and Kalia 2017).Cauliflower, so called"*Queen of winter vegetables*" is gaining popularity among cole crops on the account of its delicious taste, flavour and high nutritional value. It is also known by the name "Aristocrat of cole crops"(Nimkar and Korla 2014).

Globally, cauliflowers and broccoli are grown over an area of 1395.152 thousand ha with the annual production of 25984.758 metric tonnes and productivity is 18.62 metric tonnes/ ha. China is the leading world producer of cauliflowers and broccoli with an area of 529.98 thousand ha and annual production of 10449.39 thousand metric tonnes with the productivity of 19.72 metric tonnes/ ha (Anonymous 2017). In India, cauliflower is grown over an area of 453 thousand ha with the production of8668 thousand metric tonnes and productivity is 19.1 metric tonnes/ ha (Anonymous 2018). The top cauliflower producing countries include India, China, France, Italy, UK, USA, Spain, Poland, Germany, and Pakistan (Sharma et al. 2005).

In cauliflower, F_1 hybrids are gaining popularity over open-pollinated varieties being uniform in maturity, having high total and early yields, resistance to pest and diseases and, wider adaptability (Kucera et al. 2006; Sekhon et al. 2018). For developing hybrids, self-incompatibility (SI) and cytoplasmic male sterility (CMS) systems are widely used now a days. Further to make hybrid seed production more reliable through advanced approaches, Double Haploid (DH) technology emerged as an alternative approach. It is most widely used to develop inbred lines as well as for genome mapping. Though it is still at infancy stage in public sector institutes but has gained a lot in private sector. Reviewing about DH in cauliflower could help the young scientists to get consolidated information about appropriate media preparation and culture methods.

Marker Assisted Selection (MAS) is another approach which has proved to be more reliable over conventional systems of breeding. Here, DNA markers play vital role being reliable selection tool. DNA markers are highly stable, easily scorable in laboratory and are not influenced by environmental factors. Increased reliability, efficiency and low cost of DNA markers make them more relevant for MAS. In case of cauliflower, different maturity groups are there. Further, cauliflowers on basis of temperature requirement and morphological variation were again classified into European and Indian cauliflowers. The identified markers in one group have limited use in other group as DNA markers have poor transferability (Singh et al. 2015). Thus studies regarding to different maturity groups particularly, European and Indian

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cauliflowers, needs to be reviewed for providing information regarding available/ identified DNA markers.

Haploidy breeding

In the era of 21st century,mass multiplication of plants as well as developing disease resistant plants in *in vitro* conditions has attracted the scientific community to speed up breeding programmes for cauliflower improvement. In cauliflower, where SI and CMS lines are gaining popularity for hybrid seed production, their mass multiplication through tissue culture method emerged as the most viable option (Sharma et al. 2005). Plant part (organ, cell or piece of tissue) grown on a nutrient medium is known by name "explant". Meristem is considered to be most ideal explant for *in vitro* multiplication. Other parts like pedicel, stem, stump, anther, peduncle, tip, leaf, curd and root have also been used by many workers for *in vitro* method (Table 1). Curd explants are mostly used when to go for industrial production of parental hybrid lines through *in vitro* method. This method is generally followed for seed production (Kieffer et al. 1994). Ross (1980) provides detailed review on*in vitro* production of disease resistant plants.

Developing homozygous lines through microspore and anther culture (Sharma et al. 2005) acts as most viable option for increasing the uniformity in plant population. Though anther culture method is quick but harvesting of embryoides from culture depends on various factors (Lillo and Hansen 1987). These include varying concentrations of sucrose and growth hormones, prior high temperature treatment and donor anther's developmental stages. Simmonds and his coworkers in 1989 reported Uninucleate stage as best stage for anther culture in most of *Brassicas*. Further, Ockendon in 1988 observed significant differences in the embryoid producing efficiency different genotypes of cauliflower.Chromosome doubling through colchicine treatment (0.05%) to plant apices of haploid embryoides results in dihaploid plants (DH) which upon selfing produce homozygous uniform population.

Table 1 List of different explants (Somatic embryogenesis) used by different workers in their study

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Explants used	Reports
Peduncles	Christey and Earle 1991
Microspores and protoplasts	Zhao et al. 1996
Cotyledons	Narasimhulu and Chopra 1988
Roots	Lazzeri and Dunwell 1984; Horeau et al. 1988
Somatic embryogenesis	Pareek and Chandra (1978)
Somatic embryos	Leroy et al. (2000)
Protoplasts	Delpierre and Boccon-Gibod 1992

Micropropagation

Many workers have achieved direct regeneration from meristematic tissues and curd pieces. In cauliflower most responsive explant reported is curd pieces. Kumar et al. (2003) reviewed this aspect and reported studies as given in Table 2:

Table 2 Micropropagation in cauliflower

Explant	Medium	Response
Hypocotyl, curd pieces, branches	2.44µM BA + 5.0µM NAA + 168µM sucrose	Multiple shoot
Curd meristem	2.0 Kin liquid	Field transfer of plants
Head explant	1.0 NAA + 0.1 IAA (mg/l)	Plants with high survival rate
Internode and inflorescence axis	5.0µM BA	Regenerated plants
Leaf and stem	8.88µM BA	Regenerated plants

Breeding methods used in cauliflower are essentially conventional but less-effective, due to which not much progress has been reported in cauliflower breeding (Gu et al. 2014). They presented an efficient method of microspore culture for production of double haploid loose-curd cauliflower plants.

MARKER ASSISTED BREEDING

Molecular markers (closely linked to targeted traits) are the powerful genomic tools to increase the efficiency and precision of breeding in crop improvement (Varshney et al. 2012). DNA

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marker technology is now being increasingly realized viz., germplasm characterization and genetic diversity analysis (Lashermes et al 1996; Anthony et al 2002; Steiger et al 2002), analysis of alien genome introgression (Prakash et al 2002; Herrera et al 2002) and for marker identification having linkage with resistance genes (Noir et al 2003; Prakash et al 2004). Developing linkage maps through the use of DNA markers (Collard et al. 2005) and utilizing them for identification of chromosomal regions using QTL analysis (having both qualitative and quantitative genes) is an emerging trend nowadays.

Molecular mapping of cauliflower

Zhao et al. (2016) constructed a genetic map which will not only be useful in comparative genomics but also play important role in identifying QTLs for cauliflower improvement. Details about this linkage map are given in Table 3.

Table 3 Details about genetic map constructed by Zhao et al. (2016)

Markers Employed	Average data integrity of linkage map	Total genetic lengthof final map	Average marker interval	Reference genome	Reference
1776 high-quality SLAF markers, including 2741 SNPs,	95.68%.	890.01 cM	0.50 cM	364.9 Mb	Zhao et al. (2016)

Plant genetic resources are important valuable tool for crop improvement. An understanding of genetic diversity is essential for proper utilization of genotypes in the target oriented research programmes. Molecular markers act as viable, more efficient germplasm characterization tool being polymorphic in nature. Different studies conducted by different workers using different molecular markers are given in Table 4. RAPDs were considered as most suitable markers in 1990s and scientist's working on these markers suggested the use of large number of primers for successful cultivar identification. Best primers reported for producing polymorphic bands are listed in Table 5. Later on ISSRs were developed by Zietkiewicz et al. (1994), which were also known as semi-arbitrary markers and have advantage being quick as well as easy to develop and

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use, highly polymorphic and reproducible in nature. ISSRs are most widely used for mapping, genetic diversity evaluation and cultivar identification. Tri and tetra-nucleotide primers were used for amplification of ISSRs by Leroy et al. (2000) and Bornet at al. (2002) and they reported tri-nucleotide repeats were the most powerful primers for high level of polymorphism. RAPD, ISSR and SRAP were used in single study by Wang et al. (2008) to reveal polymorphism in 32 cultivars of cauliflower. Polymorphic rate was observed to be 78.57% in case of both RAPD and SRAP markers but it was 63.74% in case of ISSRs. SSR markers have been used for assessing diversity in cauliflower cultivars (Ram et al. 2016). RFLP markers were used by Barbeyron and Boury (1998) for mapping of nuclear recessive male sterility gene. AFLP markers in combination with SRAP were developed and used for constructing a genetic map by Li and Quiros (2001). Li and Garvin (2003) worked on finding the markers linked to "Or" gene and they found 10 AFLP markers and converted four out of these into RFLPs for mapping it.

Table 4 Studies conducted by different workers using molecular markers

Molecular markers	References
RAPDs	Hu and Quiros (1991); Demeke et al. (1992); Kresowich et al. (1992)
ISSRs	Zietkiewicz et al. (1994); Leroy et al. (2000); Bornet et al. (2002)
RAPDs, ISSRs and SRAP markers	Wang et al. (2008)
SSRs	Ram et al. (2016)
RFLPs	Barbeyron and Boury (1998)
AFLPs	Li and Quiros (2001); Li and Garvin (2003)

Table 5 List of primers producing polymorphic bands in cabbage and cauliflower cultivars

Primers	Sequence (5'-3')	Markers used	Reports
116	TACGAT GACG	RAPD	Demeke et al. (1992)
16	AACGCGCAAC	RAPD	Kresowich et al. (1992)
Tri-nt	CAACAACAACAACAA	ISSR	Leroy et al. (2000)
	CAGCAGCAGCAGCAG	ISSR	Leroy et al. (2000)
Tetra-nt	GATAGATAGATAGATA	ISSR	Leroy et al. (2000)

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GACAGACAGACAGACA	ISSR	Leroy et al. (2000)
me1-em4	RAPD, ISSR and SRAP	Wang et al. (2008)
me8-em8	RAPD, ISSR and SRAP	Wang et al. (2008)

Conclusion

DH and MAS could act as potential tools for accelerated crop improvement in cauliflower.

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