International Journal of Environment, Agriculture and Biotechnology
Vol-8, Issue-1; Jan-Feb, 2023

Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Peer Reviewed

Application of advanced molecular to select the variety of Bitter gourd (*Momordica charantia* .L) in Can Tho

Lang Thi Nguyen*, Hieu Chi Bui, Loan Hong Thi Nguyen, Phuoc Trong Nguyen

High Agricultural Technology Research Institute (HATRI); Web:hatri.org
*Corresponding author

Received: 27 Dec 2022; Received in revised form: 25 Jan 2023; Accepted: 02 Feb 2023; Available online: 09 Feb 2023

Abstract— *A study was conducted to evaluate the genetic variation in bitter ground to using SNP (single-nucleotide polymorphism) markers. Thirty -five primers showing reliable polymorphism were used .This paper mainly applies the molecular directive from the self-absorbed population of F_6 of Cho Moi/Ben Tre. The Polymorphism on two SNP directives, TP1386 and TP 1877 with Bitter gourd on LG1. A wide variation was observed for morphological traits like the number of days to the first male flower anthesis (29.33–33.67), first female flower anthesis (30.5–38.6), fruit length (19.00–22.80 cm), fruit diameter (12.20–19.60 cm), and yield per plant (933.8–1147.9 g).According to the GGT map, it is easier to determine the genetic pattern of hybrids in the population compared to the genome of the parents in the F_6 generation of the Cho Moi/Ben Tre . With 34 SNPs (single-nucleotide polymorphism) molecules directives on LG1, the genetic distance from 0-112. cM. The selected lines carried a superior homogeneity to the parent on the LG1. The result is 7 lines with F_7 with 100% genes for hight yield the same with the father variety (Ben Tre), carrying hight yield. The seven lines selected are: 1(F2-2-1-7-1); 2(F2-8-17-2-2); 5(F2 -5-3-1-5); 7(F2-25-15-8-7); 10(F2-54-4-1-10; 35(F2-10-6-5-35); 36(F2-5-2-7-36). However, after evaluating F7 lines and comparing phenotypes and genotypes, there were only two lines: 2(F2-8-17-2-2); 7(F2-25-15-8-7)good appty for breeding and hight yield . Named line 7(F2-25-15-8-7) was designated HATRI 07KQ . DNA Sequence of HATRI 07KQ were submitted to GenBank .*

Keyword— ***Bitter gourd , genetics and breeing , GGT, SNP (single-nucleotide polymorphism) molecular, hight yield***

I. INTRODUCTION

Bitter gourd (*Momordica charantia* L.; 2*n* = 22) is an economically important vegetable crop belonging to the subtribe Thalidianthinae, tribe Joliffieae, subfamily Cucurbitoideae and family Cucurbitaceae (Jeffrey, 1980; De Wilde and Duyfjes, 2002). In bitter gourds, gynoecism is under the control of a single recessive gene (gy-1) (Ram et al., 2006; Behera et al., 2009; Matsumura et al., 2014), while two pairs of genes were reported by Cui et al. (2018). The flowering traits like days to first pistillate flower appearance, node at first pistillate flower appearance and staminate: pistillate (♂:♀) flower ratio (sex ratio) are directly related to earliness and fruit yield. Production of hybrid seeds in bitter gourd is highly expensive because it is done mainly through hand pollination. Behera et al. (2020) reported that F1 hybrid was derived from the crosses between pure-line of bitter gourd having good specific combiners for yield and its components. Valyaie et al. (2021) reported that heterosis was obtained in seed quality character and yield.

But utilization of a gynoecious line would be more economical and easier method (Behera et al., 2009). Conventional phenotypic selection for high and stable yield requires the evaluation of yield in multiple environments over several seasons; which is very expensive and time consuming (Yuan et al., 2002). Application of molecular indicator combining selection by traditional hybridization method shortens time in breeding (Lang et al 2020).QTL

analysis was performed for six major yield-contributing traits such as fruit length, fruit diameter, fruit weight, fruit flesh thickness, number of fruits per plant and yield per plant. These six quantitative traits were mapped with 19 QTLs (9 QTLs with LOD > 3) using composite interval mapping (CIM). Among 19 QTLs, 12 QTLs derived from 'Pusa Do Mausami' revealed a negative additive effect when its allele increased trait score whereas 7 QTLs derived from 'DBGy-201' revealed a positive additive effect when its allele trait score increased(Rao et al.,2021).The microsatellites i.e.SSR markers are mostly preferred because of their co-dominance, repeatability and easy transferability even though the initial cost of development of these markers is very hig (Powell, et al 1996). However, the number of microsatellite markers available in *Momordica* species is few(' Saxena, S. *et al* 2015). It is established that a greater number of markers are necessary for the development of a genetic map and marker-assisted selection(Tang et al.,2007). It is applied recently as a very reliable tool for marker-assisted selection in accelerating crop improvement program (He et al.,2014). One major QTL *qYD1* and two minor QTLs *qYD15* and *qYD20* explained 23.28% of phenotyping variation for yield per plant. The QTLs identified in the present study will be helpful in marker-assisted selection and molecular breeding in bitter gourd crop improvement.(Rao et al.,2021)

In VietNam , very limited information is available for determining nature of gene effects and inheritance of yield and yield contributing attributes in bitter gourd. For this purpose the present experiment was undertaken to breeding program with component and marker assited selection (MAS)bitter gourd for the development of high yielding variety.

II. MATERIALS AND METHODS

Plant materials

A Cho Moi was crossed with Ben Tre of bitter gourd F_7 population was developed. Ten parents were crossed to develop F1 seeds .Twenty of a single F_1 plant derived from the cross Cho moi/ Ben Tre were developed. The female flowers of these plants were bagged before flowers opened and were hand-pollinated by rubbing matured anthers of the male flowers on receptive stigma of the female flowers early in the morning. The hybridized female flowers were kept bagged until formation of visually conspicuous green ovaries. The F1 plants selfed to develop F_2 population (150), the F_2 population was selfed individually to develop 150:F3 families , plants selfed continue to F_4: $F_5$and F_6 About 50 F_6:7 seeds from each family were sown in a single row with three replications, following recommended agronomic practices.

DNA isolation and molecular marker analysis

DNA extraction. The ninety varieties were grown in pots. Maximum protection was employed to ensure healthy and disease free-growth of seedlings. Leaves were collected 2-3weeks after planting for extraction of DNA.

Standard molecular grade chemicals and general techniques for preparing stock solutions, buffers, reagents and equipment were followed according to Sambrook et al., (1989). Molecular work was conducted at the Genetics and Plant Breeding Department of HATRI , Vietnam

DNA suitable for PCR analysis was prepared using a simplified procedure according to Mc Couch et al., (1988). A piece of young rice leaf (2cm) was collected and placed in labeled 1.5ml centrifuge tube in ice. The leaf was ground using a polished glass rod in a well of a Spot Test Plate (Thomas Scientific) after adding 400µl of extraction buffer .Grinding was done until the buffer turned green, an indication of cell breakage and release of chloroplasts and cell contents. Another 400µl of extraction buffer was added into the well by pipetting. Around 400µl of the lysate was transferred to the original tube of the leaf sample. The lysate was deproteinized using 400µl of chloroform. The aqueous supernatant was transferred to a new 1.5ml tube and DNA precipitated using absolute ethanol. DNA was air- dried and resuspended in 50µl of TE buffer (Lang 2002)

DNA quality checks used 1% agarose by melting 3g agarose in 300ml TAE buffer . The mixture was heated in microwave for 5-6 minutes and then cooled to around 55-60^{0}C. This was then poured on prepared electrophoresis box with combs. Gels were ready and combs removed after about 45 minutes. Seven microliters of DNA sample plus 3µl loading buffer (Tris 1M pH = 8.0, glycerol, EDTA 0.5M pH = 8.0, xylene cyanol 0.2%, bromphenol blue 0.2% and distilled water) was run at 70-80v, 60mA for 45 minutes or until loading buffer dye moved far from the wells. Gel was then taken out and stained with ethidium bromide after which was visualized under UV light.(Lang 2002)

Microsatellite Analysis

The whole microsatellite analysis included PCR assay, polyacrylamide gel electrophoresis, band detection and scoring.

PCR assay. Microsatellite primers were used to survey polymorphism on the samples. These were randomly selected from the 34 microsatellite primer pairs currently available for bitter groud(Rao et al., 2021). The PCR reaction was as follows:

Reactions were overlaid with mineral oil and processed in a Programmable Thermal Controller

programmed for 35 cycles of 1 min at 94^0C, 1 min at 55^0C and 2 min at 72^0C, with a final extension at 75^0C for 5 min. After amplification, 10µl of stop solution was added to the PCR product which was then denatured at 94^0C for 2 min. Eight microliters of each reaction were run on polyacrylamide gel.

***Band detection and scoring*.** Plates were separated using a plastic wedge and removed from the tank. The acrylamide gel was soaked in ethidium bromide staining solution for 15 to 20 minutes. Bands in the ethidium bromide-stained gels were detected and photographed under UV light. Allelic bands were scored as 1 or 0 for presence or absence, respectively. Data were entered directly into an Excel spreadsheet.

Data Analysis

Analysis of variance. The agro-morphological data collected were initially analyzed through analysis of variance to verify genetic variation in the traits measured. The few traits with insignificant genetic variation, based on the F-test, were not considered for further analyses.

Recording of trait data

Fully matured unripe fruits were picked from each F_7 plant over the duration of fruit production. Some fruits were studied in situ and left to ripen for collection of seeds. Qualitative traits including fruit color were recorded visually.

-Hight plant (m): The Hight of the plant was measured in meters from the ground level to the tip of the vine of plants

-Days to first male flowering: The number of days taken from sowing of seed to the opening of first male flower on the plant was recorded as days to first male flowering and number of days taken from sowing of seed to the opening of first female flower on the plant was recorded asdays to first female flowering.

-Days to first female flowering: The number of days taken from sowing of seed to the opening of first female flower on the plant was recorded as days to first female flowering.

-Fruit length (cm) and fruit diameter(cm): The observations regarding fruit length and diameter were measured from five fruits, randomly selected from each treatment, at second, fourth and sixth pickings, respectively. Five randomly selected fruits were taken from the harvested fruits in each replication when

it reached edible maturity. The fruits were cut longitudinally and length was measured with the help of a measuring tape and fruit diameter was measured with digital Vernier calipers.

-Number of fruits per plant: Number of edible fruits was counted at each picking and summed up for all the pickings for aplot. Number of fruits per plant was calculated after dividing total number of fruits in a plot by total number of plants in a plot.

GGT mapping assesses the genetic diversity of hybrid populations

(1) Genotype testing of hybrid populations on LG1 is based on polymorphic molecular markers between parent and parent plants. GGT mapping assesses the genetic diversity of hybrid populations, thereby selecting individuals carrying the desired target gene The GGT method proposed by Tanksley et al. (1998) and, Van Berllo (2008), Milne et al. (2010) built this useful software. GGT 2.0: "graphical genotyping" is a new method developed by the authors of Wageningen University, where alleles express dominant contraction, recessive contraction, and heterozygosity in all hybrids in a population, allowing the selection of individuals to gather the desired genes in the most effective way. GGT mapping method through the following steps: Excel data file: encoding the population gene with A, B being the homozygous genotype of the parent tree; H is a heterozygous genotype; Tumors are unidentified genotypes; (2) Import data into the GGT window: convert Excel data to GGT data; (3) data processing in GGT.

III. 3. RESULT OF DISCUSSION

3.1. Development of Bitter gourd hybrid populations

3.1.1.Diversity of gene sources on parent Bitter gourd varieties from (Cho Moi/ Ben Tre) The selection of peanut varieties using MAS has brought certain successes in recent times such as: shortening the selection time, selecting varieties resistant to adverse conditions, disease-resistant varieties, quality varieties. Therefore, the parent Bitter gourd varieties (Cho Moi and Ben Tre,) were genotype-analyzed to consider carrying hight yield genes, and at the same time, molecular markers of genes related to yield and yield components. Sixty-nine molecular directives were used to assess genetic diversity between parent Bitter gourd varieties, but only 34 for polymorphisms with parents: including 2 molecular TP 1386 and TP 1877 directives marked genes that regulate the hight yield .PCR production detected parent varieties (with gene markers involved 16 marker TP 5296, TP5205,TP5058,TP3064,TP3003,TP2693,TP2480,TP2443 . TP2345. TP2313, TP2199,TP1992, TP1877, TP1459, TP 1386 , TP 1323 on polyacrylamide gel with silver nitrate staining(figure 1)

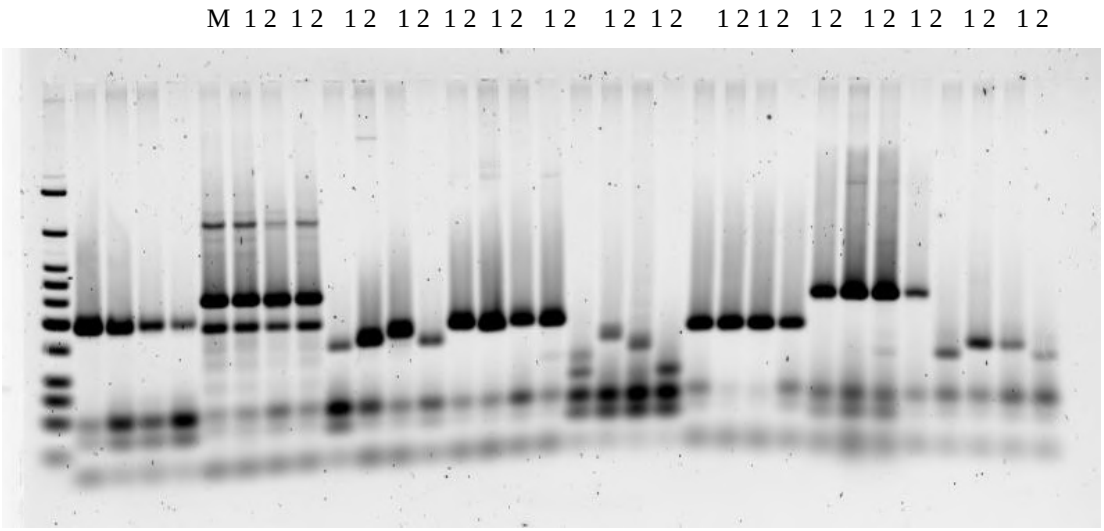

Fig.1: PCR production detected parent varieties (with gene markers involved 16 marker TP 5296, TP5205,TP5058,TP3064,TP3003,TP2693,TP2480,TP2443. TP2345. TP2313, TP2199,TP1992, TP1877, TP1459, TP 1386 , TP 1323 on polyacrylamide gel with silver nitrate staining.

Notes : M. DNA lamda , 1: Cho Moi and 2: Ben Tre.

3.1.2. Development of hybrid populations :The parent seeds were planted at the HATRI Institute's green house and resulted in 45 F_1 plants. Twelve one plants identified as hybrids actually carry both heterozygous alleles from their parents due to to molecular directives. These twelve one F_1 are used to F_2. Of the 150 F_2 Plants, thirty-two were found to carry both parent alleles in heterozygous condition. Generation F_1, F_2, F_3, F_6 were genotyped with markers, and the last four were identified as homozygous for both alleles with large particle sizes (Figure 3, Figure 4,). According to Rao et al. (2021), the nuclear indicator gene on Bitter gourd is controlled by the hight yield localized on LG 1 associated with TP1877 andTP 1459 , and TP1386. Three marker , TP1877 and TP 1459 , and TP1386 are therefore used to test genes associated with hight yield .

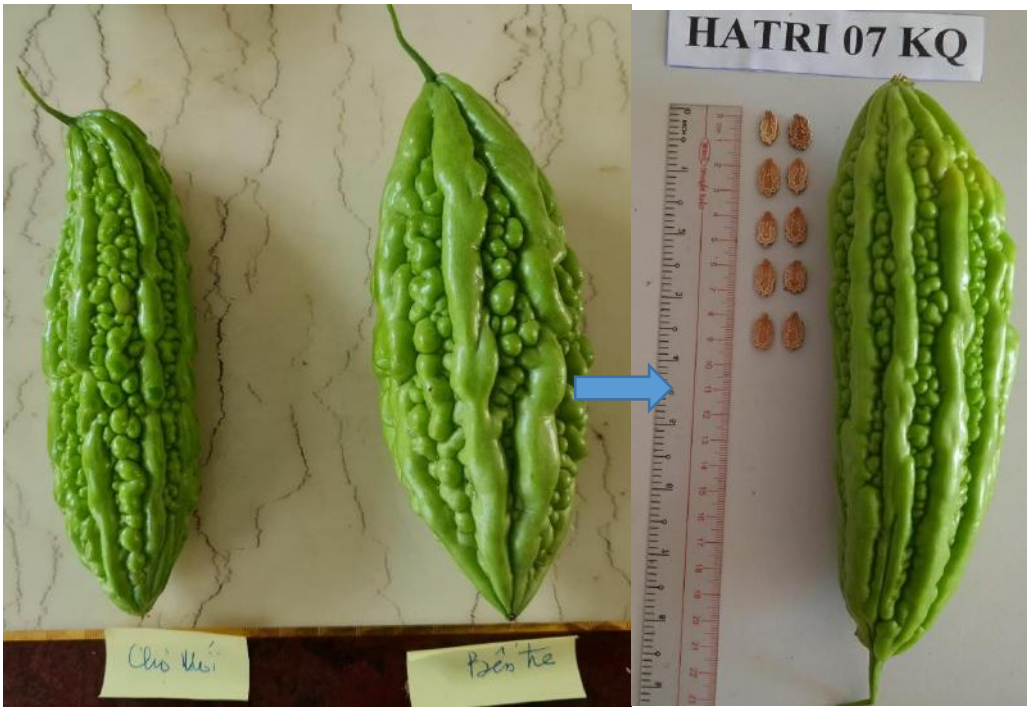

Fig.2: Cho Moi and Ben Tre for parents and F7 generation hybrids: named HATRI 07KQ

3.1.3. Application of molecular marker on F_3 populations

Closely LG1 with marked by molecular marker TP1386. This gene is associated with the particle size group according to (Rao et al. (2021). The TP1386 molecular was used on the F3 population to evaluate and select the high yield of bitter ground. DNA is extracted from a segregating population derived from a cross of Cho Moi/ Ben Tre. Marker TP1386 has a size (300-350bp)detected . In the F3 population with a common parent and the same population size of 50 plants were also compared (Figure 3). The segregation pattern (Figures 3) . The results showed that there were 4 lines the same with Ben Tre (1,29,37 and 50) . and 20 lines the same with size marker Cho Moi (14,15,16,24,25,26,27,28,30,31,32,33,34,35,36,37,38,40,41, 42(300 bp) . Other individuals carry heterozygous genotypes of the same size as their parents(300-350bp).

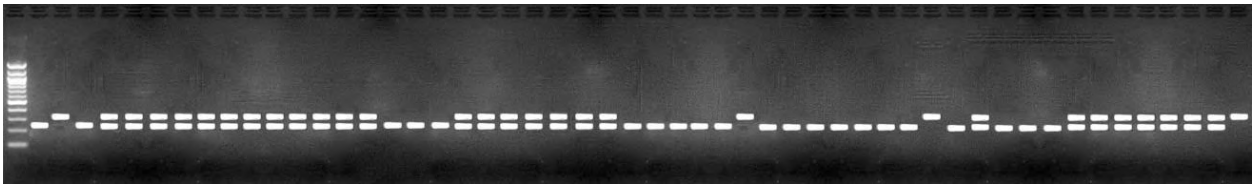

Fig.3: PCR product amplified from DNA extract F_3 population . The primers are TP 1386 with two bands position 300bp (Cho Moi) and 350bp (Ben Tre), on 3% agarose gel.

Note: M: molecure weight marker; P1: Cho Moi , P2: Ben Tre , 1-50 is F3

Similar to the results of the PCR product in Figure 4 with the molecular directive TP 1877 recorded from the population Cho Moi/ Ben Tre give 6 homozygous plants with the allele of the product such as Ben Tre variety.The results of the PCR product show that there are 6 individuals at positions 1,2,5,7 ,10 and 26 of the same size as Ben Tre corresponding to the size of 210bp. There are 17 individuals in position 31,32 ,40,41,42,44,45,46,48,49,50 of the same size as Cho Moi with 250 bp. Other individuals carry heterozygous genotypes of the same size as their parents(210-250bp). Thus, two molecular guidelines (TP 1877) on population show that the F_3generation is still quite strongly dissociated by 26-60% for the two molecular directives above in order.

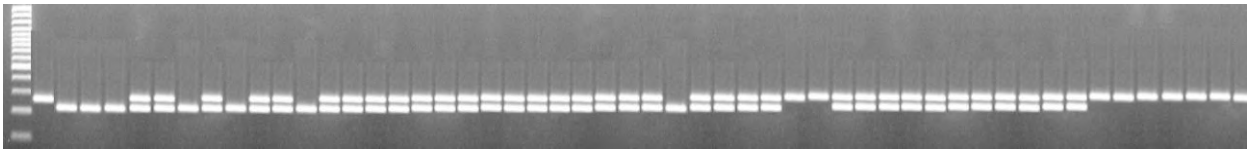

Fig.4: PCR product of the molecular TP 1877 on 50 Lines of gene on LG 1, two bands position 250 bp (Cho Moi) and 210bp (Ben Tre), on 3% agarose gel.

Note: M: is the standard marker; P1: Cho Moi , P2: Ben Tre , 1-50 is F_3

3.3. Selection of populations through GGT mapping

3.3.1. Selection of F_6 individuals of hybrid populations

The F_5 population of the Cho Moi/ Ben Tre hybrid pair gives continued self-absorption and F_6 generation selection. In the F_6 population, 27 lines were selected for genotype assessment through the chromosome LG1 map on each GGT (Graphical genotyping), which is a method that allows the expression of dominant, recessive, heterozygous alleles of a population. The GGT map makes it easier to identify the genetic pattern of hybrids in the population compared to the gene segment compared to the parent. GGT map is built on the Cho Moi/ Ben Tre platform in the F_6 generation. On this map, the gene that regulates particle size traits is marked by 22 molecular indicators on LG1, the migration distance.

The GGT map in Figure 5 shows that 7 individuals have 100% of the gene regions that coincide with the father (Ben Tre), carrying the target gene hight yield . Selected individuals are 1(F2-2-1-7-1); 2(F2-8-17-2-2); 5(F2 -5-3-1-5); 7(F2-25-15-8-7); 10(F2-54-4-1-10); 35(F2-10-6-5-35); 36(F2-5-2-7-36). This noted that on 7 generation lines selected from F_2 to F_7, there was a homogeneity of large and small particle sizes on these lines. Particularly, two individuals 41(F2-7-2-9-41) recorded above accounted for 95.2% of the large content of Ben Tre . This individuals continue to choose the F_8 generation to continues in breeding.

3.3.2.Phenotypic evaluation of the Cho Moi/ Ben Tre :the F_6 population continued lines after evaluation by molecular directive continued planting for F_7 generation dissociation,particle size of selected lines in the field on the

basis of the generation analyzed phenotype. In the generations that continued to selected the F_7generation, the indicators hight plant, days to first male flowering, days to first female flowering, fruit length, fruit diameter and number of fruits per plant were statistically significant. The tallest variety was 2(F2-8-17-2-2) (231.5 cm) and the shortest was 10(F2-54-4-1-10) (165.3 cm). For Days to first male flowering , the longest was of controlled parents trial (14.5-24.5 d) followed Days to first female flowering (19.5-28.8 d). The length(19-22.8 cm) and fruit diameter (12.2-19.6cm) .The yield of all the lines derived Cho Moi/Ben Tre were hight yield to the yield of the standard checks with parents . At Cai Răng , with eight F_7 and two checks from parents were evaluated in three replications . The highest yield was obtained from line 7(F2-25-15-8-7) (1147.9g/plant) next line 2(F2-8-17-2-2) (1145.8g/plant).

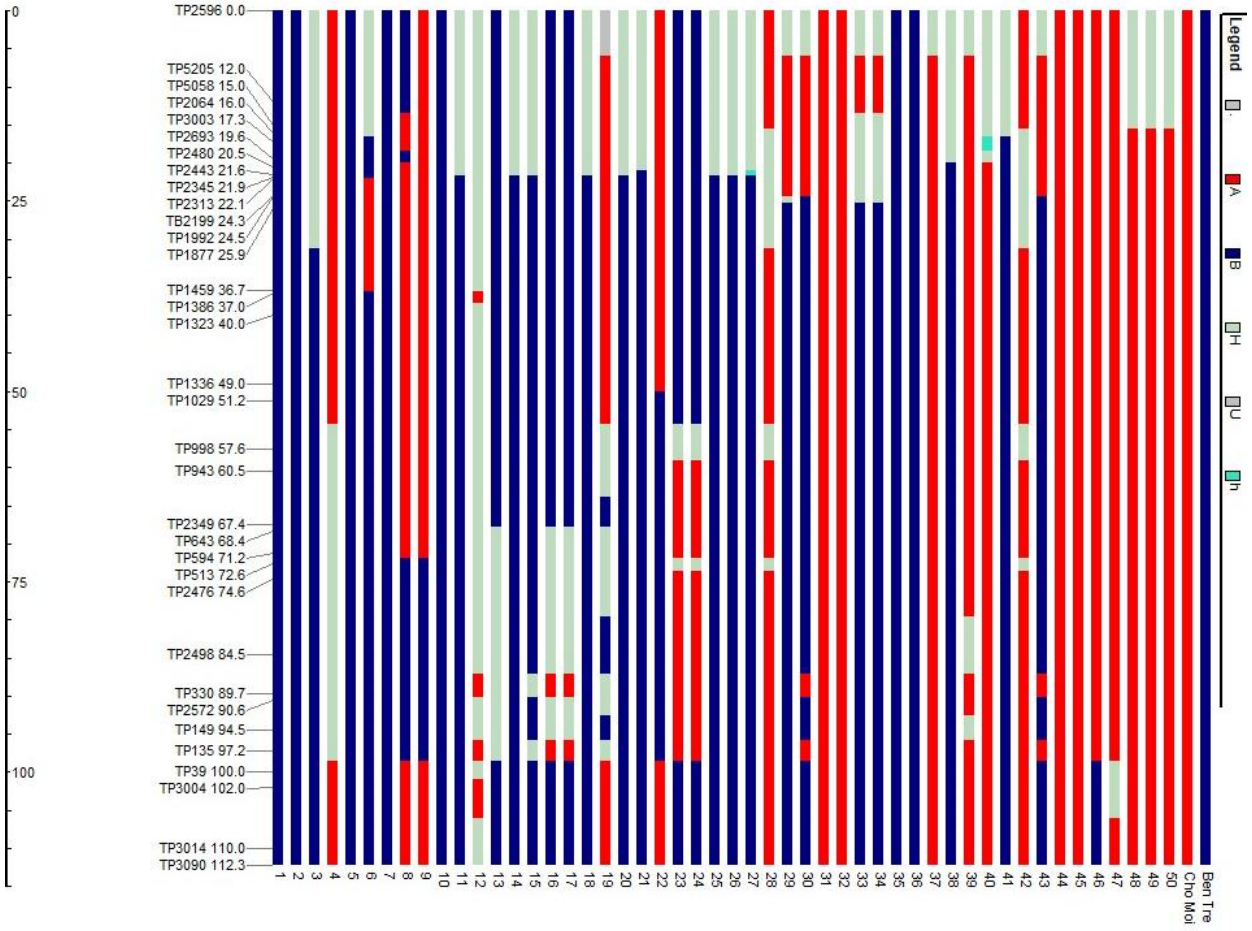

Fig.5: A. Generation F7 genetic diversity in hybrid populations of Cho Moi / Ben Tre on F7 of bitter grounder

Note: blue: genotype according to the parent (Ben Tre), red: genotype according to the parent plant (Cho Moi), gray: heterozygous genotype, greencolor : marking selected individuals, 1-50: individuals of the Cho Moi/ Ben Tre population from F6. Figure 5.B.

Table 1: Yield and component yield on bitter ground at CaiRang (CanTho)

Bitter groud	Hight plant(cm)	Days to first male flowering	Days to first female flowering	Fruit length (cm)	fruit diameter (Cm)	Number of fruits per plant
1(F2-2-1-7-1)	228,8b	21,2a	32,3c	21,1a	16,2c	1140,7b
2(F2-8-17-2-2)	231,5a	29,5a	38,67a	22,8a	15,8d	1145,8a
5(F2 -5-3-1-5)	209,9c	22,9a	32,9c	21,2a	19,8a	1140,5b
7(F2-25-15-8-7)	171,7d	22,2a	30,6c	22,2a	19,7a	1147,9a
10(F2-54-4-1-10)	165,3e	19,9b	35,6b	22,0a	16,5c	1138,4c

35(F2-10-6-5-35)	227,8b	21,1b	35,9b	21,2a	13,5e	937,6d
36(F2-5-2-7-36)	222,2b	22,1b	37,8a	19,1b	12,4f	933,8d
41(F2-7-2-9-41)	221,2b	24,5c	30,5c	19,8b	12,2f	1128,0c
Cho Moi	226,5b	23,1b	38,8a	19,0b	15,4d	910,5d
Ben Tre	201,2c	16,1b	30,8c	20,9a	19,6a	1140,7b
Cv	9,2	1,1	4,9	1,22	1,38	2,62

IV. DISCUSSION

The goal of a plant variety multiplication program is to make a change and select the desired genotype(s) for cultivation or for breeding purposes. Selection of species was based on careful consideration of a number of factors including their economic importance in targeted geographical areas, nutrient density, access to genetic resources, the improvement in the status of the crop globally, and unsolved production constraints and WorldVeg's comparative advantage in solving them versus the private sector (World Vegetable Cente,2019). Bitter ground , which are a highly self-pollinating crop, require special attention in detecting, passing high yields and selecting because these processes require special skills and can be time consuming. Genetic research assists breeders in understanding the mechanism of inheritance and improving the effectiveness of a breeding program. Significant progress has been made in the genetics and plant breeding of peanuts for many years. In recent years, the discovery of polymorphic molecular directives (SNPs) in combination with developed sequencing technologies has led to a significant improvement in fine mapping processes (Rao et al., 2021) .The yield of bitter ground is genetically controlled by polygenic factors. High-resolution mapping of quantitative loci (QTLs) with linked markers can facilitate marker-assisted selection in seed selection for target characteristics. In the current study, with the population of Cho Moi crossed with Ben Tre variety for hight yield , there is an improvement. A graphical representation of molecular marker data can be an important tool in the process of selection and evaluation of plant material. A computer program was developed that enables representation of molecular marker data by simple chromosome drawings in several ways. Commonly used marker file types that contain marker information serve as input for this program, which was named 'GGT' (an acronym of Graphical GenoTypes)(http://www.dpw.wau.nl/pv/pub/ggt/ www.plantbreeding.nl (in prep)2007. Besides representation, GGT can also be used for a diverse range of selections and analyses.Used to build GGT maps based on the 34-loci molecular directive and extending the length of 112 cM. The GGT map in Figure 5 shows that 7 individuals have 100% of the gene regions that with the father (Ben Tre variety), carrying hight yield . Selected individuals are two lines 7(F2-25-15-8-7) (1147.9g/plant) next 2(F2-8-17-2-2) (1145.8g/plant) apply in the future. Named line 7(F2-25-15-8-7) was designated HATRI 07KQ . DNA Sequence of HATRI 07KQ were submitted to GenBank .

This noted that over 4 generation lines selected from $F_3$to F_7 there was a homogeneity of large and medium sizes on these lines. Molecular indicators are marked and developed targeting marker loci associated with varieties for good dissociation and high heterozygous rates are TP 1877 . The hight yield and quality are important for bitter ground selection and production, so a more mechanical understanding of shell development and seed maturation will be conducive to improving these characteristics.

V. CONCLUSION

Selected hybrid individuals must carry the dominant homologous gene on the corresponding chromosome region obtaining 7 lines containing 100% of the gene region that with the father (Ben Tre), carrying the target gene hight yield . A wide variation was observed for morphological traits like the number of days to the first male flower anthesis (29.33–33.67), first female flower anthesis (30.5–38.6), fruit length (19.00–22.80 cm), fruit diameter (12.20–19.60 cm), and yield per plant (933.8–1147.9 g). With 34 SNPs (single-nucleotide polymorphism) molecules directives on LG1, the genetic distance from 0-112. cM. The selected lines carried a superior homogeneity to the parent on the LG1. The result is 7 lines with 100% genes for hight yield the same with the father variety (Ben Tre), carrying hight yield. The seven lines selected are: 1(F2-2-1-7-1); 2(F2-8-17-2-2); 5(F2 -5-3-1-5); 7(F2-25-15-8-7); 10(F2-54-4-1-10; 35(F2-10-6-5-35); 36(F2-5-2-7-36). However, after evaluating F_7 lines and comparing phenotypes and genotypes, there were only two lines: 2(F2-8-17-2-2); 7(F2-25-15-8-7)good appty for breeding and hight yield . Named line 7(F2-25-15-8-7) was designated HATRI 07KQ . DNA Sequence of HATRI 07KQ were submitted to GenBank .

ACKNOWLEDGEMENTS

The authors would like to thank the Provincial People's Committee and Can Tho Department of Science and Technology for providing funding to implement this project.

REFERENCES

[1] Behera, T. K., Dey, S. S., Munshi, A. D., Gaikwad, A. B., Pal, A., and Singh, I. (2009). Sex inheritance and development of gynoecious hybrids in bitter gourd (*Momordica charantia* L.). *Sci. Hort.* 120, 130–133. doi: 10.1016/j.scienta.2008.09.006

[2] BEHERA, T.K., DEY, S.S., DATTA, S. and KOLE, C., 2020. Classical genetics and traditional breeding. In: C. KOLE, H. MATSUMURA and T. BEHERA, eds. *The Bitter gourd genome: compendium of plant genomes*. Switzerland: Springer, pp. 45-59. http://dx.doi. org/10.1007/978-3-030-15062-4_4.

[3] De Wilde, W., and Duyfjes, B. (2002). Synopsis of *Momordica* (Cucurbitaceae) in SE-Asia and Malesia. *Bot Z.* 87, 132–148.

[4] Jeffrey, C. (1980). A review of the Cucurbitaceae. *J. Linn. Soc. Bot.* 81, 233–247. doi: 10.1111/j.1095-8339.1980.tb01676.x.

[5] He, J. *et al.* Genotyping-by-sequencing (GBS), an ultimate Marker-Assisted Selection (MAS) tool to accelerate plant breeding. *Front. Plant Sci.* 5, 484 (2014).

[6] Milne, I., P. Shaw, G. Stephen, M. Bayer, L. Cardle, W.T.B. Thomas, A. J. Flavell, and D. Marshall. 2010. Flapjack—graphical genotype visualization. Bioinformatics 26: 3133–3134.

[7] Nguyễn Thị Lang (2002), *Phương pháp cơ bản trong nghiên cứu Công nghệ sinh học*, NXB Nông Nghiệp, TP.HCM.

[8] N.T. LANG[1*], N.T. PHUOC, B.H. THUAN and B.C. BUU.2020.BREEDING RICE (*Oryza sativa*. L) FOR SALT TOLERANCE AND GRAIN QUALITY TRAITS. SABRAO Journal of Breeding and Genetics 52 (3) P326-340, 2020.

[9] Powell, W., Machray, G. & Provan, J. Polymorphism revealed by simple sequence repeats. *Trends Plant Sci.* 1, 215–222 (1996).

[10] Rao.P. Gangadhara , T. K. Behera, Ambika B. Gaikwad, A. D. Munshi, Arpita Srivastava, G. Boopalakrishnan & Vinod 2021. Genetic analysis and QTL mapping of yield and fruit traits in bitter gourd (*Momordica charantia* L.)*Scientific Reports* volume 11, Article number: 4109 (2021) .

[11] Saxena, S. *et al.* 2015.Development of novel simple sequence repeat markers in bitter gourd (*Momordica charantia* L.) through enriched genomic libraries and their utilization in analysis of genetic diversity and cross-species transferability. *Appl. Biochem. Biotechnol.* 175, 93–118 (2015).

[12] Tang, R. *et al.* Genetic diversity in cultivated groundnut based on SSR markers. *J. Genet. Genom.* 34, 449–459 (2007).

[13] Yuan, J., Njiti, V. N., Meksem, K., Iqbal, M. J., Triwitayakorn, K., Kassem, M. A., et al. (2002). Quantitative trait loci in two soybean recombinant inbred line populations segregating for yield and disease resistance. *Crop Sci.* 42, 271–277. doi: 10.2135/cropsci2002.2710.

[14] VALYAIE, A., AZIZI, M., KASHI, A., SATHASIVAM, R., PARK, S.U., SUGIYAMA, A., MOTOBAYASHI, T. and FUJII, Y., 2021. Evaluation of growth, yield, and biochemical attributes of bitter gourd (*Momordica charantia* L.) cultivars under Karaj conditions in Iran. *Plants*, vol. 10, no. 7, pp. 1-19. http://dx.doi.org/10.3390/ plants10071370. PMid:34371573.

[15] Van Berloo, R. 2008. GGT 2.0: Versatile software for visualization and analysis of genetic data. Journal of Heredity 99(2): 232–236. (Available online at: http://dx.doi.org/10.1093/jhered/esm109) (verified 29 Dec 2010).

[16] Young, N. D., and S. D. Tanksley. 1989. Restriction fragment length polymorphisms maps and the concept of graphical genotypes. Theoretical and Applied Genetics 77(1): 95–101. (Available online at: http://dx.doi.org/10.1007/BF00292322) (verified 29 Dec 2010).

[17] http://www.dpw.wau.nl/pv/pub/ggt/ www.plantbreeding.nl (in prep).2007.

[18] World Vegetable Center. *International Vegetable Breeding: A Strategy to Create Development Impact at Scale*; Publication No. 19-838; World Vegetable Center: Shanhua, Taiwan, 2019. [**Google Scholar**]