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Role of sterility and incompatibility and crop improvement in vegetable crops

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Abstract
Male sterility in vegetables is a never-ending process due to rapid advancement in molecular technique sand their implementation. Male sterile mutants play an important role in the utilization of heterosis and the study of development and regulation in plant reproduction Substantial progress has been made in understanding the mechanism of male sterility in selected vegetable crops. On a global level, cytoplasmic male sterility (CMS) and cytoplasmic genetic male sterility (CGMS) are the most widely utilized in the majority of vegetables. In India vegetable hybrids based on CMS and CGMS system have been limited. In India, genetic male sterility (GMS) has been exploited commercially only in the cases of chilli and muskmelon to develop F1 hybrid seed commercially. Molecular basis of self-incompatibility system in vegetable crops Self incompatibility is an important out breeding mechanism results due to morphological, genetic, physiological and bio chemical causes. So far it has been reported in about 70 families of angiosperms including several crop species. The common way of plants to avoid self-fertilization is by self-incompatibility. It is a physiological barrier making the flower difficult to fertilize itself even though it may be abundantly pollinated with its own pollen. There are two forms of self-incompatibility system includes sporophytic self-incompatibility and gametophytic self-incompatibility. Sporophytic self-Incompatibility system is a feature of crucifer family (Brassicaceae). In this review, we discuss the self-incompatibility system in molecular level, its practical application and suppression and its impact in vegetable crops.

Keywords: Self-incompatibility, vegetable crops, genetic male sterility, cytoplasmic genetic male sterility

Introduction

Vegetables are regarded as protective foods as they are rich in minerals, vitamins and antioxidants. Vegetables play an important role in the balance diet by providing not only energy but also supplying vital protective nutrients either mineral or vitamins. Thus, vegetables are getting increasing higher importance in India as well as in the world due to their relevance in achieving nutritional security from emerging nutritional problems in human beings. Today, India is the second largest producer of vegetables in worlds after China. Total Horticulture production in 2020-21 is estimated to be 326.58 million Tonnes, an increase of about 5.81 million Tonnes (increase of 1.81%) over 2019-20. (NHB Database, 2020-21).

of its crops productivity is lost due to biotic stress. Vegetable are succulent and sensitive plants. During domestication, crop plants were subjected to intense selection pressure resulting in their narrow genetic base. Abiotic stresses reduce average yield of crops upto 50%. In India also 67% of the area is rained and crops in these areas invariably experience droughts at different magnitudes. Annually about 42% of the crop productivity is lost owing to various abiotic stress factors. By 2025, 30% of crop production will be at risk due to the declining water availability. World Bank projects that the climate change will depress crop yield by 20% or more by the year 2050.

Self incompatibility is the in ability of a plant with hermaphrodite flowers producing developmentally functional male and female gametes to set seeds on self – pollination. This is, in effect, achieved by interposing an ovel physiological barrier at any stage between pollination and fertilization. The regulation of mating among- self – gametes is generally operative in the stylar region which act as a highly effective biological sieve of diploid tissues arresting the pollen tube growth and thereby preventing haploid gametes (male and female) from uniting and affecting fertilization. Thus, in self-incompatibility nature has evolved one of the most efficient mechanisms to promote out breeding and consequent heterozygosity in angiosperms. The term self-incompatibility was originally coined by Stout in 1917.

Koelreuter, in the middle of 18th century, first reported self-incompatibility in *Verbascum phoeniceum* plants.

The Ogu CMS in *B. oleracea* is derived from radish. There is no Ogu CMS restorer gene in *B. oleracea*. All offspring produced by Ogu CMS lines are male sterile. Therefore, we cannot isolate new breeding materials from some excellent Ogu CMS germplasms by selfing. Development of Ogu CMS restorer lines is of great importance for the innovation and utilization of Ogu CMS germplasm in *B. oleracea*. Using distant hybridization and embryo rescue techniques, the Ogu CMS restorer gene in *B. napus* has been successfully introduced into Chinese broccoli. Through multi-generation backcrosses combined with marker screening, a Chinese broccoli Ogu CMS restorer line with a normal number of chromosomes was developed.

Male sterility is defined as the deviant condition in normally bisexual plants with the failure of the plants to produce functional pollens, anthers or male gametes. It refers to a condition in which the plants are unable to produce or release the functional pollen grain as a result of failure of formation or development of pollen, stamens or gametes although the female gametes function normally. J.K. Koelreuter. Observed anther abortion within species & species hybrids and was first to report male sterility in flowering plants. The male sterility attains diverse forms such as:

- Absence, malformation of male or gans in bisexual plants.
- Fail to develop normal micro sporogenous tissue.
- Anomalies in micro sporogenesis yield in ginviable, deformed or aborted pollen.
- Viable pollen development but anthers in dehiscent.

Main features of self-incompatibility

- Self-incompatibility is an important out breeding mechanism which prevents autogamy and promotes allogamy.
- Self-incompatible species do not produce seed on self-pollination but leads to normal seed set on cross pollination.
- It maintains high degree of heterozygosity in a species due to outbreeding and reduces homozygosity due to elimination of inbreeding or selfing.
- Self-incompatibility results due to morphological, genetic, physiological and biochemical causes. It is not under simple genetic control.
- Self-incompatibility reaction can operate at any stage between pollination and fertilization.
- Self- incompatibility has been reported in about 70 families of angiosperms including several crop species.

Male sterility can arise in plants due to following reasons

- Barrier of tapetal layer: Delayed degeneration of tapetal cells that block the availability of nutrient to microspore.
- Improper timing of callase activity: Callase is an enzyme required for breakdown of thecal lose that surrounds the pollen mother cells, helps in release of pollen ; early or delayed callase activity lead to sterility.
- Role of Esterase: Esterase play role in the hydrolysis of Sporopollenin, the polymer required for pollen formation. Decreased activity of esterase in male sterile plant has been observed in tomato and in radish.
- Absence or malformation of male organs (stamens) in bisexual flowers: Failure to develop normal micro sporogenous tissue-anther

Mechanisms of self-incompatibility

There are two different types of events which are considered to constitute the self incompatibility system: (1) the stimulation of unlike genotypes and (2) inhibition of like genotypes. Thus two hypotheses have been proposed to explain the mechanism of self incompatibility in plants.

Complementary hypothesis

This hypothesis was proposed by Bateman in 1952. According to this hypothesis incompatibility results due to absence of stimulation by the pistil on pollen growth in the like genotypes (S1 S2 X S1 S2). In other words, self-incompatibility results due to absence of substances in the pistil or pollen which are essential for pollen tube penetration on selfing, the pollen and /or the pistil fail to produce the substance which is essential for pollen germinate or pollen tube growth in the style and the ovary. Complementary system depends on the combination of unlike alleles in the pollen and style. Such combination of alleles leads to production of either a stimulant for pollen tube growth or an antidote to the inhibition already present in the style.

Oppositional hypothesis

This hypothesis states that interaction between like alleles (S1 S2 X S1 S2) leads to production of inhibitor which inhibits the Abnormal micro sporogenesis: Deformed or in viable pollen growth of pollen tube in the pistil. In other words, as a result of interaction between like alleles a substance is produced in pollen and pistil which has the property to interfere with the normal metabolism of the pollen grain or the pollen tube. The inhibitor can act in three ways: (1) it may inhibit an enzyme or auxin necessary for pollen tube growth, (2) may block pollen tube membrane, and (3) may inhibit an enzyme necessary for the penetration of style.

Classification of self-incompatibility

Self-incompatibility can be classified on the basis of (1) flower morphology, (2) genes involved, (3) site of expression of self-incompatibility reaction, and (4) pollen cytology.

Table 1: Classification of self-incompatibility

Basis of classification	Types of self-incompatibility	Brief Description
1. Flower Morphology	(a) Heteromorphic	SI is associated with differences in flower morphology.
	(i) Distyly	Styles and stamen sare of two types, *i.e* short and long.
	(ii) Tristyly	Styles and stamens have three positions, *i.e.,* short, medium and long.
	(b) Homomorphic	The flowers do not differ in Morphology.
	(i) Sporophytic	SI is governed by genotype of pollen producing plant.
	(ii) Gametophytic	SI is governed by the genetic constitution of gametes.
2. Gens Involved	(a) Monoallelic	SI is controlled by a single gene
	(b) Diallelic	SI is governed by two genes.
	(c) Polyallelic	SI is governed by several genes

3. Site of Expression	(a) Stigmatic	SI genes express on the stigma.
	(b) Stylar	SI genes express in the style.
	(c) Ovarian	SI genes express in the ovary.
4. Pollen Cytology	(a) Binucleate	The pollen grains have two nuclei
	(b) Trinucleate	The pollen grains have three nuclei

SI = Self incompatibility Based on flower morphology, self-incompatibility system is of two types: *viz.*, (1) heteromorphic system, and (2) homomorphic system

Heteromorphic system

When self-incompatibility is associated with differences infloral morphology, it is known as heteromorphic system. Inthis system self-incompatibility results due to differences inthe length of style and stamen. This system is again of twotypes, *viz.*, (a) Distyly, and (b) Tristyly.

Distyly: It refers to two types of styles (short and long) and stamens (low and high). This system operates in the family Primulaceae. In Primula, there are two types of flowers: *viz.*, (1) Thrum type which has short style and high anthers, and (2) pin type with long style and low anthers. The crosses are compatible only between the style and stamens of matching length. In other words, crosses are compatible between pin x thrum or thrum x pin but not between pin x pin and thrum x thrum flowers.

Distyly

Table 2: Differences between thrum and pin flowers of *primula*

Particulars	Thrum(Ss)	Pin(ss)
Incompatibility reaction of style	Thrumtype(L1)	Pin type(l1)
Incompatibility reaction of pollen	Thrumtype(L2)	Pin type(l2)
Length of style	Short(G)	Long(g)
Size of stigmatic cells	Small(S)	Large(s)
Heigh to fanthers	High(A)	Low(a)
Size of pollens	Large(P)	Small(p)

Potential Application of SI Research in crop Breeding and Production

Crop type	SI System	SI/Sc[ab]	Potential Application of SI Research	Reference's
Tomato	S- RNA based GSI non self-recognition	SC	Introgression of crop wild relative traits into elite cultivar by overcoming IRBs depending on SI	Tovar – Mendez *et al.* 2017 [35]
Cabbage, Broccoli etc.	SSI	SI	Identification of new target gens conferring SC. Development of SC lines for hybrid breeding	Xiao *et al* 2019 [40]
Potato	S- RNA based GSI non –self recognition	SI /SC	Development of new CRISPR kosc diploid lines for efficient inbred /f1 hybrid strategies	Ye *et al.* 2018 [44] Enciso Rodriguez *et al.* 2019
Radish	SSI	SI	S- genotype for selecting weak Si plant as male and maintainer Lines for hybrid breeding	Wang *et al* 2014

Parent application related to self-incompatibility

Publin	Title	Major claims	Potential Uses	Crop	Reference's
CN109750061	Method for overcoming diploid potato self-incompatibility	Development of diploid Sc potatoes by knocking-out S RNA asses using CRISPR case gene editing	Breakdown of SI. Development of Scdiploid lines for potato breeding	Potato	Sanwer *et al* 2019
WO /2016 / 137029	Primer set for assessing combination purity or discriminating genotype of cabbage class –II Si Function	Class –II SRK genotyping by specific by specific PCR Amplification	Assess purity and discriminate genotype to enhance hybrid seed production efficiency	Cabbage	Kang *et al* 2016 [18]
1020090053403	Primer set detecting SLG and SRK genotype of Radish SI	A primer set for detecting SLG genotype in Radish a PCR method using the primer set determine radish the genotype identity	Detect SI genotype of radish to prevent the failure of pollination and hybridization between radish havingthe same SI genotype	Radish	Kim kit *et al* 2009 [21]
CN 102234324	Protein involving SI and cross compatibility control of phanerogram pollen, coding gene there of and application	A vector containing a promoter and an RNAi cassette with a Phssk 1pollen factor Antisense from *petunia hybrida*	Breakdown of SI	Solanaceae	Zhao *et al.* 2011

Homomorphic system

In homomorphic system, self-incompatibility results due to physiological causes rather than differences in flower morphology. In this system, the plants do not have differences in the length of style and stamens or other floral parts. This system is very much important in crop plants. It can operate in various ways as given below;

(1) Gametophytic system

When the self-incompatibility is controlled by the genetic constitution of gametes, it is known as Gametophytic self-incompatibility system.

This system was first discovered by East and Mangelsdorf (1925) in *Nicotiana sanderae*. Now this system has been reported in rye, red clover, white clover, potato, tomato and several other crop plants.

Main features of this system are

1. Self-incompatibility in majority of species is governed by a single gene S which has large number of multiple alleles. However, in rye self-incompatibility reaction is governed by two loci (Sobotka, 2000) [32].
2. In this system alleles have individual action in the style without interaction.
3. Pollen grains are unable to germinate or function on a pistil having similar alleles as that of pollen. The pollen tube growth is usually inhibited in the style or ovary.
4. This system gives rise to three types of pollinations, *viz.*, fully incompatible (S1 S2 x S1 S2) in which both allele are common in the pollen and ovule, (2) half the pollen is compatible (S1 S2 x S1 S3) in which one allele is different and (3) fully fertile (S1 S2 x S3 S4) when both alleles differ in pollen and ovule.
5. Gametophytic system permits recovery of male parent only in the partially fertile crosses which are obtained when one allele differs in the cross, *viz.*, S1 S2 x S1 S3. This cross would give rise to S1 S3 and S2 S3 progeny.
6. Plant species belonging to gametophytic self-incompatibility system have Binucleate pollen.
7. All gametophytic systems (except in Gramineae) operate with wet stigma surfaces and there is no direct interaction between one pollen grain and one surface cell because germination takes place in a common fluid medium (Heslop-Harrison, 1975).
8. The biochemical substance which is associated with the incompatibility response of the pollen develops very late, *i.e.*, during pollen formation in gametophytic system.

(2) Sporophytic system

When the self-incompatibility is governed by the genotypes of pollen producing plant (Sporophyte), it is called Sporophytic system. This system was first discovered by Hughes and Babcock (1950) in *Crepis foetida* and Gerstel (1950) in *Parthenium argentatum* (Guayule). Now this system has been reported in Radish, Cabbage, Cauliflower, Sunflower, Cosmos and several other crop plants.

Main features of this system are

- In this system also self-incompatibility is controlled by a single gene S which has multiple alleles.
- The alleles may show dominance, individual action or interaction in either pollen or style as per the allelic combinations involved.
- This system exhibits inhibition of pollen germination or pollen tube growth on the stigma of same flower.
- The Sporophytic system contains a form of dominance in which S1is dominant over all other alleles, S2 is dominant over all except S1 and so on (S1 > S2 > S3 > S4). In this system, crosses between different genotypes are either fully fertile or completely sterile.
- Pollen grains from both heterozygous or homozygous plants react in a similar fashion due to dominance effect of male parent.
- The system permits recovery of parental genotypes in some crosses
- This system of self incompatibility generally have tri nucleate pollen and operate with a dry stigma.
- The biochemical substance which is associated with the incompatibility develops very early in Sporophtic system that is before pollen development. (Nasrallah, 1993).

SI in Brassicaceae

- SRK, the female determinant spans the plasma membrane of the stigma papilla cell.
- SP-11 male determinant expression occurs in anther tapetum.
- Upon pollination SP-11 binds SRK in an S- haplotype specific manner.
- Binding induces auto- phosphorylation of SRK, triggering a signal cascade resulting in rejection of self-pollen
- SLG enhances the SI reaction in some S -haplotypes
- Positive effectors MLPK, ARC-1.
- Proteasomal degradation.

SRK (S-locus receptor kinase)

- The female determinant of SSI
- Encodes allelic forms of a receptor serine / threoninereceptor domain.
- Expressed in the epidermal cells (papillae) of the stigma.
- Transgenic gain of function mutation experiments showedthat SRK alone determine SI specificity and its ability isenhanced by SLG.

Self-Incompatibility in Cruciferae

In controlling pollination, self-incompatibility (SI) has beenused in the family Cruciferae. These include many important kinds of vegetables, such as cabbage, radish, Chinese cabbage, turnip and broccoli. The study of SI in crucifer crops began in Japan. In 1949, a Chinese cabbage F1 hybrid variety, "Nagaoka Kohai I Go", was produced by Shojiro Ito, and in1961 a radish F 1 hybrid variety, "Harumaki Minowase", was produced by a commercial seed company.

There are genetic variations in the reaction level of self-incompatibility (RLSI) to a 4% CO 2 gas treatment. Thus, theparental lines in the parental seed production in a single crosshave to show a marked reaction to CO2. In F 1seed productionon both a single and a double cross, the parental lines have toshow a high LSI. It is therefore important to know the genetic relationship between these characteristics.

SI in Brassicaceae

- SRK, the female determinant spans the plasma membrane of the stigma papilla cell.
- SP-11 male determinant expression occurs in anther tapetum.

- Upon pollination SP-11 binds SRK in an S- haplotype specific manner.
- Binding induces auto- phosphorylation of SRK, triggering a signal cascade resulting in rejection of self-pollen
- SLG enhances the SI reaction in some S -haplotypes
- Positive effectors MLPK, ARC-1.
- Proteasomal degradation.

Classification of male sterility

Classified male sterility into two major groups: genetic (spontaneous or induced) and non genetic (induced) male sterility. On a phenotypic basis, genetic male sterility has been divided into three classes (i.e. sporogenous, structural and functional) and non-genetic male sterility as chemical, physiological and ecological male sterility. In addition, on a genotypic basis, genetic male sterility has been grouped as genic, cytoplasmic and gene cytoplasmic male sterility

Genetic male sterility (GMS)

GMS is reported in about 175 plant species including important vegetable crops. As the name suggests, this type of male sterility is controlled by the gene(s) from the nuclear compartment. Most of the naturally occurring or induced male sterile mutants are recessive in nature with a few exceptions genetic male sterility (GMS). In addition, genetic engineering has produced a novel type of dominant genetic male sterility referred to as transgenic male sterility.

Genetic male sterility

Crops	Gene number/condition	Gene	Variety developed
Tomato	Single recessive gene	*ps*-2	Shalimar Tomato Hybrid-1 Shalimar Tomato Hybrid-2
Chilli	Single recessive gene	ms-12&ms-3	CH-1,CH-3
Muskmelon	Single recessive gene	ms-1	Punjab Hybrid-1

Utilization of CGMS in vegetables

Crops	Gene	Commercially utilized	Variety
Chilli	Single recessive gene	ms-2	Arka Meghna Arka Sweta Arka Harita Kashi Surkh
Onion	Single recessive gene		Arka Kirtiman Arka Lalima
Carrot	Single recessive gene		Pusa Nayanjyoti Pusa Vasuda

Chemically induced male sterility

CHA (Chemical Hybridizing Agents) is a chemical that induces artificial, non-genetic male sterility in plants so that they can be effectively used as female parent in hybrid seed production. Also called as Male gametocides, male sterilants, selective male sterilants, pollen suppressants, pollenocide, androcide etc. The first report was given by Moore and Naylor (1950) [26], they induced male sterility in Maize using maleic hydrazide (MH).

Chemical hybridizing agents used in different crops.

S. No	Chemical	Crop
1	Gibberillic acid	Lettuce, maize, onion, rice
2	Malic hydrazide	Cucurbits, onion, tomato, wheat
3	Naphthalene acetic acid	Cucurbits
4	Ethereal	Rice, sugar beet, wheat
5	Sodium methyl arsenate	Rice
6	Zinc methyl arsenate	Rice

Problems in use of self-incompatibility in hybrid seed production

Production and maintenance of inbred lines by hand pollination is tedious and costly.

- This raises the cost of hybrid seed
- Continued Selfing leads to a depression in self-incompatibility and it unintentionally, but unavoidably, selects for self-fertility.
- In the gametophytic systems, continued inbreeding gives rise to new incompatibility reactions, which may limit the usefulness of such inbreds as parents.
- Environmental factors eg. High temperature and high humidity etc. reduce or even totally overcome self-incompatibility reaction leading to a high (30% or more) proportion of selfed seed.
- Bees often prefer to stay within the parental line, particularly when the parental line differ morphologically. This in turn, increases the proportion of selfed seed.
- Transfer of S alleles from one variety or more particularly species to another variety to species is tedious and complicated. This has prevented the use of self-incompatibility in hybrid seed production in Solanaceae and Compositae.

Temporary suppression of self-incompatibility

Following measures are used for maintenance of inbred lines:

- Bud Pollination.
- Surgical Techniques – *Brassica* spp.
- End of Season Pollination.
- High Temperature – *Trifolium* spp., *Solanum* spp.
- Increased CO_2 concentration.
- High Humidity.
- Salt (NaCl) sprays
- Irradiation (Solanaceae)
- Double pollination
- Grafting (*Trifolium pratense*)

Conclusion

1. Self-incompatibility (SI) signaling represents a unique

mechanism for self/non self recognition between pollen and pistil. As a result, the self or genetically related pollen is unable to germinate or grow in the style to complete fertilization. Recent studies have shown that proteolytic events play important roles in both self-pollen rejection and compatible pollen growth during SI signaling.

2. In Brassicaceae-type SI, a U-box protein ARM-repeat-containing1 (ARC1) participates in the SI response as a functional E3 ligase after the specific recognition between pollen and pistil. In addition, its likely substrate, Exo70A, which acts as a potential pollen compatibility factor, has been identified.
3. In Papaveraceae-type SI, the pistil *S* selectively interacts with self-pollen, which in turn triggers a signaling cascade that culminates in the programmed cell death (PCD) of the selfpollen tube.
4. In Solanaceae-type SI, which represents the most Phylogenetic ally widespread form of SI both the ubiquitin-proteasome pathway and the vacuole pathway appear to take active parts in SI responses.

References

1. Brink K, Crowgey E, Dietrich N, Hondred D, Young JK, Zhong CX. Plant Genome DNA Flanking SPT Event and Methods for Identifying SPT Event. U.S. Patent No. 8,257,930, 2012, September, 4.
2. Brugiere N, Yuhai Cui, Steven Rothstein. Molecular mechanism of self recognition in *Brassica* self incompatibility. Trends in plant science. 2000;5(10):432-438.
3. Chen L, Liu Y. Male sterility and fertility restoration in crops. Ann. Rev. Plant Biol. 2014;65:579-606.
4. Chen Y, Xie J, Guo Y, Kang J. Transcriptional activation analysis of an Ogu CMS-related gene BoMYB1 in Brassica oleracea. Acta Agric. Boreal. Occident. Sin. 2014;23:120-126. (In Chinese)
5. Cheng F, Sun R, Hou X, Zheng H, Zhang F, Zhang Y, *et al*. Subgenome parallel selection is associated with morphotype diversification and convergent crop domestication in *Brassica rapa* and *Brassica oleracea*. Nat. Genet. 2016;48:1218-1224.
6. Domblides EA, Domblides AS, Zayachkovskaya TV, Bondareva LL. Identification of cytoplasm types in accessions of the family Brassicaceae (Brassicaceae Burnett) with DNA markers. Vavilov J Genet. Breed. 2015;19:529.
7. Edh K, Bjorn Widen, Alf Ceplitis. Molecular population genetics of the SRK and SCR self incompatibility genesin the wild plant species *Brassica cretica* (Brassicaceae). Genetics. 2008;181:985-995.
8. Emsweller SL, Jones HA. An interspecific hybrid in *Allium*. Hilgardia. 1935;9:265-273.
9. Georgiev H. Heterosis in tomato breeding. In: Kalloo G (Ed) Genetic Improvement of Tomato. *Monographs* on Theoretical and Applied Genetics 14, Springer-Verlag, Berlin, 83-98.
10. Guo Y, Xie J, Jian Y, Yu J, Kang J. Cloning and functional analysis of Ogu CMS-related gene BoMF1 Promoter in *Brassica oleracea*. ActaHortic. Sin. 2013;40:887-895. (In Chinese)
11. Gupta SK. Practical plant Breeding published by Agrobios (India), Jodhpur, 2005.
12. Han F, Yuan K, Kong C, Zhang X, Yang L, Zhuang M, *et al*. Fine mapping and candidate gene identification of the genic male sterile gene ms3 in cabbage 51S. Theor. Appl. Genet. 2018;131:2651-2661.
13. Han F, Zhang X, Yuan K, Fang Z, Yang L, Zhuang M, *et al*. A user-friendly KASP molecular marker developed for the DGMS-based breeding system in *Brassica oleracea* species. Mol. Breed. 2019;39:90-96.
14. Huang MD, Hsing YI, Huang AH. Transcriptomes of the anther sporophyte: Availability and use. Plant Cell Physiol. 2011;52:1459-1466.
15. Indian Horticulture Database. National Horticulture Board, Ministry of Agri., Govt. of India, 2014, 2-4.
16. Ji J, Yang L, Fang Z, Zhuang M, Zhang Y, Lv H, *et al*. Recessive male sterility in cabbage (*Brassica oleracea* var. capitata) caused by loss of function of BoCYP704B1 due to the insertion of a LTR-retrotransposon. Theor. Appl. Genet. 2017;130:1441-1451.
17. Ji J, Yang L, Fang Z, Zhuang M, Zhang Y, Lv H, *et al*. Complementary transcriptome and proteome profiling in cabbage buds of a recessive male sterile mutant provides new insights into male reproductive development. J Proteom. 2018;179:80-91.
18. Kang JG, Nou IS, Park JI, Yang KW, Hwang ID, Kim IW, *et al.* Primer set for assessing combination purity or discriminating genotype of cabbage class-II self-incompatibility factor. Patent No WO/2016/137029 A1. Seoul, KR, 2016.
19. Kang J, Guo Y, Chen Y, Li H, Zhang L, Liu H. Upregulation of the AT-hook DNA binding gene BoMF2 in Ogu CMS anthers of *Brassica oleracea* suggests that it encodes a transcriptional regulatory factor for anther development. Mol. Biol. Rep. 2014;41:512-527.
20. Kao The-Hui, Tatsuya Tsukamoto. The Molecular and Genetic Bases of S R Nase Based self incompatibility. Theplantcell. 2004;16:S72-S83.
21. Kim KT, Kim JH, Cho KH, Park SH, Lim SH, Yoon MK, *et al.* Primer set detecting SLG and SRK genotypes of radish self-incompatibility. Patent No 1020090053403 B1. Korea, 2009.
22. Li C, He S, Lan C, Ren X, Si J, Li C, *et al*. Transgenic male sterile cabbage plants induced by BcA9-Barnase transformation. J SW Univ. 2015;37:52-58. (In Chinese)
23. Liu S, Liu Y, Yang X, Tong C, Edwards D, Parkin LAP, *et al*. The Brassica oleracea genome reveals the asymmetrical evolution of polyploid genomes. Nat. Commun. 2014;5:3930.
24. Ma Y, Kang J, Wu J, Zhu Y, Wang X. Identification of tapetum-specific genes by comparing global gene expression of four different male sterile lines in *Brassica oleracea*. Plant Mol. Biol. 2015;87;541-554.
25. McCubbin GA, Tech-hui Kao. Annu. Rev. Cell. Dev. Biol. 2000;16:333-364.
26. Moore RH. Several effects of maleic hydrazide on plants. Science. 1950;112:52-53.
27. Pelletier G, Budar F. Brassica Ogu-INRA cytoplasmic male sterility: An example of successful plant somatic fusion for hybrid seed production. In Somatic Genome Manipulation; Springer: New York, NY, USA, 2015, 199-216.
28. Sanwen H, Chunzhi Z, Zhen P, Mingwang Y. Method for overcoming diploid potato self-incompatibility. Patent No CN 109750061 A. China, 2019.
29. Schopfer CR, Nasrallah ME, Nasrallah JB. The Male determinant of self incompatibility in *Brassica*. Science.

1999;286:1697-1700.
30. Sharma JR. Principles and practices of plant breeding, published by Tata McGraw – Hill publishing company limited New Delhi, 1996.
31. Singh P. Essential of plant Breeding, Kalyani publishers; Rajendranagar, Ludhiana, 1996.
32. Sobotka R, Lenka Sakova, Vladislav Curn. Molecular Mechanisms of self-incompatibility in Brassica. Current Issues. Mol. Biol. 2000;2(4):103-112.
33. Staniaszek M, Marczewski W, Habdas H, Potaczek H. Identification of RAPD markers linked to the ps gene and their usefulness for purity determination of breeding lines and F1 tomato hybrids. Acta Physiologiae Plantarum. 2000;22(33):03-06.
34. Tanaka Y, Tsuda M, Yasumoto K, Yamagishi H, Terachi T. A complete mitochondrial genome sequence of Ogura-type male-sterile cytoplasm and its comparative analysis with that of normal cytoplasm in radish (*Raphanus sativus* L.). BMC Genom. 2012;13:352.
35. Tovar-Méndez A, Lu L, McClure B. HT proteins contribute to S-RNase-independent pollen rejection in Solanum. Plant J. 2017;89:718-729. doi: 10.1111/tpj.13416.
36. Uyttewaal M, Arnal N, Quadrado M, Martin-Canadell A, Vrielynck N, Hiard S, *et al*. Characterization of *Raphanus sativus* pentatricopeptide repeat proteins encoded by the fertility restorer locus for Ogura cytoplasmic male sterility. Plant Cell. 2008;20:3331-3345.
37. Wang Q, Zheng P, Zhang L. Identification and classification of S haplotypes in radish (*Raphanus sativus*). Plant Breed. 2019;138:121-130. doi: 10.1111/pbr.12664.
38. Wang Q, Zhang Y, Fang Z, Liu Y, Yang L, Zhuang M. Chloroplast and mitochondrial SSR help to distinguish allo-cytoplasmic male sterile types in cabbage (*Brassica oleracea* L. var. capitata). Mol. Breed. 2012;30:709-716.
39. Wang Z, De W, Gao L, Mei S, Zhou Y, Xiang C, Wang T. Heterozygous alleles restore male fertility to cytoplasmic male-sterile radish (*Raphanus sativus* L.): A case of over dominance. J Exp. Bot. 2013;64:2041.
40. Xiao Z, Han F, Hu Y, Xue Y, Fang Z, Yang L, *et al.* Overcoming cabbage crossing incompatibility by the development and application of self-compatibility-QTL-specific markers and genome-wide background analysis. Front. Plant Sci. 2019;10:189. doi: 10.3389/fpls.2019.00189.
41. Xing M, Sun C, Li H, Hu S, Lei L, Kang J. Integrated analysis of transcriptome and proteome changes related to the Ogura cytoplasmic male sterility in cabbage. PLoS ONE 2018, 13, e0193462.
42. Yan H, Fang Z, Liu Y, Yang L, Zhuang M, Zhang Y. *In vitro* conservation technique for the dominant genic male sterile materials in cabbage. Chin. J Trop. Agric. 2013;33:35-39. (In Chinese)
43. Yasumoto K, Terachi T, Yamagishi H. A novel Rf gene controlling fertility restoration of Ogura male sterility by RNA processing of orf138 found in Japanese wild radish and its STS markers. Genome. 2009;52:495-504.
44. Ye M, Peng Z, Tang D, Yang Z, Li D, Xu Y, *et al*. Generation of self- compatible diploid potato by knockout of. S-R Nase. Nat. Plants. 2018;4:651-654. doi: 10.1038/s41477-018-0218-6
45. Yu H, Fang Z, Liu Y, Yang L, Zhuang M, Lv H, *et al*. Development of a novel allele-specific Rfo marker and creation of Ogura CMS fertility-restored interspecific hybrids in *Brassica oleracea*. Theor. Appl. Genet. 2016;129:1625-1637.
46. Zhang L, Kang Z, Liu H, Kang J. Cloning and expression of an Ogura cytoplasmic male sterile (Ogu CMS) related MYB transcription factor in *Brassica oleracea* var. capitata. J Agric. Biotech. 2012;20:627-635. (In Chinese)
47. Zhu Q, Kang Z, Jian Y, Ding Y, Kang J. The molecular characteristics of Ogura cytoplasmic male sterility related gene orf138 in cabbage (*Brassica oleracea* var. capitata). Chin. Agric. Sci. Bull. 2012;28:104-109. (In Chinese)