Journal of Tropical Agriculture 59 (1): 113-117, 2021

Short communication

Cross-genera transferability of microsatellite markers from Brassicaceae, Solanaceae and Cucurbitaceae to *Momordica* genus

Lavale Shivaji Ajinath[1*], Kelkar Vipul Ganesh[1], Thoppurathu Feba Jacob[1], T. Pradeepkumar[2], Jiji Joseph[2] and Deepu Mathew[1]

[1]*Centre for Plant Biotechnology and Molecular Biology, Kerala Agricultural University, Thrissur 680 656, Kerala, India*

[2]*College of Agriculture, Kerala Agricultural University, Thrissur 680 656, Kerala, India*

Received 28 July 2020; received in revised form 22 March 2021; accepted 23 March 2021

Abstract

Bitter gourd, being a rich source of phytonutrients, holds a special value in dietary habits of the consumers. Genetic improvement of this crop by inclusion of wide array of genes through inter-specific and inter-generic breeding approaches is substantially important. Microsatellites are extensively used by breeders as a molecular tool for mapping and introgression of qualitative and quantitative traits into the crop plants. This study was conducted to identify the microsatellites from Brassicaceae, Solanaceae and Cucurbitaceae family that can be efficiently used in *Momordica* genus. A total of 182 microsatellites from above families were studied for their amplification in two sub-species of *Momordica charantia*. Thirty one microsatellites were successfully amplified in bitter gourd producing 32 different alleles. The identified transferable microsatellites are useful in studying the relationships between these families and for mapping genes/QTLs in bitter gourd.

Key words: Cucurbit, Inter-genera transferability, Molecular markers, QTL, SSR.

Bitter gourd (*Momordica charantia* L.), a cucurbitaceous vegetable, is widely cultivated in Asia, Africa, and the Caribbean for medicinal purpose (Grover and Yadav, 2004; Marr et al., 2004; Van Wyk, 2015). It is a rich source of phytonutrients such as carbohydrates, essential minerals, and vitamins. The yellow fruit pulp and arils are high in carotenoids, iron, phosphorus and ascorbic acid (Behera et al., 2010). Enhanced yield, tolerance to fruit fly and mosaic virus, near-white fruit colour, higher female to male flower ratio, smoother ridges on the fruits and lower momordicin are the major breeding objectives in this crop. However, crop improvement attempts in bitter gourd are limited.

Molecular markers assist breeders to track the genes and quantitative trait loci, leading to efficient marker assisted selection. Microsatellites (simple sequence repeats, SSRs) are the repeated regions in the genome, differentially evolved in each genome due to the slippage during DNA replication. These regions associated with each allele of any gene may vary in length and this variation could be used in the development of markers named microsatellite markers, which could be well used for mapping the genes and QTL. Due to their co-dominant nature and universality in eukaryotic genomes, they are proved to be the most useful molecular markers in population genetics (Walter and Epperson, 2001). The success of MAS depends on highly saturated genetic linkage map and mapping of quantitative trait loci (QTL) for the target traits. Higher number of molecular markers spanning the entire genome is an absolute pre-requisite of linkage mapping. The

*Corresponding author: Phone: 9980665441, E-mail: shivajiraje3386@gmail.com

cost and extent of research efforts required are major obstructions for development of microsatellites. Limited attempts have been made for the development of microsatellite markers in bitter gourd (Wang et al., 2010; Ji et al., 2012; Saxena et al., 2015; Cui et al., 2017).

During the evolutionary events of crop plants, certain genome structures are conserved across genera as reported in several plant families (Bonierbale et al., 1988; Langercrantz, 1998; Ahn and Tanksley, 1993). This homology is observed using microsatellite markers as anchor points by inter-specific (Kong et al., 2007; Saxena et al., 2015; Wu et al., 2016) and inter-generic (Katzir et al., 1996; Chiba et al., 2003; Kong et al., 2007) amplification in cucurbits. These markers can be efficiently used for broadening the genetic base of crop plants. Such inter-generic microsatellite markers are shown to be efficient for the mapping of horticultural traits in bitter gourd (Wang and Xiang, 2013). The objective of this study was to test the transferability of microsatellite markers from *Luffa cylindrica*, *Cucumis melo*, *Cucumis sativus*, Chinese cabbage and *Capsicum* spp, for efficient use in bitter gourd breeding programs.

Two bitter gourd genotypes, one each from *Momordica charantia* var. *charantia* (cv. Priyanka, from Regional Agricultural Research Station, Kerala Agricultural University, Tiruvalla) and *Momordica charantia* var. *muricata* (from Regional Station, National Bureau of Plant Genetic Resources, Thrissur, India) were used to evaluate the transferability of microsatellite markers. Seeds of each accession were germinated in polybags in the greenhouse. After the emergence of 4-5 leaves, the plantlets were transplanted in the field.

Genomic DNA was isolated from the emerging leaves using the cetyl trimethyl ammonium bromide (CTAB) method (Dellaporta et al., 1983). The quality and quantity of the extracted DNA were assessed using NanoDrop spectrophotometer and agarose gel electrophoresis. The samples were diluted with TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) to 50 ng/µL and stored at -20 °C.

Isolated DNA was subjected to polymerase chain reaction using 182 microsatellite markers including 127 EST-SSRs from *L. cylindrica* (Wu et al., 2016). We chose the markers that (1) had previously amplified in *L. cylindrica* and *L. acutangula* (2) were polymorphic, and (3) co-dominant in nature. Other markers included 23 SSRs from *C. melo* (Chiba et al., 2003), 10 EST-SSRs from *C. melo* (Kong et al., 2007), 7 EST-SSRs from *C. sativus* (Kong et al., 2006), 3 EST-SSRs from Chinese cabbage (Xin et al., 2006), 12 SSRs from *Capsicum* spp. (Lee et al., 2004; Minamiyama et al., 2006). The microsatellite primers from all sources other than *L. cylindrica* were already reported to be polymorphic in bitter gourd (Wang and Xiang, 2013).

PCR amplification was carried out with 20 µL reaction mixture having 1 µl of 50 ng/µL DNA, 2 µL *Taq* assay buffer (10X), 1.5 µL dNTPs (10mM), 1 µL forward and reverse primer (10 mM) and 1 unit Taq polymerase enzyme (GeNei, India). Amplification was performed with the following thermal cycle profile: initial denaturing at 95° C for 2 min., followed by 36 cycles of the polymerization reaction, each consisting of denaturation at 95° C for 30 sec., annealing at 55° C for 45 sec and an extension step at 72° C for 2 min. A final extension step was run for 10 min. at 72° C. The PCR was performed using thermocycler (ProFlex, Life Technologies). PCR products were electrophoresed on 2-4 % agarose gel at 4 V/cm for 1 h. and visualized under UV light.

Cross-transferability was determined from the presence or absence of bands on agarose gels of PCR products. Since bitter gourd is diploid, a successful microsatellite marker is supposed to generate only one band in any homozygous locus and two bands in heterozygous locus, at the expected product sizes. Primer combinations generating more than two bands and combinations generating product size at unexpected range were

Table 1. Summary of response to thermal cycling by 182 SSR primer combinations

Response of the primer combination	Number of primers showing the response	Percentage
Failed to amplify the marker	151	82.97
Successfully amplified	31	17.03
Polymorphic	1	

considered unsuccessful.

A total of 31 primer pairs (17.03 %) generated amplification, while there was no amplification in the remaining 151 microsatellites (82.97 %) (Table 1). The 31 amplified SSRs included 18 from

Table 2. Source, transferability to bitter gourd and polymorphic status of the SSR primer combinations studied

Source of the primer combination	Number of SSR primer combinations studied	Number of SSR primer combinations successful to amplify the marker in bitter gourd	Number of polymorphic SSRs
Luffa cylindrica	127	18	1
Cucumis melo	33	8	0
Cucumis sativus	7	2	0
Chinese cabbage	3	2	0
Capsicum spp.	12	1	0

Table 3. Details on the SSR primer pairs successful in *Momordica charantia*

Source species	Marker code	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)	AT (°C)	Number of alleles	Transferability*
Luffa cylindrica	SGJ745	TCTCTGAACAAACCCCAACC	GCCGTTTTGCTGTTGATTTT	275	55	1	±
	SGJ753	GGATCGATTCCCTTCACGTA	CCTTGCCCTTCTGTTTTGAA	310	55	1	† , ±
	SGJ774	CTGGAAAAAGGGCAAAAGAA	TGGCCCATGGTTCATCTTAT	230	55	1	±
	SGJ777	CACTGCCAACCAGATTCAGA	TCATCTGGGTCCTCCTGTTC	300	55	1	† , ±
	SGK922	CGGGTTGGAGTCTATGCAGT	CCCTCTTGCTTTTGCTGTTC	180	55	1	† , ±
	SGK992	GGCTGATGGAGACATTTCGT	GACAAACAAGCTGAGACCCC	133	55	1	† , ±
	SGK1005	GCAGAAGAGCGTCCAAGTTC	GTTTCTCTCTCCCTGCCCTT	230	55	1	† , ±
	SGK1031	GCTGTTGCTGTTGCTGTTGT	CCAACAGGCCTGCTACTTTC	220	55	1	† , ±
	SGK1041	AGCTTTTCTAGGAGTCCGCC	GCCATTGACGACAATTCCAT	235	55	1	† , ±
	SGK1046	TCTGTTTGTGGCCATGAGAG	GCTTTGCTGATGATGTCTGG	460	55	1	† , ±
	SGJ663	GAAGAAGGAACAGAGGCGTG	CCCCCTGAAATTTCTTCTCC	120-130	55	2	† , ±
	SGJ669	CACACCAAATTCAAACCCAG	CAAACCCCAAATAAACGAACA	175	55	1	† , ±
	SGJ679	GGTCCCCAGTCAGTCATCTC	GGCATCCCTTAAGCTCCTTC	180	55	1	† , ±
	SGJ690	ATCGGTCGTTGGTTGATCTC	ATCAGACAGCCACTGCTCCT	140	55	1	† , ±
	SGJ696	TTGATCACTGAAATGCCTGC	CTTGCCAGATAGAAACCCCC	295	55	1	† , ±
	SGJ697	GCCGATCATAACAGAGGGAA	CACATTTGGAACTCGAGGCT	450	55	1	†
	SGJ700	AACACCATTTTGAAAGGCCA	TACAAATTCCCAAATCCCCA	455	55	1	† , ±
	SGJ726	AATCCTTCAACGACCATTCG	CAGGTGCATGAATTTTGGTG	245	55	1	† , ±
Cucumis sativus	cs37	AGTGGCCAACTCTCGATGAT	TGCTTCCACTGGGTTCTTCT	260	55	1	† , ±
	cs50	ACGGCTTCCATTAACACCTG	AAGCTTCAATGGCTTCCTCA	200	55	1	† , ±
Chinese cabbage	p004	TGCTTGCAGAAAGACGAACA	TTCTTAGTGTCAACCAGGCG	220	55	1	±
	p008	AGATTACTGGAGAAGCCGCC	AGAAGGAGCTCTTGTGAGCG	360	55	1	† , ±
Capsicum spp	cams-424	TAGCAGCAGCTGATGGAGAA	CCTTCTTCTTTGCCACCTTC	200	55	1	† , ±
Cucumis melo	cm04	CATGGCGATGTTTTCTTTCA	AAGGGAAAATTTTGGAAGTGG	350	55	1	† , ±
	cm09	GTCAAAAGCATCAGCAGCAA	CAAGTTAGGCAAACCCCAAA	400	55	1	† , ±
	cm17	CCTTCATCATCATCATCGTCA	GACCGGCAGTGGACATAGTT	150	55	1	† , ±
	cm47	ACTTTGAATCCTCCGCTCCT	TGCATGAGACCTTGTGGAAG	200	55	1	† , ±
	cm53	CTGCCGTGAAGGAGAAGAAC	AGCCTCAATCCCCAATCTCT	400	55	1	† , ±
	CMMS 30-3	TTCCCACCAGCCCAACGGACACACT	GAGATACAGAAACGACGACTAACCT	175	55	1	† , ±
	CMMS34-8	TTTCTTACTTTTTGGTTTGGTTCTG	GGCGCTGTGGTGAGTGTCGGGAGAG	200	55	1	†
	CMMS36-2	CCACACATTACAAACTAAACAAACA	CGATTCCGATTTGGTGTGGCGTTTT	155	55	1	† , ±

AT: Annealing temperature

*Transferability: marker loci were considered transferable to a given species if bands are revealed upon gel electrophoresis

† *Momordica charantia* var. *charantia*, ± *Momordica charantia* var. *muricata*

L. cylindrica, eight from melon, two from cucumber, two from Chinese cabbage and one from *Capsicum* sp. (Table 2). Only one marker (SGJ663 from *L. cylindrica*) was polymorphic between the two bitter gourd genotypes. As the number of genotypes used for microsatellite amplification was less, there was limited scope of finding polymorphic markers. However, the number of amplifiable and transferable markers was higher than the earlier studies of transferability of microsatellites in various genera (Chiba et al., 2003; Kuleung et al., 2004; Ekue et al., 2009; Azevedo et al., 2012; Santos et al., 2014; Bombonato et al., 2019).

The microsatellite markers used in this study were from other species like *L. cylindrica*, *C. melo, C. sativus*, Chinese cabbage, and *Capsicum annuum*. Fifty-five markers among these were earlier been used in mapping of horticultural traits in bitter gourd where they were successfully amplified and found polymorphic (Wang and Xiang, 2013). However, in our study, only 13 microsatellites were amplified. This is suggested to be due to single or few nucleotide polymorphisms at the annealing site of the primer between our genotypes and genotypes used by Wang and Xiang (2013).

A total of 32 alleles, ranging from 120 to 460 bp in size, were amplified. Thirty one out of 182 SSRs studied were transferable to *Momordica* species, while 151 primer pairs failed to amplify in any of the two genotypes. Twenty-six of these 31 primer pairs gave amplification products in both the *Momordica* sub-species whereas five primer pairs gave amplification in only one of the two species included in the study (Table 3). Most of the markers used in our study were derived from ESTs. The genomic SSRs were found to have more discriminating power as compared to EST-SSRs (Zhang et al., 2014; Parthiban et al., 2018). Among the 182 microsatellites studied, 147 microsatellites were derived from ESTs from different genera (Xin et al., 2006; Kong et al., 2006; Kong et al., 2007; Wu et al., 2016). This may be the possible reason for the lower amplification success and polymorphism.

The identified markers suggest the evolutionary links between these very important vegetable crops and form the basis for investigations on evolutionary relationships between them. These markers can be exploited for widening the genetic base of bitter gourd through inter-generic breeding approach as well as for mapping genes/QTLs in bitter gourd.

References

Ahn, S. and Tanksley, S.D. 1993. Comparative linkage maps of rice and maize genomes. Proc. Natl. Acad. Sci. USA, 90: 7980-7984.

Azevedo, A.L.S., Costa, P.P., Machado, J.C., Machado, M.A., Vander P.A., and da Silva Ledo, F.J. 2012. Cross species amplification of *Pennisetum glaucum* microsatellite markers in *Pennisetum purpureum* and genetic diversity of napier grass accessions. Crop Sci., 52(4): 1776.

Behera, T.K., Behera, S., Bharathi, L.K., John, K.J., Simon, P.W., and Staub, J.E. 2010. Bitter gourd: botany, horticulture, breeding. In: Janick, J. (ed), Horticultural Reviews, Wiley-Blackwell, Hoboken, New Jersey, United States, pp. 101-141.

Bombonato, J.R., Bonatelli, I.A.S., Silva, G.A.R., Moraes, E.M., Zappi, D.C., Taylor, N.P., and Franco, F.F. 2019. Cross-genera SSR transferability in cacti revealed by a case study using *Cereus* (Cereeae, Cactaceae). Genet. Mol. Biol., 42(1): 87-94.

Bonierbale, M.W., Plaisted, R.L., and Tanksley, S.D. 1988. RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics, 120: 1095-1103.

Chiba, N., Suwabe, K., Nunome, T., and Hirai, M. 2003. Development of microsatellite markers in melon (*Cucumis melo* L.) and their application to major cucurbit crops. Breed. Sci., 53: 21-27.

Cui, J., Cheng, J., Nong, D., Peng, J., Hu, Y., He, W., Zhou, Q., Dhillon, N. P. S., and Hu, K. 2017. Genome-wide analysis of simple sequence repeats in bitter gourd (*Momordica charantia*). Frontiers Plant Sci., 8:1103.

Dellaporta, S.L., Wood, J., and Hicks, J.B. 1983. A plant DNA mini preparation: Version II. Plant Mol. Biol. Rep., 1: 19-21.

Ekue, M.R., Gailing, O., and Finkeldey, R. 2009. Transferability of simple sequence repeat (SSR)

markers developed in *Litchi chinensis* to *Blighia sapida* (Sapindaceae). Plant Mol. Biol. Rep., 27(4): 570.

Grover, J. and Yadav, S. 2004. Pharmacological actions and potential uses of *Momordica charantia*: a review. J. Ethnopharmacol., 93(1):123-132.

Ji, Y., Luo, Y., Hou, B., Wang, W., Zhao, J., Yang, L., Xue, Q., and Ding, X. 2012. Development of polymorphic microsatellite loci in *Momordica charantia* (Cucurbitaceae) and their transferability to other cucurbit species. Sci. Hortic., 140:115–118.

Katzir, N., Danin-Poleg, Y., Tzuri, G., Karchi, Z., Lavi, U., and Cregan, P.B. 1996. Length polymorphism and homologies of microsatellites in several Cucurbitaceae species. Theor. Appl. Genet., 93: 1282-1290.

Kong, Q., Xiang, C., and Yu, Z. 2006. Development of EST-SSRs in *Cucumis sativus* from sequence database. Mol. Ecol. Notes, 6:1234–1236.

Kong, Q., Xiang, C., and Yu, Z. 2007. Mining and charactering microsatellites in *Cucumis melo* expressed sequence tags from sequence database. Mol. Ecol. Notes, 7:281–283.

Kuleung, C., Baenziger, P.S., and Dweikat, I. 2004. Transferability of SSR markers among wheat, rye, and triticale. Theor. Appl. Genet., 108(6): 1147-1150.

Langercrantz, U. 1998. Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that *Brassica* genomes have evolved through extensive genome replication accompanied by chromosome fusion and frequent rearrangements. Genetics, 150: 1217-1228.

Lee, J.M., Nahm, S.H., Kim, Y.M., and Kim, B.D. 2004. Characterization and molecular genetic mapping of microsatellite loci in pepper. Theor. Appl. Genet., 108:619–627.

Marr, K.L., Mei, X.Y., and Bhattarai, N.K. 2004. Allozyme, morphological and nutritional analysis bearing on the domestication of *Momordica charantia* L. (Cucurbitaceae). Econ. Bot., 58(3):435-455.

Minamiyama, Y., Tsuro, M., and Hirai, M. 2006. An SSR-based linkage map of *Capsicum annuum*. Mol. Breed., 18:157–169.

Parthiban, S., Govindaraj, P., and Senthilkumar, S. 2018. Comparison of relative efficiency of genomic SSR and EST SSR markers in estimating genetic diversity in sugarcane. 3 Biotech, 8:144.

Santos, C.P., Rocha, D.S., Bajay, M.M., Santos, F.R.C., Campos, J.B., Pinheiro, J.B., Zucchi, M.I., Silva-Mann, R., Arrigoni-Blank, M.F., and Blank, A.F. 2014. Cross-species transferability of microsatellite markers in the genus *Lippia*. Genet. Mol. Res., 13(4): 9846-9850.

Saxena, S., Singh, A., Archak, S., Behera, T.K., John, J.K., Meshram, S.U., and Gaikwad, A.B. 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(1): 93-118.

Van Wyk, B. 2015. A review of commercially important African medicinal plants. J. Ethnopharmacol., 176:118-134.

Walter, R. and Epperson, B.K. 2001. Geographic pattern of genetic variation in *Pinus resinosa*: Area of greatest diversity is not the origin of postglacial populations. Mol. Ecol., 10: 103-111.

Wang, S., Pan, L., Hu, K., Chen, C., and Ding, Y. 2010. Development and characterization of polymorphic microsatellite markers in *Momordica charantia* (Cucurbitaceae). Am. J. Bot., 97(8): e75–e78.

Wang, Z. and Xiang, C. 2013. Genetic mapping of QTLs for horticulture traits in a $F_{2:3}$ population of bitter gourd (*Momordica charantia* L.). Euphytica, 193(2):235-250.

Wu, H., He, X., Gong, H., Luo, S., Li, M., Chen, J., Zhang, C., Yu, T., Huang, W., and Luo, J. 2016. Genetic linkage map construction and QTL analysis of two interspecific reproductive isolation traits in sponge gourd. Frontiers Plant Sci., 7:980.

Xin, Y., Cui, H.R., Lu, M.Z., Yao, Y.L., Jin, J.Q., Lim, Y.P., and Choi, S.Y. 2006. Data mining for SSRs in ESTs and EST-SSR marker development in Chinese cabbage. Acta Hortic. Sin., 33:549–554.

Zhang, M., Mao, W., Zhang, G., and Wu, F. 2014. Development and characterization of polymorphic EST-SSR and genomic SSR markers for Tibetan annual wild barley. PLoS ONE, 9(4): e94881.