INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 2, p. 173-182, 2020

RESEARCH PAPER OPEN ACCESS

Assessment of genotypic variation and self-incompatibility in cauliflower (*Brassica oleracea* var. *botrytis*) genotypes

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Key words: *Brassica oleracea* Var. *Botrytis,* Cauliflower breeding, Self-incompatibility, Genetic variation, Qualitative traits, Quantitative traits.

http://dx.doi.org/10.12692/ijb/16.2.173-182 Article published on February 05, 2020

Abstract

Cauliflower is an important cool season vegetable. Knowledge of genetic variation and self incompatibility of cauliflower genotypes may be important as it provides a base for breeding strategies. In this study, genetic variation and strength of self-incompatibility were assessed in different exotic and indigenous genotypes. Mean comparison of genotypes revealed significant variability in all the genotypes regarding quantitative traits i.e. curd weight, plant weight, biomass and curd yield. Extensive variability was also observed for qualitative traits with Shannon–Weaver diversity index (H' = 0.60- 1.08) among the studied genotype. Coverage of curd by leaves, leaf attitude and curd shape in longitudinal section showed the highest degree of genetic variation. Genotypes also showed variation regarding vegetation period, in this respect HCF-12 was characterized as early maturing while HCF-23 as a late maturing genotype. Regarding self-incompatibility all the genotypes showed significant difference when assessed by artificial selfing method with coefficients of variation (CV= 40%) while less variations were observed through natural selfing method (CV= 2%). On the basis of artificial sellfing HCF-22 and HCF-23 (SI >95%) genotypes were categorized into strongly self-incompatible and HCF-12, HCF-13 into self-compatible (50%≤SI≥5%). Snow Wizard, Snow Mountain and FDIII were classified into partial self-incompatible (95%≤SI≥50%). These findings of genotypic variation in phenotypic traits and self-incompatibility expressed the usefulness of these genotypes as parents in the cauliflower breeding program.

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Introduction

Cauliflower is a popular winter vegetable among the cool season vegetable group. The other members of this group are cabbage, broccoli, brussels sprouts, kohlrabi, and kale. All these members were originated through mutation, selection and adaptation from wild cabbage (*Brassica oleracea*) also known as colewort (Purugganan *et al.,* 2000). Cole vegetables are mostly consumed for their nutritional value. Although the nutritional profile of each vegetable varies, all are rich in vitamin A, vitamin C, vitamin K, and dietary fibers (Sanlier and Saban, 2018). In recent years, higher intake of these vegetables was also associated with decreased risk of cancer i.e. colon, breast, colorectal and prostate (Higdon *et al.,* 2007; Pilatova *et al.*, 2011).

In Pakistan, Cauliflower is an important winter crop and cultivated under an extensive range of agricultural regions. During winter it is cultivated in plain areas while in summer its cultivation is carried out in highland areas. Non-availability of local seed is the biggest problem for the farming community. Due to the absence of local cultivars seed, seed requirement is fulfilled through imported seed. Recently, 15.1 tons of cauliflower seed were imported in 2017-18 to fulfill the country's demand (FSC&RD, 2017).

Hybrid cultivars are becoming popular among vegetable growers due to their higher yield and uniformity. In the past, a lot of hybrids of tomato, chillies, cucumber, onion, cabbage, melon, watermelon and bitter gourd had been imported in the country and approved for general cultivation (FSC and RD, 2006; Amin *et al.,* 2018; Tahir *et al.,* 2018). Similarly, in cauliflower hybrid cultivars are also preferred for their uniformity, consistency of vigor, yield and curd quality. But due to non-availability of information regarding genetic variation and breeding mechanism, the country is bound to import seeds of hybrid cultivars from different countries.

Caulifloweris a cross pollinated vegetable in which self-pollination is prevented by the sporophytic type of self-incompatibility. Sporophytic self-incompatibility is a type of self-incompatibility in which germination of the pollen grain hindered on stigma due to the similar S genotype of pollen and stigma. A single S locus with multiple alleles was reported to be involved in its inheritance (Bateman, 1955). Two major genes S-locus receptor kinase (*SRK*) and S-locus cysteine rich protein (*SCR*) were found to be present at this locus and involved in the recognition of self-pollen. Both the genes are tightly linked to each other and always inherited as a single unit, but perform independent action in male and female reproductive organs. *SRK* was reported as a female determinant and its protein product was found in the papilla cells of stigma while *SCR*identified as a male determinant and found in the pollen coat and tapetum of the anther (Jung *et al.,* 2013; Kitashiba and Nasrallah, 2014).

Previously sporophytic self-incompatibility based this system was used for hybrid development in cauliflower worldwide. But due to its highly non-stable nature, it was replaced with cytoplasmic male sterility (CMS) system when Seminis vegetable seed inc. published patent (US6046383A, April 4, 2000) titled Cytoplasmic male sterile *Brassica oleracea* plants which contain the polima CMS cytoplasm and are male sterile at high and low temperatures. The presence of self-incompatibility creates hindrance while developing CMS and their maintainer lines. The assessment of self-incompatibility is still necessary to perform successful self-pollination and back-crossing for male-sterile and maintainer development and also for making successful crosses among inbred lines for hybrid cultivar development. So, still, breeders have to know the extent of self-incompatibility among the breeding material to develop hybrid cultivars.

Genetic variation is the baseline for starting the breeding program of a crop as it provides information regarding diverse genotypes that could be used for the development and evaluation of breeding material and new varieties with desirable characteristics (Vanlalneihi *et al.,*2019). Comprehensive information regarding genetic variation also helps in developing

superior inbred lines to be used in hybrid breeding as parents (Singh *et al.*, 2014). Information regarding genetic variation and self-incompatibility of currently cultivated cauliflower genotypes is almost negligible and also creating hindrance for starting a cauliflower hybrid breeding program.

Keeping all these issues in view, the present study was designed to evaluate the different cauliflower genotypes regarding genetic variability and self-incompatibility. Information regarding genetic variability and self-incompatible of genotypes obtained through this study would be helpful for the breeders to start the hybrid breeding program in cauliflower.

Material and methods

The present study was carried out at the research area of vegetable research Institute Faisalabad, Pakistan, located between 73–74° E longitude and 30–31.5° N latitude. Metrological data was collected from the observatory of plant physiology section agronomic research institute, Faisalabad (Fig.1). Seven genotypes from private and public sectors were used to get a picture of genetic variation and self-incompatibility presence in cauliflower genotypes. Exotic genotypes were collected from different local private companies which are importing cauliflower seed in the country and local genotype was collected from Vegetable Research Institute, Faisalabad (Table 1).

Nursery of the genotypes was sown in the field on September 11, 2017, and four-week old seedlings were transplanted on one side of a row. The trial was arranged ina randomized complete block design with three replications keeping plant to plant distance of 45 cm and row to row 75 cm. Standard cultural practices and plant protection measures were carried out regularly. Sixteen plants of each genotype were transplanted on one side of the row. All the studied genotypes were evaluated for genetic variability using different quantitative and qualitative traits. Ten plants of each genotype were used for recording data of quantitative i.e. curd weight, plant weight, biomass, curd yield, and qualitative traits such as vegetation period, leaf attitude, curd shape, curd color, compactness of curd and coverage of curd by leaves. Data for the vegetation period was recorded from transplanting to the harvest of the genotype in the curd stage. For qualitative traits, data were recorded according to the standard cauliflower descriptor described by (Sarwar, 2008) and (Li *et al.,* 2008) (Table 2).

From the rest of the plants, three plants (plant 1= P1, plant 2= P2, plant 3= P3) were randomly selected and used for testing selfing incompatibility. Selfing and crossing were carried out during February 2018 when maximum temperature was ranged between 25-30°C. As the inflorescence behavior of cauliflower is cymose type hence pollination of all genotypes took place from February 17, 2018, until February 28, 2018. In literature two types of methods are reported for self-incompatibility testing, natural self-pollination and artificial self-pollination (Sahu, 2017; Sing and Vidyasagar 2012). For self-incompatibility testing by using both these methods, the inflorescence of each plant was partitioned into three parts at the bud stage. Forty five buds on each inflorescence part were retained to use them for selfing and crossing. After retaining uniform buds, each inflorescence part was covered with a butter paper bag immediately and tagged for artificial selfing, natural selfing and crossing respectively. Artificial selfing was carried out manually and for this freshly open flowers were used. For natural selfing, flowers let to self-pollinate in butter paper bags until all the flowers changed into silique. The third part of inflorescence was used for making crosses with other genotypes to get the cross seed data. After 60 to 70 days of pollination, mature siliques were harvested to record the data of seed set per silique.

Statistical analyses

To check variation among genotypes regarding quantitative traits, data were analyzed by analysis of variance and means of the genotype were compared with LSD test by using Statistix 8.1 software. Genetic variations regarding qualitative traits were assessed

by the Shannon-Weaver information index. The Shannon-weaver information index was computed from the phenotypic frequencies of qualitative traits. Index H' was calculated as described by (Magurran, 2004; Shannon and Weaver, 1949).

$$H' = -\sum_{n=1}^{n} pi \, ln \, pi$$

Here pi is the relative frequency and ln is the natural logarithm.

Self-incompatibility was tested on the basis of the self-incompatibility percentage. The self-incompatibility percentage is calculated by using the formula provided by Damake *et al.*,(2009). Genotypes were classified in to five groups strictly self-incompatible (SI = 100%), strongly self-incompatible (SI > 95%), partially self-incompatible (95% ≤ SI ≥ 50%), self-compatible (50% ≤SI ≥ 5%), fully self-compatible (SI = 0%) as describe by Damake *et al.*, (2009).

$$\text{Selfcompatibility (\%)} = \frac{\text{Number of seed per pod under self - pollination}}{\text{Number of seed per pod under cross pollination}} \times 100$$

$$\text{Self - incompatibility (\%)} = 100 - \text{Selfcompatibility (\%)}$$

Results and discussion

Genetic variation assessment of quantitative traits

Curd yield is an important economic trait as it is the main outcome source for the farmers. Significant variations were observed regarding curd yield in the studied genotypes (Table 3).

The highest curd yield was found in HCF-12 (46.03 t/ha), while the low yield was observed in Snow Mountain (22.01 t/ha) (Table 4). It was found that differences in the yield among the genotypes are mainly due to differences in their genetic constituents. Similar findings regarding genetic variation for curd yield were also observed by (Vanlalneihi *et al.,* 2019); while evaluating sixteen genotypes belonging to the early maturing group of cauliflowers.

Table 1. Origin and source of collection of studied cauliflower genotypes.

Sr. No.	Genotype	Origin	Genotype source
1	FD-III	Indigenous	Vegetable Research Institute, AARI, Faisalabad
2	HCF-12	Exotic	Kanzo quality seed sons PVT limited
3	HCF-13	Exotic	Kanzo quality seedsons PVT limited
4	HCF-23	Exotic	Kanzo quality seed sons PVT limited
5	HCF-22	Exotic	Kanzo quality seed sons PVT limited
6	Snow Mountain	Exotic	Mehrmohd Din & sons PVT limited
7	Snow Wizard	Exotic	Mehrmohd Din & sons PVT limited

Yield is the most integrative trait as it is influenced by many other known and unknown factors. Curd is the edible portion of the cauliflower and is the main factor that affects the yield of cauliflower genotypes. Higher the curd weight more will be the yield of the respective genotype. In the present study, genotypes are showing significant differences regarding curd weight. Curd weight in a range of 0.86 kg to 1.80 kg was observed in studied genotypes. The highest curd weight of 1.80 kg was recorded in the HCF-12 genotype while the lowest curd weight (0.86 kg) was recorded in Snow Mountain (Table 4). A range of curd weight from 0.11 kg to 1.20 kg was recorded in cauliflower inbred lines belonging to different regions of China by (Zhu *et al.,* 2018).

Plant weight and curd weight are highly correlated characters and contributed significantly to increasing the yield of a genotype (Kumar *et al.,* 2017). A significant variation regarding plant weight was also observed in the assessed genotypes. The highest plant weight (2.88 kg) was recorded in the HCF-12

genotype which also showed the highest curd weight. Similarly, the lowest plant weight was recorded in Snow Mountain (Table 4). Significant variations were also found in genotypes for biomass production. Kumar *et al.* (2017) also observed significant differences in curd weight and plant weight among the mid-season cauliflower varieties. Significant differences regarding plant weight, curd weight, and curd yield among the early season cauliflower were also reported by (Chittora and Sing, 2015).

Table 2. Code and states of the qualitative variables for cauliflower genotypes.

Quality variable	Code and characters state						
	0	1	2	3	4	5	7
Leaf attitude		Erect		Semi Erect		Horizontal	
Curd shape in longitudinal section		Circular	Transverse Broad Elliptic	Transverse Medium Elliptic	Transverse Narrow Elliptic	Triangular	
Curd color	White	Cream	Yellow	Tan	Green		
Curd compactness				Loose		Intermediate	Large
Coverage of curd by leaves		Not Covered	Partly Covered	Covered			
Curd pubescence / Hair on curd	Absent	Light	Medium	Much			

Table 3. Analyses of variance for different quantitative traits of cauliflower genotypes.

Source of variation	DF	Sum of squares			
		Plant weight (kg)	Curd weight (kg)	Biomass (t/ha)	Curd yield (t/ha)
Replication	2	0.00	0.08	2.67	7.70
Variety	6	0.80**	0.37*	502.48**	217.35**
Error	12	0.06	0.08	9.99	24.60

* = significant at 5% level of probability, * *= significant at 1% level of probability.

Genetic variation assessment of qualitative traits

Genetic variations regarding qualitative traits are highly desirable as most of the quality traits are highly desirable from the market of view. Genotypes showed extensive variability regarding all the qualitative parameters. Vegetation period showed variation among the genotypes with a range of 75-95 days. HCF12 had the lowest vegetation period of 75 days, so it was characterized as early maturing while the genotype HCF-23 with 95 days of vegetation period characterized as late maturing. Curd quality is another important trait from the market point of view as a clear white and compact curd, without pubescence is highly desirable in the market.

Table 4. Mean comparison of studied genotypes regarding different quantitative parameters.

Variety	Plant Weight	Curd Weight	Biomass	Yield
	kg	kg	t/ha	t/ha
FD-III	2.81a	1.18bc	72.38ab	30.55cd
HCF-12	2.88a	1.80a	74.12a	46.03a
HCF-13	2.24bc	1.28bc	57.57c	32.17cd
HCF-23	2.61ab	1.58ab	67.05b	40.69ab
HCF-22	2.76a	1.39bc	71.07ab	33.70bc
Snow Mountain	1.56cd	0.86c	40.23d	22.01d
Snow Wizard	1.82d	0.93c	46.81d	23.99d
LSD	0.16	0.19	11.49	4.58
	*	*	*	*

LSD = Least significant differences, * = significant at 5% level of probability.

The performance of genotypes regarding other quality parameters is presented in Table 5. The frequency of various qualitative traits along with their Shannon – Weaver index (H') of diversity estimated based on six qualitative traits of all cauliflower varieties is presented in Table 6.

Table 5. Phenotypic performance of different cauliflower genotypes regarding quality parameters.

Variety	Vegetation period*	Leaf attitude	Curd shape	Curd color	Curd compactness	Coverage of Curd by leaves	Curd pubescence
FD-III	82	5	3	2	3	2	1
HCF-12	75	3	1	2	5	1	1
HCF-13	78	3	1	2	5	1	1
HCF-23	95	3	2	1	5	3	1
HCF-22	87	3	3	1	5	3	1
Snow Mountain	82	1	1	2	5	2	2
Snow Wizard	82	1	1	2	3	2	2

The average value of the H' was 0.80. Coverage of curd, leaf attitude, and curd shape in the longitudinal section showed the H' value above than average (0.80). Highest value of H'was recoded for coverage of curd by leaves trait (1.08); trait that is highly desirable for white curd suggesting greater variation for this trait among the studied genotypes. The H' value for the rest of the traits such as curd color, compactness and curd pubescence was 0.60 indicating the lowest variation in these traits. Shannon-Weaver diversity index (H') was used in many crops such as rice and barley for the estimation of phenotypic diversity (Rabara *et al.,* 2014; Shakhatreh *et al.,* 2010). In cauliflower, Shannon-Weaver diversity index was also used as phenotypic diversity measurements of cauliflower inbred lines for qualitative traits. A range of H vale 1.12-0.69 for different qualitative traits such as plant growth habit curd shape, curd color, amount of curd hair and solidity of curd was observed in cauliflower inbred lines belonging to different regions of China (Zhu *et al.,* 2018).

Table 6. Frequency distribution and phenotypic diversity index (H) for quality characters among seven cauliflower genotypes.

Quality variable	Frequency of different grades of different traits						Shannon-Weaver index (H)
	0	1	2	3	4	5	
Leaf attitude		2		4		1	0.96
Curd shape in longitudinal section		4	1	2			0.96
Curd color	2	5					0.60
Curd compactness				2		5	0.60
Coverage of curd by leaves		2	3	2			1.08
Curd pubescence / Hair on curd	5	2					0.60
Average diversity/ variation							0.80

Assessment of self-incompatibility

Self-incompatibility is the main feature of the brassica family genotypes that create a hindrance for self-pollination of a genotype. In cauliflower, a great variation regarding self-incompatibility was recorded in previous studies. Highly to facultative self-incompatible and self-compatible types of genotypes were reported in cauliflower (Watts, 1963; Niuwhof,

1974; Gray and Crisp, 1976).In the present study, the strength of self-incompatibility was assessed by performing natural and artificial selfing. In case of natural selfing, no significant differences were seen among genotypes in respect of self-incompatibility as the coefficient of variance (CV=2%) is very low (Table 7).

Table 7. Classification of genotypes on the basis of self-incompatibility percentage.

Sr. No.	Genotype name	SI testing on the basis of natural selfing			SI testing on the basis of artificial (manual) selfing	
		SI%		Phenotype	SI%	Phenotype
1	HCF-12	P1	99.8	SSI	36.7	SC
		P2	99.5	SSI	45.0	SC
		P3	99.8	SSI	32.9	SC
2	HCF-13	P1	99.8	SSI	34.0	SC
		P2	99.5	SSI	21.3	SC
		P3	99.8	SSI	9.8	SC
3	HCF-22	P1	99.8	SSI	97.8	SSI
		P2	100.0	St.SI	95.7	SSI
		P3	100.0	St.SI	97.8	SSI
4	HCF-23	P1	100.0	St.SI	95.7	SSI
		P2	99.8	SSI	98.6	SSI
		P3	100.0	St.SI	96.6	PSI
5	Snow Mountain	P1	97.3	SSI	90.0	PSI
		P2	98.6	SSI	90.0	PSI
		P3	98.2	SSI	91.0	PSI
6	Snow wizard	P1	96.5	SSI	86.0	PSI
		P2	95.9	SSI	86.0	PSI
		P3	97.7	SSI	91.0	PSI
7	FDIII	P1	96.8	SSI	86.0	PSI
		P2	96.13	SSI	90.0	PSI
		P3	95.68	SSI	89.0	PSI
	CV%	2			40	
	SE mean	0.35			6.47	

SC= Self-compatible, SI= Self-incompatible PSI= Partial self-incompatible SSI= Strongly Self-incompatible, St. SI = Strictly Self-incompatible.

All the genotypes regarding this method were characterized as strictly to strongly self-incompatible (SI >95%) (Table7). (Sing *et al.,* 2002) tested fourteen genotypes regarding self-incompatibility by using natural and artificial selfing methods and found similar results regarding self-incompatibility by using natural selfing method. In their study, almost all the genotypes set no seed on natural selfing, hence all were characterized as self-compatible. Sahu (2017) also reported similar findings when tested Indian cauliflower on the basis of natural self-pollination carried out through bagging.

In the case of artificial selfing, significant variation was observed regarding self-incompatibility among the genotypes. The coefficient of variation CV=40% for this method is showing that genetic variation exists among the genotypes regarding self-incompatibility (Table 7). On the basis of self-incompatibility percentage, two genotypes HCF-22 and HCF-23 that showed the SI% greater than 95 characterized as strongly self-incompatible while genotype Snow Mountain, Snow Wizard and FD III as partially self-incompatible (95% ≤ SI ≥ 50%). Genotype HCF-12 and HCF-13 showed the self-

incompatibility percentage in range of (9.8-45.0%). They showed SI in the range of 50%≤SI≥5%; therefore they were categorized as highly self-compatible (Table 7). The results of this study are similar to Hadj-Arab *et al.*, (2010) who also observed strongly self-incompatible to partial self-incompatible and self-compatible plants in the advanced generation of cauliflower while testing self-incompatibility through artificial selfing method. A similar variation in cauliflower germplasm regarding self-incompatibility was also observed by (Watts, 1963; Niuwhof, 1974;Gray and Crisp, 1976).

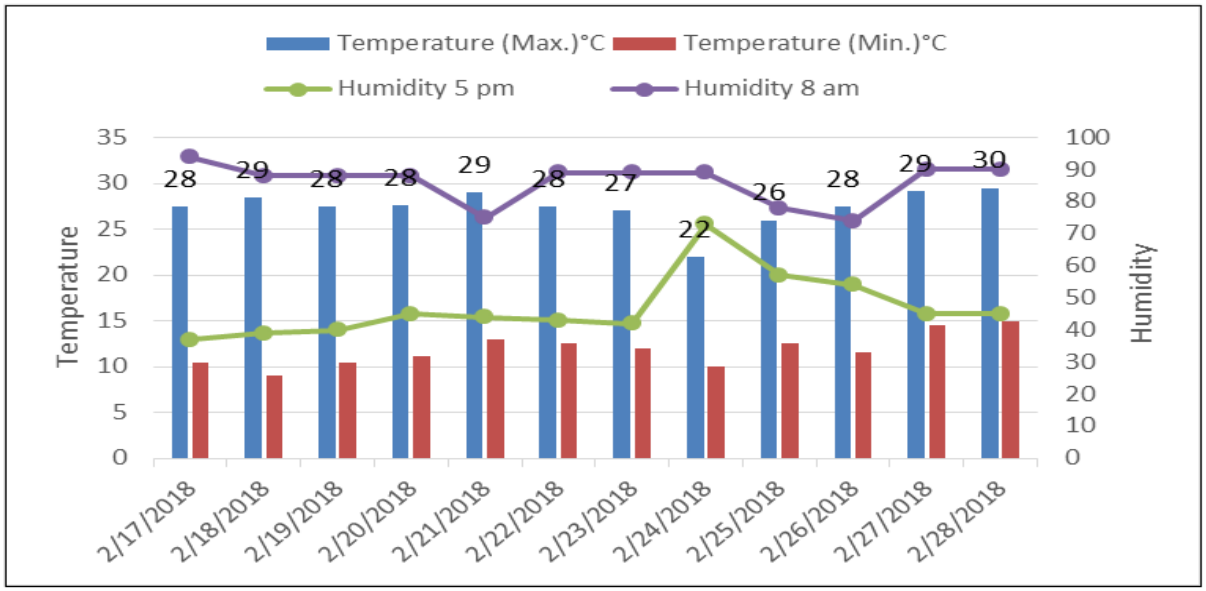

Fig. 1. Metrological data during self-incompatibility based studies in cauliflower.

Differences in genotype classification on the basis of natural and artificial pollination showed that the bagged or natural selfing may not be suitable for testing self-incompatibility. The reason that was found behind this was non-effective self-pollination due to the hypogynous nature of cauliflower flower and secondly self-seeds formation during bagged condition due to bud pollination. As bud pollination is a method that is used for making self-seed in self-incompatible genotypes.

In this method, flowers are pollinated at bud stage as at this time expression of genes responsible for self-incompatibility i.e. *SRK, SCR*was less hence no hindrance for self-pollen germination into pollen tube at the stigma were present. This reason for self-seed development through bud pollination was also observed in bagged/naturally self-pollination of the highly self-incompatible variety Pan Shubhra by (Damake *et al.,* 2004). In this genotype, stigmas were protruded out from bud and exposed to receive pollen.

In literature another method like the observation of pollen tube growth in pistil through fluorescence microscopy after staining with aniline blue also used for determination of self-incompatibility. Ruffio-Chable *et al.* (1997) reported the seed test based self-incompatible testings the most accurate measurement for the breeders to identify self-incompatible and self-compatible plants.

Conclusion

Seven cauliflower genotypes exhibited greater variations regarding quantitative, qualitative, and self-incompatibility traits. All the genotypes showed significant variations in curd weight, yield, plant weight, and biomass. Regarding qualitative traits, coverage of curd by leaves, leaf attitude and curd shape in longitudinal section showed the highest variation. From the vegetation period, it was concluded that HCF-12 is the early maturing variety while HCF-23 is late maturing. Regarding self-incompatibility all the genotypes showed significant differences when assessed by artificial selfing method.

It may be concluded that self-incompatibility assessment on the basis of natural self-pollination may be confusing so, to test the level of self-incompatibility artificial selfing should be carried out in cauliflower. Genotypes HCF-12 and HCF-13 showed stable self-compatible while the genotype HCF-22 and HCF-23 showed strongly self-incompatible expression in the study. All the genotypes were evaluated in respect of self-incompatibility when the temperature of the air was ranged between 22-30°C, so the results are not misleading in respect of temperature effect.

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