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Genetic and Molecular Aspects Encompassing Male Sterility in Onion (*Allium cepa L.*)

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Abstract

Onion is the most diversified crop and has been in cultivation since antiquity owing to its medicinal and nutritional properties. The increased acceptance of bulb onion among folks could lead to an increase in the area under cultivation with proportionate amount of bulb production. But yield per unit area gets limited owing to dearth of heterosis pertaining to bulb yield. The only possible way to achieve enhanced productivity is by exploiting heterosis using male sterility mechanisms in onion. Researchers developed numerous markers linked to male sterile cytoplasms (CMS-S and CMS-T), male-fertile normal (N) cytoplasms and nuclear-male-fertility restorers (Ms) locus. The markers discovered has proven to aid effective selection and isolation of male sterile lines, their maintainer lines and restorer-of-fertile lines. In heterosis breeding the male sterility trait is precious for its potential advantages in onion hybrid development. This artefact presents an insight on genetic and molecular aspects of male sterility in onion.

Introduction

The genus *Allium* is largest among different monocots and are comprised of 920 species [1]. Onion, among different alliums has highest potential in terms of economy, sharing a value of 70% followed by garlic (25%). Onion is a diploid crop having 2n=2x=16 chromosomes [2-4]. Its inflorescence is an umbel which bears hermaphrodite flowers with protandrous type anthers [5-7]. Due to this protandrous nature, the dehiscence of pollen occurs before stigma gets receptive which leads to high degree of cross pollination [8]. Self-pollination is seldom observed in onion, but 3-4 years of forced selfing can lead to development of near homozygous pure-bred lines [9]. Contrarily, if inbred lines are made to out-cross then heterosis for shape, size, yield, earliness and uniform maturity can be seen. Since ages, onions are nurtured owing to its massive medicinal properties such as anti-carcinogenic, anti-biotic, anti-cholesterol, anti-microbial, hypo-lipidaemic [10], hypo-glycemic, lacrimatic and anti-thrombotic [11]. Besides, round the world, consumption of onions is increasing steadily due to presence of quercetin, dietary flavonoids and nutritional benefits. Worldwide, onions are cultivated in an area of 4.45 million hectares with an optimum production and an average productivity of 85.94 million tons and 19.30 tons per hectare (t/ha). However, major onion producers like India and China encompass low productivity [12]. Such low productivity of onion bulbs could result due to extensive cultivation of open-pollinated varieties rather than F_1 hybrids which are highly heterotic for yield and growth traits. Onion responds well to heterosis breeding but a lot of intricacies are present pertaining to its improvement which involves presence of several small-sized hermaphrodite flowers, difficulty in manual emasculation and cross-pollination, biennial nature, high inbreeding depression and minimal hybrid seed production. Such inherent breeding complexities can be overcome by using molecular markers and genomic technologies linked to male sterility traits [13]. Such techniques could

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expedite onion improvement programmes. In addition, male sterility systems benefit F_1 hybrid seed production and evades barrier in breeding system like tedious hand emasculation and pollination and also prevents unrestricted sib-mating or selfing. The genetic nuclear cytoplasmic sterility systems like CMS-S and CMS-T are in use for hybrid seed production of onions. The CMS-S cytoplasm is stable across different environments and has a simple inheritance pattern for male-fertility restoration, thus, used widely in production of F_1 hybrids. On the other side, CMS-T cytoplasm possesses complex fertility restoration system. Furthermore, detection of molecular markers connected to male fertility restoration (*Ms*) locus (C/R line) and normal (N) cytoplasm type enables breeders to isolate maintainer lines in any open-pollinated population. At seedling stage A-, B- and C/R lines could be isolated in a year, but, conventionally it takes almost 2-4 years via test cross assay which are advanced further in order to transfer into desirable maintainer background. Moreover, to transfer B-line into A-line genetic background requires almost 10-12 years through 5-6 backcrosses and A-line is crossed to C/R line to produce F_1 hybrids [14]. Thus, identification of markers tightly linked to nuclear gene at *Ms* locus would thereby help in easy isolation of maintainer lines at seedling stage by marker assisted selection (MAS) by screening seedlings in a limited time phase thereby lessening expenditure on field screening, crop management and labour inputs [15]. To further add, markers serve as compelling tools in evolutionary studies, molecular genetics, marker-assisted breeding and selection in onion. The present review briefs an insight on genetic and molecular aspects governing male-sterility in onion.

Origination and prevalence of cytoplasm

The male sterility systems stemmed out either by induced or spontaneous mutation, intergeneric, interspecific, intra-or inter-specific hybridization or by protoplast culture [16]. This arose due to interaction between plasma and nuclear genes that is chloroplast and mitochondria genes [17]. The cytoplasms of onion were diversified by interspecific hybridization between onion and other *Allium* species, between onion (*Allium cepa* L.) and Japanese Bunching onion (*Allium fistulosum* L.) were found to be highly male-sterile [18]. In addition, this male-sterility could happen due to reciprocation of mitochondrial genes (responsible for male sterility) and lack in homology in plant species.

The origination of CMS or CGMS systems is primarily due to dynamic reorganization of mitochondrial genes. Thus, these male-sterile inducing genes are chimeric. The stoichiometry of these chimeric genes is maintained over generations [19,20]. Such stoichiometry of chimeric genes changes sporadically by a process known to be 'sub-stoichiometric shifting' which occurs due to mutations of nuclear genes. This situation leads to restoration of male fertility [21]. The Penta-Trico-Peptide Repeat (PPR) proteins encodes male-sterility induced genes which are suppressed by fertility-restorer (*Rf*) genes. These proteins suppress stoichiometric shifting of ssDNA binding protein-1 directed to mitochondrial DNA (mtDNA) [22]. Studies reveals that presence of large multicellular mitochondrial sequences of genome having different stoichiometry and its evolution occurs via point mutation rather than reorganization of sequences. Thus via point mutation, T-cytoplasm of onion originates from N-cytoplasm, where N-cytoplasm originates from M-cytoplasm (present in wild sp. *Allium vavilovii*) [23].

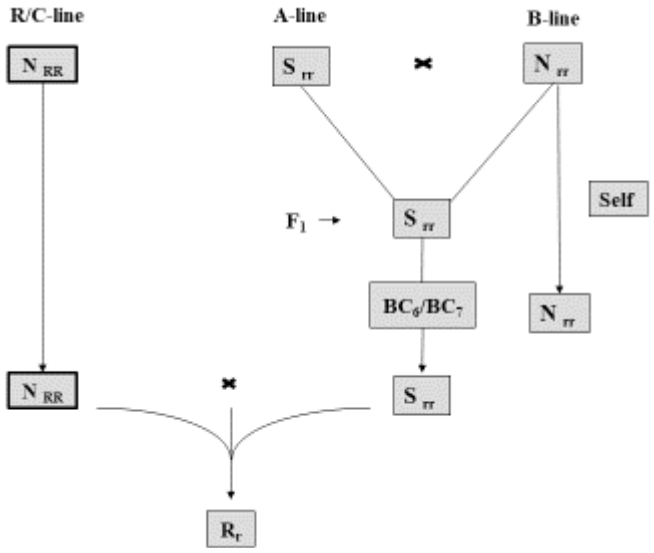

Figure 1: Flow diagram of various steps involved in the development of onion hybrids using CMS system [24].

Onion male sterility systems

Male sterility in onion was first identified in onion (*Allium cepa* L.,) during 1925 in the cultivar Italian Red and was noted as CMS-S cytoplasm [25]. Laterwards, CMS-T cytoplasm was traced in the variety Jaune-Paille-Des-Vertus [26] and CMS-C cytoplasm was identified in Rijnsburger onion [27]. Male sterility systems are classified into M-cytoplasm(s) and S-cytoplasm. These M-cytplasms(s) are sub-divided further into M_1-cytoplasm-which is a maintainer line of S-cytoplasm (male fertile) and M_2-cytoplasm-which is similar to normal (N-) cytoplasm (male fertile). M_3-cytoplasm is more CMS-T cytoplasm (male-sterile), whereas, M_4-cytoplasm is more similar to CMS-T cytoplasm (male-sterile). To establish male sterile lines (CMS-Smsms) it takes four to eight years by backcross with corresponding maintainer lines having genetic backgrounds NMsms or Nmsms or NMSMS. The interaction of the lines with sterile cytoplasm to that having nuclear dominant genes (SMSMS and SMsms) leads to male fertility [28]. They further added that by scoring of progenies evolved from the cross between male-fertile and male-sterile lines, the male sterility, their maintainers and restorers can be located. If the F_1 hybrid emerges out to be male sterile, then it is a maintainer of male sterility, whereas, if the F_1 hybrid turns out to be male-fertile, then it is the fertility restorer line of male sterility system.

In plants, male sterility systems promote outcrossing. Such system is common in nature in order to prevent inbreeding when plants are unable to produce functionally viable male gametes. The onion male sterility system was first recognized by Henry Albert Jones in 1925 in 'Italian Red' cultivar. Subsequently a pedigree male sterile line "13–53" (A line) was isolated. [25] regarded this line as S-cytoplasmic line or CMS-S-cytoplasmic line. Fertility restoration of CMS-S cytoplasmic plants was revealed after mating with R/C line (male-fertile line). Restoration of fertility is governed by *Ms* allele which is single dominant at nuclear locus, hence, the name cytoplasmic-genic male sterility (CGMS). B-line i.e., maintainer line of onion male sterility is formed by crossing normal cytoplasm or fertile cytoplasm with *ms* allele which is recessive at fertility restoration locus. Correspondingly, maintenance and reproduction of male-sterile plants by crossing with maintainer plants [28]. [26] noted an additional male-sterile type i.e., CMS-T cytoplasm from a French variety named 'Jaune Paille Des Vertus'. This CMS-T cytoplasm

had been delineated for hybrid seed production. [29] found out complex nature of inheritance for restoration of male-fertility in CMS-T cytoplasmic plants. Such complex inheritance pattern is governed by three segregating loci which involves one independent (a) and complementary genes (b and c).

(N-) cytoplasm or Normal cytoplasm

The normal cytoplasm i.e., N- cytoplasm is widely known to be male-fertile counterpart of onion evolved from M- cytoplasm. [30] confirmed paternal influence of N-(normal) cytoplasm by its prevalence in cultivated and wild *Alliums* of Central Asia. They also found out uncanny similarity between N-cytoplasm to that of M-cytoplasm. Researchers estimated frequencies of N- and S-cytoplasms through computer simulations in open-pollinated populations and observed that N- cytoplasms were predominant in open-pollinated population having high frequency of dominant (*Ms*-) allele(s). This could happen due to continuous selection of dominant alleles against recessive *ms*-alleles across several generations as population might have possessed S-cytoplasm in the past. These *ms*-alleles might disappear upon selection of alleles from male gamete of *Ms*-locus in the population having S-cytoplasm. [31] revealed in their studies that in the populations of S-cytoplasm the fixation of dominant alleles gets increased beyond fifty-generation by random mating. Thus due to the preponderance of dominant (*Ms*-) alleles in normal cytoplasmic populations, isolation of B lines (maintainer lines that is N-cytoplasm with *ms*-allele) from dominant (*Ms*-) allelic population is time-consuming and gets much more complicated and time-demanding.

S-cytoplasm or CMS-S cytoplasm

The origination of male-sterility system transfigured onion seed industry via hybrid seed production [32, 33]. In 1925, the phenomenal plant breeder Dr. Henry Albert Jones identified S-cytoplasm in onion variety 'Italian Red' and thereafter isolated a pedigree line which is male-sterile named '13-53'. The S-cytoplasm varied in Middle-East and Central Asia. In ancient past, S-cytoplasm might have introgressed into normal-cytoplasmic population and as a consequence S-cytoplasm has been distributed to rest parts of the world [34, 28]. Male sterility in onion is indebted to S-cytoplasm or CMS-S cytoplasm owing to unusual inter-specific hybridization which resulted due to disharmony between cytoplasm of female parent and nucleus of male parent. This resulted in male-sterility in progeny. The events of interspecific hybridization might have occurred naturally in yesteryears and has transcended uncalculatedly by introgression into onion population. Origination of male-sterile cytoplasm through such series of interspecific hybridization has led to the development of natural triploid 'Pran'. 'Pran' is an intermediate evolutionary species between *Allium* × *proliferum* (Moench) Schra or *Allium* × *cornutum* Clement ex Visiani, whose alien cytoplasm has been transferred into *Allium cepa.* Such event has been confirmed by southern blotting of S-cytoplasm. In addition, *mt*DNA and RFLP of *cp*DNA in S-cytoplasm differed in N- and T-cytoplasm(s). [35] reported its co-inheritance with chloroplast and mitochondrial genome and also found to have originated from other species with subsequent introgression into onion. Thus S-cytoplasm has been hinted to be alloplasmic origin. In several onion cultivars S-cytoplasm had been diversified through development of F_1 hybrids for exploitation of heterosis. The distribution of genotypic frequencies of N- and S-cytoplasms in a random mating population has been found to be equal in open pollinated populations and thus identification of maintainer lines stands important among open pollinated population using markers or by test crossing [36,37].

Genetic inheritance of S-cytoplasm

The cytoplasmic-genic male sterility stems out from interaction of homozygous recessive genotype (*msms*) with male-sterile cytoplasm (CMS-S) at nuclear male-fertility restoration locus (*Msms* or *MsMs*). Consequently, nuclear markers which are tightly linked at *Ms* locus would thereby allow analysis of molecular assisted segregation. [38] examined 188 F_2 population plants obtained from the cross 506L (male sterile line) × H6 (double haploid, male fertile line) and found out that the entire F_2 population was segregated into 3:1 Mendelian ratio of male-fertile and male-sterile phenotypes. [39] confirmed 1:1 segregation in a backcross population (BC_1) of three-way cross combination [118(S_{msms})×(118×12-10(S_{MsMs}))]. In addition, differential gene expression of *AcPME* gene marker were genotyped at flower bud stage by 112 male-fertile plants (S_{Msms}) and 128 male-sterile plants (S_{msms}). [40] revealed a segregation ratio of 1:1 of two backcross population genotyped by SCAR markers DNF-567 (*Ms*) and RNS (*ms*). The ratio obtained were fitted into the model of single-gene restorer-of-fertility (*Ms*) gene. [15] used a cross [BYG15–13(N_{MsMs})× AC43 (N_{msms})] to obtain F_2 segregates. The F_2 population was segregated into 14 N_{MsMs}: 28 N_{Msms}: 13N_{msms} with p=0.973 i.e., the population was fitted into expected segregation ratio 1:2:1. In addition, the test cross progenies exhibited significant effects (p<0.1) by RFLP analysis for nuclear restoration of male-fertility. The above studies validate the fact that for restoration of fertility the S-cytoplasm is governed by single dominant gene.

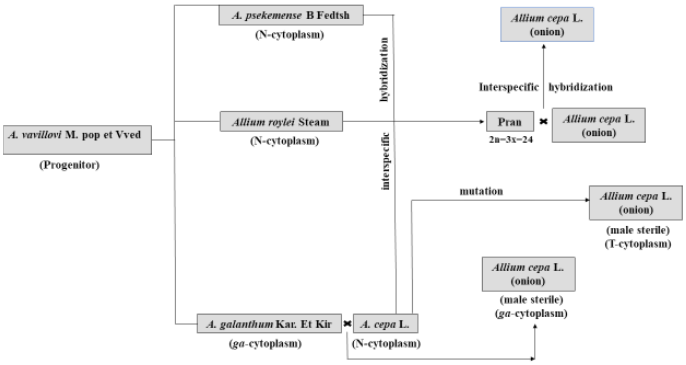

Figure 2: Origin and evaluation of male-sterile system (s) in onion.

T-cytoplasm or CMS-T cytoplasm

[26] discovered CMS-T cytoplasm in France and its characterization for commercial seed production was given by [29]. The T-cytoplasm had developed from N-cytoplasm via point mutation in the mitochondrial genome. This might have arisen either by insertion in mitochondrial genome or due to small genome change (figure 2). In European countries, T-cytoplasm is extensively used for production of hybrid onions. T-cytoplasm is unveiled to be autoplasmic origin of normal cytoplasm or N-cytoplasm, it is because the polymorphism among *cp*DNA and *mt*DNA sequences of the *orf*22 gene and *atp*6 gene does not differ from CMS-T and N-cytotypes. T-cytoplasm differed with two SNPs and a four base-pair insertion in *cp*DNA from normal cytoplasm, thus, S- and T-cytoplasms were confabulated to be of different origin. [21] reported in their molecular genetics studies that the genomic shift of the *orf*725 gene resulted in origination of CMS-T mitotype by way of increased copy number without decreasing normal *cox*I gene. Further, [41] depicted

that recent origin of CMS-T cytotype from normal or N-cytotype due to organization of nucleotide sequences in the gene.

Genetic inheritance of CMS-T or T-cytoplasm or CMS-T like cytoplasm

The restoration of male-fertility of T-cytoplasm or CMS-T cytoplasm were controlled by three *Rf* genes viz., an independent gene (a) and two complementary gene (b and c). [29] revealed complex segregation pattern of male-fertility phenotypes from 24 populations. They used sample size from 14 to 44 in each population. Presence of small sample size would result in digression from a model of single-gene inheritance in restoration of male-fertility in T-cytoplasm. Further, [42] interestingly found in their studies three F_2 population phenotypes that segregated perfectly with *jnurf*13 genotypes. The plants were fitted into segregation ratio of 3:1. Thus genotyping using markers revealed presence of single dominant gene for restoration of fertility in T-cytoplasm [42].

Cytotype-Y (CMS-T like), a novel onion cytoplasm, possesses a unique stoichiometry of *coxI* and *orf*725. Besides, cytotype-Y (CMS-T like) revealed heterozygous genetic condition (*Msms*) of accession PI273626. Upon selfing a single plant, first-generation selves (S_1) were produced and upon genotyping the result which were deduced from *Ms* locus found to be linked with *AcPMS1* gene marker (RF31446). Furthermore, male-fertility restoration controlling genotypes exhibited deviation from expected 3:1 segregation ratio of single gene inheritance. [43] explained such deviation might have stemmed out due to instability of male sterility and also by the influence of genetic factors of said accession possessing T-like cytoplasm. Studies are further needed to throw light on the inheritance pattern for restoration of male-fertility of T-cytoplasm or CMS-T cytoplasm.

***Galanthum* cytoplasm or (*ga*-) cytoplasm**

The *ga*- cytoplasm or *galanthum* cytoplasm is known to be potential source of male-sterility for heterosis breeding in onion. Such type of cytoplasm has been transferred via interspecific hybridization from *Allium galanthum* Kar et Kir into onion (*Allium cepa* L.) [44], shown in figure 2. Phenotypically, the flowers of *ga*- cytoplasmic plants differed by upward curling of perianth segments and its filaments are without anthers. Such traits can ideally be used as morphological markers to segregate S- and T- cytoplasm from CMS-*ga* cytoplasm. Furthermore, male-sterile lines of shallot developed by substitutions of *ga*-cytoplasm (*galanthum* cytoplasm) [45]. In addition, for bunching onion male-sterility occurred due to incompatibility in interspecific hybridization between cytoplasms of *Allium galanthum* Kar et Kir and nucleus of bunching onion.

Genetic inheritance of *Galanthum* cytoplasm or (*ga*-) cytoplasm

[45] introgressed breeding populations i.e., BC_3, BC_4 and BC_5. These breeding populations segregated into equal proportions of male-sterile and male-fertile phenotypes (1:1). Their studies concluded the fact that a single dominant gene was responsible for restoration of fertility in galanthum cytoplasm or *ga*- cytoplasm or CMS-*ga* cytoplasm. [46] explained that male-fertile lines could serve as fertility restorer in S-cytoplasm, but the condition does not hold true for population having *galanthum* cytoplasm or *ga*- cytoplasm. Instead, the fertility restorers have been the maintainers of *ga*-cytoplasm.

Unusual male sterile cytoplasms

Potential sources of male sterile in onion have been isolated in Dutch and Japanese populations. Such systems of male sterility of uncertain origin had been used for developing hybrids of Rijnsburger-type in Netherlands. [46] reported other types of male sterility systems in Polish and Dutch populations, which are about to characterize for inheritance pattern. The Kz1/ms line, a male sterile plant isolated from Kaizuka (Kz1 in short, a Japanese variety) to which upon RFLP analysis of T-cytoplasm and *Kz*1-cytoplasms unveiled to be members of M-cytoplasm. This *Kz*1-cytoplasms might have emanated due to mutation of N-cytoplasm and thus it is of autoplasmic by origin [46]. Besides, in India a putative CMS system have been sorted out in onion cultivar Nashik-White-Globe. The said variety has been adapted to exploit heterosis and eventually developed two hybrids, namely Hybrid-5 and Hybrid-1[47]. Different valuable source for onion population possessing S-, T-, N- and other uncommon male-sterile cytoplasms are presented in Table-1.

Table 1: List of different onion breeding lines possessing different onion cytoplasms.

Breeding lines	Cytoplasms	References
B1750B	N–cytoplasm (an inbred maintainer of B1750A)	[28]
B1750A	S–cytoplasm inbred, USDA	[28]
RJ70A	T-cytoplasm, (derived from male-sterile inbred RJ70B)	[46]
M11I1, OMI13, 5,7,8	male-sterile populations (obtained from Nasik White Globe)	[47]
614A, 8111A, 8152A (CMS-*ga*)	*ga*-cytoplasm	[46]
RJ70B	N-cytoplasm, (an inbred maintainer of RJ70A)	[46]

Peculiarity of male sterile systems

S-cytoplasm is known for its high stable nature despite idiosyncrasy of environmental conditions. The said cytoplasm possesses wide spectrum of recessive allele at the nuclear locus known for restoration of male fertility (*Ms*/*ms*). This had led to the development of F_1 hybrids than other male-sterile systems [28, 46]. Nevertheless, in European countries the T-cytoplasm are used for production of hybrid onions but its commercial utilization has been limited due to complex inheritance pattern for restoration of male fertility.

Morphological peculiarities

Male sterility is an ocular feature and male sterile plants are unable to produce functionally viable gametes (Table-2). Its polymorphic traits can easily be visualized by naked eyes than those of male fertile traits. Genetically, this male sterility (cytoplasmic-genic) is inherited maternally and it differs from male fertile phenotypes in terms of deformed and shriveled microspores. In addition, pollen grains are unable to dehisce from the anther sac owing to lack of nutritional strength in the locules of anther of tapetum tissue leading to premature autolysis. Findings say that phenotypically varieties 'Pran' and Italian-Red pedigree '13-53' line unveiled similar red-coloured spindle shaped bulbs having top-set bulbils with a mixture of flowers which does not shed pollen grains and morphologically both revealed paternity lineage having origination of S-cytoplasm.

In other words, male-sterile possesses long style. The stigmatic knob in it turns receptive sooner with decreased receptive area than those of male-fertile ones [48]. In contrast to well proliferating perianths, and translucent anthers of male-fertile flower, male sterile flowers do not open fully and possesses pellucid anthers. Furthermore, as male sterile plant ages, degeneration of pollen grains, anthers, stamens, tapetal cells of anthers and microspores at the end of meiosis. Besides, lack of pollen in anther sac, narrow stigmatic knob, curved perianth segments, lack of pollen grains in the anther sac, presence of fused sacs is also observed. The anther tissues of male sterile ones are uncloaked by three types of atypical tapetal behavior, (i) premature breakdown of tapetum tissue in tetrad stage, (ii) occurrence of hypertrophy in tapetum tissues and premature autolysis after diad stage, (iii) tapetum hypertrophied during microsporogenesis along with non-functional tapetum tissues. During pre- or post-meiotic stage the microsporogenesis can lead to different anomalies occurring due to aberration of meiosis of formation of tetrads. This led to the release of tetrad by dissipation of thick callose wall leading to degeneration and vacuolation of microspores in Italian Red '13-53' (male sterile).

Table 2: Morphological characteristics of male sterility systems in onion.

Cytoplasms	Phenotypic expression of male sterile types	References
S-cytoplasms or *CMS-S* cytoplasm		
Zittauer–Gelbe-Kasticka	Anthers with misshapen microspores, anthers are non-viable and are clumped together	[49]
Pukekohe–Longkeeper	Fused anther sacs with no pollen grains and are unable to dehisce	[52]
T-cytoplasm or *CMS-T* cytoplasm		
A. galanthum Kar. et Kir	Irregularities in microspore meiosis and tapetal developmental stage	[51,52]
	Reduced perianth size, lack of anthers	[46]

Genetical features

S- and *T*- cytoplasms were genetically well-characterized and this had happened due to diversification of onion male sterility. *S*-cytoplasm has five differential polymorphisms, namely *cp*DNA-1, -2,-4,-41 and -42. Such polymorphisms resulted due to rearrangement of genomes for unequal size of polymorphic bands with restriction digestion of other enzymes. Polymorphisms of *cp*DNA-42 revealed a 3.1kb band in N-cytoplasm and a band of 3kb in *S*-cytoplasm upon digestion with *Eco*RI and *Bg*lII, succeeded by hybridization against orchid clone-17. Besides, on restriction enzyme analysis of *Hind*III and *Bam*HI, the digestion profile of *S*-cytoplasm *mt*DNA differentiation from N-cytoplasm. In fact, restriction profiles of T-cytoplasm were also generated with *Hind*III and *Bam*HI and were found identical to N-cytoplasm. No variation of *T*-cytoplasm was actually found upon southern blotting of mitochondrial genome. Expectantly, onion *cp*DNA upon the restriction enzyme analysis were found similar with *mt*DNA for T- and N-cytoplasm, but differences among S-, T- or N-cytoplasm were found with *Hind*III and *Bam*HI digests of *mt*DNA, to that of *Hind*III, *Xba*I and *Eco*RI digestion of *cp*DNA for S- and N- cytoplasms [52]. Besides, *cp*DNA-41 polymorphisms were found identical to 'Pran' and other S-cytoplasmic [53]. [54] reported that the cytoplasms of *A. fistulosum* and CMS-S plants could differentiate due to automorphic gain of *cp*DNA-41 in S-cytoplasm. [55] developed PCR marker of chives by chimerical mitochondrial CMS_1 could also differentiate onion cytoplasms. They designated the marker as *orf*A501 marker which is quite applicable in different populations like landraces, open-pollinated varieties, F_1 hybrids and segregating populations to distinguish all of these three cytoplasms. Incidentally, this marker *orf*A501 can also augment and intensify CMS_1 of chives and also T- and S- cytoplasm of onion. The chloroplast gene *psbA* along with *Msp*I (restriction enzyme) revealed that N-cytoplasm possessed an *Msp*I restriction site but S-cytoplasm did not. Such difference in chloroplast gene (*psb*A) are able to distinguish the cytoplasm from mixed population. [21] reported that normal (N-), CMS-S and CMS-T cytoplasms could be distinguished against *orf*725 gene in S-cytoplasm, *cox*I gene in N-cytoplasm and both *orf*725 and *cox*I genes in CMS-T cytoplasm. In addition, the stoichiometries of these two genes i.e., *orf*725 and *cox*I of *mt*DNA were found to be consistent among diverse germplasm and thus development of such markers based on copy numbers (relative) to differentiate N-cytoplasm, CMS-S and T-cytoplasms within one simple PCR would be appropriate. [21] further observed that S-cytoplasm of *cox*I gene delineated homology with *orf*725 gene from T- and S- cytoplasms of *Allium cepa* L. The homolog sequence between *cox*I and *orf*725 gene might be prone to *mt*DNA recombination in *Allium* sp. Henceforth, the chimeric gene junction of *orf*501 in chive to that of *orf*725 in onion were found identical and thus this chimeric gene *orf*725 of onion can serve as candidate gene for male sterility and the gene marker (*orf*725) can be used in open-pollinated varieties for marker-assisted selection of male sterility. [56] used CAPS (Cleavage Amplified Polymorphic Sequence, a co-dominant marker) to differentiate variants of *atp6*-type-1 in N- and CMS-T cytotypes and *atp6*-type-2 in CMS-S cytotype. [57] used *atp9* gene in the marker assisted selection. The co-dominant markers were developed based on gene encoding putative *oligo-peptide transporter* (*opt*) and on EST probe sequences. Two *Ms* allele linked *opt*-alleles revealed polymorphism namely R_1 (439 bp) and R_2 (108 bp) *InDels* respectively were used and designated as *opt*-marker which could easily differentiate heterozygous and homozygous recessive, homozygous dominant genotypes in the F_2 population. [40] developed a PCR-marker linked to the *Ms*-locus by coversion of AFLP marker into SCAR. The research group opted for conversion because AFLP markers have high technological demand and are relatively costly, thus limiting extensive application in wide range of screening of population. The AFLP marker after conversion into SCAR marker was designated as DNF-566 which does not co-segregated with dominant *Ms*-allele in the populations. On the other side, the marker RNS-357 which was designed by some workers got co-segregated with *ms*-alleles. [58] and [40] described that these two above markers were used to gauge different onion cytoplasms having different genetic backgrounds of maintainer lines, male sterile lines, restorer fertility lines and F_1 hybrids. [13] reported in their studies that SNPs were linked tightly to nuclear *Ms*-loci which when further advanced for isolation of male-sterile, maintainer lines by differentiating S- and N-cytoplasms. The results were found similar to S-cytoplasms (153 355 bp) and c-DNA of N-cytoplasms (153 538 bp). [38] found out the

upstream region of the gene *PsaO* and are linked to dominant *Ms*-allele by 53 bp *InDel*, the marker then developed referred to be *PsaO* marker. [58] revealed RAPD markers to distinguish cytoplasms and were converted into CAPS in *jnurf*05 and *jnurf*17. The markers *jnurf*17, *jnurf*05 and *opt* were linked to *Ms*-locus. Recombination was found between *opt* and *Ms*-locus but no recombination was observed between *Ms*-locus and *jnurf*05 and *jnurf*17 markers. *Ms*-locus, finally, located on second chromosome of chromosome consensus map with *PsaO* and *opt* markers respectively. [59] described that the tightly linked markers are best in marker aided selection of *Ms*-alleles for isolating *Ms*-gene through map based-cloning method. The linkage between fertility restorer gene with *jnurf*12 (co-dominant marker) and *Ms*-locus got perfectly matched with all recombinants. But, application of *jnurf*12 marker has been limited due to multiple banding pattern, thus, a more reliable *InDel* based simple PCR marker called *the jnurf*13 has been developed. [48] this *jnurf*13 marker matched perfectly with phenotypes having male fertility corresponding to CMS-S, T-cytoplasms and co-segregated perfectly. Further, [43] found out marker RF31446 was correctly linked to *Ms* locus controlling restoration of fertility which indicated that the fertility restoration of male-sterility was confabulated by Y-cytotype determining *Ms*-locus. Some more molecular markers which were used for characterization of male sterility systems in onion given in Table-3.

Table 3: Molecular markers used in characterization of male sterility systems in onion.

Marker type	Genes	Name of Marker	References	Marker type
		Forward	Reverse	
CAPS	atp6 gene	CCCAAACTCTTCCCAGCCCTAACCTCA	TGGCTATCGAAAGAATGAGTCCGCAAA	[41]
RT-PCR	*cox*2 gene	GCACCCTCCGCTGCTTACCAAATTCTT	CCTTCAGTGCGGGATTCAAGATGTTCC	
PCR	*cob* gene	CGGAGCGAAAAGGGTTTTCCATGAGAT	TTGTATGTATGCCCGATCCA	
PCR	*PsaO* gene	CCTCATGCTTGCTTGGTCTT	AAGCGTGCTCGATTGTAGGTCCTTT	[38]
	Opt gene	CCTTGGAAAGGCGCAACTAAAGATTTGA	TGTGGCCCAATAATACAAACAAGCAGGA	
RFLP	*petB*(5)	CAGGTGTGGTTCTGGCTGTA	CGGCAGTAAGAAGAGGCAAT	[61]
RFLP	*atpF*(3)	TTCGGAAACAAAGGGAAAAA	TCCGACAACAAGTTTTCCAAC	
InDel	*accD* gene	AGAATGAGGAGCAGGAAAACTCT	AGTCGTGATTGTTACTCTTAGACCT	
SCAR	Sequence base	TACAGATTTGTTTATCTTCTTCTTCTTCT	TTCATTTGTTAGGATGTACTCTTACC	[40]
CAPS	*Rf* gene	GGTTCTTCGCAAAGTTCTCG AACAAATCAATCGCCTGAAAA	TGTGAAAAGATTGGACATACTGC ATTATGGCCGATTTCTCAGC	[48]
Multiplex	*AcSKP*-1 gene	GCAATACACAGCTTCTAGCTGAATT	AACACACACAGAGTGAGAAATTTTATAT	[62]
PCR	*Rf* gene	TCACCTTTTACTTGCATCTGGTT	CCATTGGTACTTGATGCAAA	
HRM	*AcPMS1* gene	GCGAAGAATATTTTAAGGTTGTCG	CAGGAGAGATACCAGACCCATT	[63]

Molecular concept of origin of male sterility

CMS-S type of male sterility was identified in cultivar Italian Red and in France the same type was spotted in a commercial lot of Rovigo Italian onions. But 'Dorata-Di-Parma', an open-pollinated variety from France revealed CMS-T cytoplasm type. In addition, several open-pollinated varieties from Italy possesses S-cytoplasm bearing nuclear restorer *Ms* allele with high allelic frequency. Thus, male sterility could arise due to voluntary crosses accidentally with different origins of *ms* alleles of onion which could be distinguished by *cp*DNA and *mt*DNA restriction patterns of RFLP markers. By restriction profiling using RFLP marker of mitochondrial and chloroplast genomes polymorphism was revealed and on southern blot analysis of profiles of *mt*DNA of S- and N-cytoplasm put forward the fact that S-cytoplasm had alloplasmic origin. Since, no polymorphism was observed between genomes of T- and N-cytoplasms indicating the fact that T-cytoplasm could be autoplasmic in origin. Polymorphisms of S-cytoplasm or CMS-S cytoplasm were similar to that of *cp*DNA of the triploid viviparous onion variety 'Pran' and was morphologically found similar to the S-cytoplasm source of 'Italian Red' cultivar. Cladistic studies suggested that CMS-S cytoplasm has been introduced from an unknown source but autoradiograms revealed that S-cytoplasm or CMS-S cytoplasm might have engulfed earlier to its discovery. Reportedly, both *orf*725 as well as *cox*I genes and their different sections of relative copy numbers induces male sterility. [21] reported that CMS-T mitotype was developed by high genomic shift of both *orf*725 and normal *cox*I genes copy numbers, whereas, CMS-S mitotype was developed by decreased genomic shift of normal *cox*I copy numbers and increased genomic shift of *orf*725 gene. Development of four dominant *exon*1 and *exon*2 variants (two in each) resulted due to interruption of group II intron in *cox*2 gene. Both of which were found identical in CMS-T and normal-N cytotypes but were subsisted as sublimons in CMS-S cytotypes. From such revelation, [41] found out that no variation was there in nucleotide sequences and arrangement of gene between CMS-T and N-cytotypes but difference was observed in S-cytotype which certainly validates the fact that origination of CMS-T type of male sterility was recently from N-cytotype. The integration of *ycf*2 gene (partial chloroplast gene) was discovered in S-cytotype and among 32 *Allium* species, 11 species were found to be after dynamic rearrangement of *mt*DNA genome from male-sterile and normal onions and other 32 species. This further emphasizes that integration of *ycf*2 gene may occur in a common ancestor and other *Allium* species. In addition, substoichiometric shifting might have caused *ycf*2 gene of *mt*DNA to disappear in normal onion cytoplasm.

A shift from cis- to trans-splicing of *cox*2 in a common ancestor of all *Allium* species has been insinuated due to the presence of a trans-splicing group II intron of *cox*2 in other *Allium* species. [64] found out existence of a chimeric *orf*725 gene in CMS-T and CMS-S cytotypes in *A. roylei* causing male sterility which further adds on the fact that *orf*725 gene was organized recently in an ancestor of onion and other related *Allium* species. [64] used hypervariable *cp*DNA IGS (Intergenic Sequences) between *rps*16 and *trnQ* hypervariable variants and revealed

N-, S-, T-cytotypes as well as phylogenetic hierarchy of 35 *Allium* species. The researcher further the sequences of *Allium dictyoprasum* and *Allium vavilovii* were identical and were close relatives of onion N- and CMS-T cytotypes. In addition, tight relationship of CMS-S cytotype with *A. roylei* and *A. galanthum* suggesting CMS-S cytotype to be of alloplasmic origin. Furthermore, alloplasmic origins of CMS- S and autoplasmic genesis of CMS-T male sterility finds its support from the findings of chloroplast genome sequence analysis [60]. [63] observed that *mt*DNA sequences of CMS- S, CMS-T and normal (N) cytoplasm types revealed that CMS-T and CMS-S cytoplasm were almost similar with an exception of *orf*725 chimeric gene which possesses an additional sequence of *cox*1, whereas, normal (N) cytoplasms differed by three SNPs. Such, SNPs were confirmed by four CMS-T lines and were found similar to N-cytoplasm lines. Besides, *orf*725 gene copy number was observed to be less as compared to CMS-T cytoplasm type indicating CMS-T male sterility might have been induced by an independent substoichiometric shifting event of *orf*725 gene. On the other side, [40] revealed that on sequence comparison of mitochondrial genome the *orf*725 emerged as causal gene for inducing N-, CMS-S and T-cytoplasms and CMS-T like cytoplasms in onion.

Mapping of *Ms* locus across different mapping population

[66] used *Ms*-locus related markers onto mitotic metaphase, pachytene (super-stretched) chromosomes and found that tyramide FISH of amplicons was located physically on chromosome 2. They observed presence of short-genomic amplicons between 846-2251 bp and a cDNA clone of 666 bp and the markers were scattered in the proximal centromere in chromosome 2 in the long arm region of lower recombination. This provided tight linkage of markers' and marker aided selection of *Ms* locus. [60] by using 110 F_2 population from a cross [506L (CMS- S) × H6 (DH line)], constructed a linkage map for *Ms* locus which was located on chromosome 2 and the CAPS marker *jnurf*17 and *jnurf*05 revealed no recombinant with *Ms* locus (Table-4). In addition, they randomly mated 15 F_2 heterozygous male-fertile plants obtaining 2927 $F_{2:4}$, 1346 $F_{2:3}$ plants. Furthermore, recombinant analysis of 4273 segregates using *jnurf*17 and *jnurf*05 markers no recombinants between *Ms* locus and *jnurf*05 were obtained revealing a strong connection. [13] by selfing fertile heterozygous (N_{Msms}) variety 'Sapporo- Ki' developed progeny populations (S_1, S_2, S_3, S_4), mapping populations (N_{MsMs} or N_{msms}), Near Isogenic lines (NILs) and also developed other S_1 families 'Sapporo- Ki' (SK), 'Mountain- Danvers' (MD), 'Brigham- Yellow- Globe' (BYG). The said researcher developed test cross progenies from MD, BYG, SK paired with male-sterile lines and screened them for 930 SNPs. The *Ms* (fertility-restorer gene) gene was mapped on chromosome 2 with three tightly linked SNPs which revealed linkage disequilibrium between *Ms* locus and the genotypes and finally for development of male-fertility restorer or maintainer lines, all these markers aided in selection of Ms, ms allele for onion hybrid breeding. [15] discovered flanking RFLP markers to the *Ms* locus at 8.6cM and 9cM (AOB272) in the F_2 and 58 F_3 mapping populations derived from [BYG15– 13 (N_{Msms}) × AC 43(N_{msms})(AOB186)]. The workers found that as these marker sequences were homologous to Rf_2 locus (aldehyde dehydrogenase) of maize, the *Ms* genes were assigned to linkage group I.

Table 4: Varied molecular markers in mapping Ms locus across different mapping population.

Mapping populations	Molecular markers	Marker distance	Location	Reference
F_2, 58 families of F_3 population, BYG15-13 (N_{Msms}) × AC43 (N_{msms})	RFLPs (AOB272: probe used)	0.9cM	Chromosome 2	[15]
S_1 families of Brigham Yellow Globe (BYG), Mountain Vanvers (MD), Sapporo-Ki (SK) Test cross families BYG,MD,SK paired with male-sterile lines NILs from SK heterozygous-N_{Msms}(S_1,S_2,S_3,S_4)	SNPs (isotig29186_1830, isotig34671_610, isotig30856_1351)	0.9cM	Chromosome 2	[13]
F_2, $F_{2:3}$ and $F_{2:4}$ from heterozygous plants 506L (S_{msms})×H6(N_{MSMS})	CAPS (jnurf05)	0.05cM	Chromosome 2	[60]
Tyramide FISH	Five cDNAs of onion are linked to *Ms*	Mapped physically on long arm near centromere	Chromosome 2	[66]

Markers used for trait linkage in improvement programmes

Occurrence of linked polymorphic markers to the cytoplasm

CMS-S cytoplasm which finds its source from cytoplasmicgenic male sterility has simple mode of inheritance. For this reason CMS-S cytoplasm is the most widely used cytoplasm. On the other side, CMS-T cytoplasm possesses a complex mode of fertility restoration due to inheritance of nuclear genes. It generally requires 4-8 years to identify cytoplasms using testcrosses and traditional phenotyping. Thus, in order to speed up onion breeding, a detectable PCR polymorphism by an autapomorphic insertion of 100bp from *cp*DNA character-42 sequence of N-cytoplasm was discovered and generated as first PCR marker. This helps *cp*DNA amplicon fragments to distinguish N-cytoplasm from S-cytoplasm. Thus this method proves to be cheaper, significantly faster and a good replacement for southern blot analysis or testcrossing assays. [58] developed a PCR-RFLP marker to distinguish N-cytoplasm (male-fertile) from S-cytoplasm (male-sterile) using *MspI* as restriction enzyme of the chloroplast *psbA* gene. They noted that polymorphism in N cytoplasm plants was due to *MspI* enzyme restriction site (CCGG) and observed no *MspI* target in S-cytoplasm plants as it was CTGG sequence which was found mismatched with restriction site of *MspI.* This certainly validates the fact that the marker PCR-RFLP can accurately distinguish and identify N- and CMS-S cytoplasm in onion. In earlier studies, *cob* gene has been reported to differentiate N-cytoplasms and CMS-S cytoplasms in *mt*DNA polymorphisms. The *cob* gene is a determinant of CMS-cytoplasm possessing an atypical transcript pattern. The PCR primers flanking the *cob* gene upstream region which could distinguish N- and CMS-T cytoplasms from CMS-S cytoplasms based on *mt*DNA differences. Thus this marker is easy to use and reveals quickly the results. In addition, this *cob* gene marker is able to isolate the CMS-S cytoplasm in any population but is unable to distinguish T-cytoplasm from N-cytoplasm. Thus, by using the chimerical mitochondrial sequence (CMS1) of chives

which anchors the upstream region of *cob* gene of mitochondria an *orf*501 gene-specific PCR marker was generated to distinguish CMS-S from T-cytoplasm in onion. It could also differentiate S-, T- (male sterile) cytoplasm from normal (N-) cytoplasm in onion. [55] validated the *cob* gene and *orf*501 marker in open-pollinated varieties, F_1 hybrids and different Turkey landraces. The revelation of *orf*725 (a chimeric gene) and *coxI* gene in *mt*DNA can regulate cytoplasmic fate in plants due to the isolation of *orf*A501 homolog and sequences flanking it. Both of these *coxI* and *orf*725 genes were used to develop effective and inexpensive molecular markers to differentiate N-, S- and T-cytoplasm. [21] described that using MK-F as common marker binding to the *coxI* coding sequence and reverse primers MK-R1 binding to *orf*725 and MK-R2 binding to coxI gene; such markers could differentiate such cytoplasms in one PCR having high reliability. [67] from their studies found that the NGS data of *cp*DNA from CMS-S cytoplasmic and N-cytoplasmic onions disclosed 28SNPs, *petB* gene ((*Bam*HI)), restriction enzyme polymorphic sites (*atp*F gene (SacI)) and an *InDel* (*accD* gene) were scattered among 20 chloroplast genes validating N- and S-cytoplasmic plants. Further, [63] proved that SNPs were validated and confirmed in four CMS-T lines at *orf*725, *cox*1 genes and 243 different breeding lines (N-, CMS-S, CMS-T cytoplasm), which thereby allowed easy differentiation of male-sterile and normal cytoplasms.

Occurrence of molecular markers linked to the fertility restoration

Ms/*ms* alleles were used in fertility restoration (R/C) and in maintainer (B) lines, which are eventually used in creating male-sterile (A) lines. Thus in onion hybrid development identifying maintainer (N_{msms}) and restorer (N_{MsMs}) lines are very crucial and critical. Hence efficient molecular markers are required in the marker-assisted breeding of onions to distinguish genotypes at a nuclear locus (*Ms*). [15] investigated *Ms* allelic diversity using AOB272 genomic region (RFLP loci) governed by SNPs in germplasms of onion taken under study. This had resulted in SSCPs (Single-stranded Conformational Polymorphisms) at AOB272-EcoRI facilitating maintainer line selection. In spite of the linkage between *Ms* locus and RFLPs, the linkage equilibrium leads to the difficulty of spotting maintainer lines in an open-pollinated population. Hence markers with linkage disequilibrium with *Ms* locus would be precise in marker-assisted selection in *Ms* locus. [15] described that previously a simple PCR-dominant marker specific to *Ms*-locus was created. In addition, RACE (Rapid amplification of cDNA ends) was employed and the putative oligopeptide-transporter (OPT) encoding gene was isolated with polymorphic 108 and 439 bp *InDels* was spotted and developed by tandem repeats between the *Ms* allele linked OPT gene (1.5cM) leading to the development of simple PCR-based OPT marker. [38] designed another PCR marker using tandem repeats (14 and 39 bp) based on *photosystem-I-subunit-O (PsaO) gene.* This was isolated by genome walking of EST-RFLP probe and was found to be linked at a distance of 6.5 cM to *Ms* locus. [42] observed in their studies that simple PCR marker with relatively large 34 bp *InDel* based ILPs for encoding the *AcPMS1* gene (RF31446) for the repair of DNA mismatch in protein PMS1. This is responsible for fertility restoration in onions and is highly reliable for generating polymorphic molecular markers for genotyping of *Ms*. This greatly enhances efficiency in breeding onions or rather in the F_1 development of onions. [60] converted polymorphic RAPD markers into CAPS markers (jnurf05, jnurf06, jnurf10, jnurf17) which possessed tight linkage with *Ms* locus. These markers were of co-dominant nature which could easily differentiate homozygous from heterozygous plants, dominant from recessive alleles. In addition, genome walking of an RAPD marker flanking sequence leads to the development of dominant simple PCR markers (jnurf20).

Marker-aided selection of CGMS lines in onion

In onion, male sterility is the most feasible way to produce hybrid seeds at a commercial scale. To achieve such production, maintainer lines (N_{msms}), and dominant homozygous fertility restorer lines (N_{MsMs}) are required alongside male sterile lines. Thus, markers linked to *Ms* genotypes and cytoplasm types render in expanding F_1's breeding and development. The *cob* marker which is a cytochrome-b (cob) protein mitochondrial DNA marker was utilized along with phenotypic evaluation to isolate maintainer and male-sterile lines. [68] used *cob* marker and observed frequencies of male-sterile plants (S_{msms}) to be 0.015 in the genotype Punjab Naroya, 0.006 in Punjab white, 0.020 in Punjab Selection, whereas, frequencies of maintainer plants were noted to be 0.232 in Punjab Selection, 0.182 in Punjab White, 0.133 in Punjab Naroya. [69] exploited *Ms*/*ms* allelic markers of *AcSKP1* and AcPMS1 genes and *orfA*501, *cob*, *orf*725 gene-specific markers among Brazilian onion germplasms. They observed frequencies of *Ms* and *ms* allele to be 0.52 and 0.48, respectively, whereas, frequencies of CMS-T, -S, and N-cytoplasm were 0.28, 0.47, and 0.25, respectively. [70] isolated cytoplasmic genotypes in some Poland Breeding lines by RFLPs (*Xba*I) using mtDNA among mitochondrial genes viz. *cox1*, *cob*, *atp6*, *atp9*, *atpA,* and *nad3*, *nad4,* and *nad6.* The results revealed that polymorphisms were shared among cytoplasmic and germplasm lines owing to heteroplasmy in mitochondrial genes. [71] deployed marker-assisted selection of *Ms* locus and *Ms* locus co-segregated *jnurf*13 marker. In addition, they observed heterozygous dominant, homozygous dominant, homozygous recessive, homozygous, and heterozygous among cytoplasm types and among 100 breeding lines 89 maintainer (N_{msms}) lines were identified. Studies by [69] explained that N_{msms}/S_{msms} condition was confirmed by accessions EHCEB-20112006/EHCEB-20111006, EHCEB-20142040/EHCEB-20141040, EHCEB- 20142028/EHCEB-20141028, whereas, N_{msms}/T_{msms} condition were confirmed by the genotypes Alfa- SF/Alfa-SF, EHCEB-20142027/EHCEB-20141027 and EHCEB-20102019/EHCEB-20101019. These lines can be the possible resource for heterosis breeding. [72] identified fertility-restorer locus (*Ms*) using two sets: SCAR markers (FN1, RN1, F3S2 and R3S2) for *Ms* and *ms* allelic plants and nuclear markers [novel chimeric gene, *orf*725 gene (N/S) MK marker] among open-pollinated varieties (OPVs). The results revealed that 70% of OPVs exhibited male-sterile cytoplasm along with recessive alleles at *Ms* locus and are thus male-sterile (A), whereas, normal cytoplasm were exhibited by 20% plants with recessive alleles (*ms*) at *Ms* locus which is known as maintainer line or B-line. Hence, identifying such A-, -B and R/C (restorer line) lines from open-pollinated varieties can help in developing high-yielding F_1 hybrids in onion using hybrid seeds having low cost of production.

Limitations in male-sterile line development in onion

From past four decades, very few countries especially the Netherlands, USA, Japan and Korea has exploited heterosis in onion. Besides, very few research group has attempted in exploiting heterosis in onion using male sterile systems. In fact, some male sterile lines in long day type onion were mostly unstable and unsuitable under short day conditions. Onion hybrids which outshined in terms of bulb yield and quality influenced the onion seed market in UK, Germany and the Neth-

Table 4: Marker-assisted separation of *Ms* locus and cytoplasmic male-sterile lines for breeding F1's in onion.

Loci	Mapping population	References
Fertile N- cytoplasm, CMS- S, - T cytoplasm	176 cultivars and breeding lines using *orf*725 marker	[21]
Ms locus	F_2 populations generated from 506L male- sterile line and H6 male- fertile line using PSAO and OPT marker	[38]
Ms locus	301 plants of F_2 and F_3 populations generated from 506L male- sterile line and H6 male- fertile line using CAPS marker	[56]
Ms locus	BC_1 population, self-cross progeny and breeding lines using SCAR markers	[40]
Ms locus	Open-pollinated populations using *AcSKP1* marker	[62]
Ms locus	59 genotypes taken from Embrapa Onion Germplasm Bank using *AcSKP1* and *AcPMS1* markers	[69]
Fertile N-cytoplasm and CMS- S and -T cytoplasm	Brazilian onion germplasm using *orf*725 marker	[73]
Ms locus and CMS-S cytoplasm	Crosses, selves and testcrosses onion lines were utilized for multiplex-PCR molecular marker (AcCN)	[74]
Fertile N-cytoplasm, CMS-S and -T cytoplasm	OPV gene bank from Punjab province using *orf*725 markers	[72]

erlands surpassed to contemporary open pollinated varieties. The reasons stand out to be owing to absence of maintainer genotypes with respect to male sterile genotypes, instability in male sterility, high labour-demanding, high time-demanding in terms of seed production and hybrid breeding. In addition, complex genetics, rare occurrence of N_{msms} (which is almost rare event among local genotypes), genetic complication in breeding onion hybrids, technological gap in the knowledge for breeding hybrid onions are some of the reasons behind less exploitation of male sterile lines in onion hybrid development.

Conclusion

Since antiquity CMS-S cytoplasm is in extensive use for introgression of alien cytoplasm into *Allium cepa* L. The classical hybridization of biennial generation of onion usually takes at least 4-8 years to classify male sterile cytoplasms (CMS-S) or normal cytoplasms (N-cytoplasms). Molecular marker eases in time of classification in distinguishing N- and S-cytoplasm. Thus efforts are needed to initiate validation of other molecular markers like SSR, SNP, CAPS and *InDel* to serve purposes like haplotype analysis, allelic variation and establishment of handy co-dominant markers. In fact, marker assisted selection can be effectively utilized in isolating male-sterile, their maintainer parts and fertility-restorer lines particularly in large populations. Thus, use of molecular markers saves resources from being exhausted on crop establishment and evades the complexity involved in phenotypic screening. The development of physical map, effect of restorer genes and its role in translation and post-translational event in inducing male-sterility may further be studied to derive more information on onion male sterility systems.

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