MOLECULAR MAPPING OF NUCLEAR MALE STERILITY GENE *ms-1* IN MUSKMELON (*Cucumis melo* L.)

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
HORTICULTURE (Vegetable Science)
(Minor Subject: Plant Breeding and Genetics)

By

Manpreet Singh
(L-2016-A-159-M)

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CERTIFICATE - I

This is to certify that the thesis entitled **"Molecular mapping of nuclear male sterility gene *ms-1* in muskmelon (*Cucumis melo* L.)"** submitted for the degree of **Master of Science** in the subject of **Horticulture (Vegetable Science)** (Minor subject: **Plant Breeding and Genetics**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Manpreet Singh** (**L-2016-A-159-M**) under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

This is to certify that the thesis entitled, **"Molecular mapping of nuclear male sterility gene *ms-1* in muskmelon (*Cucumis melo* L.)"** submitted by **Manpreet Singh** (Admn. No. **L-2016-A-159-M**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Sc.** in the subject of **Horticulture (Vegetable Science)** (Minor subject: **Plant Breeding and Genetics**) has been approved by the Student's Advisory Committee after an oral examination on the same.

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ABSTRACT

Nuclear male sterility (NMS) is one of the most extensively exploited pollination control mechanisms for hybrid breeding in muskmelon. Punjab Agricultural University, Ludhiana has developed three hybrids, Punjab Hybrid, Punjab Anmol, MH-27 using MS-1 male sterile genotype possessing *ms-1* gene. Male sterility reduces the cost of hybrid seed production, but the 50% heterozygous male fertile plants in the female line need to be rouged out before pollination. A codominant molecular marker closely linked to *ms-1* gene would not only facilitate its rapid transfer to new inbred lines, but it will also assist the removal of 50 % fertile plants in the female line before transplanting in the hybrid seed production block. Therefore, the present study was undertaken to identify codominant simple sequence repeat (SSR) marker(s) linked to *ms-1* gene in an F_2 population derived from a cross 'MS-1 × KP_4HM-15'.Segregation analysis of F_2 population confirmed the monogenic recessive inheritance of the male sterility gene *ms-1*. Total 498 SSR primers were used for analysis of polymorphism between the parents followed by bulk segregant analysis (BSA). The primers differentiating the parents as well as the sterile and fertile bulks were further used for screening of F_2 population to assess the linkage distance between the *ms-1* gene and the putative markers. It was found that two SSR markers, DM0187 and DM0038 were linked to the *ms-1* gene. The marker, DM0187 was closely linked at a genetic distance of 6.6 cM while the distance of *ms-1* locus and DM0038 marker was 21.1 cM. Linkage analysis placed *ms-1* locus between the two marker loci on chromosome 6 of muskmelon. The closely linked marker, DM0187 can be used to speed up the transfer of *ms-1* gene into new desired lines. It will also aid the elimination of fertile plants in the female line at seedling stage. Further, it provides perspective for the fine mapping and cloning of *ms-1* gene.

Keywords: *Cucumis*, Heterosis, Molecular marker, Linkage map, SSR

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Signature of Major Advisor **Signature of the Student**

ਖੋਜ ਦਾ ਸਿਰਲੇਖ	:	ਖਰਬੂਜੇ (*Cucumis melo* L.) ਦੇ ਆਣਵਿਕ ਨਰ ਜੀਵਾਣੂਹੀਨਤਾ ਜੀਨ *ms-1* ਦਾ ਅਣੂਕਣਿਕਾ ਪ੍ਰਤੀਚਿਤਰਣ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਂ ਅਤੇ ਦਾਖ਼ਲਾ ਨੰਬਰ	:	ਮਨਪ੍ਰੀਤ ਸਿੰਘ (ਐੱਲ-2016-ਏ-159-ਐੱਮ)
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ਸਾਰ-ਅੰਸ਼

ਖਰਬੂਜੇ ਵਿੱਚ ਹਾਈਬ੍ਰਿਡ ਪ੍ਰਜਨਨ ਲਈ ਆਣਵਿਕ ਨਰ ਜੀਵਾਣੂਹੀਨਤਾ ਸਭ ਤੋਂ ਵੱਧ ਵਰਤੀ ਜਾਣ ਵਾਲੀ ਪਰ-ਪਰਾਗਨ ਨਿਯੰਤਰਣ ਕਰਨ ਵਾਲੀ ਵਿਧੀ ਹੈ। ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ ਨੇ *ms-1* ਜੀਨ ਵਾਲੇ ਪੁਰਸ਼ ਜੀਵਾਣੂਹੀਨ ਕਿਸਮ MS-1 ਦੀ ਵਰਤੋਂ ਨਾਲ ਤਿੰਨ ਹਾਈਬ੍ਰਿਡ, ਪੰਜਾਬ ਅਨਮੋਲ, MH-27 ਵਿਕਸਤ ਕੀਤੇ ਹਨ। ਨਰ ਜੀਵਾਣੂਹੀਨਤਾ ਹਾਈਬ੍ਰਿਡ ਬੀਜ ਉਤਪਾਦਨ ਦੀ ਲਾਗਤ ਘਟਾਉਂਦੀ ਹੈ, ਲੇਕਿਨ 50% ਅਸਮਰੂਪ ਨਰ ਉਪਜਾਊ ਪੌਦੇ ਪਰਾਗਿਤ ਕਰਨ ਤੋਂ ਪਹਿਲਾਂ ਬਾਹਰ ਕੱਢਣੇ ਜਰੂਰੀ ਹਨ। *ms-1* ਜੀਨ ਨਾਲ ਜੁੜਿਆ ਇਕ ਕੋਡੌਮੀਨੈਂਟ ਆਣੂਵਿਕ ਚਿੰਨ ਨਾ ਸਿਰਫ ਨਵੀਆਂ ਇਨਬ੍ਰਿਡ ਕਿਸਮਾਂ ਵਿੱਚ ਤੇਜ਼ੀ ਨਾਲ ਇਸ ਜੀਨ ਨੂੰ ਤਬਦੀਲ ਕਰਨ ਦੀ ਸਹੂਲਤ ਪਰਦਾਨ ਕਰੇਗਾ, ਪਰ ਪੌਦਿਆਂ ਨੂੰ ਹਾਈਬ੍ਰਿਡ ਬੀਜ ਉਤਪਾਦਨ ਬਲਾਕ ਵਿੱਚ ਤਬਦੀਲ ਕਰਨ ਤੋਂ ਪਹਿਲਾਂ ਇਹ ਮਾਦਾ ਕਤਾਰ ਵਿੱਚੋਂ 50% ਨਰ ਉਪਜਾਊ ਪੌਦਿਆਂ ਨੂੰ ਕੱਢਣ ਵਿੱਚ ਵੀ ਸਹਾਇਤਾ ਕਰੇਗਾ। ਇਸ ਲਈ ਮੌਜੂਦਾ ਅਧਿਐਨ 'MS-1 x KP_4HM-15' ਦੇ ਸੰਕਰਨ ਤੋਂ ਲਈ F_2 ਆਬਾਦੀ ਵਿੱਚ *ms-1* ਜੀਨ ਨਾਲ ਜੁੜੇ SSR ਚਿੰਨਾਂ ਨੂੰ ਲੱਭਣ ਲਈ ਕੀਤਾ ਗਿਆ ਸੀ। F_2 ਆਬਾਦੀ ਦੇ ਸੈਗਰੀਗੇਸ਼ਨ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਪੁਸ਼ਟੀ ਕੀਤੀ ਕਿ ਨਰ ਜੀਵਾਣੂਹੀਨਤਾ ਇਕ ਅਪ੍ਰਭਾਵੀ ਜੀਨ *ms-1* ਦੁਆਰਾ ਸੰਚਾਲਿਤ ਕੀਤੀ ਜਾਂਦੀ ਹੈ। ਕੁੱਲ 498 SSR ਪਰਾਈਮਰ ਪਿੱਤਰੀ ਕਿਸਮਾਂ ਦੇ ਵਿਚਕਾਰ ਬਹੁਰੂਪੀਕਰਨ ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਲਈ ਵਰਤੇ ਗਏ ਅਤੇ ਉਸ ਤੋਂ ਬਾਅਦ ਸੈਗਰੀਗੈਂਟ ਵਿਸ਼ਲੇਸ਼ਣ (BSA) ਕੀਤਾ ਗਿਆ। ਪਿੱਤਰੀ ਕਿਸਮਾਂ ਦੇ ਨਾਲ ਨਾਲ ਜੀਵਾਣੂਹੀਨ ਅਤੇ ਉਪਜਾਊ ਬਲਕਾਂ ਵਿੱਚ ਭਿੰਨਤਾ ਦਖਾਉਣ ਵਾਲੇ ਪਰਾਈਮਰਾ ਦਾ ਸਰਵੇਖਣ F_2 ਅਬਾਦੀ ਵਿੱਚ ਕੀਤਾ ਗਿਆ ਤਾਂ ਜੋ *ms-1* ਜੀਨ ਅਤੇ ਪੁਟੇਟਿਵ ਚਿੰਨ੍ਹਾਂ ਵਿਚਕਾਰ ਸੰਯੋਜਨ ਦੂਰੀ ਦਾ ਮੁਲਾਂਕਣ ਕੀਤਾ ਜਾ ਸਕੇ। ਇਹ ਪਾਇਆ ਗਿਆ ਕਿ ਦੋ SSR ਚਿੰਨ, DM0187 ਅਤੇ DM0038 *ms-1* ਜੀਨ ਨਾਲ ਜੁੜੇ ਹੋਏ ਹਨ। ਚਿੰਨ, DM0187 ਨੂੰ 6.6 cM ਦੀ ਅਨੁਵੰਸ਼ਕ ਦੂਰੀ ਨਾਲ ਨੇੜਿਓਂ ਜੁੜਿਆ ਹੋਇਆਂ ਦੇਖਿਆ ਗਿਆ ਜਦੋਂ ਕਿ *ms-1* ਲੋਕਸ ਅਤੇ DM0038 ਦੀ ਦੂਰੀ 21.1 cM ਸੀ। ਸੰਯੋਜਨ ਵਿਸ਼ਲੇਸ਼ਣ ਦੁਆਰਾ *ms-1* ਲੋਕਸ ਨੂੰ ਗੁਣਸੂਤਰ 6 ਉੱਤੇ ਦੋ ਚਿੰਨਾਂ ਦੇ ਵਿਚਕਾਰ ਰੱਖਿਆਂ ਗਿਆ। ਨਜ਼ਦੀਕੀ ਨਾਲ ਜੁੜਿਆ ਚਿੰਨ DM0187 *ms-1* ਜੀਨ ਨੂੰ ਨਵੀਆਂ ਲੋੜੀਂਦੀਆਂ ਕਿਸਮਾਂ ਵਿੱਚ ਤੇਜ਼ੀ ਨਾਲ ਤਬਦੀਲ ਕਰਨ ਲਈ ਵਰਤਿਆ ਜਾ ਸਕਦਾ ਹੈ। ਇਹ ਮਾਦਾ ਕਤਾਰ ਵਿੱਚੋਂ ਨਰ ਉਪਜਾਊ ਪੌਦਿਆਂ ਨੂੰ ਪਨੀਰੀ ਲਗਾਉਣ ਦੇ ਪੜਾਅ 'ਤੇ ਕੱਢਣ ਵਿੱਚ ਵੀ ਸਹਾਇਤਾ ਕਰੇਗਾ। ਇਸ ਤੋਂ ਇਲਾਵਾ, ਇਹ *ms-1* ਜੀਨ ਦੇ ਸੂਖਮ ਪ੍ਰਤੀਚਿਤਰਣ ਅਤੇ ਪ੍ਰਤੀਰੂਪਣ ਲਈ ਦ੍ਰਿਸ਼ਟੀਕੋਣ ਮੁਹੱਈਆ ਕਰਦਾ ਹੈ।

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CHAPTER I

INTRODUCTION

The genus *Cucumis* belongs to family *Cucurbitaceae* and is represented by as many as 66 species (Sebastian *et al* 2010). Muskmelon (*Cucumis melo* L.) and Cucumber (*Cucumis sativus* L.) are the two major commercially cultivated vegetable crops of this genus (Kirkbride 1993). Muskmelon is one of the most economically as well as nutritionally important cucurbits, cultivated in many tropical and subtropical regions of the world. It is a diploid plant species (2n=2x=24) with a small genome size of approximately 450 Mb (Arumuganathan and Earle 1991). It is the most diverse *Cucumis* species, especially for the fruit related traits such as size, shape, colour, taste and texture (Kirkbride 1993, Whitaker and Davis 1962).

Muskmelon fruit is valued among consumers for its unique aroma, sweetness, texture and high phytonutrient content (Lester 2008). Besides the sensorial attributes, it is an excellent source of vitamin A (β-carotene), a good source of vitamin C (Ascorbic acid), folic acid and potassium. It has low fat, cholesterol and sodium. Additionally, the phytochemicals present in muskmelon such as, cucurbitacin-β, lithium and zinc provides protection against cancer, depression, ulcers, dandruff and stimulate the immune system (Lester 1997).

In 2016, the global production of muskmelon was 31.2 million tonnes from an area of 1.2 million ha (Anonymous 2016). China, Turkey, Iran, Egypt and India are the leading muskmelon producing countries (Anonymous 2016). In India, during 2017 melons were cultivated on an area of 50 thousand ha with production of 1.1 million tonnes and the average productivity was 21.9 tonnes ha^{-1} (Anonymous 2017a). The major muskmelon producing states in India are Uttar Pradesh, Andhra Pradesh, Punjab, Madhya Pradesh and Harayana (Anonymous 2017b). In Punjab, muskmelon was cultivated on 5.2 thousand ha area, with 93.1 thousand tonnes of total annual production (Anonymous 2017c).

The geographical origin of muskmelon has remained controversial as both Africa and Asia are suggested as its possible origins. Based on species richness of wild *Cucumis* in Africa, muskmelon is suggested to be of African origin (Whitaker and Davis 1962, Kerje and Grum 2000, Pitrat 2008). However, recent phylogenetic studies, molecular data and broader species sampling support Asian origin (Schaefer *et al* 2009, Sebastian *et al* 2010). The wild progenitor of *C. melo* is in India and the closest relative (*Cucumis picrocarpus* F. von Mueller) occurs in Australia (Sebastian *et al* 2010).

Muskmelon is predominantly a cross pollinated crop possessing great variability. Its ability to produce a good quantity of seeds per fruit facilitates hybrid breeding (Dhaliwal 1995). Heterosis breeding is preferred in muskmelon as hybrids are uniform, more stable, higher yielding and early maturing. Heterosis over the better parent has been observed as higher for fruit weight, fruits per plant, fruit yield per plant, total soluble sugars and moisture

content (Tomar and Bhalala 2006). Artificial emasculation and pollination for producing F_1 hybrids is a costly venture and causes seed contamination through self-pollination. The F_1 hybrid seeds cost twelve to thirty times higher as compared to open-pollinated cultivars (McCreight and Elmstrom 1984). Different cross pollination promoting mechanisms, such as, gynoecism, monoecism and genic male sterility are present in muskmelon which can be utilized effectively for economical and feasible production of F_1 hybrids (Kumar and Singh 2005). Genetic male sterility, being a potentially stable out-crossing system can curtail such a high cost by eliminating hand emasculation and also maintains genetic purity of hybrid seeds (McCreight and Elmstrom 1984).

At present, five nuclear male sterility genes *viz. ms-1, ms-2, ms-3, ms-4, and ms-5* are known (Dhillon and Kumar 2008). All of them are recessive and non-allelic and possess unique phenotypes (Pitrat 1991). The first male sterility gene was reported by Bohn and Whitaker (1949). In India, the MS-1 genotype having *ms-1* gene was introduced in 1978 (Nagaraju *et al* 2017). Thereafter, it was used to develop three commercial muskmelon hybrids, Punjab Hybrid (Nandapuri *et al* 1982), Punjab Anmol (Lal *et al* 2007) and MH-27 (Anonymous 2018). Besides *ms-1*, the male sterility gene *ms-5* has been recently utilized to develop a hybrid, MH-51(Anonymous 2018). Over time, 'MS-1' has survived its utility. With increasing consumer awareness, the demand for muskmelon hybrids with specialty traits is emerging. Thus, for developing specialty muskmelon hybrids, there is an urgent need to transfer this male sterility gene in new inbred lines possessing special horticultural traits.

Genetic male sterility is governed by a recessive gene in muskmelon. To produce hybrid seed, male sterile line (*msms*) used as female parent is crossed with homozygous male fertile line (*MsMs*) used as male parent. The male sterile line (*msms*) is maintained by crossing it with a heterozygous male fertile line (*Msms*). The resulting progeny segregates into Male Sterile (*msms*) : Male fertile (*Msms*) :: 1:1 ratio. The male fertile plants in the female line are identified and removed at anthesis from the hybrid seed production block to produce 100 percent pure hybrid seeds.

Since a single recessive gene governs male sterility in muskmelon, generation of new GMS lines using classical backcross technique is time consuming and strenuous task. This is because the homozygous male sterile plants appear only in segregating generations. The homozygous (*MsMs*) and heterozygous male fertile (*Msms*) plants in the backcross population cannot be distinguished phenotypically, as both genotypes exhibit male fertile phenotypes. Progeny testing *via* self-pollination needs to be done for identifying heterozygous male fertile plants in the backcross generations.

Molecular mapping of *ms*-3 and *ms*-5 genes controlling male sterility has been reported. Park *et al* (2004) developed an SCAR marker, SOAM08.644 linked to *ms-3* gene at 5.2 cM. The male sterility gene *ms-5* has been mapped to a 30-kb association region spanning

2,522,791 to 2,555,104 bp positioned on scaffold 000048 on chromosome 9 (Sheng *et al* 2017). However, no such report related to the first male sterility gene, *ms-1* has been published. Thus, identification of molecular markers associated with *ms-1* gene will be very useful, as it can be used to easily differentiate homozygous and heterozygous male fertile plants in the backcross population without testing the progeny. Hence, it can be utilized for developing new GMS lines. Moreover, the linked markers will be used to identify male sterile plants in the hybrid seed production block at an early seedling stage to establish 100 percent pure stand of female plants. Consequently, there is an urgent need to generate information on genetic markers related to *ms-1*, the male sterility gene. Therefore, the present study is intended to accomplish following objectives:

1. To map male sterility gene *ms-1* on the molecular linkage map of muskmelon
2. To identify SSR marker closely linked to male sterility gene *ms-1*

CHAPTER II

REVIEW OF LITERATURE

2.1 Classification of muskmelon

The name of genus *Cucumis* was coined by Linne in 1783, who gave the description of five cultivated melon species (Stepansky *et al* 1999). Naudin (1859) merged these into one species, *Cucumis melo*, comprising 10 melon varieties. Pangalo (1929) proposed a multi-level classification wherein each of the four *C. melo* varieties were subdivided into two subspecioids, agrestis (wild ones) and cultus (cultivated types). Each subspecioid had further sub-types. Munger and Robinson (1991) simplified Naudin's classification by dividing *C. melo* into 7 groups, including *C. melo* var. *agrestis* Naud. (Wild melon), *C. melo* var. *flexuosus* Naud. (Snake melon), *C. melo* var. *conomon* Mak. (Pickling melon, Chinese white cucumber), *C. melo* var. *cantalupensis* Naud. (Cantaloupe or muskmelon), *C. melo* var. *inodorus* Naud. (Winter melons, honeydew or casaba), *C. melo* var. *chito* (Mango melon) and *dudaim* Naud. (Queen's pocket melon) and *C. melo* var. *momordica* (Phoot or snap melon). Robinson and Decker-Walters (1997) merged the *reticulatus* and *cantalupensis* groups of Whitaker and Davis (1962) into *cantalupensis* group and *dudaim and chito* in the *duduaim* group. The plant material used in this study belong to the *reticulatus* group.

2.2 Male Sterility in muskmelon and its utilization

On the basis of genotype, Kaul (1988) classified male sterility into three major groups i.e.

a) Genetic male sterility (GMS)
b) Cytoplasmic male sterility (CMS)
c) Cytoplasmic-genic male sterility (CGMS)

However, only genetic male sterility (GMS) has been reported in muskmelon to date. As the name indicates, genetic or nuclear male sterility is governed by the nuclear genes. Most of the induced or naturally occurring male sterile mutants are recessive with exception of Brassica crops such as rapeseed, cabbage and broccoli (Li *et al* 1988, Fang *et al* 1997, Kim and Lee 2012). The Predominance of recessive male sterility implies that mutation in any nuclear gene(s) is the cause of GMS by altering microsporogenesis, microgametogenesis or stamen development and varying the GMS mutant phenotypes accordingly (Kumar *et al* 2000).

Till date, five nuclear male sterility genes have been reported in muskmelon (Table 2.1). Each of these genes is recessive in nature and display unique phenotype. These five male sterility genes are located on five different linkage groups and no allelism has been reported (Pitrat 1991). Bohn and Whitaker (1949) identified first male sterility gene in a powdery mildew resistant line. It was controlled by a recessive gene hindering the development of pollen in the tetrad stage. The second male sterility gene (*ms-2*) was reported by Bohn and Principe (1964) who observed three male sterile mutants in a breeding line La Jolla 40460. It was a triple cross (powdery mildew race 2-resistant P3 × PMR 45) × PMR 450 derived F_7

progeny. Progeny tests revealed that the *ms-2* gene was present in the parent of La Jolla 40460. Neither its grandparent nor the 10 F_6 sibs or the former's eight sibs had this gene. It was an obvious indication that mutation occurred in either one of the two germ cells of the grandparent or the somatic cells of parent itself. The third gene for male sterility was observed by McCreight and Elmstrom (1983) in PI321005 (Georgia 47 × Smith's Perfect) at Leesburg, Florida. Its reciprocal crosses with lines having *ms-1* and *ms-2* genes produced fertile progenies indicating that *ms-3* is non-allelic to the former male sterility genes. Segregation ratios of four F_2 progenies confirmed monogenic recessive gene inheritance. The fourth gene for male sterility was reported by Lozanov (1983). A fifth male sterile mutant had been first encountered by Lecouviour in 1966 while introducing the PMR 45 powdery mildew resistance in the Charentais-type muskmelon, though the findings had not been published. Subsequently, it was utilized by Clause Seed Company for producing F_1 hybrid seeds. But its inheritance and description was published by Pitrat and Risser in 1990 (Lecouviour *et al* 1990). The number of blossoming male flowers on male sterile plants is less than that on male fertile plants due to the bud stage abortion. The anthers are empty and reduced in size. The pollen degeneration begins at meiosis. The male sterile mutant produced fertile F_1 hybrids with male fertile plants of different types including American Cantaloupes, Charentais and Indian lines (MR-1). The F_2 progenies advanced from these hybrids always segregated into 3:1 monogenic phenotypic ratio confirming recessive gene control. Allelism tests with the first four male sterility genes revealed that *ms-5* is not allelic to any of these genes. Park *et al* (2004) confirmed that the andromonoecious (*a*) gene controlling stamen presence or absence is genetically unlinked to *ms-3* gene. This is in consensus with the reports of Pitrat (1991) and Dogimont (2011) that the '*a*' gene is not linked with any of the five male sterility genes.

Table 2.1: Male sterility genes in muskmelon

Preferred Gene Symbol	Synonym	Character	Reference(s)
ms-1	ms^1	*male sterile-1*; Indehiscent anthers with empty pollen walls in the tetrad stage.	Bohn and Whitaker (1949)
ms-2	ms^2	*male sterile-2*; Anthers indehiscent, containing mostly empty pollen walls, growth rate reduced.	Bohn and Principe (1964)
ms-3	*ms-L*	*male sterile-3*; waxy and translucent indehiscent anthers, containing two types of empty pollen sacs	McCreight and Elmstrom (1983)
ms-4	-	*male sterile-4*; small indehiscent anthers, first male flowers abort at bud stage.	Lozanov (1983)
ms-5	-	*male sterile-5*; abortion of male flowes at the bud stage, small indehiscent anthers	Lecouviour *et al* (1990)

Dogimont 2011

Despite the availability of five nuclear male sterility genes in muskmelon, the commercial exploitation of male sterility is still limited. Though, the first male sterility gene, *ms-1* has been successfully utilized for hybrid seed production at commercial scale in India (Nandapuri *et al* 1982, Lal *et al* 2007, Anonymous 2018). One of the reasons for limited utilization of male sterility can be the non-availability of superior male sterile lines possessing good horticultural traits. The development of new GMS lines by transferring male sterile gene through conventional back-cross method is time-consuming. Since a single recessive gene governs male sterility, homozygous (*MsMs*) and heterozygous (*Msms*) male fertile plants in the backcross population cannot be distinguished phenotypically, as both genotypes display male fertile phenotypes. Selfing has to be done for the expression and selection of plants with *ms* allele.

The male sterility is maintained by crossing male sterile line with an isogenic heterozygous male fertile line (*Msms*). The resulting progeny segregates into Male Sterile (*msms*) : Male fertile (*Msms*) :: 1:1 ratio. The male sterile plants are identified at anthesis. Identification is done by rubbing the anther of freshly opened flower across the fingertip. Adherence of yellowish/creamy powder denotes male fertile plant whereas the absence of pollen shows that the plant is sterile.

Some plant breeders have reported difficulty in identification of *ms-1ms-1* plants stating that the male sterile phenotype is unstable. However, in a study conducted on the stability of expression of *ms-1* gene, McCreight (1980) concluded that phenotypic expression of *ms-1ms-1* is stable. Two to four flowers per plant were scored for the presence/absence of pollen. Pollen presence was assessed by rubbing anther on the fingertip and by observing the anthers stained by methyl blue or acetocarmine under 100 X magnification. It was suggested that a plant should be tagged as male fertile as soon as a single male fertile flower is identified. A plant should be confirmed as male sterile only after scoring at least 3-4 flowers for the absence of pollen.

Another limitation of GMS system is that it does not produce 100 percent sterile progeny. Imprecise identification of male sterile plants in the female line and slackness in other measures such as the removal of pre-set fruits after completing the identification process can cause contamination of hybrid seed. The effectiveness of GMS system could be enhanced with the availability of morphological markers preferably in seedlings or prior to flowering. McCreight (1983) and Pitrat (1991) reported loose linkage between red stem (*r*) and the *ms-1* gene, and between yellow-green leaves (*yg*) and *ms-2* gene, respectively. However, the use of such markers in any hybrid seed production program has not been reported. Molecular markers linked to two male sterility genes *ms-3* and *ms-5* have been reported by Park *et al* (2004) and Sheng *et al* (2017) respectively. These markers can be used to identify the sterile plants at seedling stage before transplanting in the hybrid seed production block.

2.3 SSR marker development

Genetic marker or molecular marker may be a gene or DNA sequence of known chromosomal location. A genetic marker that is in close proximity to the target gene is said to be tightly linked to that gene and serve as a sign or flag (Collard *et al* 2005). On the basis of various polymorphism-detecting techniques (PCR-polymerase chain reaction, Southern blotting and DNA sequencing) many DNA markers, including RAPD (Randomly Amplified Polymorphic DNA), RFLP (Restricted Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), SNP (Single Nucleotide Polymorphism), SSR (Simple Sequence Repeat), etc. have been developed and genetic maps for some crops have been constructed in the recent years (Collard *et al* 2005). SSRs, also known as short tandem repeats (STRs) or microsatellites are repetitive DNA sequences of 1-6 base pairs distributed throughout the coding as well non-coding nuclear and cytoplasmic DNA of most of the eukaryotic organisms. Different individuals show SSR polymorphism due to the variable number of tandem repeats resulting from replication slippage or recombination errors (Vieira *et al* 2016). SSR genotyping has turned out to be the most widely used technique in plants for the last 20 years due to codominant, multi-allelic and highly informative nature of SSR markers that exhibit high reproducibility and transferability among related species (Mason 2015). These SSR markers have been employed for genetic mapping, variety identification, phylogenetic studies, marker-assisted selection, QTL mapping and construction of high-density genetic maps (Chistiakov 2006).

Bhawna *et al* (2015) identified microsatellites from available whole genome sequences of melon using microsatellite identification tools (MISA) and subsequently, mapped with BLAST software. An SSR database encompassing 39072 SSR markers, comprised of information regarding motif sequence, motif length, motif type, marker ID and chromosomal locations was developed. An average frequency of nearly 123 microsatellites for every mega base (Mb) sequences was observed. The most frequent were dinucleotide repeats (61.13%), followed by trinucleotides (30.66%), tetra-nucleotides (5.59%), penta-nucleotides (1.83%) and hexa-nucleotides (0.77%). Chromosome 1 and chromosome 10 contained the highest (4103) and lowest (1960) number of microsatellite markers, respectively. Synteny analysis of melon with cucumber and watermelon revealed that 44.46% (17375) of these markers were also present in cucumber whereas only 18.67% (7298) of these microsatellite markers were syntenic to the watermelon genome.

Zhu *et al* (2016) identified 44265 microsatellites from DHL92 melon genome assembly available at https://melonomics.net/genome/. It was approximately 0.31% of the surveyed sequence with a mean of about 108.8 SSR/Mb. Among different types of repeat motifs, the most prevalent were dinucleotides (49.13%), followed by tri-nucleotides (20.60%), tetra-nucleotides (9.01%), while the least occurring repeat types were octa-

nucleotides (1.46%). Also, the frequency of SSRs decreased with an increase in the number of repeated units. Among the motif sequence types, the rate of occurrence of AT was significantly higher among dinucleotide motifs as well as in the total melon genome occupying 39.23% of the total microsatellite loci encountered. Likewise, the AAT, AAAT, AAAAG, AAAAAG, AAAAAAG, and AAAAAAAG were predominant repeats within respective classes. No correlation between frequency of SSR loci and chromosome size was observed. It was observed that chromosome 1 and chromosome 10 have the highest and lowest number of microsatellites, respectively. By employing Primer3 software, 28570 SSR primers were designed. Information illustrating chromosomal positions, repeat motifs and expected size of PCR product of these markers was also generated. To evaluate transferability, comparative analysis of these melon markers was conducted in cucumber and watermelon. It was revealed that 4002 (14.01%) SSR markers produced single amplicon, 289 displayed at least two amplicons and six had multiple amplicons on the seven chromosomes of cucumber. In case of watermelon, 1085 markers displayed one amplicon and 36 had two or more amplicons. These results were in accord with the evolutionary distances of melon from the two cucurbitaceous species.

2.4 Construction of genetic maps in muskmelon

Saturated genetic maps are prerequisite for effective utilization of markers in genomic analysis and plant breeding. Linkage maps have several applications including gene mapping, QTL analysis, positional cloning, MAS, linkage disequilibrium analysis, genome assembly, comparative genomics and many more (Diaz *et al* 2011). Pitrat (1991) developed a genetic map of muskmelon containing genes for flower biology, disease resistance and vegetative characters. Later, Baudracco-Arnas and Pitrat (1996) constructed first molecular map of melon using RFLP, RAPD, four disease resistance markers and a morphological trait in an F_2 population of cross between two variable genotypes, 'Vedrantais' and 'Songwhan Charmi'. Total 1390 cM genetic distance was covered across 14 linkage groups in contrast with the expected 12 chromosomes. Also, this map was based on dominant markers (RAPD) with low transferability, thus limiting their application in genetics and breeding.

Oliver *et al* (2001) provided codominant marker (SSR, RFLP and AFLP) based saturated map of melon using F_2 population advanced from cross of Korean accession 'PI161375' with Spanish melon 'Pinyonet Piel de Sapo'. Out of 411 markers, 391 loci were mapped to 12 major linkage groups, covering 1197 cM genetic distance, with 3 cM/marker map density. The remaining 21 markers were observed as unlinked. Perin *et al* (2002a) construced a composite linkage map of melon by merging maps developed using two recombinant inbred line resulting from the crosses of a French line, Vedrantais with Korean line PI161375 and Indian line PI414723. Vedrantais possess good agronomic whereas PI161375 and PI414723 are multi-disease resistant genotypes. This integrated map consisted

of 668 loci including thirty-two genes and 23 morphological traits spanning 1,654 cM distributed over 12 linkage groups of melon. Quantitative trait loci (QTLs) related to fruit shape (Perin *et al* 2002b) and fruit quality traits (Monforte *et al* 2004) were identified using markers from the available genetic maps, proving the usefulness of these maps in gene mapping and QTL analysis for economically important traits.

However, majority of markers used for constructing these melon maps were dominant in nature (RAPD, ISSR, AFLP) with few codominant (SSR, RFLP) markers (Baudracco and Pitrat 1996, Oliver *et al* 2001, Perin *et al* 2002a). To increase the saturation of existing melon maps with codominant markers, Gonzalo *et al* (2005) constructed an SSR based composite melon map, by merging the maps developed using an F_2 population and double haploid lines derived from same parents, Piel de Sapo (PS) and PI161375. The merged map comprised of 327 markers spanning 1,021 cM on 12 linkage groups with average interval of 3.11 cM/marker. Deleu *et al* (2009) saturated the existing PI161375 (SC) × Piel de Sapo (PS) genetic maps with 200 Single nucleotide polymorphic (SNPs) markers, reducing the average map interval to 2.3 cM/marker. Analysis 45 SNPs in 48 diverse accessions of melon correctly predicted the genetic relationship and differentiated all accessions compared with existing markers.

Gonzalez *et al* (2010) constructed the first physical melon map using bacterial artificial chromosome (BAC) library (Luo *et al* 2001, Leeuwen *et al* 2003) and previously reported genetic maps. The map consisted of 441 singeltons, 1355 contigs with an estimated length of 407 Mb. A physical map is valuable tool for comparative genomics, map-based cloning and genome sequencing.

Harel-Beja *et al* (2010) developed a genetic melon map enriched with EST markers and QTLs for fruit quality traits including carotenoid genes and sugars. Under International Cucurbit Genomics Initiative (http://www.icugi.org), Diaz *et al* (2011) integrated 8 previously reported genetic maps to develop a highly saturated consensus map of muskmelon. This consensus map consisted of 1592 markers (330 SNPs, 640 SSRs, 239 RFLPs, 252 AFLPs, 89 RAPDs, 15 ISSRs, 11 morphological traits and 16 indels) spanning 1150 cM distributed over 12 linkage groups with marker density of 0.72 cM/marker. Among these markers, 196 were newly developed or contributed by company representatives. Syngenta Seeds provided 822 SSRs of which only 85 were incorporated in the consensus map. The construction of this high density integrated melon map involved diverse melon genotypes from different horticultural groups (cantalupensis, reticulatus and inodorus), ensuring the utility of available markers in a wide range of experimental crosses.

Garcia-mas *et al* (2012) reported melon genome sequence assembling 375 Mb of DHL92, covering 83.3% of the estimated genome of melon available at http://melonomics.net. More recently, Argyris *et al* (2015) improved anchoring of available

melon assembly using resequencing data from parents of DHL92 (Piel de Sapo and PI161375) and F_2 mapping population. Newly developed 580 SNPs were used in anchoring 354.8 Mb of melon sequence covering 141 scaffolds, representing 98.2% of the genome assembly published by Garcia-Mas *et al* (2012).

Diaz *et al* (2015) anchored SNP and SSR markers available in the ICuGI consensus map to the physical melon map assembled by Garcia-Mas *et al* (2012) and Argyris *et al* (2015). The information regarding most of these SNPs and SSRs was gathered from the integrated melon genetic map developed by Diaz *et al* (2011), by merging 8 previously reported independent genetic maps. The SSR and SNP positions in the ICuGI consensus map showed a decent concordance with physical position. Though, 3% of the ICuGI markers exhibited strong discordance. Perusal of original linkage map revealed that the marker positions in the original maps fit better in the physical map as compared to the ICuGI positions. Thus, misplacement while merging the genetic maps is the likely reason for inconsistency between ICuGI consensus map and the physical maps. The integrated physical map is available at https://melonomics.net/files/integrated%20consensus%20ICuGI% 20genetic%20%20physical%20map/.

2.5 Bulk Segregant Analysis (BSA)

Michelmore *et al* (1991) introduced bulk segregant analysis as a rapid method for detecting molecular markers linked to any target gene or genomic region of interest. Two DNA bulks are constructed from segregating population advanced from a single cross. Each bulk consists of individuals that are identical for a specific genomic region or trait but random at all other regions. Thus, the bulks are genetically dissimilar in the target region and seemingly heterozygous at all unlinked regions. The principle underlying BSA is that pooling of known individuals allows the analysis of a specific genomic region against an arbitrary genetic background. In this study, the two bulks were generated from an F_2 population of lettuce by pooling individuals that were homozygous dominant and homozygous recessive for resistance to downy mildew, separately. Homozygous dominant and heterozygous individuals were distinguished by analyzing F_3 families. The bulks were screened using random amplified polymorphic DNA (RAPD) primers and restriction fragment length polymorphism (RFLP) probes of known distance from the target gene. They displayed that the markers polymorphic between the bulks will be genetically linked to the gene determining the trait used to form the bulks. Markers within 25-centimorgan on either side of the target locus can be reliably identified using this technique. This approach was expected to have extensive application in self-pollinated as well as obligatorily outbreeding species. BSA is simple and rapid as compared to the use of near-isogenic lines as only a segregating population from a single cross is required. In muskmelon, this technique has been used to map two male sterility genes (Park *et al* 2004, Sheng *et al* 2017). The nuclear male sterility gene *ms10* in Chilli was

mapped at 7.2 cM distance from SSR marker AVRDC-PP12 on chromosome 1 using SSR-BSA technique (Aulakh *et al* 2016).

2.6 Mapping of GMS genes in muskmelon

RAPD markers linked to *ms-3* gene governing male sterility in muskmelon were identified by Park *et al* (2004). The most tightly linked RAPD marker was converted into SCAR (Sequence Characterized Amplified Region) marker. Bulk segregant analysis was performed to screen 680 RAPD primers between parental lines *ms*-3 (male-sterile) and 'TAM Dulce' (male-fertile) and male sterile and male fertile DNA bulks of F_2 plants, simultaneously. A single recessive gene inheritance for male sterility was detected in F_2 population and affirmed in the F_3 families. Out of 680 RAPD primers, two markers were found to be linked to *ms*-3 gene in the F_2 population. Among these two markers, the marker, OAM08.650 was closest to *ms-3* gene at 2.1 cM. This marker sequence was further used to design a specific primer pair. Based on this specific primer pair, SCAR marker SOAM08.644 was developed. Analysis of the linked markers in *ms*-3 × 'Mission (male-fertile), F_2 population provided the confirmation that these SCAR and RAPD markers have consistent linkage with *ms-3* gene at 5.2 cM. The reason for the difference in linkage distances in the two F_2 populations may be marker sampling variation or chromosomal differences and varying recombination frequencies. The above markers were also found in 22 fertile heterozygous F_1 plants having male sterility gene *ms-3*.

Recently, Sheng *et al* (2017) employed a combination of BSA and Specific length amplified fragment (SLAF) sequencing to map a nuclear male sterility gene, *ms*-5. They identified a 30-kb association region spanning 2,522,791 to 2,555,104 bp positioned on scaffold 000048 on chromosome 9. Further, screening of 252 F_2 plants using 23 cleavage amplified polymorphic markers from chromosome 9 revealed that the markers, BSA 16 and BSA 3-3 are closely linked to *ms*-5 gene at 0.2 cM and 0.4 cM, respectively. As revealed by quantitative RT-PCR, LOC103498166 ABORTED MICROSPORES (AMS) gene displayed a significant difference in the level of expression between male sterile (*ms*-5) lines and male fertile lines (HM1-1) in their 2-mm tetrad and 5-mm first pollen mitosis stages. Based on data analysis, it was concluded that the transcription factor AMS is highly probable candidate gene underlying nuclear male sterility of *ms*-5.

Linked markers offer a perspective to speed up the development of new GMS lines by resolving the problem of identifying plants containing recessive male sterile (*ms*) gene among the backcross populations with *MsMs* and *Msms* genotypes. Also, the fertile plants can be identified and eradicated at seedling stage before transplanting the female line in hybrid seed production block, thus making the seed production process more cost-effective. Molecular markers associated with the other genes for male sterility are not available yet. The salient

features of molecular markers associated with the two male sterility genes (*ms*-3 and *ms*-5) are listed in table 2.23

Table 2.2: Characteristics of molecular markers linked to GMS genes in muskmelon

Marker	Type	Gene	Marker Sequence	Size (bp)	Distance (cM)	Reference(s)
OAM08.650	RAPD	*ms*-3	ACCACGAGTGTCGAGAAGAA (F) ACCACGAGTGAGGGATCTTC (R)	644	5.2	Park *et al* (2004)
SOAM08.644	SCAR	*ms*-3			5.2	Park *et al* (2004)
BSA 16	CAPS	*ms*-5	TCACTCTTCCTCTTCTCCTTCTCCA (F) TCTCCTCACCACGCCCAATCA (R)		0.2	Sheng *et al* (2017)
BSA 3-3	CAPS	*ms*-5			0.4	Sheng *et al* (2017)

2.7 Marker development and mapping of other genes

Wechter *et al* (1995) combined BSA with RAPD analysis to detect molecular markers linked to *Fom* 2, a dominant gene conferring resistance against race 1 Fusarium wilt. Three DNA bulks *viz,* homozygous resistant bulk comprising 7 plants, a mixed bulk of 22 homozygous resistant and heterozygous resistant plants and homozygous susceptible bulk comprising 11 plants were constructed from an AY (susceptible) × MR-1 (resistant), F_2 population. Screening of PCR amplified DNA bulks using 320 decamer primers revealed that one primer, 5' CCC CTC GAA T 3' generated a 1.6-kb fragment in resistant bulks. However, this fragment was absent in the bulked susceptible plants. Homozygous and heterozygous resistant F_2 plants were distinguished through progeny testing. Further, this primer correctly predicted the resistance reaction of 92 plants from a population 94 individually analyzed F_2 plants. To simplify the procedure of its utilization in the breeding program, Wetcher *et al* (1998) developed two 24-mer SCAR primers, MUSKFOM I and MUSKFOM II, manipulating the sequence of 1.6 kb RAPD fragment. Evaluation of this primer pair in the bulked DNA samples, MR-1 derived F_2 population and other unrelated melon lines revealed that 1.5 kb SCAR fragment amplified in resistant material was not displayed in the any of the susceptible lines. Through Southern hybridization, it was confirmed that the same sized amplification product obtained from the other resistant lines was homologous to that of MR-1. This information will be useful in the marker-assisted transfer of *Fom 2* gene in desired muskmelon cultivars.

Another dominant gene *Fom-1* confers resistant against race 0 and 2 of *Fusarium oxysporum* f. sp. *melonis*. Oumouloud *et al* 2008 developed molecular markers linked to this resistance gene using BSA-RAPD technique in an F_2 mapping population advanced from cross, 'Charentais-Fom1' × 'TRG-1551'. Out of total 400 RAPD primers, 320 primers

displayed polymorphism between the parents. These 320 polymorphic primers were analyzed in resistant and susceptible DNA bulks prepared by mixing DNA of 10 homozygous resistant and 10 homozygous susceptible F_2 plants, respectively. It was observed that three primers located on chromosome 9, $V01_{578}$, $B17_{649}$ and $V06_{1092}$ distinguished the two DNA bulks. $V01_{578}$ and $B17_{649}$ primers amplified 578 bp and 649 bp fragments of DNA, respectively, only in 'Charentais-Fom1' and resistant bulk. Primer $V06_{1092}$ produced a 1092 bp DNA band only in 'TRG-1551' and susceptible bulk. Screening of these three primers in 116 F_2 plants revealed that the *Fom-1* gene was located at distance of 4.0, 3.5 and 15.1 cM from the RAPD markers, $V01_{578}$, $B17_{649}$ and $V06_{1092}$ respectively. Primers $V01_{578}$ and $B17_{649}$ were in coupling whereas $V06_{1092}$ primer was in repulsion to *Fom-1* dominant allele. These three linked RAPD markers were sequenced to develop SCAR markers $SV01_{574}$, $SB17_{645}$ and $SV06_{1092}$. Analysis of linked markers in 24 melon accessions from different melon types revealed that the developed markers behaved differently with various melon types endorsing the hypothesis of multiple and independent origin of genes conferring resistance against the race 0 and 2 of *Fusarium oxysporum* f. sp. *melonis.* Earlier Brotman *et al* (2005) developed cleaved amplified polymorphic sequence (CAPS) markers tightly linked to *Fom-1* gene. However, the RAPD and SCAR markers developed by Oumouloud *et al* 2008 were suggested to be more universal than the CAPS markers on the basis of analysis of these markers in different types of melon accessions.

Monoecism is an important trait for hybrid breeding of melon as it eliminates hand emasculation step in developing F_1 hybrids, thus reducing the cost and increasing purity of hybrid seed. In muskmelon, sex expression depends on interaction of three genes, andromonoecious (*a*), gynomonoecious (*g*), and maleness (*M*) (Poole and Grimball 1939, Kenigsbuch and Cohen 1990). Monoecious (A-GG) phenotype is determined by the dominant allele of '*a*' gene, whereas plants with recessive alleles are andromonoecious (aaGG). Noguera *et al* (2005) mapped '*a*' gene using a combination of BSA and AFLP technique. Two Charentais type homozygous lines of melon, CM (monoecious) and CA1 (andromonoecious) were used to generate double haploid (DH) population comprised of 38 individuals which was subjected to BSA. A third andromonoecious line (CA2) was used to generate backcross population (BC_1) comprising 530 individuals {(CM×CA2) ×CA2}. Chi-square analysis confirmed that monoecy/andromonoecy is controlled by dominant/recessive alleles of a single gene. Total 79 AFLP primers used to screen the parents (CM and CA1) and four bulked DNA samples (two monoecious and two andromonoecious). Out of these, one AFLP primer amplified a DNA fragment M3A in monoecious parent (CM) and both monoecious bulked DNA samples but absent in andromonoecious parent (CA1) as well as andromonoecious bulks. The same primer produced a banding pattern M3a complementary to M3A in andromonoecious parent and bulks but no amplification was observed in monoecious

parent (CM) and one monoecious DNA bulk. Linkage analysis in DH population placed the marker M3 at 3.3 cM from the '*a*' gene. This AFLP marker (M3) displayed two types of banding patterns, M3A and M3a, with monoecious (A) and andromonoecious (a) types, respectively, thus behaving as a codominant marker. A codominant SCAR marker was developed using the sequence of M3A and M3a DNA fragments and subsequently tested for linkage in 530 BC_1 plants. Genetic distance between the 'a' gene and SCAR marker was observed as 5.5 cM using Kosambi mapping function.

Sinclair *et al* (2006) identified RAPD markers linked to quantitative trait loci (QTLs) for vitamin C (ascorbic acid) content in a TAM Dulce (high ascorbic acid) × TGR1551 (low ascorbic acid), F_2 population employing bulk segregant analysis. A continuous dispersion for ascorbic acid content in the F_2 population indicated quantitative nature of its inheritance. High and low ascorbic acid DNA bulks along with the parents, TAM Dulce and TGR1551 were screened with 500 RAPD primers. Out of 9 markers linked to ascorbic acid QTLs, four displayed amplification only in high bulks and five in low bulks. Three markers, OAT03.250, OAT03.1600 and OAW06.1100 amplified in TAM Dulce explained 14% of the total variation and two unlinked markers OAW06.600 and OAW10.400 amplified in TGR1551 explained 12% variation using stepwise multiple-regression analysis. These linked RAPD markers could be utilized for enhancing ascorbic acid levels of melon cultivars *via* marker assisted breeding.

Powdery mildew is considered among the most destructive diseases of muskmelon throughout the world. A number of dominant genes (*Pm-1* to *Pm-8*, *Pm-E*, *Pm-F* etc.) conferring resistance against powdery mildew in muskmelon have been reported (Dogimont 2011). Wang *et al* (2011) mapped a powdery mildew resistance (*Pm-AN*) gene combining BSA with Resistance Gene Analogues (RGA) mapping and comparative genomics techniques in two F_2 populations obtained from the cross of resistant line Ano2 with two susceptible lines Hami413 and Queen. Linkage analysis placed *Pm-AN* between two codominant markers MRGH63B and RPW on linkage group 5 at distances 1.6–2 cM and 1.4–1.8 cM, respectively. The markers, ME/E1, SRAP23 displayed no recombination with *Pm-AN* gene. The virus-resistant gene *Vat* co-segregated with *Pm-AN*. Collinearity of melon with cucumber in the association region was observed in synteny analysis.

2.8 Mapping of male sterility genes in other crops

Hayashi *et al* (2011) employed amplified fragment length polymorphism (AFLP) technique and BSA to map a recessive male sterility gene *ms-S* in an F_2 population advanced from a cross between male sterile mutant MS1024 and fertile cultivar Patriot in lettuce. Four thousand ninety six AFLP primers were screened between homozygous dominant fertile bulk and homozygous recessive sterile bulk. Out of these, 63 markers distinguishing the two bulks were considered as linked. Nine linked AFLP markers were successfully subjected to SCAR

and CAPS marker conversions. Analysis of 122 F_2 plants revealed that these nine markers were all placed on one side of the *ms-S* locus. The marker, LMS0624 was closest at 3.1 cM from the *ms*-S locus. Four dominant dCAPS and CAPS markers, LMS5103, LMS3123, LMS5659, and LMS3036 were all positioned at 3.6 cM from the target gene.

A dominant gene for male sterility, DGMs79-399-3 resulting from a spontaneous mutation in cabbage was transferred to broccoli by Shu *et al* (2016). Subsequently, molecular mapping of this gene was carried out in a BC_{10} population, DGMs8554 comprised of 747 individuals, developed from broccoli inbred line 8554 as the recipient and a cabbage dominant GMS line as the donor. A total of 2585 primers, including 2570 newly designed SSRs and some previously reported SCAR, SRAP, and SSR linked markers were screened between the male sterile and male fertile DNA bulks. Three SSR markers and one previously reported SCAR marker that distinguished the bulks were analyzed in 747 BC_{10} plants. Among these three markers, scaffold10312a and scaffold129_2012 were found to be closest to DGMs79-399-3 gene on both sides at 0.563 cM and 0.328 cM, respectively.

Recently, Aulakh *et al* (2016) reported SSR markers associated with male sterility gene (*ms10*) in chilli pepper (*Capsicum annum* L.). In this study, the male sterile inbred MS-12 having *ms10* gene and the male fertile inbred VR-16 were selected on the basis of high genetic diversity between these two lines. Screening of 558 primer pairs between parental lines was followed by bulk segregant analysis. It was found that the markers, AVRDC-PP54, AVRDC-PP12 and AVRDC_MD997* were able to differentiate the genotypes of male-sterile (*ms10ms10*) and male-fertile (*Ms10*) bulks. Linkage analysis of these three markers in 210 F_2 plants showed that two markers, AVRDC_MD997* and AVRDC-PP12 were linked to *ms10* gene and the later was found to be closest to male sterility gene at 7.2 cM distance.

Additionally, molecular markers linked to two more male sterility genes, *ms1* in chilli pepper (Lee *et al* 2010) and *ms8* in sweet pepper (Bartoszewski *et al* 2012) are also available. The AFLP marker, E-AGC/M-GTG was mapped as closely linked to the *ms1* at 3 cM, using AFLP-BSA technique. The linked AFLP marker was converted to a dominant SCAR marker exploiting its internal sequence. By employing a combination of RAPD and BSA techniques along with comparative mapping with available reference maps of pepper, the *ms8* gene was mapped to the lower arm of pepper chromosome P4. These linked male sterility markers will be useful in the development of new male sterile lines by rapid gene transfer and MAS in the breeding programs of respective crops.

2.9 Marker-assisted breeding in muskmelon

Two sex determination genes, *WIP*1 (*G*) and *ACS*7 (*A*) have been reported in muskmelon (Martin *et al* 2009). Methylation silences *WIP*1 gene, promoting female flowers. This suppression of *WIP*1 gene activates another gene, *ACS*7**,** which encodes ethylene

synthase, preventing male organ development. Thus, the resulting plants having flowers with purely female organs are termed as gynoecious. Gao *et al* (2015) designed molecular markers based on *ACS*7 (*A*) and *WIP*1 (*G*) genes and the LRR domain in the *Fom-2* (*F*), conferring resistance against Fusarium wilt, by utilizing sequence information from GenBank (U S National Center for Biotechnology Information). The objective was to select of *Fusarium oxysporum*-resistant gynoecious (*AAggFF*) genotype through marker assisted selection (MAS). Genotyping of 20 F_1 and 1863 F_2 plants, from a cross, WI998 (gynoecious line) × MR-1 (Fusarium wilt-resistant line) was done using the markers. Consequently, 35 plants in the F_2 population were designated as wilt-resistant and gynoecious by MAS. *F. oxysporum* (race 1) inoulation and disease grading was done to identify resistant and susceptible plants. The results displayed high consistency between the data obtained from marker-based genotypes and phenotypes observed in the greenhouse, indicating that these molecular markers can be reliably used to detect Fusarium wilt-resistant gynoecious (*AAggFF*) melon genotypes.

2.10 Production of F_1 hybrids using male sterility

Hybrid seeds production in muskmelon can be carried out through emasculation and pollination because each fruit produces a good quantity of seed (Munshi and Alvarez 2005). Seedling requirement for one acre can be sufficiently achieved with 3000 good quality seeds which in turn can be obtained from maximum 10 fruits (Munger 1942). However, availability of pollination control mechanisms such as monoecism, gynoecism and genic male sterility provides better perspective for hybrid seed production.

Although male sterility in muskmelon has been identified as early as 1949 (Bohn and Whitaker 1949), there are few reports on its utilization in hybrid seed production. The first male sterile gene, *ms-1* has been commercially exploited for the first time in India by Nandapuri *et al* (1982) to develop Punjab Hybrid (MS-1 × Hara Madhu). Thereafter, two more hybrids, Punjab Anmol (Lal *et al* 2007) and MH-27 (Anonymous 2018) were developed utilizing male sterile genotype, MS-1. The fifth gene for male sterility, *ms-5* is said to have been employed for the production of F_1 hybrid seeds by Clause Seed Company prior to its publication (Lecouviour *et al* 1990). More recently, a new muskmelon Hybrid, MH-51 (Anonymous 2018) has been released by Punjab Agricultural University using MS-5, male sterility line.

The 50 per cent fertile plants in female line with heterozygous (*Msms*) genotype can be identified and roughed only after anthesis. After the completion of identification work, the opened flowers and the already set fruits on male sterile plants are also pinched off. This process of identification and removal of male fertile plants makes the GMS system labor-intensive and less cost-effective.

Another male sterility system, Cytoplasmic-Genic male sterility (CGMS) has an advantage over the GMS system for its ability to give cent percent male sterile plants which can be used directly as a female line. The male sterility expression is determined by the interaction of cytoplasm and nuclear genes. The maintenance of male sterile line, S*rfrf* (A-line) is carried out by crossing it with an isogenic maintainer line, N*rfrf* (B-line). For developing F_1 hybrid, A-line is intercrossed with C line, N*RfRf* that restores the fertility of the resulting hybrid. This system of male sterility has been reported in many vegetable crops, such as carrot, onion, sugarbeet, chilli and capsicum (Dhall 2010). However, no report on CGMS in muskmelon has been published.

Similarly, photoperiod or thermo-sensitive GMS system appears more convenient and workable approach. In this system, one can maintain GMS line in one environment and carry out hybrid seed production in a different environment. Sawhney (2004) reported a photoperiod sensitive mutant (7B-1) in tomato which displayed 100 per cent male sterility in long day conditions (minimum 12hr day length) but restored fertility under short day conditions (8hr). But, until the present, no such system is available in muskmelon.

Most of the muskmelon genotypes are andromonoecious, but few monoecious sex forms are also present. Since the employment of monoecism provides natural emasculation, it reduces the time for hybrid seed production by hand pollination up to 50% and enhances fruit setting by 40-70% in comparison to 5-10% for andromonoecious forms. (More *et al* 1980). Also, use of monoecious lines as female parent reduces self-pollination when male and female line are planted in adjacent rows under open pollinated conditions (Foster 1968). However, an undesirable linkage of gene governing monoecism with fruit shape has been reported (Risser 1984), resulting in deviation from the desirable round shape. This problem of undesired fruit shape was apparent in Pusa Rasraj, rendering it commercially unacceptable (Sandha and Lal 1999).

A gynoecious line produces only female flowers ensuring 100% hybrid seed production. This is a major advantage of gynoecism over GMS system where 50% male fertile plants in female line have to be identified and roughed out. In muskmelon, first gynoecious line, Wisconsin 998 was developed from 'monoecious' × 'hermaphrodite' cross by Peterson *et al* (1983). Evaluation of Wisconsin 998 in hybrid production revealed good combining ability for earliness and yield but average combing ability for TSS (Lal and Dhaliwal 1993 and Dhaliwal and Lal 1996). A hybrid (MHL-10) derived from 'W1 998' × 'Punjab Sunheri' cross was reported to have good shipping quality along with earliness and high yield. It was released in Punjab for commercial cultivation during 1995. A gynoecious line can be maintained by the use of chemicals inducing perfect flowers and maleness such as, silver nitrate ($AgNO_3$) @ 100-200 ppm, silver thiosulphate (More and Seshadri 1987) and ethral (Rudich *et al* 1969). One of the limitation of this system is the instability of gynoecious

line at high temperature. It was observed that a gynoecious line produce cent percent female flowers under low temperature and short day conditions, but becomes gynomonoecious in long day and high temperature (Kubicki 1969). Therefore, more stable gynoecious line with good TSS needs to be developed for utilization in hybrid seed production programs.

CHAPTER III

MATERIALS AND METHODS

The present investigation entitled, "Molecular mapping of nuclear male sterility gene *ms-1* in muskmelon (*Cucumis melo* L.)" was undertaken at Vegetable Research Farm and School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana during 2016-2018. PAU, Ludhiana is located at 30.9019° N latitude and 75.8078° E longitude.

The experimental material used and the methods adopted to conduct this study are discussed in this chapter. The investigation was divided primarily into two experiments,

3.1 Phenotyping and segregation analysis of F_2 population

3.2 Identification of SSR markers linked to male sterility gene *ms-1*

These experiments were carried out as follows:

3.1 Phenotyping and segregation analysis of F_2 population

3.1.1 Location of work

Vegetable Research Farm, Department of Vegetable Science, PAU, Ludhiana

3.1.2 Experimental methodology

3.1.2.1 Plant Material

In this investigation the F_2 population of cross 'MS-1 × KP_4HM-15' was used. 'MS-1' is a male sterile line having *ms-1* gene, which is responsible for its sterility and KP_4HM-15' is a male fertile line.

3.1.2.2 Raising of nursery and transplanting

To raise the nursery of F_2 plants, seeds obtained through self-pollination of 'MS-1 × KP_4HM-15', F_1 generation were sown along with the parents in polythene bags of 15 cm × 10 cm size and 100 gauge thickness in February 2017. Plants were transplanted in the field in March 2017. The crop was raised following the cultural practices recommended by PAU, Ludhiana. Seeds of F_3 generation produced by self-pollinating the fertile F_2 plants were directly sown in the green house in July 2017 to raise F_3 single plant progenies (30 each).

3.1.2.3 Phenotyping of the F_2 population

Male fertile and male sterile plants were distinguished by presence/absence of pollen. Two to four flowers from each plant were checked for the presence of pollen from 6-9 AM. Pollen presence was determined by rubbing the anthers on finger-tip. In male fertile plants with *MsMs/Msms* genotype, pollen adhered to the finger-tip whereas no pollen was found in male sterile plants with *msms* genotype. A plant was tagged as male sterile with aluminum tag only after confirming 3-4 flowers on that plant without pollen.

3.1.3 Observations recorded

- **Number of male fertile (*MsMs/Msms*) plants in F_2 population**
- **Number of male sterile (*msms*) plants in F_2 population**
- **Number of homozygous (*MsMs*) and heterozygous (*Msms*) male fertile plants in F_2 population:** This was estimated on the basis of segregation for male sterility in F_3 progenies advanced from individual F_2 male fertile plants. The F_2 plants which segregated for male sterility in F_3 generation were tagged as heterozygous (*Msms*) plants while the plants producing 100 per cent male fertile progenies were tagged as homozygous (*MsMs*) plants.

3.1.4 Statistical analysis

To confirm the recessive mode of inheritance of the *ms-1* gene, goodness of fit of the F_2 population in accordance with the expected phenotypic ratio of 3:1 was determined using chi-square analysis. The chi-square value was calculated as follows:

$$\chi^2_{(n-1)\,df} = \sum \{(O-E)^2/E\}$$

Where,

O = Observed number of plants

E = Expected number of plants

n = number of classes

df = Degree of freedom

3.2 Identification of molecular markers linked to male sterility gene *ms-1*

3.2.1 Location of work

School of Agricultural Biotechnology and Department of Vegetable Science, PAU, Ludhiana.

3.2.2 Experimental methodology

3.2.2.1 DNA Extraction

DNA of the two parental lines (MS-1 and KP_4HM-15) and 150 individual F_2 plants was isolated from immature leaf tissues in April, 2017. Steps followed for DNA isolation are as under:

1. Leaf samples collected in separate butter paper bags were transported from field to lab in ice box and stored at -80˚C till extraction.
2. Samples were grinded with liquid nitrogen using pastel mortar and the resulting fine powder was placed in properly labelled 2 ml eppendorf tubes.

3. 900 µl of extraction buffer, 2X CTAB (Table 3.1) was added and mixed by inverting the tubes to homogenize.
4. Tubes were incubated in water bath for 45 minutes at 65˚ C following gentle stirring at 10 minutes interval.
5. 800 µl of chloroform: isoamylalcohol (24:1) was added and tubes were placed on shaker for 20 min.
6. Tubes were spun for 7 minutes at 13000 rpm in a microcentrifuge.
7. Supernatant was transferred into clean 1.5 ml labelled eppendorf tubes and 600 µl of chilled isopropyl alcohol was added following mixing by inverting the tubes until the DNA precipitated.
8. Tubes were centrifuged for 5 min at 10,000 rpm to settle the DNA pellet at the bottom.
9. Supernatant was poured out and DNA pellet was rinsed with 200µl ethanol (70%).
10. DNA pellets were then air dried by inverting the tubes on a clean filter paper and 100 µl of 1X TE was added following storage at -20˚C.

Table 3.1: Composition of 2X CTAB extraction buffer

Component	**Final Concentration**
CTAB	2.0%
1M Tris (pH 8.0)	100mM
NaCl	1.4 M
0.5M EDTA	20mM
B-Mercaptoethanol*	2.0%
ddH_2O	-

*β-Mercaptoethanol was added just before use

3.2.2.2 DNA quantification

DNA quality and quantity was checked using Thermo Scientific Nanodrop™ 8000 Spectrophotometer, USA following the procedure as under:

1. A blank measurement was made with 1µl of 1X TE.
2. DNA quantity was estimated in ng/µl of samples and quality was determined in terms of 260/280 absorbance ratio using 1µl of each DNA samples.
3. On the basis of quantification, dilution were prepared so that the final concentration of the DNA to be used in the PCR amplification was 50 ng/µl.

3.2.2.3 Selection of primers

Total 498 SSR primer pairs well distributed and spanning all 12 chromosomes of muskmelon were selected from **ftp://cucurbitgenomics.org/pub/cucurbit/marker/.** Most of these primers were provided by Syngenta and few were taken from linkage maps developed by Perin *et al* (2002a), Harel-Beja *et al* (2010), Fernandez-Silva *et al* (2008) and Oliver *et al* (2001). These SSR primer pairs are enlisted in table 3.3.

3.2.2.4 Parental Polymorphism

498 SSR primer pairs were assayed between the parental lines, MS-1 and KP_4HM-15. For detection of parental polymorphism, PCR amplification was followed by gel electrophoresis.

PCR amplification

Genomic DNA was amplified using 498 pairs of SSR primers. A 15 µl reaction mixture was used with following composition:

Reaction Components	Concentration	Volume (µl)
DNA template	50 ng/µl	1.5
Forward primer	5 µM	1.5
Reverse primer	5 µM	1.5
Master-mix (Promega, USA)	-	5.25
Nuclease free water	-	5.25
Total	-	15

For PCR amplification, following temperature profile was standardized:

Steps	Temperature (°C)	Time (minutes)
1. Initial denaturation	94	4
2. Denaturation	94	1
3. Annealing	48-60*	1
4. Extension	72	2
Steps 2 to 4 repeated 35 times		
5. Final extension	72	7
6. Storage	4	

*Annealing temperature ranged from 48-60 °C depending upon the primer

Gel electrophoresis

The PCR products were run on 3% agarose gel containing 0.05 µl/ml ethidium bromide. As polymorphism was not clear between many samples, polyacrylamide gel electrophoresis (PAGE) was employed for clear separation of the PCR products.

The ingredients used and the procedure followed for preparation of 6% polyacrylamide gel are described as under:

1. The glass plates were washed with detergent solution, kept for drying and then cleaned using spirit for sterilizing the surface of the plates.
2. A rubber gasket was fixed to the edges of the rectangular plate and spacers were placed along both sides of the rectangular plate. The notched plate was then carefully placed on the rectangular plate.
3. The plates were tightened with clips to avoid leakage and the assembled apparatus was kept in vertical position.
4. The ingredients enlisted in table 3.2 were mixed in a container, but the TEMED and APS were added just prior to the pouring of solution.
5. After pouring the gel solution, a 100 well comb was inserted between the plates and the gel was allowed to solidify for one hour.
6. After solidification of gel, the gasket and comb were removed carefully, so that the wells remain intact.
7. The glass plates were then placed in PAGE assembly and the upper and lower chambers of the assembly were filled with 0.5 X TBE buffer.
8. After adding 80 µl of ethidium bromide to the lower chamber, the gel was pre-run at 300 volts for 3 hours for staining.
9. 10 µl PCR product from each sample was loaded in the wells along with 50 bp ladder and subjected to 150 volts run for 2-3 hours to resolve the PCR products.
10. Finally, the gel was visualized under UV light and photographed using gel documentation system (Alpha Imager HP, USA)

Table 3.2: Composition of 6 % polyacrylamide gel

Component	Concentration	Quantity
*Acrylamide bisacrylamide solution	40.0%	22.5 ml
#TBE	10X	7.5 ml
Ammonium persulphate in 20 ml of ddH_2O	0.07% (W/V)	0.105 g
TEMED	0.08% (W/V)	120 µl
Double distilled water (ddH_2O)		99.88 ml
Total	6%	150 ml

[*]*Preparation of 40.0% Acrylamide bisacrylamide solution*

For preparation of 100 ml solution, 38.0g acrylamide and 2.0g bisacryamide were dissolved in 60 ml ddH_2O and the final volume was made 100 ml.

[#]*Preparation 1 litre of 10X TBE*

108g Tris-base (890 mM), 55g Boric acid (890 mM) and 7.44 g EDTA was dissolved in 800 ml ddH_2O. The pH was then adjusted at 8.0 and the final volume was made 1 litre. After this, solution was autoclaved.

3.2.2.5 Bulk Segregant Analysis (BSA)

Two DNA bulks, male sterile bulk and male fertile bulk were prepared by compositing equal amounts of DNA of 10 homozygous sterile and 10 homozygous fertile plants, respectively. The markers, which discriminated MS-1 and KP_4HM-15 were used in PCR amplification of the bulks along with the parents. For comparison, PCR product of the two parents was loaded alongside the bulks in the gel.

3.2.2.6 Genotyping of F_2 plants

SSR markers that detected polymorphism between the two parents and the DNA bulks were used to genotype F_2 plants, to determine linkage with the male sterility gene.

3.2.2.6 Observations recorded

The markers that showed upper and lower DNA amlicon were considered as polymorphic. The marker data of bulks and the individual F_2 plants was recorded as MS-1 type (A), KP_4HM-15 type (B) and heterozygote type (H).

3.2.2.7 Statistical analysis

Goodness of fit of the observed segregation ratio of the co-dominant SSR markers in accordance with single recessive gene inheritance ratio of 1:2:1 was assessed using chi-square analysis. Linkage distance between male sterility gene *ms-1* and putative SSR markers was determined using MapMaker 3.0 software (Lander *et al* 1987) and a linkage map was generated.

Table 3.3: SSR Primers selected for mapping *ms-1* gene

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
1	CMTTCN273	ATGACCATGATGACTCGC	CTCCAAATAAACGCAAAG	124	1
2	DE1256	GCTCCAAAGTCAAAACACC	TTGAGACCCAGAAGGAGAG	192	1
3	DM0300	CATTATTGAAGTTAGGTCCC	GGGGGTTGAGTTAGAAAAG	301	1
4	DM0803	CCTTTGAAGTGAATGTTTCC	CTCCTTCCATTTAACTTGAAC	167	1
5	DE1374	CGTGTTTCGTTCCTACACC	CACAATCACACAACTCAAAAAG	216	1
6	TJ27	AAGCGGAACAAGCTCATCTC	CAAAAGCATCAATTGCTTGAA	171	1
7	DE1337	CTTCATCTTCTCGCAGAGC	ATAGACCTAGTCGCCCTCC	204	1
8	DM0060	AAAACAGAGGCAGGAAATC	TTTGTGGGATAAGAATTGC	191	1
9	CMMS35_3	CGGAGAAGAAGGAAGGGTTTTAAGA	ATTCGTAGTTCATACTCTCTTTCTC	312	1
10	CMCTN4	AAAACAAAAGCTCTCCACGA	CTTTCCTTTATTATGCCTACG	126	1
11	DE1177	CTTCCGCAGTTAAAACAGG	GAGCCTGTTTCGTTCACTC	181	2
12	DE2033	AGCTTTGAGAACAAGCCAC	CATCAAAATTAACTTCATGC	135	2
13	DM0298	GTTCGACGTTTACTCATCC	AGTGAAAGATGGGTGCTTC	281	2
14	CMBR120	CTGGCCCCCTCCTAAACTAA	CAAAAAGCATCAAAATGGTTG	167	2
15	CMAAGN283	GCAACAAAGAAGAAGAAG	GGAGAAGAAATTGGAAACG	91	2
16	CMCGGN210	GTCAGCTCCCTTCAAAGTC	GTCTAGTGGGCGTTGTTG	135	2
17	CMBR066	TCAAGCAAAAACCATAATCAGAA	TCCCTTTTCATCATTTCTCTTCA	116	2

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
18	CMCTTN179	CCCACCATGAATTTCCTC	CTTGAATTCCTTGGAGACG	186	2
19	CMGCTN187	GTCTACTCTCTGCCTTTCAAC	TAATGCCTCTATCTTCTCG	138	2
20	DE1329	AATGCCACCTTTTTACTCATC	AAACCAAACTGATTTCCCC	209	2
21	DE1630	ACAGATGGTTGCGAAAAAG	TCAATCTGGAGAGTGGGAC	193	2
22	DE1187	CACTCCTTTTCCGTTTCAC	GAAAAGCAGGGATCTAGGG	143	3
23	CSWCT10	AGATCGGAATTGAAAAAG	AAAGGGGCTTCCTCTCTA	183	3
24	DE1239	TTTTCGTCCAACATCAACC	TTTTCGGGTTGATGAAATC	194	3
25	CMBR026	CCAAAAGAAAAACCAAACGA	ATCACAAGCCTTTGCACTCA	120	3
26	CMBR023	TTTAACCCCAGCAGATGACC	CAACGTTATGGGGATGAAGG	157	
27	DE1462	TGATTCCCGTTCTTGAGTC	AAATTCACATTATCCATAAAAGG	216	3
28	DM0487	TTTCCGTTTGGTTAATTTG	AGAAGAATAGAGAAGCGCC	201	3
29	TJ125	GGAAAACGCAAAATCAGTGAG	CTGAACGTGGACGACATTTTT	135	3
30	DE1753	CGCTTCAAGATTAAGGGAG	TTCGCTGATTCCTTTCTTC	239	3
31	DM0854	GCACCCAAAATTGTAATGG	AGAAGGGATCAAAGTTAATATCAC	124	3
32	DM0369	AGAGCTAAAGGAGAGGCAG	AAATAGGGTGAAGAATACGC	245	4
33	CSCCT571	CCTTTCTGCTGTTTCTTCTTC	GAAGGAAGGAGTGAGGGGAAG	209	4
34	CMTTTGN209	CCATTCATTAGCTTTCCTC	GCCATTGAAACTCTGAAAC	166	4
35	DM0551	CTTTCTAGCTAATTCCCGC	TTATCGAGTATTTGGCGAG	342	4

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
36	DM0055	GATGAAGCTTTGGAGGATAC	CAAATGAGGAATCTGAGTTTAG	160	4
37	CMBR106	GTACCTCCGCCGTTGATCT	TGAGATAATAAGAAATCCAACCCA	147	4
38	CMTTCN270	CAGTGTTAATTCCTCTCTTC	GAGATGACTGCGATGTAAG	142	4
39	DM0107	GCTTTTGTTGATTTGTTGG	TGTAGATGAAGTAATTTGTATTTG	242	4
40	CMTC168	ATCATTGGATGTGGGATTCTC	ACAGATGGATGAAACCTTAGG	200	4
41	CMGAN59	CCAAATATTTGTTGAGAGAG	CCCTTATTTCAGCCAATTTC	69	4
42	DM0934	TGTTAGCTGTACTGCCACG	AAAGTTAAATTTGGTTATGTCCC	216	5
43	CMMS2_3	ATCACCCACCCCACCACTGCCAAAA	CCTTGAAAAACCACCAACATAACAC	213	5
44	DE1279	TAAACCTCACCCCAAAAAC	AGGATGAGGGTGGAAAGAG	202	5
45	DE1840	TGAAAGAAAGGTGACCGAC	ATGATTCAATTTCGGGTTG	166	5
46	DE1557	CAAAGACATAAGCCCGATG	AAAAGAAAGATACAAGTTAGGGC	217	5
47	DE1354	AGAGAATTTGGAATTCGGC	CGTTAAAATTCCCAACGG	221	5
48	DM0454	GCCAATACAAAACTGGTG	TCCGCTTAAACTAAACTCC	94	5
49	DM0561	AGGTTGGTTACCTGGAGTC	CTCCCTTCCCTAGAACAAC	197	5
50	DM0214	TTCTCCTGAGGTCACATTC	TTTGTTCAAGGGATGCTAC	272	5
51	CMBR123	TCCGAAGTAAACATCAAAGACA	GGTCAGTCAAGATAGTTACGGTTG	160	5
52	DM0159	TTATTGACGAAATGAGCTG	TTTGTATTTTTGGAAAGGG	271	6
53	DE1875	AACGTACAAAATGTACAAAACAC	ACCGTTGGATTGCATTAAC	157	6

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
54	DE1345	GACTGGTTCAGCTGATAAGG	CTAAGAGGGCTTTGACACG	219	6
55	DM0550	AGTTAGGGCAACTCTCCTC	TTCTTTCCCTTTGAAATCC	156	6
56	CMCTN85	TGATGTGTCTGGCAAGAACC	GGTAAGAAACTTGGCAGTTGC	185	6
57	CSCT335	CCTTCACTTCCATCTTCATC	CGGTCCTTCATTTCATAGAC	120	6
58	CMTCN41	CCCCAAGATTCGTATTAATC	TGGTAGTAGAGATGATATAC	129	6
59	DM0749	TTTTTCCCCTAACATCATTC	TTTTCTTTTGTCTTAGCGG	324	6
60	DE1487	TCTAAAATCCCAAAACCCC	AAAACCCAATAAGGATCGG	217	6
61	DM0145	ATCTGAGGTTGAAGCAAAG	GTCGAAGATATTGTCAGGC	247	6
62	DE1585	GCACTGTGAAACACTCACAC	AAAGCGTAAGAGCAACACG	202	7
63	DM0104	TCTTGGACACATGGAAGTC	CGAGATGCACATAAACTTTC	202	7
64	CMTAAN87	TGACATCAGTCTTTGGGAGATC	GCAGTCAGGATAATATGGTTGG	125	7
65	DE1181	ATCCCGCAAATTAAAAATG	AAAAACAAAAATTGCAGCC	182	7
66	DE1836	GTTTCAGGGACAGATGTGG	CCTTGATTCTATGTAGGCTGG	175	7
67	DE1378	TGTTGTTCTTCATTGCGAC	ACTCTGTACATTGCCCAAC	146	7
68	DE1083	TATGACCAATTGGAGAATG	GATACCGAGAAAAAGCTTCC	198	7
69	CMGA15	CGGCAAGACGATTGGCAGC	ATCACCGTAGCGAAGCACC	150	7
70	DM0024	AAGGCCAAGAGATAATAGTG	TCCAACTCAATTTTACGAAC	191	7
71	DE1457	AGGATGCAAAGGTAGTTGC	CGACCAAACCTAAACCAAG	222	7

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
72	DM0196	GTCAACTGCGTTACTGTTG	TAGTGCTGAAAGCAATGTC	254	8
73	DE1292	AGGGAGAGTATTTTAAGTTAATTG	ACAAAGGAAGCTAAAGCCC	192	8
74	DE1101	AGGAAAATACAAAATGGGTTG	AATTAAATCAGGGGGTTGG	155	8
75	DM0463	AGTAATCGGTAAACTAGGAGG	TTTCATTCACCTCTTGTGG	275	8
76	DE1073	TGGAATTGAAGAGCATTTTG	AAGAGAGGGGAGGTGTGTC	135	8
77	DE1231	TATGCGTCTTACCGAAACC	AATTTTTCATCAAGATTTGC	201	8
78	DE1853	AAAAGGGGTAAAAGAATTGC	CATCAAACAGAACAAATGTACG	248	8
79	CMTTCN163	TTTACTCCCAATACTTTCATCG	AACCTTTGAAGAATCTCCGTG	131	8
80	CNGAN224	AATCGAAATCCATCTCAC	TCTAAGCCACGACATCAC	170	8
81	CMTCN56	CTTTTCTCTTCTTCTATTCTC	ATCCAAAAGGAATCGGAAAG	96	8
82	DM0220	GAAGAGGGGTTGGTAGAAG	AAGGGTTTGAGCACATAAG	226	9
83	CMTC47	GCATAAAAGAATTTGCAGAC	AGAATTGAGAAGAGATAGAG	168	9
84	TJ150	ACACACCTAATCTCCCTACCTTC	CTCAAACAACGTCAGCTGGT	134	9
85	DE1215	TTTCTCTTTTTGGAACTCCC	TTGGGAGATCGTAAGGTTG	224	9
86	DM0468	CCCCTCTTATCTTTTCCTG	CATCAAGAAGTCACGGAAG	227	9
87	DM0490	TAGCAAACGACAACTAGGC	GTGGAAAAGAGAGGAAAGG	322	9
88	DM0500	TTTGCTCTCTTTCTTGGTG	TTTGATGTGTTGCAATGTC	204	9
89	CMATN22	CGGCAATCATCTTATCTTTC	AAGATTGAAGTGGGAAAATG	166	9

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
90	DE1326	TCGTTTAGGAAGCCTTTTG	GACGGGAAAGAACACAATG	149	9
91	CMMS35_5	AACGGGATTTTGGAGGCATATTCGG	CTCCCCAGTGTATCAGCCAAATCTC	174	9
92	DM0706	GAAAGGAAACGAGAAAAGG	TCTATCTTGCAGGCTATGG	347	10
93	CMBR115	AGGGTGGAAAGACCCCTATG	TGTGAATGTATCTTTTCTGATACTGC	151	10
94	CMGAAN233	TGCAGGCTTTTTCATAAC	TGTTTATCAATGGCAGCG	145	10
95	DE1172	CACATTGCAGAAGATGCAG	ATGAATGATACTCGGGCTG	221	10
96	DM0757	TAGAAAAGCAGCCAACAAC	GCCACTCCTCTAGAACTCC	200	10
97	DM0932	CAAATTAAAAGAACGTAGAAATAG	TCCCAAAAACAATAACTCTCC	142	10
98	DM0570	TATCTTCTGGGCTGAGTTG	AAAGGAAACCGGAAGAAC	336	10
99	CMTA134a	ACGTGCTTCAGTAAACATG	CCGACATTGAAAACCAACTTC	159	10
100	DM0098	CTATTCCCACTAGAACGAAG	GTCATGATTGAGTTCTTTGC	112	10
101	DM0618	ATATAGCAGCCGAGTGATG	GCGAATCATGTTTACATCC	301	10
102	DM0913	GCTCTGTTATAACCGTAACTGG	ACGTGGCTAAATCTCGTTC	192	11
103	DM0752	TTCGGTCAATAGAAACTGC	CCCCATTTCACTGTCTTTC	134	11
104	DE1410	AACTCATCACATGGAGAAGC	GAGGGAGCTGTTGTTTTTG	180	11
105	DE1321	GAAGAAAACGGCTGCCAC	ACGGACTGTTGGTGTTTTC	126	11
106	CMAGN45	CCCACAAGAGAGAGAGAGAG	GTGTGACAGGTAGATTGTTGG	100	11
107	CMGAN51	AAACCTTAACGATCTATTCG	TCAAGAAGACGAAACTATTC	188	11

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
108	DM0229	GACTTGACGAAAATTCCAC	TCTTCTTGTCCACCATCTC	175	11
109	CMAAAGN148	CCATGAGGAACTAAATAGAGCC	CGGTCTCTGCTTGCTTTC	157	11
110	TJ147	GAAAGGTAGGAAGAAAGTGAAGA	ACTCTTGAAGCTGACCGATG	110	11
111	DM0503	GTGTGTATGAGATAGGCGG	AGAAAGGATGGAGAACTGG	182	11
112	DE1534	CACAAGTTGCGAGTGTCAG	TCGTTGCTGGTTAGTTTTTC	207	12
113	CMAAGN255	GAAGAAGAAGAAGAAGAAAAGC	GATTCAAAACAAAAAGAAAGAG	152	12
114	DM0634	AGAGTCGGAAATTGAGAGG	TTCCTTCAAGCATCTTTTG	163	12
115	CMACGN289	TCATGTCAACCGAAGCTAG	CAGATACTGTCCGAACGTG	137	12
116	DM0555	CACAAGAAAAGTCCGACAC	GCAATTTGTTCTCATTTCG	253	12
117	DE1957	CAATAAAGGAAAAACTAGAAATG	TTGGATTTTCTCATACCCG	112	12
118	DE1610	AACCATGGAGACGAGATTG	ACGACTCCTCCCCAGCTC	146	12
119	DE1980	ACGAAGGGGATCTTTTGAG	GAGCTATTCCCTTTCACCC	231	12
120	DE1081	CAAACAAAAGAAGTTGAAAATTG	AAAACCATGGACTTTGGC	224	12
121	DM0839	AACACCATCGAGGTAGTGC	GTTAGGGACGAAAGGAAGG	234	12
122	DE1294	ATTGGGGGAGAAGTGTAGC	CTCCAAATAAACGCAAAGC	218	1
123	CMAGN10	CCTCAAAAGAATCCCAAGCA	TTAGCCCATGTCTTGTTTGG	110	1
124	DM0076	GAGTAAGAAATCAGCGCAAC	TAAATTCGCATTGCCATAC	274	1
125	CMATN240	GAACACACACAAAAATGAG	AAAGGGAAGATTCTAGTTG	152	1

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
126	DE1582	TATTCATATGCAAGCCAGC	GCACAATTATTTAAAAGTTTAGGG	135	1
127	CMBR147	GCTTCAATGATGCTGATAAAGA	CGTCACAATGTGCTTATGAGA	158	1
128	DE1497	ATTTCCATTCCCCCATAAC	AAGATACAATCCTTCTTGATTGAG	126	1
129	CMCTN86	GTGACAGTTATCAAGGATGC	AAGGGAATGCATGTGGAC	184	1
130	DM0699	CTTGACTGTGATTCCAAGG	TCACCTAGCGTACCAAATC	200	1
131	DM0325	AGCTGAGTTTTGGTTGTTG	AAGGAATCCCAAGAGAATG	284	1
132	DE1823	TTGTTGAACATGGAAATGG	CTTTAGGACCAACTACCAAATG	197	1
133	DM0137	TAGAACAGAAATGGCGAAG	CGGCATATCAATACAAACC	260	1
134	DM0135	AAGAAAAGTAACCAATCCAC	AACAAAACCGAACTGATAC	263	1
135	CMGAN92	GAGAGAGAGAGAGAGATG	GGTTGGGTACTCCGAGTTA	140	1
136	DM0073	CTCATCGCAAAACCATATC	AGTTTGTGGATCGTTTAGG	131	1
137	CMCT505	GACAGTAATCACCTCATCAAC	GGGAATGTAAATTGGATATG	219	1
138	DM0675	ACCTCAAGATCTCCTCCAG	CAAGACATTTTTGCCTCTC	261	1
139	DE1507	CGATCGTTGCTAGGAGAAC	TTGAGCACATCTCTTTCCC	186	1
140	DM0144	TTAGCCAACAACAGTTTCC	GAGAAACTTGATACAAAACTCG	289	1
141	DM0040	ATATTCACCAAACCCCTAAG	GGATAGCAATTAACCCACC	157	1
142	DE1607	TTGGTAGCTTCACCTGCTC	AAAAACCCAATTTCCACATAC	186	1
143	DE1069	CCCACTAACCACTCCCTTC	GCAATATTTCAGCCCAATC	222	1

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
144	CMCTN53	CCACATTTGATGGAAATCTT	CATTTTATAGCTTATCTTCCG	149	1
145	DM0661	AGTTATTGACTTGAGGACTGC	AGGGAGAAATTGTCTTTGG	344	1
146	DM0198	GTGTAGGCCATGAAAATG	TTCCTTCTCTTCCTTCATC	159	1
147	DE1151	CTTTAGAATGGAGGAGGGG	CACATCACCAAAGAGAAAGG	168	1
148	CMTTAAN244	CTAAAGCTCTAAATGAAATCG	CAAAGCCTAAAGAGATTCG	152	1
149	DM0417	GCCCATGAAACTAGGAAAC	AAAGGGCAATAATGAAAGG	200	1
150	DM0339	TAATAAATACGTCCCTGCG	ATACAATGGTCAATGCGAG	285	1
151	DE1765	CACGGCATAAACCTGAAAG	ATCTTGAGAAGATGTCGGC	243	2
152	DE1265	CAGAAATTTTGGCCTGAAC	CCTGAGTTATTGCAGAGGG	175	2
153	CMAGGN188	TCTGGGAAACATGCTGAG	GTGTCCATATGTTGTCTTTTC	172	2
154	CMAGN39	GGGCCATTTTCTTTTTACAT	TCTCTTAACTTTCTCTCTCC	158	2
155	DE1135	TTCAGCAAAAGAACCGAAC	TTGCTCTTTAGGTTTCATCG	166	2
156	TJ61	TTTCAAAAAGCGAACCAGCTA	TCGGACTCGATTACCAAACA	113	2
157	DM0052	GTTGGCCTTAGATTTTGTG	TTTAAACCCATCAAATCCTC	173	2
158	CMAGN16	CGATAAATGTTGATGAAAGTC	TTCCTCAGGTCATATTCTTC	165	2
159	DE1338	AAGCGACCCTCCTGTAGTC	TGAGCTGAGCTGAGTTGTG	117	2
160	DE1902	TATTGAATGGTTCGGTTCC	AAGATCTGGGTTGTGTTGG	182	2
161	DM0473	TTAAAGCAACTCCAACCAC	TTTAGCCACTACATTTGGC	261	2

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
162	DE1463	CTAAGACCAAAGGACACCG	CCAAGTCTGAGGCTCGTAG	114	2
163	DE1392	ATTTGAAGACCCAAACACG	AGAACAGGAGCCGTAGAATC	198	2
164	DM0019	TCTACCCACCTAGTTAGCTG	CATCGAGTGCTACCATTAG	150	2
165	DM0464	TCTTTTCCTTTTCCTTTCG	CCGTACGTTTCATTGTTTC	318	2
166	DE1291	CAACTCCCTCCCTTTCTTC	CAAAATCTCCAAACCCAAC	117	2
167	DE1411	TGGAGAAGAGAAAGTGAGAAC	TTCCTTCCTCATTTTGCAG	163	2
168	DE1514	GAAACCTTCGGTTCAAATG	CGTAAATTTAAGTTTGTTGGAATC	153	2
169	DM0601	AAAGCCCCAAATTAATCG	AAATTATCCCGATTTACCG	144	2
170	DM0062	CGTTTGTTTACCTTCTTAG	ATTACACAGAAAAGTCAAGC	134	2
171	DM0290	CAAGGGAAAATGAGAAATATG	TATGCAGTGGTTCACAATG	302	2
172	DM0784	TTGAATTATGTCCCCAATG	TATCTTTTTCTTCGCTTCG	237	2
173	DE1271	TTGGGGTTTTGTTGAGAAC	CTGCTCAAAATTTACGACG	215	2
174	DE1240	CATCCCAATTTCTCTTTGC	GACTTTACGATCATTCCGC	192	2
175	CMAGN180	GAGAAAAATGATGATAATACAAG	GCCCACTGGATTTATAAAG	171	2
176	M548	AACAGGTAGAGGAAAGCATG	TGACCCACTAGTACATCTCTC	145	2
177	DM0189	TTGCGTAAAAACCACTTG	TCCCATCAAAAATTCAAAG	296	2
178	CMCT44	TCAACTGTCCATTTCTCGCTG	CCGTAAAGACGAAAACCCTTC	104	2
179	DM0711	GGAACTAATAGCCCACCTC	CTGTCTCACAGAATTATGCC	260	2

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
180	DE2020	AGTCACAATTCCACAACCC	TCGCCACACTAATCAACTG	213	2
181	DM0536	CCAAATCGAAACAAAAGTC	TGTTAGATTTGTTGCAGGC	297	3
182	DM0589	AGAGAAAGGATTAATGTCGG	TTAGAATGTCGTCAAACCC	337	3
183	DM0651	TGTTTTCTTCCCTTAGTGC	TCCTACCACGTCAAAACC	345	3
184	DE1206	GCTCATTTTTGGGTGTAGTG	AAGTCCAAAAACACAAGCG	213	3
185	DM0765	GCGTCATAGCGTACTTAGC	ATTTGTTTTGCCATTTCTG	323	3
186	CMTCN66	CTCCGATCAATTTTACATCT	GAATAAACTTGGTGTCCAAC	127	3
187	DM0244	CTTATGCTCTTTGGCTTTG	CACTTCAGAACAAAGGGTG	176	3
188	DM0526	CGAAACCTTTTGTCACATC	AGGAATACTTCATCCTTTTCC	273	3
189	CMGA128	ATGAAGAAGGGATATTCAAAG	ACTCCATTGTTGCTAACCTTT		3
190	DM0034	AAGCTCAAACCTAAATCGTC	GGTATTGCCCACTGAATTG	184	3
191	CMBR095	TTGACCTTTTACGGTGGTCC	CGGACAAATCCCTCTCTGAA	117	3
192	CSWCT03a	TCTACGTCGATGAATCA	CTCAGATAACCCAAAATA	409	3
193	DE1602	CCTCCTCAAGAACTCATCG	GTCGCAGAACAGAAATCAAC	222	3
194	DE1563	TCTTGATAGGGTCGACTGG	TTCCAAAATCAAACAACCC	192	3
195	DE1590	GCCAAACTCAGAAGCAAAG	ACAACAGCCATTCATCGAC	153	3
196	DM0071	AAGAAAGTCCCTCAGTTCAC	CAATACGTTGTGGCCTAAG	139	3
197	DE1288	TCCCAGGGTATTTTGGTC	GAGAATTAAGGGTTTGGGG	225	3

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
198	CMCTN5	CACCTTAAAGTTTAGCCCC	AAAAATGCAATGAACTGAGCGC	211	3
199	DM0477	TGTGGGAAGAGAACTTTTG	GAAGAAGGGTTTGAATCTTG	214	3
200	DE1533	CTGTTTCGAGAAAGGATGC	GGCAACTCATTTATCAGGC	164	3
201	DM0188	TTTTTAAGCCTTCTCTGTG	ATCAAGTGTCAATGGTTCC	118	3
202	DE1667	AATTAATTTAAGTGTTGAGAATCC	CTCATAATCACGAGTCTGCC	218	3
203	DE1344	ATCCCATTTCCCCATTTAG	ACCCACACATACAAAATCAAC	160	3
204	DE1300	TCCACAACGAATTTAACCC	TTCAAATCTTACCATCGGG	166	3
205	DM0192	ACACCATCACCAAACTCTC	TGGGATTTTCCATACCTC	114	3
206	DE1511	TGTTGCAAATGTAAGTGCC	AATGGAAAAGGAGCATTAGG	181	4
207	DM0263	AAGCCATTGTCCCACAAG	CAGTGGTTCTGTAGCCATC	114	4
208	DM0773	AACGGCTCAAAAATAATCC	TTGTTTTAGCGTTAAATAGTG	339	4
209	DE1234	CGGGACATAATTTGCTGAC	AGGGGTTAATAATTGTGTTGTG	171	4
210	DE1578	GATCGTTCTTCCTTTGTTC	GTTTGGTGAGGATTTGACG	211	4
211	CMTGGN99	TAGTTGGTGGTGGTGGTGGT	CGGTCACGTCGTGAAGTTAG	175	4
212	CMN238	CTTTTTCCTAGCATGTTTG	CAGTTCGTTCCTCTTTTC	280	4
213	CMN21_06	AGGAGGTCGAGATTGCAAAA	AAAAACTGGAAAGATGTTCGC	222	4
214	DE1885	CGCAAAGGAAATGTGATTC	TTGGTGGGTATTGAATTTTG	182	4
215	DM0508	CAACCAAAGTATTGATAAAAG	TCGTCTTGAATCATTAATTG	256	4

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
216	CMBR089	TCATCTCATTCTCATTCTTCCTCT	TGAGGTTTATGAGTGTGTGGTTTT	134	4
217	DM0085	AGACAATTAGGTGACGAGG	ACAGAACACAGAACGCAAC	154	4
218	DM0620	ATAAGCAGACACGTGGAAG	TCAATGTCGGAAAAAGAAG	282	4
219	DM0051	AAAAACAAACAACGTCTCG	CAATCTATGACTGCCAAGG	159	4
220	DE1130	AAACCTGTGATAAACAAGCG	TCATATGTTGCCTGACATTG	199	4
221	DE1834	TTTTCGCCTAAAATCATGC	CTTTTAGGTAGAATACCGAAGTC	170	4
222	DE1408	GGGGGAAGATGGTATTTTG	GAAGCTTATTCAAGTGGCG	166	4
223	DM0758	TGCTGGCTCATAAAAACTC	ATTGGGGAGATAATCCAAG	221	4
224	DE1529	CATTGAAATTGAAACACCG	GCTCCATCGTAATTGACAAC	195	4
225	DM0662	TCAGACGACGGAGATTAAC	TGTCTGATAGGTTTTTCCG	349	4
226	DE1584	AATGAAAGACTGCATTGGG	CAGTTTGTGAGACCCATCC	122	4
227	DE1458	AATACAGGAAACGCATTGG	TGGTGATTACCAATAAACGG	179	4
228	DE1072	TTGTTGTGTGTTGGTGAGG	ACTGCTAACCCCGCTACAC	117	4
229	DM0556	TCCCCACTTACAAAGAAAAG	TTGTGAGTCGTTGTCACTG	345	4
230	DE1368	GCGGAACCTGATTTTTCTG	AATCCTCAAATACACATTTCC	215	4
231	CMAGN73	ATCCAACTCGACCAAGAAAC	CAGCTCTACAACAACATCTC	130	4
232	DE1839	TTCACAAAACGACAACCAC	GTCGAGCGTGAGAAGAAAC	223	4
233	DM0152	GCAAAATCATAAACTTCCC	TTTGTGGTGTAAATGGTTC	102	4

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
234	CMACN213	AGAGGTAGATTGTGTTCCG	GGGTAAAGATCATATAAAATC	184	4
235	CMBR090	GTACCTCCGCCGTTGATCT	TGAGATAATAAGAAATCCAACCCA	147	4
236	DE1220	TGACAAACCTTAATTTGAAAAAG	GCATGTTTGATTCATTAATTTTG	217	4
237	CMTCCN194	CATCGACAACATTCCAATC	GACGATTCACTTGACACG	167	4
238	DM0933	AGTCCACTCCTCCAATAAAAC	GGCCTTTAAATTGCCATAAC	142	4
239	DM0922	TGGTGTGCTGATTTGAGAG	CTCTGATCCTCACCGTCAC	115	4
240	DE1524	ATCGTGGAGAACAGGATTG	CTCTTCTTAAACCCGACCC	168	4
241	DE1645	AGAGGAAAGAAGAGTTGAGTG	GGAAAAGGAGAAATGGGAG	219	4
242	DM0168	CATTTCATTTCATCACTTCC	GCGGCTGTTAATGATAAAG	261	4
243	DM0606	ATATCGGTATTGAGAGGGC	TAGTCAAGCATTGGCTACC	336	4
244	DE1156	AAAAGACCCATTAAAACCTTG	TTTTGACCAAGATGAAGCC	223	4
245	DE1856	CTGCATGAGGATCAAGTACC	TTGGGTTTTTCTTTTCTTTTC	132	4
246	DE1824	TTTCAACCTTCACCACTACC	AAGCAGTCGGTATCTGTGG	250	4
247	DE1059	TTCAGCTTCTTGACTTCCG	AGAGGACGAGGAGGAAGAG	218	4
248	DE1810	AAAAGAAGACAAGGAAAGCAG	TAATTAGGATGCCTTTGCC	189	4
249	CMBR116	ACCTCAAATCTCACCGTTGG	CTACGGTTCCATTTCCCTGA	205	4
250	CMTCN44	CAACTTAGATAACGACCTCT	TTCAAAGCCTCCAAAGAAAC	169	4
251	DE1035	TCTTCATTTTCTTTGCCTTTC	CTTCCAAGGGTCTTCAATG	161	5

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
252	TJ181	CCCCATCTCAATTTGTCACC	AGGTGGAGGAAGGGGTTAAA	116	5
253	CMCTN35	CCAATAATGTAATCGTCTTGG	GTTCCAAACTTTCTACCAATCA	186	5
254	DE1884	CTTGCGTCAACACATTCAC	GATTGAAGGATTGAGTGGG	212	5
255	CMAGN52	CCACCAACATAACACACAAC	CTCTCACACTGTTGGGAAGA	135	5
256	CMTCN9	CCCCCATATTCATCAAAACT	CTTCCTTTTTTTCACACCCT	207	5
257	DE1404	AAAATCTCATTTACCATAAGATCC	GCAACACAATCTTTTGCAG	191	5
258	CMATN101	GCTTGTCTTTGTGTTTGC	GAGAACAAGACTCCTTAATCC	167	5
259	CMTCCN195	GCTACTTCAATGGCGGAC	CACTGTCGAATTCGGAAG	178	5
260	DE1608	AAAATTTGGATGCGATGAG	GCAGCGGTTCTAGCATTAC	191	5
261	DM0287	TTTGGTCAGTGTGATTGTG	AAGAAGTTGTGGCAGATTG	319	5
262	CMGAN3	GTTAAAGGCTATGGTATAGAAC	AGAATAAGGTCCACATAAGG	203	5
263	DM0096	GAAAGATTCATTCACGTCG	AAAGGTCAAAATATTGGATTG	112	5
264	DE1415	TGATGCATAGCTCAAGTCG	AAAAACGCTAAAAGATGAAAATC	208	5
265	DM0552	TTTTAATGCCCATGGTATC	AAAAGGAACAGAACAAGGG	301	5
266	CMCTN2	CTGAAAGCAGTTTGTGTCGA	AAAGAAGGAAGAGGCTGAGA	172	5
267	CMGAAN144	CCGATCATACACCGAATG	CTTATGATGCCGGATACG	138	5
268	DE1469	GAGAGGAGGACGAAAGACC	ACTCTCCGCTTTCTTTTCC	224	5
269	DM0638	ATGAATGATCTGGTTGGTG	CTAAGATCACACCACTGCC	107	5

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
270	CMTCN227	CCTCCGAACTCTCTCATC	ACCATCGTCATAGCCTTG	155	5
271	DE1644	AGCCATTTTCAAGTTGTGC	GTTGTATAACCAAAAATGCAAG	182	5
272	DE1500	GGACATATGGTTACTGAACTTTG	CACAGAACTTTTTCTTCTCTCAG	167	5
273	DE1402	TTGTTAGTTTAGCGTTTAGGG	CAGAGAATCAAATCTCCGC	198	6
274	CMTCN50	TCTACTTCCATGAATCCATC	TAGAATGGTTAGGAAACCCT	134	6
275	DE1417	ACAATCCGCAAAGATGTTC	GAAATTAACTTTCAATTTTGTG	181	6
276	CSWCT02B	TTCTGCATACCCTCTCCT	CACACTTCCAGATGGTTG	203	6
277	DM0867	GAGCAATAGAGACCGTTGG	AAACTCAACCACTCCCCTC	168	6
278	DM0546	AAACGAAGTGCAAAAAGTG	GCCCCTTTTAATGGAGTAG	226	6
279	DE1686	GGCCAGTCAATTCTTCTTG	CGATGATAAAGGCAAAAGG	193	6
280	DM0894	ACAAAATGGTAATGAAAACTTG	AACAAGAAAGCTACCACGC	234	6
281	DM0109	TGAGGATTACGAGAAAAGC	TGAAAATGGAAATTGAAGG	249	6
282	CMATN239	TCTTTCGGTCACACGTTG	CCTACAAGAATGGCCCTC	140	6
283	CMTAN263	CAAAACTCTAAACAACGAC	ATGTAAATAGCAAAGGAAC	115	6
284	DE1036	CCCATGAAAGAAAATGGAG	TTCATCTTCCATCAAACCC	193	6
285	DE2044	TACCGTTCCCCACTATCAC	GTTGGCCCTTTGTTAAATG	246	6
286	DE1581	CGCTGTACGGAGTTTCTTC	CATTCTTTACAACTATACGCTGC	223	6
287	DE1077	GAGCTCGAAAATGGCTTG	ATAGGCAACAAGTTCCGTC	225	6

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
288	DM0084	AAAACATACGAGTATTGAAACC	ATATGTCTCAATGCCAAGG	101	6
289	DE1250	AGGGGAGGCTCTGTAGAAG	ACACACCCTTCCCTAAACC	209	6
290	DE1132	TTGATTGAGGAAATGTAGGG	TGGGTCCCCTCATGTAAC	225	6
291	DM0132	CCCCAGATTTATATCCACC	ACCCACCTTATCCTTTACG	165	6
292	DM0557	AATTCAACATGGTGCTTTG	ACACCCTTAGGTGGTGGG	320	6
293	DE1591	AACTTTTCCCATTTCCGAC	TTTGTTACCTTAAATGATTGGTG	219	6
294	DE1981	TGTGGTTTATGGTTGGACC	TTGCGATGCATCTTGATAG	197	6
295	DM0738	TTTCTCCCTCATATCATCG	TTTCCCGAAAATAATATGC	290	6
296	DE1124	GGGAAAAATGATTAAGGGG	CTTGCTCCGTGATTTTCTC	225	6
297	CMGAAN275	AATACTTGCCCTGTTTGC	CAAGTTCCACCATCCATTC	147	6
298	DM0574	TTCGAATAAAACACTTGCC	TCGAGTAGGCTGAAATGAG	194	6
299	CMGAN13	GTGAGCTAAATTCAATGACCC	TCAGAGAGCTTTGGATCAGA	119	6
300	DE1488	ATTGGAATTCGTGATCTGC	GCATTTTGTTTACGCCATC	210	6
301	DE1103	CACATGACTTTTCACAAACG	GAATTCTATCTCTGTCTATCAAAG	204	6
302	DM0564	GCCATGGTAAGTGACAATC	GAAGGTCATGCAGTATTGG	244	6
303	DM0064	GCAAAGAAAGAAGTGATCG	CGTTGAATTCTGGTATTTTATG	198	6
304	DE1076	CCCTTGAATAATGCAGACG	AATGTCATTTAGATCTTGGACC	215	6
305	DE2016	GGGAGAAAGAATGAGAAGATG	AAATTCTAGATTTGGCCCG	181	6

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
306	DM0187	TTTTTAGGTTTCCTTCGAC	CATGGGTAGGTTAGGAATG	264	6
307	DM0710	GAAATATATAATTAGTCACAAACCAAC	ATCTCCACTTTATCCTCCG	277	6
308	DE1736	CCGTCGGTCTAGAAGAATG	CTAAGGGAAAAAGGCAAGG	135	6
309	CMN61_14	TGCAGGATCAAGAATCAAGTTC	ACGAACTCCGGCATAATCAC	163	6
310	DM0753	CAGATTTGAAAGGAAGTCG	TAACGACGAAGACCAGATG	170	6
311	DE1432	TGGATCCAAACTTACTACG	AGACATCCAAAAATAATATTCCAG	211	6
312	DM0554	AATGTCGAAATTTGTATGC	TCTTTGTCTTTGGGGCAC	283	6
313	DM0038	CATGTTTATACACCTATTCAGC	CTATGTTGTGCACTTTTAATG	122	6
314	M112	GCTTATCCCTCACCGAACAA	AATCGCCGATCTTCTTCAAA	206	6
315	DE1180	CTTCCCCCTCAAAATTACC	AAATTAACCGAAATCGAAATG	130	6
316	CMCTN38	TAAAACACTCTCGTGACTCC	GATCTGAGGTTGAAGCAAAG	141	6
317	DM0228	GACGAGAATTTGTTGGAAG	AGTGCCAGAGATGATGAAC	279	7
318	DM0498	ATCGTGAATCGCAAAAAC	ATTTCGCTTATGCAGTTTG	349	7
319	DM0108	TAGCTTGAACTTCGTCCTG	GAAGCGTACTCCCTATTGC	246	7
320	M181	TGGTTAGTTAAAGAAGAGAGAG	ACGCAGCCATACACAGAC	248	7
321	DE1295	AAGGTCCAAACTTTGAGGG	TATGCCCAATGGTACTTCC	113	7
322	CMCTTN174	CACGAGGCTTCTTCTTCTTC	GCACGGTTACTTTCTCGC	146	7
323	DM0309	GGCAGTAAATGACCATGAC	GGTGACGAACAAACTGAAG	230	7

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
324	DE1477	GAATGGAGCGTAAGAGGAC	CTTCCACGCTTGATTCTTG	123	7
325	CMAGN75	TGGGTTTTCTTCTACTACTG	TGCTTTTACTCTCATTCAAC	157	7
326	CMMS004	GCCCAACGGACACACTCACTCACAC	GAGGGAGTAAGAATAAGAAGAAGAA	190	7
327	DE1048	ATCCCATTTTCCCTACCAC	TTGACATTTCCATTTTCCAC	183	7
328	DM0777	AGAAGGAAGGATGGGTATG	TCTTCTGCTTTGATTACGG	209	7
329	DE1406	GGTCAGGATAGAGGGCAG	CCCAAATTTCGAGGTAAG	225	7
330	DM0206	TGTAACACCCCAAAGTTTC	AGGAAGAAGATTGAAATGG	297	7
331	DM0283	GATTGACGACGAAGATCG	CGTACATAAAGGCATCAGG	255	7
332	DM0584	CAAAGAAATATGAGAGGATGG	AAGCTCTTCCTTCAACTCC	291	7
333	DE1139	TTTAGGGGTTGCTACAATG	CCTTCACATCCTCTCTTCTTC	154	7
334	DM0560	CATTTCAATATCCATTGGC	CATACACTTCGAATCAGGG	268	7
335	DM0653	CATCAAAATAGACATGCCAC	TACTGACCCAAACACCAAC	235	7
336	DE1126	TTGAATGATATACGAGTCAAAATG	GTGTTCATGAATTAGGGCG	181	7
337	DE1564	TCAGGATTTCAAATTTTATTTTTC	GATCTCCATGCAGTTCCAG	150	7
338	DM0572	ATATTTTGACCCCAATTCC	CCCAAGAGAAAACACAATG	332	7
339	CMAGN141	TTTGTGAGGGTGCAGCTAG	CAACACAACACATGGCAATTC	172	7
340	CMBR012	AAACAAACATGGAAATAGCTTTCA	GCCTTTTGTGATGCTCCAAT	134	7
341	CMTCN30	GGAGGGAAAGGAAAGAGAGA	GGCAAGAAGATGGCAAAGAT	193	7

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
342	CMBR058	CAGCGATGATCAACAGAAACA	TACCATTCAGGGGGACACTC	123	7
343	DE1174	TCGAACATATCCAGTGCAG	TCCCTTCACTCTCTGCTTC	192	7
344	TJ204	CTCTCTTCATTTCCCCTCGTT	TGGCCTGGAAAGTAAGGGTAT	225	7
345	DE1092	TTACCAAAATTCCATTCCC	AGAAGGAGAGAATGAGTGGC	185	7
346	DE1350	AGGGTGATTTTTGTGCAAC	AGTTTCACGTCCGAATCTG	202	7
347	CMGAN48	TTTAGGTTACGAAAACCCAG	ACTTATTCTTCCCGAAAACG	120	7
348	DE1414	AAACTGCCTTCTTTCACCC	TGGTTGAATTAAACAAAAACC	205	7
349	DE1878	AGGTATGCTAAGTTCCCCC	AGAACGGGAAAAGAAAAGG	101	8
350	DM0043	GGTATTTGGTAATCGTATCC	GACAGTGAAGACCGTTACAG	138	8
351	DE1236	GCCATTGGTATTGGGTAAG	TGTGTTGGTGTTCGTGTTC	186	8
352	DE1353	AAGAAGGCCTCCAATCAAG	AGAGCGAGAGGAACAACAG	220	8
353	CMAGN249	TCTCCAGCTCTTCCTAGG	CCATTGTTTTCTCTTCTTC	213	8
354	DM0471	TCCAAACCAAGGTATGAAG	TGCTGATCAAGAAAGAACG	338	8
355	CMCTT144	CAAAAGGTTTCGATTGGTGGG	AAATGGTGGGGGTTGAATAGG	192	8
356	DM0047	AACTTTAAGTATCAATGCAAC	AGATTATATTCAGAAGTTTAGG	268	8
357	DE1543	AGTGAAACAAAAGGGTATTAAC	AACTCGCGTGGTGATTAAG	206	8
358	CMBR109	TGGAATGTACCGTGATGGGT	ATACAGCAGATCCACAGGGG	148	8
359	DE1643	CAAGTTGGGTTGTGGTCAG	AGGAGAGGACAACCAGAAG	217	8

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
360	DE1461	GCTGTTTTAAACCATTTTTCC	AAGGTCAAATTTAGCTTCTTATTC	154	8
361	DE1302	TGATGGATAGGACGAGAGG	AGCTTCCTCACCATCATTG	219	8
362	CMAGN47	CTTCATGTTTTATTTCCGTGG	CAACGAGAGGTACATAATTC	184	8
363	DE1490	CCCAATTTTGACTTTGCAC	CCTCTCTCAAGTCTTCCATTAG	199	8
364	DM0637	ATCTGCAACATCAAAAACC	TTGATCAAGAAAAGCATCC	157	8
365	CMBR145	TGTGACAATGTGCAACCAG	AAAAATGGTGTTAAACGACATGG	153	8
366	TJ2	GAGGAATCCGAGACCACAAC	GCCAAGTGTGTGTTGGAAAA	152	8
367	CMATTTN262	ATTGGTTCGCATCTTTAC	TCATTTCTACACCACTTACC	139	8
368	DE1170	CACAGACAAAGTATCGAAAAGAG	TAGCGAGTCGAAACCTCTC	153	8
369	DE1826	TCAATCCGTTGGTAAAACC	CCCCAGAAATATGTATCCAG	226	8
370	CMTCCN157	GCTATTTAAACCTCTTCCTC	GATTGGACCTGATTGCTC	180	8
371	CMBR022	CCAAAACGACCAAATGTTCC	ATACAGACACGCCTTCCACC	177	8
372	DE1175	ATGGTGACGGGATTGTAAG	GATTGGAAACTGTAACGCC	216	8
373	DE1468	TTGATCTCCGCCATAGAAG	TCTTCTACCATTTGCCAGC	192	8
374	DM0069	CGAGATTTTGACTGCTGAG	ACCGAAGAAAGATGAACAG	176	8
375	DE1333	TCACAAGCACATACATCCAG	TCCATTGAAGGTTCCTTTG	179	8
376	DM0091	ACTTGTAGTTAACCGCTGG	TTCTTTCACTGTGTTGATAGG	115	8
377	DE1434	TGGATTATTCAAACAACGAAC	GTCGTTTCTCCGATGGTC	224	8

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
378	DM0103	CACCCTCAACTTCTCTTCAC	TACAAACTAATTTATAATGCAGAC	196	8
379	CMACC146	CAACCACCGACTACTAAGTC	CGACCAAACCCATCCGATAA	152	8
380	DM0766	GGAAAAATTCCCTCTCAAG	CTGTGTTGCTTTGCTTCTC	347	8
381	DM0640	CTGGAGAAGACAACAATGG	TTCATTCCCTATTTTTCCC	321	8
382	CMBR042	AACCAACTTCCATTCCCCTC	GAGGCACTCATTTTCTCTGAATG	136	8
383	DM0367	GAACACAAATGGAAAGCAC	CATCATTTGACCATCCATC	343	8
384	CMAG59	TTGGGTGGCAATGAGGAA	ATATGATCTTCCATTTCCA	-	8
385	CMCTTN181	CTCTCTGCAATTCTCGCC	CAACCATCCGCTTCACTC	190	8
386	DE1366	TTCTCCGATGTGTCCTCTC	GTCGCTTGGAATATATCGG	144	8
387	DM0289	GTATCATGTCGGAGAAACG	CCTTTATCCCCACTTTTTC	237	8
388	CMTTCN222	GTAATCAGCCGTGCAATG	TTGCTCTTTTCGTCTTTTAC	180	8
389	DM0020	GCGATCTTGAAGTTTGTAAG	CAAAACCTAACAATCCACC	109	8
390	DM0540	TTGAGGGTGATGAGAAATG	TGAAGAAAATGTGGTGAAAG	210	8
391	CMCCTN226	CCCCTCTTCTCTCTTCTC	CATACCCACAAGCACCAC	196	8
392	DM0353	GTTTTTGACATGAACAGCC	AAGCATTTTGAACCTCCTC	314	8
393	DM0767	AAAATCGAAAAATCTTAAGAAATAG	GGCATTACCAAACACAAAC	333	8
394	DE1752	CCAAAGCATACTCGAGACC	CGCCTTCTTGAATCAGTTC	212	9
395	DE1400	AACTTTTGCTTTCCCTTCC	TGGGGAATTAGGGTTAGATG	198	9

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
396	DE1475	AATTAAACCACACCCCTCC	GGTTTCTTGGTATTGACGG	210	9
397	DE1467	GATGTCTTGAGCGAGAAGG	ACCATCACATTCCACAACC	210	9
398	CMN53_68a	AGAATCGTCGAACCCCTTTT	ACCATACTCCGTTGTCGGAA	289	9
399	DE1549	GGGGTCAAGATAGGGAGAC	ACAGGTGAGGAAGCTAGGG	181	9
400	DE1102	GACTAACTTCGCCTGATGG	CAATAACCAAAATGATTTAAACG	108	9
401	DE1232	TCAATCTCACTTGCCTTTTC	CCAATAAACGCATGGAAAC	223	9
402	TJ66	TGAACTAAACCCATGTGGAAAA	TTGAATTGGGGTTTTGAGTTG	183	9
403	DM0644	CACTCATTGTAATGCCCTG	TCCACCATACAATATCTTTCC	324	9
404	DM0611	AGAACCCAATTGAAAAACC	GCACCTAAAAAGGTAACCC	256	9
405	DE1330	TTCTTCCTTTTCAACGAGC	AGCAAGAATCTGTGGGTTG	182	9
406	DM0030	CCAAAGTAAAAGTGAAGTCC	CTTGAAATGAATTTGAGGTG	148	9
407	CMTATTCN260	GCACGAGGCTCAACTACC	GAGACCGAAATTGAAGAATAG	145	9
408	DM0431	AGATGGTGATTGGATGATG	AAAATTAAATATTAGCCTTTTGG	128	9
409	CMTCN1b	CCCTTCATTTTCATCATCC	GAAGACGGCAAATTGAGCT	155	9
410	DM0545	GGGTATGCTTCATAACACC	TATCTCACCCTTCATGTGC	261	9
411	CMCCTTTN217	CCACCTTGTAATCACTCATC	AGCCTTCATTCCCTCTTC	~300	9
412	DE1670	AAAGAAAATTGGATTGGGG	GCAAATGTGATATGGTCCC	138	9
413	DE1372	GTTCCAATTGGGGAGATG	CAAATCCAACGATTCATAAAC	218	9

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
414	DE1219	ATACGAAAGAGCAAGCAGG	CTAAAGCTCTTCGCCAGTG	160	9
415	DM0231	AGCCACTTGAATGAGTGAG	TGGAGGAATAGGACTCTCC	226	9
416	DM0580	CCACGAAATCAAAAATCAC	TTTTTCAACTTGCTTCGAC	218	10
417	DE1287	TTGGAAAAGAATTTTAGGACC	TGGATCAAACTTAGCCACG	179	10
418	DE1751	ACCCACCATGCATTCTAAC	GAGCCAGTTGGGGTTTTAG	225	10
419	DE1887	CGTGCTTCCCTCTTAAATC	GAAGGGAGTATTGAGTTGGG	193	10
420	CSWCT01	TTCTGATCAACGACGAAG	GAAACAAAAGCCTCCATTG	242	10
421	CMAAAAGN178	ATTCCACACTCCATCCTCC	TTTGACGATTGGATGTGG	194	10
422	DM0507	TTCATGAAAGGAGACTTGC	ACCCCCAAGGTATACAAAC	276	10
423	DE1868	GCAAATTGATTTTGACTAATAGC	TTGATGTATGAAAAGTAGAGTTGC	239	10
424	DM0830	TGTAGGGCTTGTTGGAAAC	TTCATGACAGCATGCCTAC	206	10
425	CMBR055	GAGGCCTTTGTGGTTCGTAA	AAAGAAATGGATAAAGGAAACAGA	107	10
426	CMTCN214	TGTGTGTGTGAGAGAGAGAG	GTGTTTGCCGATTCTACC	122	10
427	CMTCN196	GGTCGTATGTTCTGCAGC	TAATGGTGAAGAAGATAAGG	174	10
428	DE1495	GTGAACGAAGAGACGAAGC	AAAACCCCAAACCCTAGTC	207	10
429	DE1750	TTCGGGTACTCTTGACTGG	GCACATAGAGCACATTCTTTC	230	10
430	DM0083	TGATTTGGTGAATGTAAGTC	TCAAACTCCAATTCCTCAC	133	10
431	DM0582	ACTGAGGTCTTGAAGGTCC	AAGTCGAAATTGAATGCTG	258	10

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
432	DM0173	ATCGTCAGTCACCTTTTTC	AAGGAGGAGTTTGTTGAAG	267	10
433	DM0365	CTGTTCCTCTTCTGCTTCC	GAGAAAGAAATGGGAATTTG	326	10
434	DE2011	TGGAGGGGAATGTAGTTTG	GATGAAGGTTTGAAAGGGAG	230	10
435	DM0673	TCTACGGACCATGTGAATC	TCAAACAAAGTTGAAATTAGG	192	11
436	DE1274	GAAACGATGTCGGAGAAAG	GGGTGAAATGAAAGGGAAG	211	11
437	DE1921	AAAGGTTTGTGATTGTCTTCTC	GAGCCTTCTTGTTCATTCG	237	11
438	DM0425	AATTGGTGCTTTGATGAAG	CCAACACCAACAACCTAAC	344	11
439	DE1163	GCTACTCAGAATGGCGAAC	CCTTTTATTGGCGATCTTG	192	11
440	CMBR057	GCTCTGAAGAGTGGAATGAGAGA	CCATTTGGGAAGTAGGCATC	118	11
441	DM0022	AATCAATTATTGTGAATAGTCG	CAAAATTCAGATGGATTCAG	161	11
442	DE1282	TATGCCACCTAAAGGGATG	CATAGTTTGTGCCTATTGCC	207	11
443	CMAAGN230	GAAAGTCGAGGAACAACC	CTTTAATCCTCAACACTCC	173	11
444	DM0804	GGAGTCCTTTTGCAACAG	CAAAAACTGATTGTTAACTTAAC	250	11
445	CMGAN12	TTTTTGTCGTTATATGAGGG	GTTGCATAATGCTAATTTGG	179	11
446	DE1348	CACACGTTTTTCTTCATTCC	GCAACTTTGTATGTTGCCTAC	211	11
447	CMAGAN268	ATGGATGGATTCAACAGG	CAATCATCAAATGAGGAAG	182	11
448	DM0569	TGCACATGGACTACTTTGG	GAATGCACGTATGCTAACC	258	11
449	DE1055	ACAATTCTATAACATCAAAAGTTG	CTTGAGAGATGGAGGCAAG	165	11

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
450	CMN06_19_1	GCTCTCCCAAGCCTTCTCTT	CAGACCAACAATAGAATGCACA	157	11
451	DM0203	AAGTCGTAAGAACTCGCAC	TAACGGCCTCATAATTTTC	159	11
452	DE1863	CAAATCAAACACACATAAATCC	CAAAAACGAGTTTGTTGGG	147	11
453	CSWCT18B	AAGTAGTCCTCAATGCCACAT	GGAGAGAAATGGGGAAAA	182	11
454	DM0015	TGGCAAGTTTTGTCGATATG	GGAATGGAAAAGGGAATTG	243	11
455	DE1123	AAACCCAAATTGAAAACCC	TCCGCTTCATAATACAATGG	219	11
456	DM0331	AAACAGTGGCCAAATACTG	CTCAAACAAACTCCTCCAC	274	11
457	CMATN89	CACTACCTTAAAACAGAATTG	GGACAATTTAGGGAGGATC	138	11
458	DE1034	AAACTGATCGACGCATCC	AATTGAATTGGCATTTTGG	212	11
459	DE1763	TCTTTACGTGGGCTTTGAG	TTGGACCCAAAACAAAGTC	178	11
460	CMTTCN88	CTCCATATTATGCAGCGTG	TGTCAAATGGGTTCTCTC	184	11
461	DE1409	GAACGTGAATCAAGATGAGG	GTAACTTCCGGTGCGTTAG	222	11
462	DM0088	TGTTGCTTTCCCAAGTTAG	TTGCATTCATAAAGGGAAC	196	11
463	DE1167	CTGAATGGCAAGAAAGAGG	TCAAGTTTGGGAATTTTGC	160	11
464	CMTTCN264	CATCACTACATCAATTTCCC	TGCAAATCCCCATTAGAG	147	11
465	DM0685	GCAAAATTTAAAAACTCCATAG	AAAAGAATGTACACGGCAG	310	11
466	DE1074	AAGAAGTCCTGAGTGTGAGAG	CACCTCCTTCTTCATCTTCC	225	11
467	DM0614	ATGAGTTAGGCTACCGAGC	GTTGGAAGCAGCATAATAAG	114	12

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
468	DE1113	TTATCATTGGAAACCAAAGC	CCAACACTCTTAACCGCTC	217	12
469	DE1917	TCCTAATACCTTTGAATTTTGC	AAGTTAATGCAACATCCTTTTG	249	12
470	DE1299	GTGAGCCTTTCTCATAGTTGAC	GTCGTCCCAACAAATGAAG	163	12
471	CMBR034	TCTCTTTGTTTCCTCCCCCT	GTGGGGCTTGGTTCTTTTG	147	12
472	DM0819	ACCCACTCACTTGATCGTC	TGACGAAACGGAGGTAATC	241	12
473	DM0707	TCACTTACTGACAGCGAGG	CAGATCTGAAAGAAAACCG	151	12
474	DE1343	GAAACAACCATTGTCTGCC	AACCCATGAAAAAGAAAAAG	160	12
475	DE1970	TGTAACGGCTATTTTTCGG	TCCTTATAAAAGAAAAGGAGAAAG	205	12
476	M206	TGGGCTACCTCTATCCTTTCTT	AATCCCCAAAATCTCAACCA	176	12
477	DE1422	TCTTAGCTGTTGAGCATGTG	AGCAGATTCTTTGTCTTTGC	193	12
478	CMMS35_4	ACGGATACATCGAGGAGACTTCATG	GTCAGCTTCAACCCTTTACTTTTTC	105	12
479	DE1178	CCAAACACTCCCTAAACCC	CCTCCAACAATAGAATGCC	177	12
480	5A6U	TCCATTGGTTAAAAAGAAAGACG	TTCATTTTTGTATTCACTGCATTT	213	12
481	DE1531	GCATGCATGTAATTTGATGAG	ATCACATAACAGCCGCCAC	223	12
482	DE1843	TCGATCCCATGTTTTTATATC	TTAATTGAGGCACTCAGGC	238	12
483	DE1814	CAGGTTTGTCCAAAACCAC	CCAAGGTGGAATCAGCTAC	194	12
484	DE1851	CACCCTTCATTATCCCAAC	CCAGAGACGAAAAGAAATTG	114	12
485	DE1545	TTGGCCCATTTTCAATATC	AAATTTGTTCGATTCTCCG	137	12

S. No.	Marker	Forward sequence	Reverse sequence	Size (bp)	Linkage group
486	CMBR150	TTTTTACTGTGTGTTTTGATTTGTT	TTGGTGGACTGGAATCCATA	250	12
487	CSAT425	TAGGGCAGGTATTATTTCAG	ACGGACTGATTTAGTATAGGC	93	12
488	DM0191	TTGAAGGTCTTTGCTGAAG	GCCTACCACATTTAATTCC	160	12
489	DM0093	TCCACCCATCATCAATTAC	CTCTCCCTGAATTCCCTAC	171	12
490	CMTCN14	TATATTGGCTTTGGCTCTCG	GATTCGTTATCTCGACCAAC	165	12
491	DE1185	GTCGCAACACACAACATTC	ATCCGTTCCAAACACAAAC	127	12
492	DE1913	TGCCTAGCTATTCCTGTGC	AAAATGGTCATGTTAACCC	248	12
493	DE1370	AATGTAGGGATTCCAGCAG	CGTTTCTTTTGTCTCAATGC	217	12
494	CMGCAN278	CGATAACTTGGACAACTTG	GGAACATCATCATCATCATC	126	12
495	CMCTTN259	GCCAAAAGTAACGAATTC	TCCATTGTAGCCGTATATC	112	12
496	CMGAN80	ATATTGATTGCTGGGAAAGG	CTTTTTTGGCTTTATTGGGTC	159	12
497	CMAGN33	CTGTCTGCTATTCTCCACTTGG	TGTATGCCACGTAGCGAAAC	122	12
498	DM0839	AACACCATCGAGGTAGTGC	GTTAGGGACGAAAGGAAGG	234	12

CHAPTER IV

RESULTS AND DISCUSSION

Hybrid seed production of melon *via* hand emasculation and pollination costs 12-30 times higher as compared to open pollinated varieties (McCreight and Elmstrom 1984). Exploitation of male sterility reduce such high costs. Male sterile line, MS-1 having *ms-1* gene has been utilized to develop three commercial hybrids, Punjab Hybrid, Punjab Anmol and MH-27. For developing muskmelon hybrids with special horticultural traits, the male sterility gene *ms-1* needs to be transferred into new inbred lines. However, the conventional backcross method for transferring a recessive gene is very time demanding because the homozygous (*MsMs*) and heterozygous (*Msms*) male fertile plants cannot be distinguished phenotypically. Due to its codominant nature, an SSR maker linked to male sterility gene will assist the transfer this gene by differentiating homozygous and heterozygous male fertile plants in the backcross generations. Furthermore, the 50% male fertile plants in the female line could be identified and eliminated at an early seedling stage, before transplanting in hybrid seed production block. Molecular markers linked to male sterility genes, *ms-3* and *ms-5* have already been identified. This study is intended for identification of codominant molecular markers linked to male sterility gene *ms-1*.

The results are presented and discussed under following sub-headings:

4.1 Experiment 1: Phenotyping and segregation analysis of mapping population

4.1.1 Assessment of male sterile and male fertile phenotypes

The seedlings of F_2 population transplanted in the field in March 2017 produced flowers in April 2017. Male fertile and male sterile plants were distinguished visually based on presence/absence of pollen. In male fertile (*MsMs/Msms*) plants, anther lobes contained an ample amount pollen while no pollen could be seen in any of the flowers of male sterile (*msms*) plants (Fig 4.1). Further, the pollen shedding was determined by rubbing anthers of freshly opened flower on finger-tip. The plants in which yellowish/creamy pollen mass adhered to the finger were tagged as male fertile whereas those with no pollen were tagged as male sterile.

4.1.2 Segregation analysis

The F_2 population segregated into homozygous dominant (*MsMs*), heterozygous (*Msms*) male fertile and homozygous recessive (*msms*) male sterile plants. Out of 150 plants assessed for sterility, 117 plants were identified as male fertile (*MsMs/Msms*) and 33 plants were male sterile (*msms*). This phenotypic data was subjected to chi-square analysis to test the

goodness of fit of the observed phenotypic segregation of male fertile and male sterile plants to the expected phenotypic ratio of 3:1 for single recessive gene inheritance.

Table 4.1: Phenotypic segregation pattern of F_2 population

Cross	Fertility Status	Observed	Expected	$(O-E)^2/E$	$\{(O-E)^2/E\}$
MS-1× KP_4 HM-15	Male Fertile	117	112.5	0.18	0.72
	Male Sterile	33	37.5	0.54	

The calculated chi-square value of 0.72 is non-significant at 5% level of significance and 1 degree of freedom (Table 4.1). Thus, the non-significant chi-square value showed that the observed segregation of F_2 population did not deviate from the expected 3:1 ratio. The segregation analysis confirmed that male sterility in melon is governed by single recessive gene *ms-1*.

Among the fertile F_2 plants, 75 plants were self-pollinated to generate F_3 individual plant progenies. Each F_3 progeny comprised of 20-25 plants. Flowers from each plant of every progeny were carefully visualized to determine the segregation of progenies for male sterility. In 23 F_3 progenies, no male sterile flower was found. The corresponding F_2 plants giving rise to these progenies were designated as homozygous dominant (*MsMs*) male fertile. Similarly, the remaining 52 progenies which segregated into male sterile and male fertile plants were considered to have originated from heterozygous (*Msms*) male fertile F_2 plants. Thus, 108 F_2 plants (75 male fertile and 33 male sterile) from the mapping population were successfully genotyped. The genotyping of the remaining 42 F_2 plants could not be done either due to lack of fruit set or poor germination of the seed obtained from these individual F_2 plants. These remaining plants were either homozygous dominant or heterozygous male fertile plants.

4.2 Experiment 2: Identification of SSR markers linked to male sterility gene *ms-1*

4.2.1 Analysis of parental polymorphism

Four hundred ninety eight SSR primer pairs covering all 12 linkage groups of melon were used to assess polymorphism between the parental lines. After PCR amplification, the product was first run on 3% agarose gel (Fig 4.2). The primers that resolved parental DNA on gel into upper and lower amplicons were considered as polymorphic. It was observed that the polymorphism between many samples was not clear on agarose gel. Therefore, 6% polyacrylamide gel electrophoresis (PAGE) was used for better resolution of DNA amplicons (Fig 4.3).

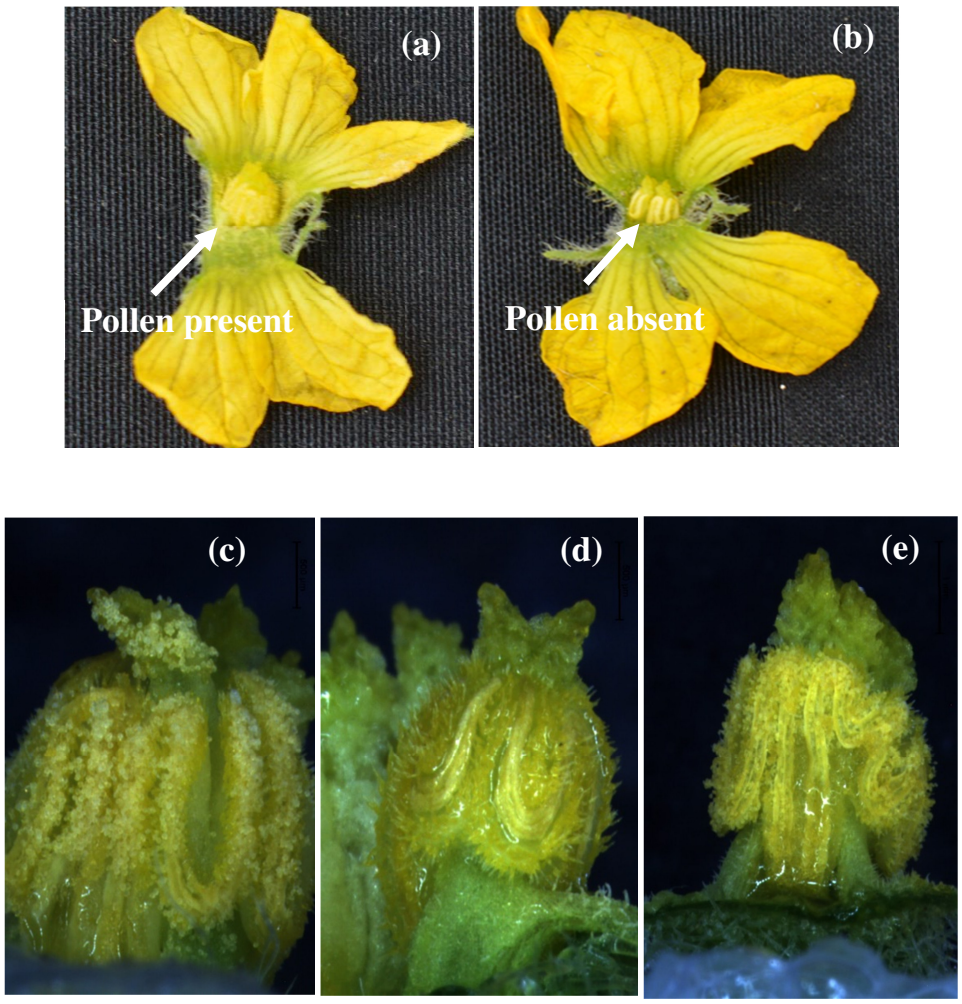


Fig 4.1: Fertility assessment based on absence/presence of pollen

(a) Male fertile flower showing presence of pollen
(b) Male sterile flower without pollen
(c) Male fertile KP_4HM-15 flower showing pollen mass adhering to anther lobes
(d) Male sterile MS-1 flower showing empty anther walls
(e) Male fertile MS-1 flower showing pollen mass adhering to anther lobes

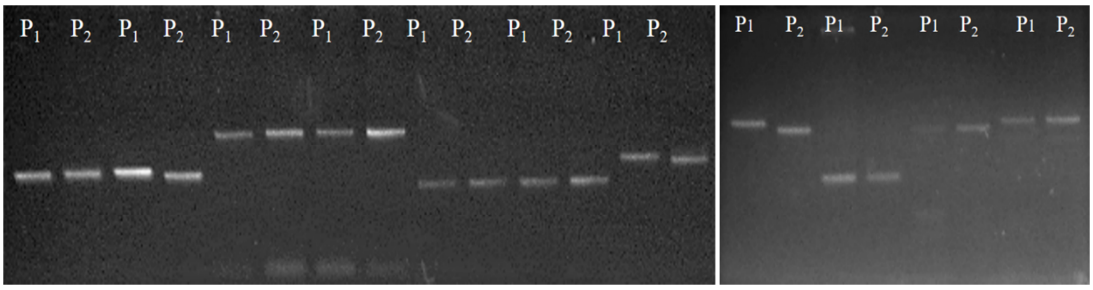


Fig 4.2: Analysis of parental polymorphism using 3% agarose gel
P_1: MS-1; P_2: KP_4HM-15

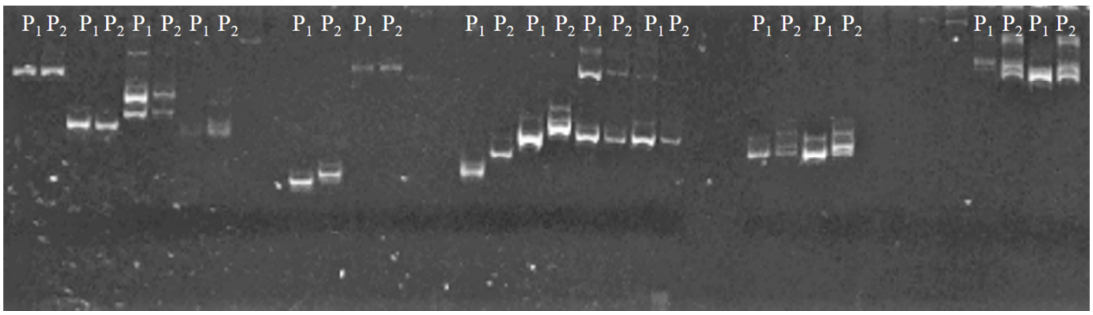


Fig 4.3: Analysis of parental polymorphism using 6% polyacrylamide gel
P_1: MS-1; P_2: KP_4HM-15

Among these SSR primer pairs, 100 primers displayed polymorphism between parental DNA using PAGE, accounting to 20.37% polymorphism (Table 4.3). The primers showing polymorphism between the parents are enlisted in Table 4.2.

Table 4.2: List of polymorphic SSR markers between MS-1 and KP_4HM-15

Sr. no.	Marker	Linkage group	Sr. no.	Marker	Linkage group	Sr. no.	Marker	Linkage group
1	DM0300	1	35	DM0468	9	69	DE1220	4
2	DE1374	1	36	DM0490	9	70	DM0933	4
3	TJ27	1	37	DM0757	10	71	CMCTN35	5
4	DE1337	1	38	CMTA134a	10	72	DM0096	5
5	DM0060	1	39	DM0098	10	73	DM0552	5
6	CMBR120	2	40	DM0913	11	74	DE1500	5
7	CMCGGN210	2	41	CMAGN45	11	75	CSWCT02B	6
8	CMCTTN179	2	42	CMGAN51	11	76	DM0546	6
9	CMGCTN187	2	43	CMAAAGN148	11	77	DE1686	6
10	DE1630	2	44	DM0503	11	78	DM0894	6
11	CMBR026	3	45	DE1534	12	79	DE1581	6
12	DE1462	3	46	DE1980	12	80	DE1077	6
13	TJ125	3	47	DE1081	12	81	DM0738	6
14	DE1753	3	48	DM0839	12	82	DM0187	6
15	DM0854	3	49	DM0699	1	83	DM0038	6
16	DM0551	4	50	DM0073	1	84	M112	6
17	DM0934	5	51	DM0675	1	85	DE1295	7
18	CMMS2_3	5	52	DM0661	1	86	DM0309	7
19	DE1840	5	53	DM0198	1	87	CMCTT144	8
20	DE1557	5	54	DE1765	2	88	DE1643	8
21	DM0214	5	55	DE1265	2	89	TJ2	8
22	DE1875	6	56	DM0473	2	90	DM0766	8
23	CMTCN41	6	57	DE1291	2	91	DM0289	8
24	DM0749	6	58	DE1411	2	92	CMTCN1b	9
25	DM0145	6	59	DM0290	2	93	DM0507	10
26	DE1585	7	60	DM0711	2	94	DE1921	11
27	DE1378	7	61	DM0536	3	95	DE1282	11
28	DE1083	7	62	DE1206	3	96	DM0569	11
29	DM0196	8	63	DE1590	3	97	DM0331	11
30	DE1101	8	64	DM0188	3	98	DE1917	12
31	CMTCN56	8	65	DM0773	4	99	DM0707	12
32	DM0220	9	66	DE1578	4	100	CMMS35_4	12
33	CMTC47	9	67	DE1834	4			
34	TJ150	9	68	DM0662	4			

Table 4.3: Per cent polymorphism between MS-1 and KP_4HM-15 per chromosome

Chromosome no.	Markers used	Polymorphic markers	% polymorphism
1	39	10	25.64
2	41	12	29.27
3	35	9	25.71
4	55	7	12.73
5	32	9	28.13
6	54	14	25.93
7	42	5	11.90
8	55	8	14.55
9	32	6	18.75
10	29	4	13.79
11	42	9	21.43
12	42	7	16.67
Total	**498**	**100**	**20.37**

4.2.2 Bulk Segregant Analysis (BSA)

The primers that distinguished parental DNA displaying upper and lower DNA amplicons were tested in BSA (Fig 4.4). The two DNA bulks, i.e. sterile and fertile bulks comprised of 10 plants each from homozygous recessive and homozygous dominant F_2 plants, respectively. Out of 100 primer pairs that showed polymorphism between the parents, five primers, DM0038, DM0187, DM0839, M112 and DE1500 differentiated the DNA bulks (Fig 4.5). PCR products of parental DNA was run along with the bulked DNA samples. With these five primers, the sterile bulk produced same amplicon size as that of MS-1 parent and the fertile bulk produced amplicon size same as that of KP_4HM-15. DM0038, DM0187 and DM0839 displayed clear polymorphism, whereas the intensity of polymorphism was low with M112 and DE1500. The primers distinguishing the DNA bulks were then tested on individual sterile and fertile plants that constituted the bulks. It was found that the segregation of primers, DM0839, M112 and DE1500 was not in conformity with the phenotypic data rendering these primers as non-candidate markers for screening mapping population. Only two primers, DM0038 and DM0187 (Table 4.4) distinguished the individual sterile and fertile F_2 plants (Fig 4.6).

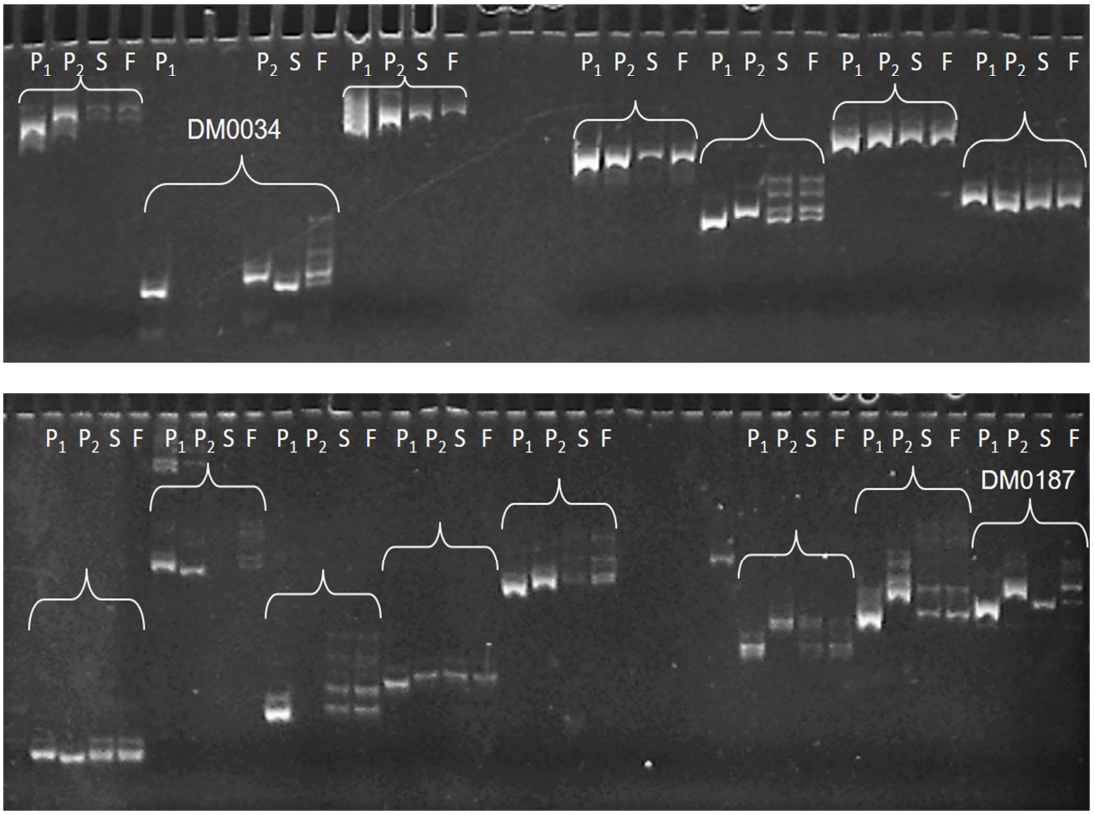


Fig 4.4: Bulk segregant analysis of polymorphic SSR markers
P_1: MS-1, P_2: KP_4HM-15; S: Sterile bulk; F: Fertile bulk

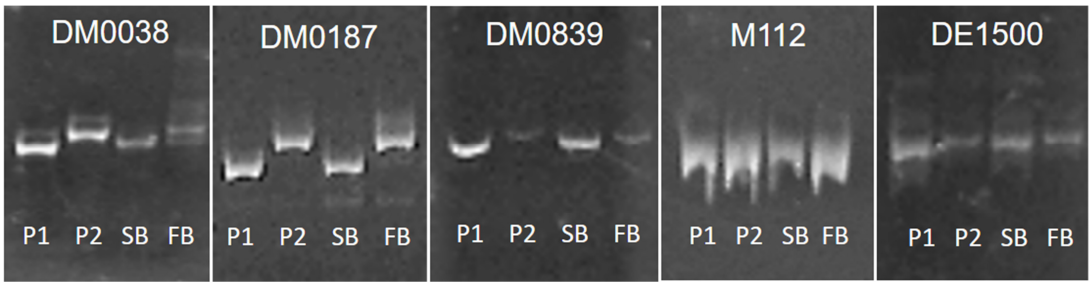
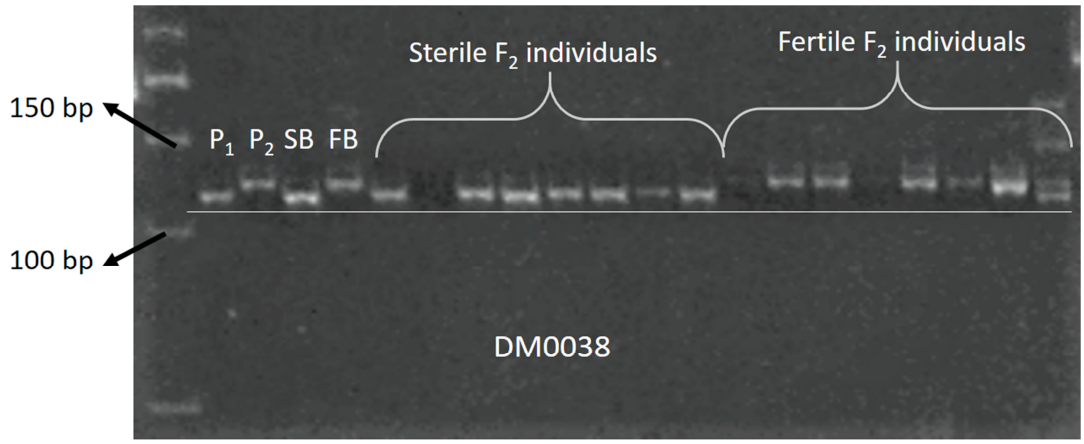
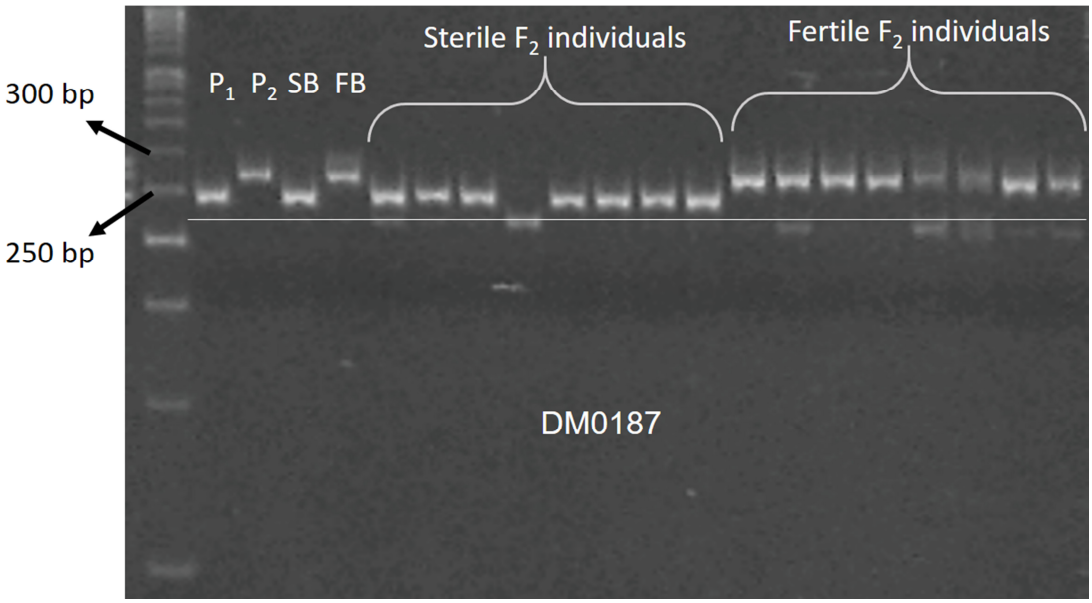


Fig 4.5: Primers differntiating sterile and fertile bulks
P1: MS-1; P2: KP_4HM-15; SB: Sterile bulk; FB: Fertile bulk

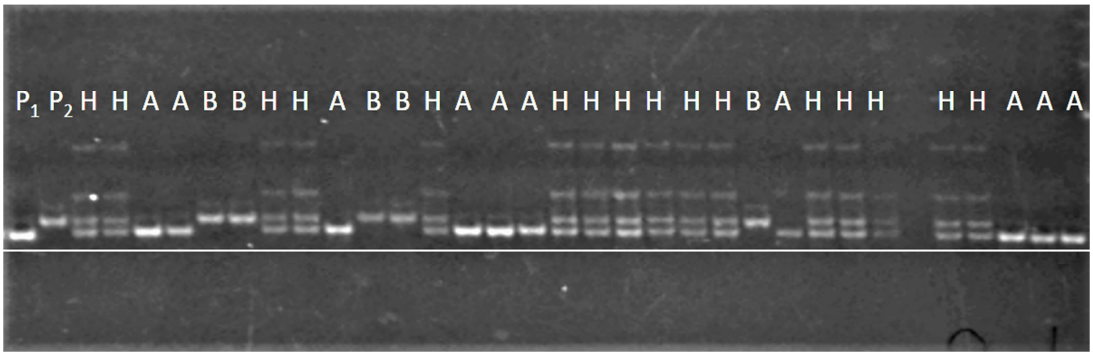


(a)



(b)

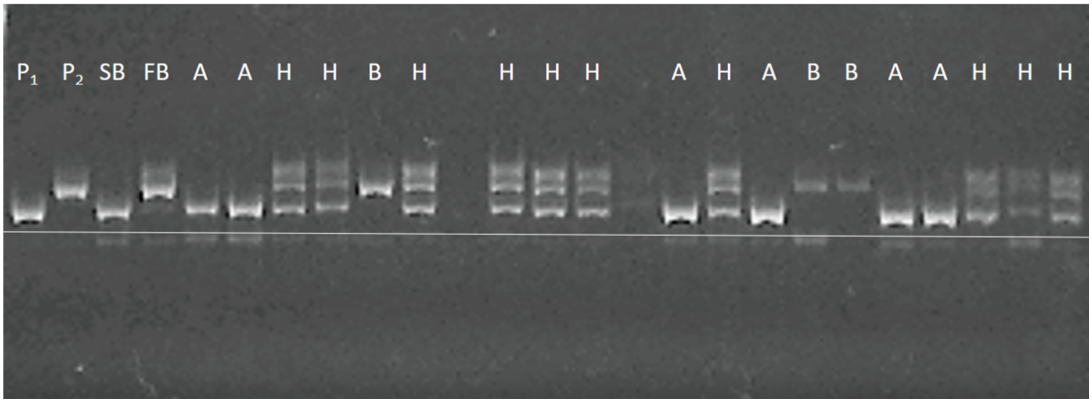
Fig 4.6: Separation of individual sterile and fertile F_2 plants using SSR markers along with 50 bp DNA ladder
(a) DM0038
(b) DM0187
P_1: MS-1; P_2: KP_4HM-15; SB: Sterile bulk; FB: Fertile bulk



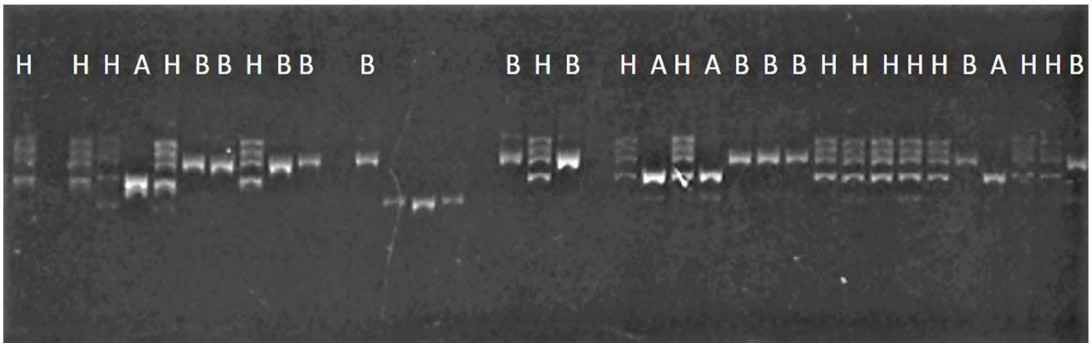
(a)

(b)

Fig 4.7: Genotyping of F_2 population using SSR marker DM0038 (a, b)
P_1: MS-1; P_2: KP_4HM-15; A: MS-1 type; B: KP_4HM-15 type; H: Heterozygote type



(a)



(b)

Fig 4.8: Genotyping of F_2 population using SSR marker DM0187 (a, b)
P_1: MS-1; P_2: KP_4HM-15; SB: Sterile bulk; FB: Fertile bulk; A: MS-1 type; B: KP_4HM-15 type; H: Heterozygote type

Table 4.4: Characteristics of the SSR markers used for screening of mapping population

Marker	Primer sequence	LG	Size (bp)	Annealing Temperature (˚C)	Source
DM0187	TTTTTAGGTTTCCTTCGAC (F) CATGGGTAGGTTAGGAATG (R)	6	264	50.4	Syngenta
DM0038	CATGTTTATACACCTATTCAGC (F) CTATGTTGTGCACTTTTAATG (R)	6	122	51.4	Syngenta

4.2.3 Genotyping of mapping population using SSR markers

The mapping F_2 population was screened using two SSR markers, DM0038 (Fig 4.7) and DM0187 (Fig 4.8). The marker data of the individual F_2 plants was recorded as MS-1 type (A), KP_4HM-15 type (B) and heterozygote type (H). Number of A, B and H type amplicons on PAGE gel were recorded. The marker data was analyzed for segregation in the mapping population.

4.2.4 Analysis of molecular marker data for mapping of *ms-1* gene

Since male sterility in muskmelon is governed by single recessive gene, the linked SSR markers were expected to segregate into 1(A):2(H):1(B) genotypic ratio. Chi-square analysis was conducted to determine the goodness of fit of the observed marker data to the expected genotypic ratio for single recessive gene inheritance. The chi-square values observed for the markers, DM0038 (Table 4.5) and DM0187 (Table 4.6) were 2.64 and 0.51, respectively. The observed chi-square values for both markers were less than the tabulated value of 5.99 at 5% level of significance and 2 degrees of freedom. Thus, chi-square analysis confirmed that the segregation data of both candidate SSR markers was in conformity with the expected 1:2:1 genotypic ratio.

Table 4.5: Segregation analysis of DM0038 marker in F_2 population

Cross	Genotype	Observed	Expected	$(O-E)^2/E$	$\chi^2 = \{(O-E)^2/E\}$
MS-1 × KP_4HM-15	A	44	37.5	1.13	2.64
	H	76	75	0.013	
	B	30	37.5	1.5	

Table 4.6: Segregation analysis of DM0187 marker in F_2 population

Cross	Genotype	Observed	Expected	$(O-E)^2/E$	$\chi^2 = \{(O-E)^2/E\}$
MS-1 × KP_4HM-15	A	35	37.5	0.17	0.51
	H	74	75	0.01	
	B	41	37.5	0.33	

4.2.5 Construction of linkage map

The linkage distance of the two SSR markers, DM0038 and DM0187 from the male sterility gene, *ms-1* was determined using MapMaker 3.0 software. The linkage group having *ms-1* gene was scanned at minimum LOD score 3.0 with maximum mapping distance of 50 cM using Kosambi mapping function. Phenotypic data of the F_2 population was arranged as male sterile (A), heterozygous male fertile (H), homozygous male fertile (B), and homozygous/heterozygous male fertile (C). The phenotypic data along with the marker data was analyzed in Mapmaker software to determine the linkage distance between the candidate markers and the *ms-1* gene. Linkage analysis established that the two putative SSR markers were linked to *ms-1* gene. The marker, DM0187 was found to be closely linked to *ms-1* gene at a genetic distance of 6.6 cM. The linkage distance between *ms-1* gene and DM0038 was assessed as 21.1 cM. The *ms-1* gene was placed between the two SSR markers located on chromosome 6 of muskmelon (Fig 4.9).

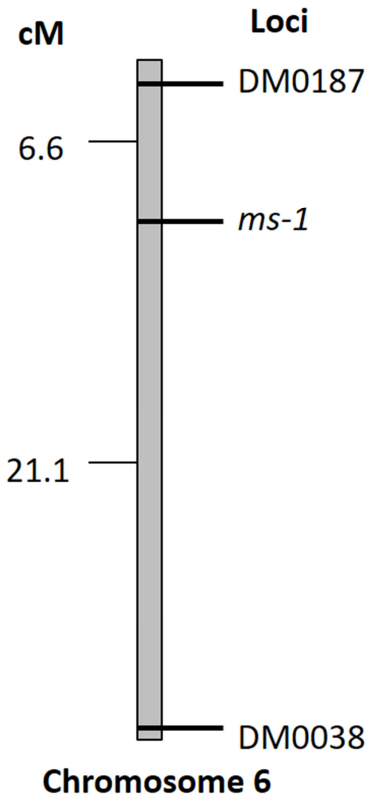


Fig 4.9: Linkage map of *ms-1* gene and SSR marker, DM0038 and DM0187

CHAPTER V

SUMMARY

Muskmelon (*Cucumis melo* L.) is an important *Cucurbitaceous* crop cultivated in many tropical and subtropical regions of the world. It is a diploid (2n=2x=24) species with small genome size of approximately 450 Mb. The origin of muskmelon is controversial as Africa and Asia are suggested as its origin. Muskmelon fruit is valued among consumers for its flavor, sweetness and high phytonutrient content. In addition to the sensorial attributes, it is an excellent source of vitamin A (β-carotene), vitamin C (Ascorbic acid), folic acid and potassium.

Muskmelon is a cross pollinated crop possessing great variability. Heterosis breeding is extensively exploited in melon for uniformity, higher yield and early maturity. Production of hybrid seeds through hand emasculation and pollination is costlier. Various cross pollination promoting mechanisms available in muskmelon *viz* gynoecism, monoecism and genic male sterility (GMS) can be utilized to cut down the high cost of producing hybrid seed. The genic/nuclear male sterility in muskmelon is stable and can be utilized for economical and feasible production of hybrid seeds. At present, five nuclear male sterility genes *viz ms-1*, *ms-2*, *ms-3*, *ms-4* and *ms-5* are known. All of them are recessive and non-allelic and possess unique phenotypes. The male sterility gene *ms-1* was introduced in India in 1978. Thereafter, it was used to develop three commercial muskmelon hybrids, Punjab Hybrid, Punjab Anmol and MH-27. Besides *ms-1*, the male sterility gene *ms-5* has been utilized recently to develop a hybrid, MH-51.

Over time, 'MS-1' has survived its utility. With increasing consumer awareness, the demand for muskmelon hybrids with specialty traits is emerging. Thus, for developing specialty muskmelon hybrids, there is an urgent need to transfer this male sterility gene in new inbred line possessing special horticultural traits. Since a single recessive gene governs male sterility in muskmelon, generation of new GMS lines using classical backcross technique is time consuming and strenuous task. This is because the homozygous male sterile plants appear only in segregating generations. The homozygous (*MsMs*) and heterozygous male fertile plants (*Msms*) in the backcross population cannot be distinguished phenotypically, as both genotypes exhibit male fertile phenotypes. Progeny testing *via* self-pollination needs to be done for identifying heterozygous male fertile plants in the backcross generations. Due to this limitation, no new GMS line could have been developed by gene transfer.

Availability of molecular markers linked to male sterility genes can speed up the transfer of GMS genes into desired inbred lines by marker assisted backcross breeding. Molecular markers linked to two male sterility genes, *ms-3* (Park *et al* 2004) and *ms-5* (Sheng

et al 2017) are available. However, no marker associated with the first male sterile gene *ms-1* has been reported. Identification of molecular markers associated with *ms-1* will speed up its transfer by differentiate homozygous and heterozygous male fertile plants in the backcross population without testing the progeny. Hence, it can be utilized for developing new GMS lines. Additionally, the markers will be used to identify male sterile plants in hybrid seed production block at an early seedling stage to establish 100 percent pure stand of female plants. Consequently, generation of information on genetic markers related to *ms-1* gene will strengthen the hybrid seed production program of muskmelon.

Thus, the present study was undertaken at Vegetable Research Farm and School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana during 2016-2018 to identify codominant molecular markers closely linked to *ms-1*, the male sterility gene.

An F_2 population advanced from a cross 'MS-1 × KP_4HM-15' was used for the mapping of *ms-1* gene. 'MS-1' is a male sterile line having *ms-1* gene, which is responsible for its sterility and 'KP_4HM-15' is a male fertile line. Male fertile and male sterile plants were distinguished visually based on presence/absence of pollen. In male fertile (*MsMs/Msms*) plants, anther lobes contained an ample amount pollen while no pollen could be seen in any of the flowers of male sterile (*msms*) plants. Further, the pollen shedding was determined by rubbing anthers of freshly opened flower on finger-tip. The plants in which yellowish/creamy pollen mass adhered to the finger were tagged as male fertile whereas those with no pollen were tagged as male sterile.

Out of total 150 F_2 plants, 117 plants were found to be male fertile (*MsMs/Msms*) and 33 male sterile (*msms*). Chi-square analysis was conducted to test the goodness of fit of the observed number of male fertile and male sterile plants to the expected 3:1 phenotypic ratio for single recessive gene inheritance. The calculated chi-square value of 0.72 was non-significant at 5% significance level and 1 degree of freedom confirming monogenic recessive mode of inheritance of male sterility gene *ms-1*. Among 117 male fertile plants, genotypes of 75 plants (23 homozygous dominant and 52 heterozygous) were determined from the phenotypes of F_3 individual plant progenies. The remaining 42 fertile plants were either heterozygous (*Msms*) or homozygous dominant (*MsMs*).

DNA of the two parental lines and 150 individual F_2 plants was isolated from immature leaf tissues. Four hundred ninety-eight SSR primer pairs covering all 12 linkage groups of melon were used to assess polymorphism between the parental lines. PCR product was resolved using 6% polyacrylamide gel electrophoresis. The primers polymorphic between parental DNA displaying upper and lower DNA amplicons were tested in sterile and fertile DNA bulks comprising 10 plants each from homozygous recessive and homozygous dominant F_2 plants, respectively. The primers that distinguished sterile and fertile bulks were used screening of F_2 population. The marker data along with phenotypic data was analyzed in

MapMaker 3.0 software to assess the genetic distance between *ms-1* gene and the codominant SSR markers.

Among 498 SSR primer pairs, 100 primers displayed polymorphism between parental DNA using PAGE, accounting to 20.37% polymorphism. Out of 100 primer pairs that showed polymorphism between the parents, five primers differentiated the sterile and fertile DNA bulks. Screening of these five primer pairs in individual sterile and fertile F_2 plants constituting the bulks revealed that only two primers, DM0038 and DM0187 were able to separate individual sterile and fertile plants. These two candidate markers were further used to genotype whole F_2 population. The other three primers were found unsuitable for scoring F_2 population due to non-conformity with the corresponding phenotypic data.

The marker data obtained from screening of F_2 population using the markers, DM0038 and DM0187 was recorded as MS-1 type (A), KP_4HM-15 type (B) and heterozygote type (H). The marker data and phenotypic data was analyzed in MapMaker 3.0 software at minimum LOD score 3.0 with maximum mapping distance of 50 cM. Linkage analysis established that the two putative SSR markers were linked to *ms-1* gene. The marker, DM0187 was found to be closely linked to *ms-1* gene at a genetic distance of 6.6 cM. The linkage distance between *ms-1* gene and DM0038 was assessed as 21.1 cM. The *ms-1* gene was placed between the two SSR markers located on chromosome 6 of muskmelon.

Chi-square test was conducted to determine the goodness of fit of the observed marker data to the expected genotypic ratio of 1:2:1 for single recessive gene inheritance. The chi-square values observed for the markers, DM0038 and DM0187 were 2.64 and 0.51, respectively. The observed chi-square values for both markers were lesser than the tabulated value of 5.99 at 5% significance level and 2 degrees of freedom. Thus, chi-square analysis confirmed that the segregation data of both linked SSR markers was in conformity with the expected 1:2:1 genotypic ratio.

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