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Molecular breeding for gynoecy trait in Bitter gourd (*Momordica charantia* L.)

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Abstract
Gynoecy is the condition where all the flowering nodes produce only pistillate flowers, which can be exploited for improving yield and economizing hybrid production. Though Bitter gourd is a typical monoecious member of Cucurbitaceae family, it also exhibits various sex forms. Gynoecious sex form has been reported in Indian germplasm which is governed by a single recessive gene '*gy-1'*. These lines have been maintained at the beginning through sib-mating. Treatment of gynoecious lines of bitter gourd with ethylene perception inhibitor i.e. silver thiosulfate induces hermaphrodite flower buds. Micro-propagation for maintenance and mass multiplication of gynoecious lines in bitter gourd for hybrid seed production was standardized. However, early phenotypic detection of gynoecy in breeding lines is difficult as it highly influenced by environment. The mode of inheritance of gynoecy and the position of the gene governing it, can be analyzed by identifying a molecular marker closely linked to the gynoecy locus which can further be used in the marker assisted selection of gynoecious line. But in India molecular breeding in bitter gourd is in its infancy, due to limited molecular resources, particularly on functional markers for traits such as gynoecy. The available molecular markers which are summarized in this review can be utilized for incorporation of gynoecy traits into genetic background of cultivated Bitter gourd. This review also covers the implication of gynoecy trait towards the sustainable development in the genetic improvement of Bitter gourd.

Keywords: gynoecy, bitter gourd, molecular marker, marker assisted selection

Introduction
Bitter gourd or bitter melon (*Momordica charantia* L.) is an economically important cucurbitaceous vegetable in terms of both culinary and medicinal usage. Heterosis is well exploited in bitter gourd for early maturity, higher yield and other agronomic traits through development of hybrids. Heterosis in bitter gourd for yield per vine ranges from 27.3 to 86.1% over the better parent (Behera, 2008, Singh *et al*., 2013) [1, 26]. However, hybrid seed production in bitter gourd is labor-intensive requiring manual bagging and hand pollination thereby increasing the cost of seed production. In addition, genetic purity is compromised in the flowers that escape bagging. Use of gynoecious parental lines that produce only pistillate flowers, reduces the labour requirement; increases seed yield and helps to maintain hybrid genetic purity. Gynoecious lines have better genetic combining ability and gynoecious X monoecious hybrids mature early with higher yield potential (Dey *et al*., 2010) [5]. Therefore, gynoecious line can be used to produce hybrids economically in bitter gourd by sparing tedious manual emasculation and pollination. The subsequent generations developed using gynoecious line as one parent shows positive impact on hybrid in terms of increase in yield and earliness.

Why gynoecism is important?
Gynoecy, condition where all the flowering nodes produce only pistillate flowers, can be exploited for improving yield and economizing hybrid production. Regardless of genetic control, gynoecious or predominantly female lines hold promise for the development of gynoecious F_1 hybrids as it avoids manual emasculation and pollination and simultaneously increase the yield and earliness of the hybrid. In Cucumber and Muskmelon hybrids are developed using gynoecious lines as female parent. Increasing number of fruit per plant reported in gynoecious cucumber lines (Fan *et al*. 2006) [6] and high yield per plant in bitter gourd (Behera *et al*. 2008) [1]. Behera *et al*. (2009) [3] also reported significant heterosis in desirable directions for yield and yield attributed traits like sex ratio, days to first picking, number of fruit per plant, yield per plant and vine length. Gynoecious lines are suitable for mechanical harvesting (concentrated fruit set during first picking) in cucumber (Staub and Bacher 1995) [27].

Genetic control of sex expression in cucurbits

Inheritance and molecular mechanisms of sex expression in cucurbits are well characterized in cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.). Sex expression in cucumber is controlled by two loci; dominant F (Femaleness) locus, in combination with another dominant M (monoecious) locus, which determines the gynoecy (Dey *et al*., 2010) [5]. Both the loci play major roles in ethylene biosynthesis. The 'F' locus encodes for an additional copy of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) gene, CsACS1G, a key regulator of ethylene biosynthesis (Matsumura *et al*., 2014, Trebitsh *et al*., 1997) [17, 28]. The other co-expressing locus 'M', induced by ethylene, further enhances ethylene production by positive feedback regulation (Mibus *et al*., 2004) [18]. Increased ethylene in the flower primordia arrests the stamens, which ultimately produce gynoecious flowers.

Similar to cucumber, sex expression in melon is also governed by two major loci; A (andromonoecious) and G (gynoecious) (Kenigsbuch *et al*., 1990) [12]. The dominant 'A' allele that suppresses the stamens development in carpel bearing flowers and co-expressing with dominant 'G' allele determine the gynoecy. Further molecular characterizations of 'A' and 'G' loci have established that sex expression in melon is also determined by ethylene. Stamen inhibiting 'A' locus encodes a key ethylene biosynthesis gene CmACS7 (Roy *et al*., 1990) [23]. Other major 'G' locus encodes a WIP transcription factor gene CmWIP1 that was localized to carpel primordia of future stamen flowers. It was reported that expression of CmWIP1 is antagonistic with CmACS7 but doesn't regulate the expression of CmACS7. Molecular characterization of 'F' and 'M' loci in cucumber and 'A' and 'G' loci in melon demonstrate that ethylene plays a significant role in sex determination in cucurbits. In addition to ethylene biosynthesis genes, several ethylene perceptors; CsETR1, CsETR2 and CsERS gene were also reported to play a significant role in cucurbit sex expression. A transposon-induced epigenetic change leads to sex determination in melon. The insertion of transposable element gyno-hAT downstream the CmWIP1, which encodes a C_2H_2 zinc finger transcription factor of the WIP protein subfamily induce DNA methylation caused by gyno-hAT insertion supress the expression of CmWIP1 and produce gynoecious flowers. Monoecious and andro-monoecious germplasm of muskmelon are lack of gyno-hAT, while the recessive hermaphrodite and gynoecious lines possessed the insertion (Martin *et al*., 2009) [15]. Taking clues from cucumber and melon, an in-silico differential gene expression analysis between a gynoecious and monoecious bitter gourd lines was performed to identify potential candidate genes in ethylene biosynthesis (Gunnaiah *et al*., 2014) [8]. Meenu (2015) observed that McACS2 mediated biosynthesis of ethylene in individual bud of gynoecious line (DBGy-201) is associated with differentiation of floral sex organ in Bitter gourd. A putative hypothesis for action of silver ion: Ag+ reduces ethylene perception resulting in less accumulation of McACS2 mRNA via ethylene signalling pathway and endogenous GA_3 cooperation supports the formation of hermaphrodite buds.

Breeding for Gynoecism

- Identification of gynoecious lines
- Study for maintenance of gynoecious lines
- Genetic inheritance of gynoecious traits
- Identification of predominantly gynoecious line
- Identification of molecular marker linked to (gy) trait
- Transfer gy trait to elite background.

Gynoecy in Bitter Gourd (*Momordica charantia L.*)

Bitter gourd is a typical monoecious crop, but being a member of Cucurbitaceae, it also exhibits different sex forms. For the first time, bitter gourd plants with a complete expression of gynoecious flowering habit have been located in naturally occurring 3 independent lines (Ram *et al*., 2002) [20]. Two gynoecious lines (DBGy-201, DBGy-202) isolated from indigenous source, from its related wild form *M. charantia* var. *muricata* L. (Behera *et al*., 2006) [6]. Two gynoecious line (IIHRBTGy-491 and IIHRBTGy-492) also identified in bitter gourd. (Varalakshmi *et al*., 2014) [29].

Maintenance of gynoecism trait

Chemicals Application (induction of hermaphrodite flowers) - GA3, silver nitrate and silver thiosulfate @ after 1st female flower (Behera *et al*., 2008) [1]. They reported effectiveness of Silver Thiosulfate 6M for sex modification in bitter gourd. Silver nitrate ($AgNO_3$) performs well as compared to GA_3 and Silver Thiosulphate [$Ag(S_2O_2)_2$] for induction of male flowers in gynoecious line.

Micro-propagation for maintenance and mass multiplication of gynoecious lines in bitter gourd for hybrid seed production has been developed (Swati Saha and T. K. Behera, 2015). *In vitro* multiplication through shoot tip culture will help in development of double haploid plants, which may enhance the ability to develop homozygosity in short period particularly for gynoecious traits. With the help of *in vitro* techniques, a large number of plants has been generated for sex modification study using DBGy 201 genotype.

Genetic Inheritance of (Gy) trait in bitter gourd

Gynoecious sex form has been reported in Indian germplasm which is governed by a single recessive gene 'gy-1' whereas Iwamoto and Ishida (2006) [9] reported that gynoecious sex expression in bitter gourd is partially dominant.

Genetic inheritance of (Gy) trait	Reference
few recessive major genes	(Ram *et al*., 2002) [20]
controlled by certain major gene(s)	(Behera *et al*., 2006) [6]
A Single recessive gene (gy-1)	(Ram *et al*., 2006) [21] (Behera *et al*., 2009) [3]

Different systems for hybrid seed production by using Gynoecy

- Gynoecious x Monoecious hybrids
- Gynoecious x Gynoecious hybrids
- Gynoecious x Hermaphrodite hybrids
- Gynoecious x Andromonoecious hybrids

The hybrids in cucurbits are produced mainly by crossing gynoecious lines with monoecious lines. The other systems of producing gynoecious hybrid seed are gynoecious × gynoecious but gynoecious × monoecious hybrids are still widely grown because this offers advantages like earliness (Jat *et al*., 2015) [10], high degree of female sex expression (Jat *et al*., 2017) [11], with uniform and concentrated fruit

formation, which was especially advantageous for mechanical harvest (Robinson, 2000) [22].

For hybrid seed production female and male rows are planted in 4:1 ratio. The female (seed parent) bears only female flowers and pollination is done by insects (honeybee). To ensure the good fruit and seed recovery, the sufficient population of honeybee 1 to 1½ colony of midium size has to be kept at the boundry of seed production plot to boost the amount of crossing. The parental lines i.e. male parent maintained by selfing (mixed pollination) and rouge out undesirable plants before contaimation take place. The female lines i.e. gynoecious lines maintained by inducing the staminate flower through the sprays of silver nitrate 200 ppm at two to four true leaf stage and then selfing is carried out. It was observed that 10-11 male flowers appear per 100 nodes. The gynoecious sex form have been commercially exploited in hybrid seed production of cucumber (Pusa Sanyog) at IARI R.S. Katrain and in muskmelon (MH10) at PAU, Ludhiana. But there is a challenge to transfer gynoecious trait from exotic gynoecious lines to Indian genotypes and selection should be made for stability of gynoecious behaviour at higher temperature along with fruit quality of Indian genotypes. Even, early phenotypic detection of gynoecy in breeding lines is difficult as gynoecy is highly influenced by environment. The mode of inheritance of gynoecy and the position of the gene governing it can be analyzed by identifying a molecular marker closely linked to the gynoecy locus which can further be used in the marker assisted selection of gynoecious line.

Molecular breeding for gynoecy in bitter gourd

Identification of molecular marker(s) associated with the targeted trait has enormous potential for the crop improvement through molecular breeding. These molecular or DNA markers are free from any epistatic interactions and environmental effects so that they can be used further in marker-assisted selection (MAS) to confirm the presence of gene of interest. Another advantage of this gene targeting or gene tagging is, if the marker is closely linked to the desired locus, it can be easily cloned and then introgressed in other genetic back ground either through direct gene transfer or molecular breeding.

- One RAPD marker (OPZ 13700bp) identified linked to (gy-1) gene at 22 cM distance (Mishra *et al*., 2014) [19].
- One inter-simple sequence repeats marker (ISSR) associated with the gynoecious trait in bitter gourd. A 1000 base pair fragment specific to the gynoecious plants was amplified by the primer $(AC)_8T$ (Gaikwad *et al*., 2014) [7].
- Several SNP loci identified which are genetically linked to gynoecy in bitter gourd (GTFL-1,2,3,11,13) of which GTFL-1 is located at 5.46 cM distsnce (Matsumura *et al*., 2014) [14].
- Ethylene responsive proteins were identified as putative candidate genes for gynoecy in bitter gourd (Gunnaiah *et al*., 2014) [8].
- Two accessions of bitter gourd, gynoecious (Gy323) and monoecious (DRAR1) lines developed at Indian Institute of Vegetable Research, Varanasi, were selected for transcriptome sequencing. Based on transcriptome sequencing, maximum 2,440 SSR primers for Gyno and 2,404 for Mono were designed. Patterns of SSR variation between the Gyno Gy323 line and the Mono DRAR1line can be used to screen and develop markers to identify lines for the gynoecism trait. (Shukla *et al*., 2015) [25].
- The QTL/gene, gy/fffn/ffn, have been identified for gynoecy/ first female flower node and female flower number respectively, which control the sex expression in bitter gourd (Cui *et al*., 2018) [4].

These markers will also be helpful for studying the mechanism affecting sex expression in bitter gourd.

Conclusion

Molecular markers could be use in marker-assisted selection of plant breeding program as well as in identifying gene at any stage of the crop growth period to reduce time and cost of traditional breeding programme. The identification of more closely linked makers will lead to saturation of the map, thereby, facilitating map based cloning and introgression of gynoecy trait into commercial inbreds in bitter gourd.

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