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Research Article

Genotyping of Bangladeshi Onion (*Allium cepa* L.) Germplasm for Male Sterility Using PCR-Based Molecular Markers

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ABSTRACT

Increasing onion productivity requires the development of hybrid varieties. Identification of sterile and maintainer lines is essential for successful hybrid development. This study aimed to identify cytoplasmic types: fertile (N) or sterile (S) and genotypic conditions at the *Ms* locus within the Bangladeshi onion germplasm, including certain mutant lines. The cytoplasmic marker *accD* was employed to differentiate between sterile and fertile cytoplasm, while three nuclear molecular markers (*OPT*, *PsaO*, and *jnurf13*) were used for genotyping the *Ms* alleles. The findings of the *accD* marker indicated that the frequency of fertile (N) cytoplasm was significantly higher than that of the sterile (S) cytoplasm. The result of nuclear markers also showed that the molecular markers used in this experiment are not sufficiently effective for accurate genotyping of the *Ms* locus due to the lack of linkage disequilibrium, suggesting the need to assess more closely linked markers for improved results, or the compromised genotypic purity of the collected germplasm may also be the reason of inconclusive genotyping. Nonetheless, molecular markers have proven highly effective for distinguishing between S and N cytoplasm. Utilizing these markers for cytotype (N/S) identification in onions will be helpful for the development of hybrid onions in the future.

Introduction

Onion (*Allium cepa* L., 2x=2n=16) is one the most important vegetable crops of Bangladesh, which is used as a condiment in practically all of our daily meals (Kumar Barmon and Anjum, 2018; Chakraborty et al., 2019). It stands first among the spice crops grown in the country, both in terms of area (5.08 lakh acres) and production (25.17 lakh metric tons) (BBS, 2022). However, the acreage production of onion is very low compared with other countries (Islan et al., 2011). The limited availability of high-quality onion seeds is a major contributing factor to the low yield of onions in Bangladesh (Islam et al., 2011; Bokshi et al., 1989). The development of hybrids is recommended to achieve the higher productivity potential.

Hybrid onion production relies on cytoplasmic-genic male sterility (CGMS), where male sterility results from the interaction between a sterile cytoplasm and a recessive nuclear allele (*msms*) (Yu and Kim, 2021). It is well established that all the genes responsible for sterility belong to the mitochondrial genome (Fauron et al., 1990; Budar et al., 2003; Hanson and Bentolila, 2004; Knoop, 2004). The identification of cytoplasmic male sterility in the onion cultivar 'Italian Red', which is controlled by S cytoplasm, led to the discovery of sterility in onions (Jones and Emsweller, 1936). This sterility could be fully restored to fertility by a single nuclear restorer gene (*Ms*) (Jones, 1943). After that another type of male sterility cytoplasm, CMS-T was found in the 'Jaune paille des Vertus' variety (Berninger, 1965), where one independent gene and two complementary genes were found to control the fertility restoration (Schweisguth, 1973). These findings facilitated the development of hybrid onion varieties in the United States and several European nations with

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high yields, and their uniformity in size, shape, color, and maturation (Khar et al., 2016).

The hybrid onion development requires a two-line system. A-lines, characterized by male sterility, function as the female parental line, and B-lines, known as maintainer lines, are employed to restore fertility in the hybrid progeny (Dehghani et al., 2021). The development of hybrid onions remains challenging due to their biennial nature and the complexities of the male sterility system. Traditional methods to identify these lines are time-consuming, often requiring several years of progeny testing (Havey, 2000; Shigyo and Kik, 2008; Khrustaleva et al., 2023). To accelerate the breeding process, molecular markers have become essential tools for distinguishing between sterile and normal cytoplasm and identifying the *Ms* locus associated with fertility restoration (Meena et al., 2023).

Numerous molecular markers have been reported to distinguish normal (N) and sterile (S) cytoplasm (Havey, 1995; Sato, 1998; Cho et al., 2006; Von Kohn et al., 2013). It is easy to identify male sterility through visual or microscopic examination, whereas determining the maintainer line requires progeny testing for genotypic analysis of the *Ms* locus (Khar et al., 2016). Markers tightly linked to the *Ms* locus were initially identified by Gökçe and Havey (2002) in the United States, with subsequent studies employing PCR markers (Bang et al., 2011; Kim, 2014; Huo et al., 2015; Kim et al., 2015) and SNPs (Havey, 2013; Von Kohn et al., 2013) confirming these findings.

Marker-assisted selection using PCR-based markers was used to determine cytoplasm types as well as the *Ms* locus in Indian onion germplasm (Khar et al., 2016). Similar applications were also reported in Brazilian germplasm (Ferreira and Santos, 2018) and Iranian germplasm (Dehghani et al., 2021). While extensive research has been conducted on the use of molecular markers in various germplasms, similar studies in Bangladeshi germplasm are lacking. This study aims to fill this gap by genotyping Bangladeshi onion germplasm using PCR-based molecular markers to identify male sterile and maintainer lines. This study may contribute to the development of high-yielding onion hybrids suited to the local context.

Materials and Methods

Plant materials

Thirty-two (32) accessions from different sources were collected, and 84 plants were evaluated for the identification of cytoplasm type and *Ms* locus. These accessions included two BINA-released varieties, ten commercial cultivars, one commercial hybrid line, and nineteen mutant lines. For mutant lines, DNA samples were collected directly from individual plants and five replications were used for other accessions. Among these, eight accessions and mutant lines were grown from seeds, and five others were grown from bulbs (Table 1).

Assessment of cytoplasm type and the male-fertility restorer locus (Ms)

Total genomic DNA was extracted from the leaves of each genotype using the modified CTAB mini-prep method (Schenk et al., 2023)**.** DNA quality and concentrations were quantified using spectrophotometric methods (Additional File 1) and the extracted DNA was stored at -20°C for PCR amplification. Locus specific primers were used for performing PCR amplification (Table 2). According to the previous literatures, PCR conditions were applied (Table 3). UV-trans-illuminator visualized DNA bands on 1.2% agarose gels after ethidium bromide staining. PCR and gel electrophoresis were conducted more than once for few samples to get complete and accurate results.

Visual evaluation of anthers

Anthers were collected when 40–50% of the flowers in the umbels had undergone dehiscence. Previous research indicates that there is a correlation between the coloration of anthers and their fertility status in male plants. Specifically, anthers that exhibit a light green are associated with male sterility, whereas those that are dark green are indicative of male fertility (Santos et al., 2010).

Table 1. Onion germplasms used in the experiment along with their cultivar types and sources

Sl. No.	Genotypes	Types	Grown from	No. of Plant	Sources
1.	Goran	Cultivar	Seed	5	Khan Seed and Agro. Limited.
2.	King onion	Cultivar	Seed	5	Khan Seed and Agro. Limited.
3.	Fursangi	Cultivar	Seed	5	Khan Seed and Agro. Limited.
4.	Taherpuri	Cultivar	Seed	5	Rayhan Onion Seed
5.	Hybrid onion	Hybrid	Seed	5	Khan Seed and Agro. Limited.
6.	Golden Queen	Cultivar	Seed	5	Khan Seed and Agro. Limited.
7.	Gujrat 911	Cultivar	Seed	5	Khan Seed and Agro. Limited.
8.	Faridpuri	Cultivar	Seed	5	Chand Seed Company
9.	Binapiaz-1	Variety	Bulb	5	BINA, BAU Campus
10.	Binapiaz-2	Variety	Bulb	5	BINA, BAU Campus
11.	BO-1	Cultivar	Bulb	5	GPB, BAU.
12.	BO-2	Cultivar	Bulb	5	GPB, BAU.
13.	BO-3	Cultivar	Bulb	5	GPB, BAU.
14.	ML-1	Mutant Line	Seed	1	GPB, BAU.
15.	ML-2	Mutant Line	Seed	1	GPB, BAU.
16.	ML-3	Mutant Line	Seed	1	GPB, BAU.
17.	ML-4	Mutant Line	Seed	1	GPB, BAU.
18.	ML-5	Mutant Line	Seed	1	GPB, BAU.
19.	ML-6	Mutant Line	Seed	1	GPB, BAU.
20.	ML-7	Mutant Line	Seed	1	GPB, BAU.
21.	ML-8	Mutant Line	Seed	1	GPB, BAU.
22.	ML-9	Mutant Line	Seed	1	GPB, BAU.
23.	ML-10	Mutant Line	Seed	1	GPB, BAU.
24.	ML-11	Mutant Line	Seed	1	GPB, BAU.
25.	ML-12	Mutant Line	Seed	1	GPB, BAU.
26.	ML-13	Mutant Line	Seed	1	GPB, BAU.
27.	ML-14	Mutant Line	Seed	1	GPB, BAU.
28.	ML-15	Mutant Line	Seed	1	GPB, BAU.
29.	ML-16	Mutant Line	Seed	1	GPB, BAU.
30.	ML-17	Mutant Line	Seed	1	GPB, BAU.
31.	ML-18	Mutant Line	Seed	1	GPB, BAU.
32.	ML-19	Mutant Line	Seed	1	GPB, BAU.

BINA: Bangladesh Institute of Nuclear Agriculture; GPB: Genetics and Plant Breeding; BAU: Bangladesh Agricultural University

Table 2. List of the primers along with their sequences, target locus and locus specific product size

Marker Name	Marker Sequence (5'-3')	Target Locus	Locus Specific Products Size (bp)	References
accD	F: AGAATGAGGAGCAGGAAAACTCT R: AGTCGTGATTGTTACTCTTAGACCT	Cytoplasmic Locus	N (375bp), S (420bp)	Von Kohn et al., 2013
OPT	F: CCTTGGAAAGGCGCAACTAAAGATTTGA R: TGTGGCCCAATAATACAAACAAGCAGGA	Nuclear *Ms* Locus	*MsMs* (659 bp), *Msms* (526 bp and 659 bp), *msms* (526 bp)	Bang et al., 2011
jnurf13	F: TGCAAGCTTGGAACTTACGC R: TTGCCAAAGGTTGCAATACA	Nuclear *Ms* Locus	*MsMs* (241 bp), *Msms* (229 bp and 241 bp), *msms* (229 bp)	Kim, 2014
PasO	F: CCTCATGCTTGCTTGGTCTT R: AAGCGTGATCGATTGTAGGTCCTTT	Nuclear *Ms* Locus	*MsMs* (490 bp), *Msms* (437 bp and 490 bp), *msms* (437 bp)	Bang et al., 2011

Table 3. PCR conditions for amplification of cytoplasmic and nuclear *Ms* locus

Marker	Denaturation (Time)	Cycles	Denaturation (Time)	Annealing (Time)	Extension (Time)	Final Extension
accD	94ºC (5 min)	35	94ºC (30s)	55ºC (30s)	72ºC (45s)	72ºC (5 min)
OPT	95ºC (5 min)	40	94ºC (30s)	60ºC (30s)	72ºC (45s)	72ºC (5 min)
Jnurf13	95ºC (5 min)	35	95ºC (30s)	65ºC (30s)	72ºC (45s)	72ºC (5 min)
PsaO	95ºC (5 min)	35	95ºC (30s)	65ºC (30s)	72ºC (60s)	72ºC (7 min)

Results

Genotyping of cytoplasmic type and nuclear male sterility locus

PCR marker *accd* was used to determine the cytoplasm and nuclear markers *jnurf13*, *OPT*, and *PsaO* for nuclear genotype at *Ms* locus. The amplicons visualized on the gels were successfully identified for these markers, with the observed fragments corresponding to the anticipated product sizes.

The frequencies of the types of cytoplasm and genotype were estimated for each marker among the replicated plants for each accession. In Binapiaz-1 and Binapiaz-2, all the plants were genotyped as N cytoplasm according to the *accd* marker. For *Ms* locus in Binapiaz-1, nuclear marker *jnurf13* genotyped 20% of plants as homozygous recessive, and 60% as homozygous dominant, where *OPT* marker genotyped the same result as *accd*, and *PsaO* genotyped 40% of plants as *msms* genotype. In Binapiaz-2, markers *jnurf13* and *PsaO* genotyped all plants as homozygous dominant at the *Ms* locus, but *OPT* genotyped 20% of the plants as homozygous recessive (*msms*).

In Goran, all the plants carried N cytoplasm. For the *Ms* locus, the *jnurf13* marker genotyped 100% plants as homozygous dominant (*MsMs*) genotypes, whereas *OPT* and *PsaO* genotyped 80% plants as *MsMs* genotype. All the plants were genotyped as N cytoplasm for the PCR marker in King onion. Markers *jnurf13* and *PsaO* genotyped that all the plants as homozygous dominant at *Ms* locus, but *OPT* predicted 60% of plants to be homozygous dominant genotype. Cytoplasm in all the plants of Fursangi was normal N type and marker *jnurf13* genotyped 80% of plants as homozygous dominant (*MsMs*), OPT genotyped 40% as homozygous dominant, and 20% as heterozygous (*Msms*), whereas *PsaO* genotyped all the plants with homozygous dominant *Ms* locus. In the Taherpuri cultivar, the N-type cytoplasm was genotyped across all plants analyzed. The molecular markers *jnurf13* and *PsaO* genotyped that approximately 80% of the plants possessed the homozygous dominant genotype. Additionally, the *OPT* marker genotyped that 60% of the plants would carry the homozygous dominant *Ms* allele. Cytoplasm was normal N type in all the plants of Golden Queen and markers *jnurf13* and *OPT* genotyped all the plants as homozygous dominant (*MsMs*), whereas it was about 60% of plants for *PsaO* marker. In Gujrat 991, all plants possessed N cytoplasm for the cytoplasmic *accd* PCR marker. Markers *jnurf13* and *PsaO* genotyped all the plants as homozygous dominant at *Ms* locus, but *OPT* genotyped 80% of plants with this type. All the plants belonging to the Faridpuri were genotyped as N cytoplasm. The nuclear marker *jnurf13* genotyped 100% homozygous recessive genotypes, where *OPT* and *PsaO* were genotyped 20% and 60% respectively.

In the BO-1, N cytoplasm was found in all the plants. The nuclear marker *jnurf13* and *OPT* genotyped 60% of plants with homozygous dominant genotype, while it was 80% for *PsaO*. In BO-2, all the plants possessed S cytoplasm, and markers *jnurf13* and *PsaO* genotyped 60% of plants as homozygous recessive genotypes, whereas *OPT* genotyped 80% of plants as *msms* genotype. N cytoplasm was genotyped for all the plants of the BO-3 genotype.

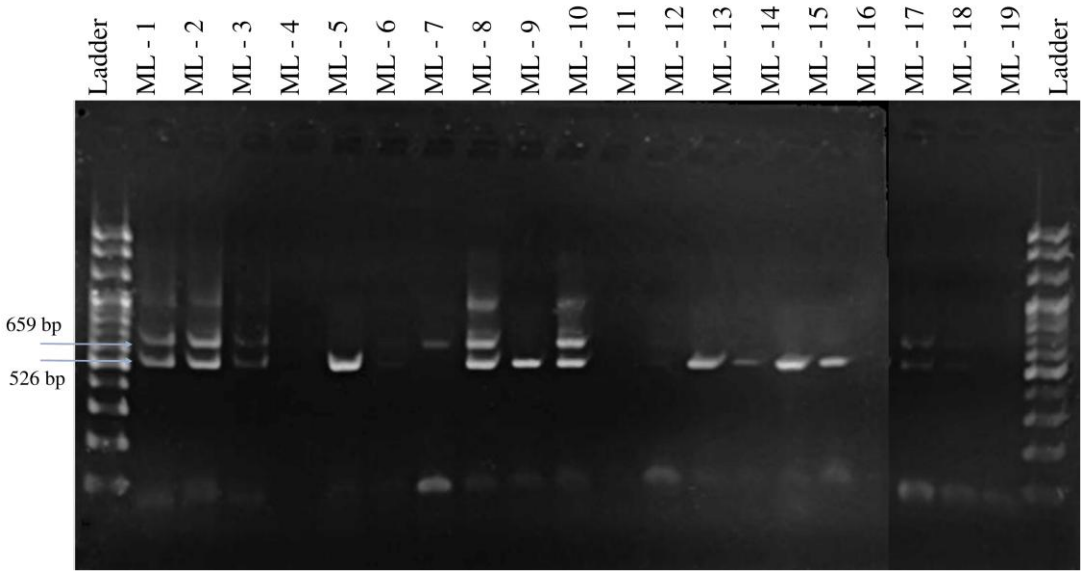

Figure 1. Genotyping of the nineteen mutant lines of onion for *Ms* locus (*MsMs* at 659 bp, *Msms* at 526 bp and 659 bp, *msms* at 526 bp) using *OPT* marker.

Markers *jnurf13* genotyped that 60% of the plants should be heterozygous dominant for *Ms* locus, but *OPT* genotyped 80% of plants as heterozygous dominant genotype.

In hybrid onion, all plants possessed N cytoplasm, and Markers *jnurf13* and *PsaO* genotyped all the plants as heterozygous (*Msms*) at *Ms* locus, but *OPT* genotyped 60% of plants as this type.

Three of the 19 mutant lines genotyped exhibited S cytoplasm, while the remaining lines showed N cytoplasm for the *accD* marker. Nuclear marker *jnurf13* genotyped 4 lines as homozygous recessive at *Ms,* whereas *OPT* genotyped 6 lines and *PsaO* genotyped 3 lines as homozygous recessive for *Ms* locus.

Visual evaluation of anthers

The visual assessment of anthers did not reveal a definitive correlation between anther color and male sterility. Both male sterile and male fertile flowers were found with light green, dark green, or yellow anthers (Figure 2).

Figure 2. Variations in anther colour: (a) dark green anthers, (b) yellowish light green anthers (c) mixture of dark green and yellowish light green anthers.

Table 4. Frequency of genotype in diverse onion germplasm for cytoplasmic and nuclear *Ms* locus as genotyped by PCR-based marker

Sl	Accession	Cytoplasmic Marker		Nuclear Markers								
		accd		*jnurf13*			*OPT*			*PsaO*		
		N	S	*MsMs*	*Msms*	*msms*	*MsMs*	*Msms*	*msms*	*MsMs*	*Msms*	*msms*
1	Binapiaz-1	1	0	0.6	0.2	0.2	0.6	0.2	0.2	0.6	0	0.4
2	Binapiaz-2	1	0	0	0	1	0.02	0	0.8	0	0	1
3	Goran	1	0	1	0	0	0.8	0	0.2	0.8	0.2	0
4	King onion	1	0	1	0	0	0.6	0.2	0.2	1	0	0
5	Fursangi	1	0	0.8	0	0.2	0.4	0.2	0.4	1	0	0
6	Taherpuri	1	0	0.8	0.2	0	0.6	0.2	0.2	0.8	0.2	0
7	Golden Queen	1	0	1	0	0	1	0	0	0.6	0	0.4
8	Gujrat 911	1	0	1	0	0	0.8	0	0.2	1	0	0
9	Faridpuri	1	0	1	0	0	0.2	0.2	0.6	0.8	0.2	0
10	BO-1	1	0	0.6	0.4	0	0.6	0.2	0.2	0.8	0.2	0
11	BO-2	0	1	0.4	0	0.6	0	0.2	0.8	0.4	0	0.6
12	BO-3	0	1	0	0	1	0.2	0	0.8	0	0	1
13	Hybrid onion	1	0	0	1	0	0.2	0.6	0.2	0	1	0
14	ML-1	1	0	1	0	0	0	1	0	0	0	0
15	ML-2	1	0	0	1	0	0	1	0	0	1	0
16	ML-3	1	0	0	1	0	0	1	0	1	0	0
17	ML-4	1	0	0	1	0	1	0	0	0	0	1
18	ML-5	1	0	0	0	1	0	0	1	1	0	0
19	ML-6	1	0	1	0	0	1	0	0	1	0	0
20	ML-7	0	1	0	0	1	1	0	0	0	1	0
21	ML-8	1	0	1	0	0	0	1	0	0	1	0
22	ML-9	1	0	1	0	0	0	0	1	1	0	0
23	ML-10	1	0	0	1	0	0	1	0	0	1	0
24	ML-11	1	0	0	1	0	1	0	0	0	1	0
25	ML-12	0	1	1	0	0	0	1	0	0	0	1
26	ML-13	1	0	0	1	0	0	0	1	0	1	0
27	ML-14	1	0	0	1	0	0	0	1	1	0	0
28	ML-15	1	0	0	0	1	0	0	1	1	0	0
29	ML-16	0	1	0	0	1	0	0	1	0	0	1
30	ML-17	1	0	1	0	0	0	1	0	0	1	0
31	ML-18	1	0	1	0	0	0	1	0	1	0	0
32	ML-19	1	0	0	1	0	1	0	0	1	0	0

Discussion

The comparatively low yields of onion in Bangladesh, relative to other countries, indicate the need for improvement of onion production (Islan et al., 2011). The reliance on local varieties limits productivity, thereby causing acute shortages in Bangladesh, especially during the off-peak season. Addressing this issue through the development of hybrid onion varieties, which may provide enhanced disease resistance, greater adaptability, and increased yield potential, could significantly enhance onion productivity.

For over a century, the concept of heterosis, commonly referred to as hybrid vigor, fascinated geneticists and made a substantial contribution to enhancing crop productivity. Heterosis refers to the phenomenon where hybrid offspring demonstrate superior agronomic performance compared to their parental lines (Schnable and Springer, 2013). For the past 50 years, F_1 hybrids have been employed in onion breeding programs across the United States, Europe, Japan etc. (Abbasi, 2023). The economic viability of hybrid onion seed production has been achieved through the use of cytoplasmic male sterility (CMS) sources. The CMS-based hybrid seed production system requires two distinct lines: a male-sterile line (A-line), and a male-sterility maintainer line (B-line, which is male fertile). The fertile cytoplasm (N) and two types of sterile cytoplasm (S and T) were found in onion (Dehghani et al., 2021). There are molecular markers that can differentiate between normal (N) and sterile (S) cytoplasm (Havey, 1995; Sato, 1998; Von Kohn et al., 2013), while other markers can differentiate all three cytoplasmic types namely, S, T, and N (Kim et al., 2009). Besides, the development of locus-specific PCR co-dominant markers enabled the differentiation between restorer lines (N_{MsMs}) and maintainer lines (N_{msms}) as well (Abbasi, 2023).

The resources needed to produce the maintainer (B) line within a population can be greatly reduced using marker-assisted selection (MAS) for cytotype. This can reduce the number of test crosses necessary to identify maintainers. By selectively advancing only the N-cytoplasmic plants to the flowering stage, these plants can subsequently be used for test crossing with a male sterile (A) line. Many of the findings from the markers used for cytoplasmic identification contributed to reducing the number of test crosses needed to distinguish between "A" and "B" lines (Santos et al., 2010; Engelke et al., 2003; Patil et al., 2016; Ragassi et al., 2012; Khar et al., 2016).

In a study on Indian germplasm, three cytoplasmic molecular markers (*accD*, *cob*, and *MK*) were used for cytoplasm identification of cytoplasmic type (Khar et al., 2016). The use of an *accD* marker for the determination of cytoplasm type is recommended due to the relative simplicity of employing just two primers and the ease of visualization (Khar et al., 2016). In this study, *accd* is used to identify the S and N-type cytoplasm among the Bangladeshi germplasms. The marker result indicated that the proportion of N-cytoplasm was significantly higher than that of S-cytoplasm. Since most onion populations have N cytoplasm, the S cytoplasm is likely an introduced alien cytoplasm in onions (Havey and Genetics, 2000; Havey, 1993). The organellar DNA of the S cytoplasm differs from that of the N cytoplasm due to several polymorphisms (Havey and Genetics, 2000; Havey, 1995; Sato, 1998; Engelke et al., 2003; Kim et al., 2009). The probability of finding maintainers is higher in populations with high levels of N-cytoplasm than in plants that are male-sterile. In these instances, the frequency of maintainer plants can be determined by conducting test crosses with a recognized CMS line in two years, utilizing the Seed-to-Seed method. In the previous study, it was found that the result of the *accd* marker was accurate in the identification of N and S-type cytoplasm (Dehghani et al., 2021).

The application of PCR markers for accurate genotyping of the *Ms* locus is crucial for the rapid development of onion hybrids. In this study, genotyping of the *Ms* locus in the onion accessions using three different markers, namely *OPT*, *jnurf13*, and *PsaO* was promising. However, there was little discrepancy in the genotyping outcome of the same accession by these markers. This may be due to the absence of complete linkage between the marker and the respective allele. The linkage disequilibrium between the *Ms* locus and nuclear markers is crucial for determining the minimum distance between markers necessary for their effective use in genotype detection. The study started with the use of the PCR marker *OPT*, during which discrepancies were noted between molecular genotyping results and field inspection observations. This demonstrated that the *OPT* marker probably is not in linkage disequilibrium with the *Ms* locus. Although the *OPT* marker is located close to the *Ms* locus at a distance of 1.5 cM, both this study and other research have demonstrated no significant linkage disequilibrium between the marker and the *Ms* locus (Dehghani et al., 2021). This suggests that crossing-over events near the *Ms* locus have occurred frequently throughout the history of onion breeding. Again, no such information was found for the other two co-dominant makers, *jnurf13* and *PsaO* whether these markers are in complete linkage disequilibrium with the *Ms* locus in these varieties or not. Similarly, no marker showed complete linkage disequilibrium with the *Ms* locus in the Indian onion population (Khar et al., 2016). On the other hand,

linkage equilibrium between the *Ms* locus and closely associated markers has also been demonstrated in onion breeding lines developed within the United States (Gökçe and Havey, 2002).

Previous research indicated that flowers possessing light green anthers exhibited male sterility (Pathak, 1997; Santos et al., 2010). However, our findings differ, as we observed male sterility in the flowers having light green, dark green, or yellow anthers. The findings of the non-correlated relationship between anther color and male sterility by Saini et al. (2015) are consistent with our observation (Saini et al., 2015).

As we used locally available open-pollinated genotypes, the genetic purity of these accessions might not be up to mark indicating a potential genetic admixture among these populations at the field level. Such variation in the pollen viability on the same plants may be an effect of high temperature as well as indicated by Khar et al., (2016). To further investigate this issue, a pollen viability test could be employed to correlate the marker-based genotyping data with actual pollen viability outcomes. In Bangladesh, the number of onion germplasm is really limited. For any future experiments, a large number of genetically pure germplasm should be genotyped in a strictly maintained condition. All these will be helpful in determining the efficiency of these markers in genotyping male sterility locus in Bangladeshi germplasm.

Based on the current findings, predicting the genotypes at the *Ms* locus precisely using these molecular markers in onion germplasm is not feasible. Nevertheless, genotyping the *Ms* locus through PCR markers remains critical to accelerate the development of onion hybrids. To ensure accurate genotyping of the *Ms* locus, the development of more tightly linked markers is essential.

Conclusion

Molecular markers successfully genotyped cytoplasmic type and nuclear male sterility locus in Bangladeshi onion germplasm. For almost all the accessions, the nuclear male sterility locus seemed to be segregated, indicating potential loss of genetic purity which may be due to open pollination at field level for several generations. The use of these markers, especially with larger population sizes and pure genetic lines will be helpful to validate the efficacy of these markers in predicting sterile and maintainer lines which will further greatly contribute to hybrid development programs.

Authors Contribution

M.M.M.: Performing the field and lab experiments, preparation of the first draft**. M.N.A.S. and M.J.H.J.:** Analysis of data, preparation of tables and figures, finalizing the draft**. K.B.Z.K and P.B.:** Collection of data**. M.R.H.:** Conceptualization, design, acquisition of funds, supervision of experiments, and finalizing the manuscript**.**

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

Abbasi, Z. 2023. Molecular markers associated with male-sterile cytoplasms and male-fertility restorer locus in onion (*Allium cepa*): a review. *Journal of Horticulture and Postharvest Research*, 6: 371-382.

Bang, H., Cho, D. Y., Yoo, K. S., Yoon, M. K., Patil, B. S. and Kim, S. J. E. 2011. Development of simple PCR-based markers linked to the Ms locus, a restorer-of-fertility gene in onion (*Allium cepa* L.). *Euphytica*, 179: 439-449.

BBS. 2022. Year Book of Agricultural Statistics of Bangladesh. Bangladesh Bureau of Statistics, Statistics and Informatics Division, Ministry of Planning, Government of the People's Republic of Bangladesh.

Berninger, E. 1965. Contribution to the study of male sterility in the onion (*Allium cepa* L.). *Annales de l'Amélioration des Plantes*, 15: 183-99.

Bokshi, A., Mondal, M. and Pramanik, M. 1989. Effect of nitrogen and phosphorus nutrition on the yield and quality of onion seeds. *Pakistan Journal of Biological Sciences*, 2(3): 752-754.

Budar, F., Touzet, P. and De Paepe, R. 2003. The nucleo-mitochondrial conflict in cytoplasmic male sterilities revisited. *Genetica,* 117: 3-16.

Chakraborty, M., Azad, M. A. and Haque, M. A. 2019. Chromosome Structural Rearrangements in Onion (*Allium cepa* L.) through Induced Mutation. *Journal of Agricultural Science and Technology B,* 9: 110-118. 10.17265/2161-6264/2019.02.004.

Cho, K. S., Yang, T. J., Hong, S. Y., Kwon, Y. S., Woo, J. G. and Park, H. G. 2006. Determination of cytoplasmic male sterile factors in onion plants (*Allium cepa* L.) using PCR-RFLP and SNP markers. *Molecular Cells,* 21: 411-7.

Dehghani, R., Abbasi, Z., Talebi, M., Shahmansouri, E. and Tabatabaei, B. E. S. 2021. Identification of male sterile (T, S) of fertility (N) cytoplasm by PCR-based molecular markers to access maintainer lines in onion. *Journal of Horticulture and Postharvest Research*, 4: 277-288.

Engelke, T., Terefe, D. and Tatlioglu, T. 2003. A PCR-based marker system monitoring CMS-(S), CMS-(T) and (N)-cytoplasm in the onion (*Allium cepa* L.). *Theoretical and Applied Genetics,* 107: 162-7.

Fauron, C. M., Havlik, M. and Brettell, R. I. 1990. The mitochondrial genome organization of a maize fertile cmsT revertant line is

generated through recombination between two sets of repeats. *Genetics,* 124: 423-428.

Ferreira, R. and Santos, C. J. S. H. 2018. Partial success of marker-assisted selection of 'A'and 'B'onion lines in Brazilian germplasm. *Scientifica Horticulturae*, 242: 110-115.

Gökçe, A. F. and Havey, M. J. 2002. Linkage equilibrium among tightly linked RFLPs and the Ms locus in open-pollinated onion populations. *Journal of the American Society for Horticultural Science*, 127: 944-946.

Hanson, M. R. and Bentolila, S. 2004. Interactions of Mitochondrial and Nuclear Genes That Affect Male Gametophyte Development. *The Plant Cell,* 16: S154-S169.

Havey, M. J. 1993. A putative donor of S-cytoplasm and its distribution among open-pollinated populations of onion. *Theoretical and Applied Genetics,* 86: 128-34.

Havey, M. J. 1995. Identification of cytoplasms using the polymerase chain reaction to aid in the extraction of maintainer lines from open-pollinated populations of onion. *Theoretical and Applied Genetics*, 90: 263-8.

Havey, M. J. 2013. Single nucleotide polymorphisms in linkage disequilibrium with the male-fertility restoration (*Ms*) locus in open-pollinated and inbred populations of onion. *Journal of the American Society for Horticultural Science*, 138: 306-309.

Havey, M. J. 2000. Diversity among male-sterility-inducing and male-fertile cytoplasms of onion. *Theoretical and Applied Genetics*, 101: 778-782.

Huo, Y. M., Liu, B. J., Yang, Y. Y., Miao, J., Gao, L. M., Kong, S. P., Wang, Z. B., Kitano, H. and Wu, X. J. E. 2015. AcSKP1, a multiplex PCR-based co-dominant marker in complete linkage disequilibrium with the male-fertility restoration (*Ms*) locus, and its application in open-pollinated populations of onion. *Euphytica*, 204: 711-722.

Islan, M. S., Rahman, K. M., Hasan, M. K. and Adnan, K. M. 2011. Present Status and Potentiality of Onion Production in Bangladesh. *Bangladesh Journal of Political Economy*, 27: 253-264.

Jones, H. A. 1943. Inheritance of male-sterility in the onion and the production of hybrid seed. *Proceedings of the American Society for Horticultural Science*. 189-194.

Jones, H. A. and Emsweller, S. 1936. A male-sterile onion. *American Society for Horticultural Science*, 34: 582-85.

Khar, A., Saini, N. and Havey, M. 2016. Limitations of PCR-based molecular markers to identify male-sterile and maintainer plants from Indian onion (*Allium cepa* L.) populations. *Plant Breeding,* 135: 519-524.

Khrustaleva, L., Nzeha, M., Ermolaev, A., Nikitina, E. and Romanov, V. 2023. Two-Step Identification of N-, S-, R- and T-Cytoplasm Types in Onion Breeding Lines Using High-Resolution Melting (HRM)-Based Markers. *International Journal of Molecular Sciences,* 24: 1605.

Kim, S., Kim, C. W., Park, M. and Choi, D. 2015. Identification of candidate genes associated with fertility restoration of cytoplasmic male-sterility in onion (*Allium cepa* L.) using a combination of bulked segregant analysis and RNA-seq. *Theoretical and Applied Genetics,* 128: 2289-99.

Kim, S., Lee, E. T., Cho, D. Y., Han, T., Bang, H., Patil, B. S., Ahn, Y. K. and Yoon, M. K. 2009. Identification of a novel chimeric gene, *orf725*, and its use in development of a molecular marker for distinguishing among three cytoplasm types in onion (*Allium cepa* L.). *Theoretical and Applied Genetics,* 118: 433-41.

Kim, S. 2014. A codominant molecular marker in linkage disequilibrium with a restorer-of-fertility gene (*Ms*) and its application in reevaluation of inheritance of fertility restoration in onions. *Molecular Breeding*, 34: 769-778.

Knoop, V. 2004. The mitochondrial DNA of land plants: peculiarities in phylogenetic perspective. *Current Genetics,* 46: 123-39.

Kumar Barmon, B. and Anjum, A. 2018. Profitability and Comparative Advantage of Onion (*Allium cepa*) Production in Bangladesh: An Economic Study in Some Selected Areas. *The Agriculturists,* 15: 66-78.

Pathak, C. S. 1997. *A Possible New Source of Male Sterility in Onion*. International Society for Horticultural Science (ISHS), Leuven, Belgium, 313-316.

Patil, Y. A., Dalvi, V. S., Borole, V. K., Krishna, B., Dhake, A. V., Sane, P. V. and Sane, A. P. 2016. *Molecular characterization of CMS lines in short-day white onions*. International Society for Horticultural Science (ISHS), Leuven, Belgium, 259-268.

Ragassi, C. F., Santos, M. D. D. M. D., Fonseca, M. E. D. N., Oliveira, V. R., Buzar, A. G. R., Costa, C. P. D. and Boiteux, L. S. 2012. Genotipagem de polimorfismos associados com sistemas de macho-esterilidade em acessos de cebola adaptados ao Brasil. *Horticultura Brasileira,* 30.

Saini, N., Hedau, N. K., Khar, A., Yadav, S., Bhatt, J. C. and Agrawal, P. K. 2015. Successful deployment of marker assisted selection (MAS) for inbred and hybrid development in long-day onion (*Allium cepa* L.). *Indian Journal of Genetics and Plant Breeding,* 75: 93-98.

Santos, C. A. F., Leite, D. L., Oliveira, V. R. and Rodrigues, M. A. 2010. Marker-assisted selection of maintainer lines within an onion tropical population. *Scientia Agricola,* 67: 223-227.

Sato, Y. 1998. PCR amplification of CMS-specific mitochondrial nucleotide sequences to identify cytoplasmic genotypes of onion (*Allium cepa* L.). *Theoretical and Applied Genetics,* 96: 367-70.

Schenk, J. J., Becklund, L. E., Carey, S. J. and Fabre, P. P. 2023. What is the "modified" CTAB protocol? Characterizing modifications to the CTAB DNA extraction protocol. *Applications in Plant Sciences,* 11: e11517.

Schnable, P. S. and Springer, N. M. 2013. Progress toward understanding heterosis in crop plants. *Annual Review of Plant Biology,* 64: 71-88.

Schweisguth, B. 1973. Etude d'un nouveau type de stérilité mâle chez l'oignon, *Allium cepa* L. *Annales de l'Amélioration des Plantes*, 23(3): 221-253.

Shigyo, M. and Kik, C. 2008. Onion. *Vegetables II: Fabaceae, Liliaceae, Solanaceae, and Umbelliferae.* Springer. 121–159.

Meena, V. K., Shekhawat, H. V. S., Chand, S., Choudhary, K., Sharma, J. K. and Lekha, L. 2023. Advances in Molecular Marker Technology and their Significance in Plant Improvement Strategies. *In:* MOHAMED, A. E.-E. (ed.) *Recent Trends in Plant Breeding and Genetic Improvement*.

Von Kohn, C., Kielkowska, A. and Havey, M. J. 2013. Sequencing and annotation of the chloroplast DNAs and identification of polymorphisms distinguishing normal male-fertile and male-sterile cytoplasms of onion. *Genome,* 56: 737-42.

Yu, N. and Kim, S. 2021. Identification of *Ms2*, a novel locus controlling male-fertility restoration of cytoplasmic male-sterility in onion (*Allium cepa* L.), and development of tightly linked molecular markers. *Euphytica,* 217: 191.