Advances in melon breeding (*Cucumis melo* L.): An update

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**Abstract** Melon (*Cucumis melo* L.) a member of family Cucurbitaceae is extensively cultivated for its fleshy fruits. Based upon the agroclimatic zones of cultivation as well as concerning the regional preferences, melon displays significant variability phenotypic and biochemical attributes. Below, an effort is put forth to considerably evaluate the scope of achievements while in the growth as well as the enactment of melon breeding programs by employing the newest solutions. Melon breeding has achieved critical milestones throughout the previous century, and we hope this trend will go on to persist down the road. However, experiments have to understand new genetic information for genes associated with the challenges imposed by climate change. The identification of valuable hereditary and also metabolic variability in the form of landraces and melon wild relatives will be useful for harvest diversification and also for the broadening of the cultivated melon genetic base. Whereas, considerable information on genomics, and melon metabolomics, is beneficial for dissecting the basis of the inheritance of important traits. Overall, we hope the manuscript is going to serve as a crucial resource for the melon breeders.

**Keywords:** Breeding, Diversity, Genetic engineering, Genomics, Male sterility, Melon, QTLs

**INTRODUCTION**

Melon (*Cucumis melo* L.) (2n = 2x = 20 four) a member of family Cucurbitaceae is a crucial eudicot diploid with a genome size of 454 Mb (Garcia-Mas et al., 2012). It is extensively cultivated all over the globe, in temperate, subtropical and tropical areas. The prominent melon producing nations are China, USA, Spain, Turkey and Iran (FAO, 2019). Based on the climatic zones and also concerning the local preferences, melon displays extreme variability in physical,
biochemical and phenotypic characteristics (Silberstein et al., 2003). The wild relatives of melon are distributed in Asia, Africa and Australia (Luan et al., 2008; Kerje and Grum, 2000; Sebastian et al., 2010; Endl et al., 2018). There is an extensive perturbation in the morphology of the fresh fruits of melon, in shape, colour, texture and flavour (Kirkbride, 1993). Melon fruit can reach a size of up to 20 kg, while the shape can vary from spherical to long type. Whereas, in flavour, fruits can be from bland to sweet, sour, or maybe bitter (Kirkbride, 1993; Stepansky et al., 1999; Liu et al., 2004; Monforte et al., 2004).

The recent breeding effort has developed several hybrids aiming to enhance storage and shelf life, disease resistance and resistance to abiotic stresses. Generally, cultivars with a longer shelf life (LSL types) are thought of by customers as having low fruit quality and therefore, present minimal acceptability (Wolff and Dunlap, 1995). Although, most of the breeders concur that powdery mildew resistance and fusarium are essential and fundamental demands of farmers for the modern melon varieties (Capel et al., 2010; Branham et al., 2018). Also, insect pest management is a significant component which benefits commercial yield by providing a much better yield safety measures, reduced waste along with much better fruit quality (Ramamurthy and Waters, 2015). Melon leaf curl New Delhi virus, which is transmitted by whiteflies is causing a considerable yield loss throughout Asia and Europe. More recently, a QTLs are identified for the disease resistance in melon (Saez et al., 2017).

Similarly, the quantitative genes of yield and also yield-related traits in melon are also thoroughly studied. Additionally, many researchers have reported on the merging ability and heterosis benefits in fruit yield along with other morphological characteristics of melon under even under biotic and abiotic stress conditions (Kamer et al., 2015; Varinder and Vashisht, 2018). Recently, due to its very affordable cost and high sensitivity, the next-generation sequencing is possible for melon. Especially GBS (genotyping by sequencing) and RAD sequencing of multiplexed samples are reproducible, fast, and accessible (Ganal et al., 2014; Shang et al., 2020; Zhang et al., 2016). Below, an attempt is put forth to significantly assess the scope of accomplishments while in the development and enactment of melon breeding programs by employing the latest technologies. We hope this manuscript will serve as an important resource for the melon breeders.
DIVERSITY

_Cucumis melo_ belongs to family Cucurbitaceae is a horticultural crop of great economic importance and showed a wide range of diversity for agro-morphological and fruit traits (Zitter et al., 1996; Silberstein et al., 2003). Leaves are simple, three or five-lobed, borne singly at nodes, have significant variation for colour, shape and size (Kirkbride, 1993). Tendrils are simple and borne on leaf axils. Melons fruits are classified as Pepo with three ovary sections (Font-Quer, 1979). Variations in melon fruits were observed for shape, size, internal and external colour. Flesh color varied from orange, light orange, pink, white, green; rind color varied from green, white, orange, yellow and red grey; rind texture as smooth, striped, warty, rough and netted; shape from round, elongated, flattened; size 4cm in _C. melo var. agrestis_ to 200 cm in _C. melo var. flexuosus_ (Kirkbride, 1993). Melon fruits are generally climacteric type, but non-climacteric type was observed in inodorus variety (Aggelis et al., 1997; Zheng and Wolf, 2000; Perin et al., 2002; Liu et al., 2004).

Almost all melon fruits have 1:1 ratio of length/breadth, while, measurements recognize flexuosus types to width ratio of around 4:1 (Burger et al., 2010). Because so many wild species of Cucumis appeared in Africa, it has been suggested the centre of origin of cultivated melon is Africa. Nevertheless, recent reports suggested the closest wild relatives of melon are found in India and Australia (Luan et al., 2008; Kerje et al., 2000; Sebastian et al., 2010; Endl et al., 2018). Melons are divided _C. melo_ ssp. _melo_ along with _C. melo_ ssp. _agrestis_ reliant on ovary pubescence (Jeffrey, 1980). It includes fifteen different groups or varieties of which ten variations are belonging to the ssp. _melo_, for example, cantalupensis, reticulatus, adana, chandalak, ameri, inodorus, chate, flexuosus, dudaim and tibish and five variations are belonging to the ssp. _agrestis_ like momordica, conomon, chinensis, makuwa, as well as acidulous (Zhao et al., 2019).

FLORAL BIOLOGY AND MALE STERILITY

Muskmelon produces several kind of flowers structures such as hermaphrodite or perfect (bisexual) flowers, andromonoecious (staminate and perfect flowers), gynomonoecious (pistillate and perfect flowers), monoecious (both staminate and pistillate) and gynoecious (pistillate) flowers (Munshi and Alvarez, 2005; Choudhary and Pandey, 2016). However, andromonoecious is the predominant sex form in muskmelon. Staminate flowers are borne either singly, or clusters
of two or rarely three but hermaphrodite flowers appear separately (Kiill et al., 2016; Revanasidda and Belavadi, 2019; Munshi and Alvarez, 2005). The ratio of staminate to hermaphrodite flowers ranged from 6 to 19:1 (Siqueira et al., 2011 and Tschoecke et al., 2015). Flowers are yellow, epigynous and actinomorphic. Flowering in muskmelon starts 40-45 days after sowing (Choudhary and Pandey, 2016; Revanasidda and Belavadi, 2019). Anthesis takes place in the early morning between 5.30 to 6.30 am, and anther dehiscence occurs 5.00 to 6.00 am. Pollens remains viable between 5.00 am to 2 pm (Choudhary and Pandey, 2016) and stigma become receptive 2 hr before and 2 -3 hr after anthesis (Munshi and Alvarez, 2005). Two significant genes A and G determine the sex expression in musk melon. Allele G is responsible for the production of unisexual male flower, either andromonoecious or monoecious (Kubicki, 1969; Kenigsbuch and Cohen 1990). Allele A with G produces monoecious flowers and with gg has gynoecious flowers (Kubicki 1962; Kenigsbuch and Cohen 1990). According to Choudhary and Pandey, the different genotypes produced by the combination of two genes can be represented as aagg-hermaphrodite, aaG_-andromonoecious, A_gg-gynomonoecious and A_G_monoecious.

Now a day’s use male sterility for heterosis exploitation is a major area of interest for plant breeders to develop high yielding commercial hybrids. Use of male sterility reduces the total cost of hybrid seed production by eliminating the need for labors for the emasculation of female parents (Dhillon and Kumar 2008). All of these five genes are recessive, non-allelic and have unique phenotype (Pitrat, 1991). The first gene for genetic male sterility was reported in 1949 by Bohn and Whitaker. The second gene for male sterility was identified by Bohn and Principe (1964) in powdery mildew resistant line La Jolla 40460. Plants with ms-2 gene produced hermaphrodite and staminate flowers are with small anthers having empty pollen walls. It differed from ms-1 for spindle orientation during meiotic metaphase-2, cell size and shape (Bohn and Principe, 1964). The third male sterility gene was reported by McCreight and Elmstrom (1983) in PI321005 a cross of Georgia 47 × Smith’s perfect. The segregation ratio in F2 indicated monogenic recessive inheritance of this gene. The anthers of ms-3 plants are waxy, dull, and translucent. The fifth male sterility gene was observed first in 1966 while developing a powdery mildew resistant line. However, its inheritance was published by Lecouviour et al., (1990). The fourth male sterility gene was identified by Lozanov (1983). This gene was used for hybrid seed production by Clause Seed Company. On hermaphrodite and staminate flowers, the
anthers are empty and of reduced size. Two hybrids namely Punjab hybrid (MS-1 x Hara Madhu) and Punjab Anmol were developed in India by using male sterility ms-1 gene (Nandpuri et al., 1982; Lal et al., 2007).

Five Male sterility genes have been identified, but commercialization of ms- based hybrid to a significant level is still waiting. There are some reasons why GMS based hybrids are utilized a limited extent. Transfer of male sterility to a new line by the conventional back cross method is time taking. As the inheritance of male sterility gene is monogenic recessive, the identification of homozygous (MsMs) and heterozygous (Msms) plants in the backcross population is difficult. One generation of selfing is required to select the plants homozygous for ms allele.

Male fertile plants are identified and removed, and remaining plants are used for hybrid seed production. The identification of the male fertile plant is very tedious and laborious. However, the efficiency of the GMS system can be enhanced by the use of morphological markers linked with the male sterile genes. A linkage between ms-1 and red stem gene was reported by McCreight (1983) and between ms-2 and yellow-green by Pitrat (1991). Similarly, Whereas, 3 SSRs on chromosome 6 linked to ms-1 gene were reported by Singh et al. (2019) and a SCAR marker SOAM08.644 linked to ms-3 gene was developed by Park et al. (2004). These molecular markers can be employed to select the male-sterile plants at the seedling stage.

GYNOECIOUS LINE

Gynoecious breeding lines provides several advantages for hybrid seed production as it reduces the cost of emasculation, manual pollination, rouging and use of chemical treatment and ensures 100% purity of hybrid seed production. Gynoecious lines also found advantageous over genetic male sterile lines as it avoids the need for identification and rouging of 50% male fertile plants. The use of gynoecious lines for hybrid seed production was also suggested by Frankel and Galun (1977) and Loy et al. (1979). Kenigsbuch and Cohen (1990) proposed possible genotype and sex expression. Kenigsbuch and Cohen (1990) suggested that for stable gynoecious condition, genotype AAgg and recessive mm combination is needed. At very first, Peterson et al. (1983) identified durable gynoecious plants from Wisconsin-998 population. Three gynoecious breeding lines were developed by More et al., (1987) at IARI, New Delhi (India). An improved gynoecious line named as ‘Gylan’ was developed from a cross of line 36 and GY-4. Fruits of Gylan are round to oval with orange flesh and weight from 0.9 to 1.1 kg. This line produced
good F1 with an andromonoecious Vedrantais and Tam-Uvalde (Cohen et al., 1993). A cross between W321 (gynoecious line) and N233 showed earliness in picking and 50% higher yield than Punjab hybrid (Dhaliwal and Lal, 1996). To induce perfect flowers for the maintenance of gynoecious lines, silver thiosulphate, Ethral and Alar had been suggested by Rudich et al. 1970 and More and Seshadri, 1987. Ma et al., (2010) identified two SSR markers MU5549-1 and MU14723-2 linked with gene ‘a’ and ‘m’, respectively. However, instability of gynoecious lines under high-temperature conditions limits their utilization for hybrid seed production.

HETEROSIS

The primary breeding objectives in muskmelon include early maturity, medium to long vine length, high female flower to male flower ratio, high total fruit yield, fruit size, fruit shape, i.e., round to flattish to oval, outer skin colour, hard netted skin, small seed cavity, adequate flesh thickness, flesh texture, attractive flesh colour, total soluble solids, good resistance against downy mildew, powdery mildew, viral disease, fruit fly, red pumpkin beetle etc. Due to vigorous growth, early maturity, high yield and good quality of hybrids, heterosis breeding is the commonly used method in muskmelon (Banga and Banga, 2000). A good level of heterosis for different traits has been reported by various researchers working on muskmelon. The first study for heterosis in muskmelon was done by Munger (1942) who noticed uniformity for fruits and 30% increment in yield of hybrid in comparison to parents. Heterosis for earliness was reported by several research workers in melon (Dhaliwal and Lal, 1996; Gaurav et al., 2000; Choudhary et al., 2003; Vishwanatha, 2003; Kamer et al., 2015; Choudhary et al., 2018), flesh thickness (Chadha and Nandpuri, 1980; Choudhary et al., 2003; Tomar and Bhalala, 2006; Kamer et al., 2015) fruit yield (Chadha and Nandpuri, 1977; Munshi and Verma, 1997; Tomar and Bhalala, 2006; Subramanian, 2008; Kamer et al., 2015; Selim, 2019) vine length, number of leaves and number of branches (Kamer et al., 2015; Duradundi et al., 2018) average fruit (Kamer et al., 2015 and Selim, 2019), fruit length, fruit girth (Tomar and Bhalala, 2006; Subramanian, 2008) leaf area index, netting, seed cavity (Selim, 2019), total soluble solids (Subramanian, 2008; Selim, 2019) number of fruit per plant (Tomar and Bhalala, 2006; Subramanian, 2008; Kamer et al., 2015) fruit shape index, fruit skin color, b-carotene, vitamin C (Kamer et al., 2015) rind thickness (Varinder and Vashisht, 2018) germination percentage, seed number per fruit and empty seeds (Nerson, 2012) powdery mildew resistance (Kesavan and More 1991).
**QTLian BREEDING**

In muskmelon, many economically important characters such as yield, quality traits and resistance against diseases showed polygenic inheritance, and the genetic variation for these characters is controlled by several genes known as quantitative trait loci. Identification of QTLs for these traits using conventional breeding approaches is not so easy due to complex nature and their interaction with the environment (Lynch and Walsh, 1998). However, with the advent of DNA marker techniques and linkage mapping, it is possible to dissect the complex nature of quantitatively inherited characters. Different mapping populations such as F2 generation, F2:3, recombinant inbred lines (RILs), near-isogenic lines (NILs) and Double haploids (DHs) are used for QTL mapping. These populations are created by crossing two parents differing for the trait(s) of interest. Each mapping populations have their advantage and disadvantage. Earlier several QTLs have been mapped in the different genetic background of muskmelon using diverse mapping populations and molecular markers. In muskmelon QTLs for different traits have been mapped using molecular markers such as cleaved amplified polymorphism sequence AFLP, RFLP (CAPS) Simple sequence repeat (SSR), Random amplification of polymorphic DNA (RAPD) (Baudracco-Arnas and Pitrat, 1996; Oliver et al., 2001; Zalapa et al., 2007; Cuevas et al., 2008, 2009; Wang et al., 2016; Baloch et al., 2016; Amanullah et al., 2018).

Earlier linkage maps in muskmelon are prepared on using segregating population such as F2 and BC1 which are unsaturated and related with few economically important characters (Baudracco-Arnas and Pitrat 1996; Liou et al. 1998; Oliver et al. 2001; Silberstein et al. 2003). However, recently linkages maps are constructed using immortal population such as RILs (Perin et al. 2002a, b; Cuevas et al., 2008; Paris et al., 2008; Fukino et al. 2008; Zalapa et al. 2007b; Pereira et al., 2018), NILs (Moreno et al., 2008) and DHs (Monforte et al. 2004). A wide range of variation was observed for fruit shape, size, external skin colour, acidity, flesh colour and fruit weight which provide ample scope for investigation of genetic and molecular basis fruit external and quality traits (Burger et al., 2006). Flesh colour in muskmelon is controlled by two genes gf (green flesh) and wf (white flesh) which interacts with each other to produce green, white and orange flesh colour (Hughes 1948; Iman et al. 1972; Clayberg 1992). Monforte et al., (2004) hypothesized that three putative loci control expression of orange flesh colour in muskmelon, i.e. ofc2.1 and ofc12.1 and ofc3.1 on linkages group LG2, LG12 and LG3, a however large
population is required to know the complexity of this trait (Gross, 1987; Navazio, 1994). For beta-carotene, seven QTLs were identified using RIL and three by using F3 population (Cuevas et al., 2008; Cuevas et al., 2009).

For fruit shape two QTL were identified by Paris et al., (2008), eight QTL by Moreno et al., (2008), two by Baloch et al., (2016), five by Pereira et al., (2018) and four by Amanullaha et al. (2018); for fruit weight four QTL by Amanullaha et al., (2018), two QTL by Wang et al., (2016), two by Zalapa et al., (2007), six by Monforte et al., (2004) and two by Pereira et al., (2018); for fruit diameter two QTL were detected by Wang et al., (2016) two by Diaz et al., 2014 and two by Pereira et al., (2018); for fruit length five QTL were identified by Diaz et al., 2014 three by Wang et al., (2016) and 4 by Pereira et al., (2018). Fruit maturity is an important trait in muskmelon as early maturity increase the total number of harvests. However, its inheritance is complex and affected favourably by environmental conditions (Monforte et al. 2004; Zalapa et al. 2006). Monforte et al identified nine QTLs for fruit maturity; three QTL for flesh thickness, four for Netting density and four for netting width; five QTL for fruit firmness and two QTL for flesh firmness; one QTL for seed number and 4 for seed weight., (2004), Moreno et al., (2008), Cuevas et al. (2009); Wang et al., (2016), Amanullaha et al. (2018) and Pereira et al., (2018), respectively. Fukino et al identified two QTL for resistance against powdery mildew., (2008), one by Capel et al., 2010 one QTL by Yuste-Lisbona et al., (2011), one by Wang et al., (2016) and one by Li et al., (2017). Two QTLs were identified against Alternaria leaf blight, four QTL for Fusarium oxysporum f. sp. Melonis, three QTL for Sulfur tolerance and three QTL for Tomato leaf curl Daley et al identified new Delhi virus (ToLCNDV)., 2017, Branham et al., 2018, Branham et al., 2020 and Saez et al., 2017, respectively. Major QTLs for different traits can be used for the genetic improvement of muskmelon using marker-assisted breeding approaches. The list of QTLs identified for the important traits of melon is provided in Table 1.

Table 1. QTLs identified for important morphological and biochemical traits along with important disease resistance traits.

| Trait       | Generation | Parents           | No. of QTLs | Linkage Group | References          |
|-------------|------------|-------------------|-------------|---------------|---------------------|
| Fruit weight| F2:3       | M4-7 × MR-1       | 4           | LG2, LG5, LG10| Amanullaha et al., (2018) |
| Trait                          | Generation | RIL/Marker Information | LG | Reference                  |
|-------------------------------|------------|-------------------------|----|----------------------------|
| Fruit firmness                |            |                         | 5  | LG2, LG3, LG5, LG8         |                             |
| Fruit width                   |            |                         | 6  | LG5, LG8, LG9              |                             |
| Flesh firmness                |            |                         | 2  | LG3, LG6                   |                             |
| Brix                          |            |                         | 3  | LG7, LG9, LG10             |                             |
| Fruit shape                   |            |                         | 4  | LG5, LG8, LG9              |                             |
| beta-carotene mesocarp        | F3         | Q 3-2-2 × Top Mark      | 3  | LG, LG8, LG9               | Cuevas et al., (2009)       |
| Fruit maturity                |            |                         | 3  | LG2, LG6, LG11             |                             |
| beta-carotene                 | RIL        | USDA 846-1 × Top Mark   | 7  | LG1, LG2, LG4, LG6         | Cuevas et al., (2008)       |
| Powdery mildew                | RIL        | AR 5 × Earl’s Favourite | 2  | LG IIA, LGXII              | Fukino et al., (2008)       |
| Fruit weight                  | F2         |                         | 2  | LG5, LG11                  | Wang et al., (2016)         |
| Fruit diameter                |            |                         | 2  | LG5, LG11                  |                             |
| Fruit length                  |            |                         | 3  | LG2, LG7, LG8              |                             |
| Flesh thickness               |            |                         | 3  | LG5, LG11, LG12            |                             |
| Netting density               |            |                         | 4  | LG2, LG4, LG6, LG7         |                             |
| Netting width                 |            |                         | 4  | LG2, LG4, LG6, LG7         |                             |
| Powdery mildew                | RIL        |                         | 1  | LG2                        | Zalapa et al., (2007)       |
| Primary branches number       | RIL        | USDA 846-1 × Top Mark   | 4  | LG1, LG2, LG10             |                             |
| Trait                           | Type | Parental combinations                        | Value | LGs                                      |
|--------------------------------|------|---------------------------------------------|-------|-----------------------------------------|
| Fruit number per plot          |      |                                             | 5     | LG1, LG2, LG8                           |
| Average weight per fruit       |      |                                             | 2     | LG1, LG8                                |
| Percentage of mature fruit     |      |                                             | 1     | LG10                                    |
| Powdery mildew                 | F2   | TGR-1551 × ‘Bola de Oro                    | 1     | LG5                                     |
| Soluble solids content         | RIL  | USDA-846-1 × Top Mark                      | 2     | LG7, LG10                               |
| Mesocarp pressure              |      |                                             | 3     | LG7, LG10                               |
| Seed cell diameter             |      |                                             | 3     | LG1, LG5, LG8                           |
| Seed cell diameter: Fruit      |      |                                             | 1     | LG2                                     |
| diameter                       |      |                                             |       |                                         |
| Percent netting at full-slip   |      |                                             | 1     | LG2                                     |
| Fruit shape                    |      |                                             | 2     | LG1, LG2                                |
| Climacteric ripening           | NIL  | PI 161375 × Piel de Sapo                   | 1     | LG3                                     |
|                               |      |                                             |       |                                         |
| Earliness                      | F2   | Piel de Sapo × PI 161375                   | 9     | LG1, LG2, LG9, LG10, LG12               |
| Fruit shape                    |      |                                             | 8     | LG1, LG3, LG5, LG6, LG7, LG9, LG11, LG12 | Monforte et al., (2004) |
| Fruit weight                   |      |                                             | 6     | LG3, LG4,                               |
| Trait                        | Population | Source                  | LGs                              |
|------------------------------|------------|-------------------------|----------------------------------|
| Sugar content                |            |                         | LG5, LG12                        |
| Powdery mildew resistance   | F2         | MR-1 × Top Mark         | LG1, LG2, LG4, LG8               |
| Fruit shape                  | F2         | MR-1 × Top mark         | LG4, LG12                        |
| SSC                          | RIL        | Védrantais × Piel de Sapo| LG8, LG9, LG10                   |
| Fruit weight                 |            |                         | LG5, LG8                        |
| Fruit diameter               |            |                         | LG2, LG5                        |
| Fruit shape                  |            |                         | LG2, LG6, LG11                   |
| Fruit length                 |            |                         | LG5, LG6, LG11                   |
| Fruit perimeter              |            |                         | LG5, LG6, LG7, LG11              |
| Seed number                  |            |                         | LG5                              |
| Seed weight                  |            |                         | LG3, LG5, LG7, LG8               |
| Fruit length                 | F2         | Piel de Sapo × PI124112 | LG2, LG3b, LG6a, LG8, LG10b      |
| Fruit diameter               |            |                         | LG3a, LG12                       |
| Fruit shape                  |            |                         | LG2, LG8, LG12                   |
| Days to maturity             | Introgression lines (ILs) | Ginsen makuwa × Védrantais | LG1, LG6                         |
| Fruit weight                 |            |                         | LG1, LG2, LG4, LG8               |
| Characteristic          | Method  | Count | Examples          |
|-------------------------|---------|-------|-------------------|
| Fruit length            |         | 5     | LG1, LG2, LG6, LG7, LG11 |
| Fruit diameter          |         | 5     | LG1, LG5, LG6, LG11 |
| Fruit shape             |         | 2     | LG7, LG11         |
| Cavity width            |         | 1     | LG2               |
| Fresh firmness          |         | 2     | LG7, LG10         |
| Aroma                   |         | 2     | LG7, LG10         |
| Rind thickness          |         | 2     | LG2, LG6          |
| Netting                 |         | 3     | LG5, LG6, LG7     |
| Color of the inner rind |         | 3     | LG6, LG8, LG12    |
| Soluble solids content  |         | 1     | LG10              |
| Sucrose content         |         | 4     | LG1, LG5, LG10, LG11 |
| Glucose content         |         | 3     | LG1, LG5, LG11    |
| Fructose content        |         | 3     | LG5, LG10, LG11   |
| Fruit area              | F2      | 4     | LG2, LG4, LG6, LG8 |
|                        | PS × TRI|       |                   |
| Diaz et al., 2017       |         |       |                   |
| Trait                        | Count | Loci                              | Reference                                      |
|-----------------------------|-------|-----------------------------------|------------------------------------------------|
| Fruit weight                | 4     | LG2, LG4, LG6, LG8                |                                                |
| Distal fruit blockiness     | 1     | LG11                              |                                                |
| Pulp area                   | 3     | LG5, LG6, LG8                     |                                                |
| Pulp thickness              | 2     | LG5, LG8                          |                                                |
| Total fruit weight per plant| 1     | LG2                               | Ramamurthy and Waters, 2015                    |
| Seed cell diameter          | 1     | LG4                               |                                                |
| Fruit shape                 | 2     | LG4, LG12                         |                                                |
| Ovary shape                 | 1     | LG11                              |                                                |
| Flesh color                 | 1     | LG4                               |                                                |
| Soluble solid concentration | 1     | LG2                               |                                                |
| Chlorosis                   | 1     | LG8                               |                                                |
| Alternaria leaf blight      | RIL   | MR-1 × Ananas Yokneum             | Daley et al., 2017                             |
| Fusarium oxysporum f. sp. melonis | RIL | MR-1 × AY                         | Branham et al., 2018                           |
| Powdery mildew              | F2    | TGR-1551 × Bola de Oro            | Capel et al., 2010                             |
| Sulfur tolerance            | RIL   | MR-1 × AY                         | Branham et al., 2020                           |
| Tomato leaf curl New Delhi virus (ToLCNDV) | F2 and BC1 | WM-7 × PS | Saez et al., 2017 |
GENETIC ENGINEERING

Traditional breeding played a significant role in the genetic improvement of musk melon for yield, quality improvements, resistance against diseases and insects pests. It made substantial progress in the varietal development process. However, there are some limitations, such as sexual incompatibility for inter-specific and inter-generic crosses (Robinson and Decker-Walters, 1999) more time taking programs (Pitrat et al., 1999) production of interspecific hybrids with low-quality characters (Seshadri and More, 2002) etc. Now a day’s most popularizing technology such marker-assisted breeding and genetic engineering contributed significantly to genetic improvement and varietal development by reducing the risks and limitations of traditional breeding approaches (Li et al., 2009). Transgenic technology has been used successfully in many crops, including musk melon (Bezirganoglu et al., 2014).

Muskmelon is affected by several fungal diseases, viral diseases and insects-pests leads to a considerable loss in production (Kwon et al. 2001). Leaf fungal diseases such downy mildew and powdery mildew and soil-borne fungus such Rhizoctonia solani causing damping-off, root and stem rot and Fusarium oxysporum causing wilting are severe threats to melon production (Gaba et al., 2004; Bezirganoglu et al., 2013). Viruses are the major limitation to cause colossal damage to melon crops. From the past three decades, two most common approaches have been used in muskmelon to develop resistance against viruses one is coat-protein mediated resistance.

Transgenic genotypes in muskmelon have been developed by overexpressing the using coat protein gene of WMV, PRSV, ZYMV and WMV. Coat-protein mediated protection or resistance against different viruses have reported by several workers (Yoshioka et al., 1992; Fang and Grumet, 1993; Gonsalves et al., 1994; Clough and Hamm, 1995; Wu et al., 2010). The expression of amino-truncated core portion or antisense CP-ZYMV provides limited protection while plants expressing the full length of CP-ZYMV gene were highly resistant (Fang and Grumet, 1993). Similarly, ribozyme-mediated resistance in musk melon was observed by Plages et al., 1997 against CMV and by Huttner et al., 2001 against ZYMV and WMV. This type of resistance is based on the ability of ribozymes, small RNA molecules, to cleave viral RNA with high specificity. An enhanced level of salt tolerance was observed in muskmelon by the use of a transgene HAL 1 from Saccharomyces cerevisiae (Bordas et al., 1997). Overexpression of HAL 1 maintained a high K, low Na content and increased K/Na ratio (Serrano, 1996).
Melon fruit is rich in vitamins and folic acid and these nutrients act as potent antioxidants and essential compounds in human metabolic reactions. Ripening process in musk melon involves a series of changes in texture, colour, aroma, firmness, flavour (Lelievre et al., 1997; Jiang and Fu, 2000) and ethylene is known as ripening factor in climacteric fruits (Giovannoni, 2001). Two critical enzymes involve in ethylene biosynthesis pathway, enzyme ACC synthase (ACS) converts the S-adenosyl methionine into 1-aminocyclopropane-1-carboxylate (ACC) which is then oxidized to ethylene by ACC oxidase (ACO) (Fluhr and Mattoo 1996; Johnson and Ecker 1998). To improve the fruit quality, reduced ethylene production and delayed ripening in muskmelon was achieved by using antisense technology for ACS and ACO gene by (Ayub et al., 1996; Guis et al., 1997; Silva et al., 2004; Nunez-Palenius et al., 2006). The transgenic approach has also been utilized for the genetic improvement of other important traits such as fruit development, sucrose content, aroma and female flower development (Yu et al., 2008; Tian et al., 2010; Zhang et al., 2014; Kocaman et al., 2016). The list of transgenics developed in melon is provided in Table 2.

Table 2. List of transgenics developed in melon for quality, biotic and abiotic stress related traits.

| Trait(s) improved       | Transgene                        | Transformation method | References          |
|-------------------------|----------------------------------|-----------------------|---------------------|
| **Quality traits**      |                                  |                       |                     |
| Ripening behavior       | ACC oxidase gene                 | *Agrobacterium tumefaciens* | Silva et al., 2004 |
| Sex expression          | ACS synthase                     | *Agrobacterium tumefaciens* | Papadopoulou et al., 2005 |
| Improved shelf life     | ACC oxidase gene                 | *Agrobacterium tumefaciens* | Ayub et al., 1996 |
| Ripening behavior       | SAMase (S-adenosylmethionine hydrolase) | *Agrobacterium tumefaciens* | Clendennen et al., 1999 |
| Reduced ethylene production | ACC Oxidase gene                | *Agrobacterium tumefaciens* | Guis et al., 2000 |
| Function                        | Gene/Protein                        | Organism                       | Reference                  |
|--------------------------------|-------------------------------------|--------------------------------|---------------------------|
| Reduced ethylene production    | pAP4 gene                           | Agrobacterium tumefaciens     | Nora et al., 2001         |
| Ripening behavior              | ACC oxidase gene                    | Agrobacterium tumefaciens     | Silva et al., 2004        |
| Sex expression                 | ACS synthase                        | Agrobacterium tumefaciens     | Papadopoulou et al., 2005 |
| Improved shelf life            | ACC oxidase gene                    | Agrobacterium tumefaciens     | Nunez-Palenius et al., 2006|
| Fruit development and sucrrose content | MAI1 (acid invertase gene) | Agrobacterium tumefaciens | Yu et al., 2008 |
| Bisexual and female flowers    | CmACS-7                             | Agrobacterium tumefaciens     | Zhang et al., 2014        |
| Aroma                          | Two ADH genes                       | Agrobacterium tumefaciens     | Kocaman et al., 2016      |

**Biotic stress**

| Function                        | Gene/Protein                        | Organism                       | Reference                  |
|--------------------------------|-------------------------------------|--------------------------------|---------------------------|
| CMV resistance                 | CP gene of CMV                      | Agrobacterium tumefaciens     | Yoshioka et al., 1992     |
| Potyvirus resistance           | ZYMV-CP gene                        | Agrobacterium tumefaciens     | Fang and Grumet, 1993     |
| CMV resistance                 | CP gene of CMV–WL                   | Agrobacterium tumefaciens     | Gonsalves et al., 1994    |
| Resistance against ZYMV and WMV| CP gene of ZYMV, WMV and CMV        |                                | Clough and Hamm, 1995     |
| CMV resistance                 | Polyribozyme against CMV            | Agrobacterium tumefaciens     | Plages et al., 1997       |
| Potyvirus resistance           | Polyribozyme                        |                                | Huttner et al., 2001      |
| Downy mildew resistance        | eR genes At1 and At2                | Agrobacterium tumefaciens     | Taler et al., 2004        |
| Potyvirus resistance           | CP genes of ZYMV and                | Agrobacterium tumefaciens     | Wu et al., 2010           |
|                         | PRSV-W                        | tumefaciens                      |
|-------------------------|-------------------------------|----------------------------------|
| Fungal disease resistance | Chitinase and β-Glucanase genes | Agrobacterium tumefaciens        |
|                         | Akbari et al., 2013           |                                  |
| Resistance against glufosinate ammonium-based herbicides. | bar gene | ZYMV-AGII (Potyvirus based vector) |
|                         | Shiboleth et al., 2001        |                                  |

**Abiotic stress**

|                         | HAL1 gene | tumefaciens |
|-------------------------|-----------|-------------|
| Salt tolerance          |           | Boradas et al., 1997 |
| Salt tolerance          |           | Serrano et al., 1999 |

**GENOMICS**

Genomics is widely used in melon research because of its affordable price and very high sensitivity, and the next-generation sequencing is doable for melon and its diverse wild relatives (Ganal et al., 2014). Especially RAD-sequencing and GBS (genotyping by sequencing) of multiplexed samples is easy, fast, and reproducible (Shang et al., 2020; Zhang et al., 2016). Furthermore, low cost and the flexibility of these tools in creating high-density genetic maps rendered their usage in genome-wide association studies (GWAS) (Nimmakayala et al., 2016). The melon genome was sequenced and annotated for the first time in the year 2012 (Garcia-Mas et al., 2012). A genomic platform had been built in an attempt to host the genome sequence (available at http://melonomics.net). Over the years, the melon genome was eventually changed to improve the anchoring and orientation of the scaffold assembly with a particular SNP selection strategy, acquiring the v3.5.1 assembly (available at http://melonomics.net). In this new genome version, 98.2 % together with 90 % of the scaffold assembly was anchored and oriented to some SNP genetic chart, respectively, which stand for a considerable enhancement of the pseudomolecules. 27.8 Mb of scaffold assembly remained unanchored in the genome and pseudomolecule zero annotation utilized in the melon genome discharge v3.5.1 was the same as in v3.5 (Castanera et al., 2020).
CONCLUSIONS AND FUTURE DIRECTIONS

Melon breeding has achieved several milestones during the last century, and we hope this trend will continue to persist in the future. Although, studies are required to recognize new genetic resources for genes related to the unique challenges imposed by climate change. Researchers need to dissect and elucidate the molecular basis for resistance, yield traits and fruit quality by gaining insight into the regulatory factors, that synchronize at different development stages for contributing traits. Nevertheless, a sturdy, durable resistance strategy is required against plant pathogens as pathogens can overcome resistance by building new races. The recent sequencing of the melon genomes at a large scale is going to provide the ability to get, reliable information for disease resistance genes to different diseases and also genes for important biochemical attributes. Latest techniques like speed breeding have to be tailored for the cucurbits keep in view the different sex forms temperature requirement for flowering induction.

As the genome sequencing costs decreasing the use of RAD-sequencing, and DNA microarrays, will accelerate genome mapping and tagging of new QTLs. These QTLs might be used to send the resistance into high yielding melon genotypes and combine different QTL with substantial resistance genes to the other races. Moreover, in melon, GWAS (genome-wide association studies) are used for mapping traits to the one candidate gene level. This procedure continues to be recently called mGWAS (metabolite genome-wide association study). The identification of useful genetic and also metabolic variability forms the foundation for directed harvest diversification as well as genetic enhancement by breeding.

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