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Original Research Paper

Genetic analysis and identification of SSR marker linked to powdery mildew resistance in bitter gourd (*Momordica charantia* L.)

Prasanth K.[1]*, Lakshmana Reddy D.C.[2], Sriram S.[3], Venugopalan R.[4], Sadashiva A.T.[5], Pitchaimuthu M.[6], Hima Bindu K.[7] and Varalakshmi B.[6]

[1]Department of Vegetable Science, Kerala Agricultural University, Thrissur - 680 656, Kerala, India
[2]Division of Basic Sciences, [3]Division of Crop Protection, [4]Division of Social Sciences & Training,
[5, 6]Division of Vegetable Crops, [7]Division of Flower and Medicinal Crops
ICAR- Indian Institute of Horticultural Research, Bengaluru - 560 089, India
*Corresponding author Email: prasanth.k@kau.in

ABSTRACT

The inheritance of powdery mildew resistance in bitter gourd resistant line IIHR-144-1 was studied in cross with two susceptible lines Arka Harit and IIHR Sel-5-8. The segregation ratios in F_2 and backcross progenies indicated that resistance is governed by dominant-recessive epistasis or dominant inhibitory epistasis, indicating the involvement of more than one pairs of genes, one dominant and one recessive for resistance along with other background minor genes in the resistant line IIHR-144-1. Bulk segregant analysis of F_2 population of cross between IIHR-144-1 and Arka Harit using SSR markers identified McSSR 57 produced a polymorphic DNA fragment that co-segregated with disease reaction. Considering the cross compatibility between *Momordica charantia* L. and *M. charantia* var. *muricata* (IIHR-144-1) the resistance trait can be transferred to cultivated species through hybridization and selection.

Keywords: Bitter gourd, inheritance, powdery mildew, resistance, SSR marker

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) is an economically important and nutritionally rich vegetable cultivated in Asia. It is grown on approximately 340,000 hectares across Asia, with India and China accounting for more than half the production. India produces about 10.83 lakh metric tonnes of bitter gourd from an area of 0.96 lakh hectare (Saxena et al., 2017). The fruit contains at least three active substances with anti-diabetic properties, including charantin, which has been confirmed to have a blood glucose-lowering effect, vicine, and an insulin-like compound known as polypeptide-*p*. These substances work individually or together to help reduce blood sugar levels, which has inspired the nickname 'vegetable insulin' for bitter gourd (Yang et al., 2015; Tan et al., 2016).

The productivity levels of commercial bitter gourd varieties are constrained by the occurrence of various diseases. Powdery mildew is one of the most important production constraints in bitter gourd growing areas of India and incidence occurs throughout the year with a crop loss of up to 50 per cent (Dhillon et al., 2016). Genetic mechanism of powdery mildew resistance varies with the genotypes and pathogen races. Not many genetic studies on resistance to powdery mildew in bitter gourd have been reported. The development of new bitter gourd cultivars with desirable agronomic traits and resistance to powdery mildew through classic improvement techniques is quite slow and laborious, apart from undergoing environmental influence. The techniques, such as marker-assisted selection (MAS) can achieve quick and effective selection of desired traits and significantly speed up the breeding process.

Bulked segeregant analysis (BSA) is an efficient approach to trait linked marker screening especially in crops where genetic maps are not available (Michelmore et al., 1991; Giovanni et al., 2004; Huang & Roder, 2004). The current experiment was taken up to understand the genetics of resistance in IIHR-144-1 and identifying molecular marker linked to the resistance which will be useful for marker assisted selection.

MATERIALS AND METHODS

Plant material and population development

Two highly susceptible genotypes Arka Harit and IIHR Sel-5-8 (as female) and one resistant genotype IIHR-144-1 (as male) maintained as true breeding inbreds in the Division of Vegetable Crops, ICAR- IIHR, Bengaluru, were used to develop two populations segregating for resistance to powdery mildew. The seeds were planted and crossing was done in December 2016 to April 2017 to produce F_1. In the following season (from June 2017 to October 2017) F_1s (cross between Arka Harit x IIHR-144-1 and IIHR Sel-5-8 x IIHR-144-1) were self pollinated to produce F_2 seeds and back crossed to parents to develop BC_1 and BC_2, respectively.

Six populations of both the crosses were planted during December 2017 to March 2018, in a randomized complete block design (RCBD) with three replications in two separate experiments. Each set consisted of 20 plants each of P_1, P_2 and F_1, 30 plants each of BC_1 and BC_2 generations and 100 plants of F_2 generation per replication. All plants were observed for reaction to the disease throughout the growing season.

Disease assessment

The plants were allowed to get infestation with powdery mildew. Disease scoring was carried out on the basis of per cent leaf area infected by the powdery mildew lesions and 0-9 rating scale was adopted for disease ratings as suggested by Jenkins & Wehner (1983). The PDI was calculated (Fanourakis, 1990) and individual plants in the population were grouped into five categories namely resistant (0-10%), moderately resistant (10-20%), moderately susceptible (20-40%), susceptible (40-60%) and highly susceptible (> 60%).

The area under disease progress curve (AUDPC) (Cambell & Madden, 1990) and apparent rate of disease development (r) were calculated (Vanderplank, 1963). For the genetic model of inheritance, the total number of plants falling into different classes was counted and subjected to chi-square analysis for goodness of fit to various classical Mendelian ratios as suggested by Panse & Sukhatme (1985).

Identification of linked SSR marker for resistance

Bulk segregant analysis approach was implemented to identify linked SSR markers. DNA isolated from 10 highly susceptible and 10 highly resistant plants from F_2 segregating population was used to constitute the susceptible and resistant bulks respectively. Genomic DNA extraction from plants was done as described by Doyle & Doyle (1987). Quantification and quality analysis of the purified DNA was carried out using spectrophotometer (Nanodrop 8000, Thermo Scientific) and DNA working stocks were prepared to make the final concentration of DNA as 30 ng/µl. The DNA of parental lines *viz*., Arka Harit and IIHR-144-1 along with susceptible and resistant bulks from F_2 population of the cross Arka Harit × IIHR-144-1 were screened with identified parental polymorphic primers.

A total of 300 SSR markers (genomic SSRs and ESTs SSRs) which are specific to bitter gourd (Ji et al., 2012; Guo et al., 2012; Wang & Xiang, 2013; Saxena et al., 2015; Shukla et al., 2015) were synthesized by Bioserve, Hyderabad, India. PCR reaction components were obtained from Bangalore Genei Pvt. Ltd., Bengaluru. DNA amplification for SSR primers was performed in a 15 µl reaction mixture containing 30 ng of genomic DNA, each forward and reverse primer at 5.0 pmol, 1X PCR buffer, 1.0 mMdNTPs, and 1.0 U of Taq polymerase with the following touchdown cycling profile: 1 cycle of 5 min at 94˚C, 10 cycles of 20 s at 94˚C, 20 s at 65˚C (decreasing in steps of 1˚C/cycle for cycles 2-10) and 90 s at 72˚C, followed by 35 cycles of 20 s at 94˚C, 20 s at 55˚C, 90 s at 72˚C and 8 min. at 72˚C using Eppendorf Thermal Cyclers. SSR primers designed from genomic SSRs and EST SSRs were used for detection of polymorphism between two parents and bulked resistant and susceptible DNA samples. The amplification products of SSR were resolved by agarose gel electrophoresis in 4% agarose gel. The sizes of amplified fragments were determined by using standard 100bp DNA ladder (Juniper life sciences). DNA fragments were visualized under UV light and photographed using a Gel documentation system (UVITec, Cleaver Scientific, UK). The primer detecting a polymorphism between both susceptible and resistant parents and the susceptible and resistant bulks was tested on the individual samples of F_2 genomic DNA.

RESULTS AND DISCUSSION

Mean performance of different generations

Analysis of variance revealed significant differences among the generations for both the crosses.

Performance of different generations based on mean PDI for the cross Arka Harit × IIHR-144-1 (Table 1) revealed that, P_1 (77.92) recorded highly susceptible reaction to the disease and BC_1 (40.36) was found to be susceptible. P_2 (0.09), F_1 (0.14), F_2 (6.81) and BC_2 (0.12) exhibited resistance throughout the growing period. In the cross IIHR Sel-5-8× IIHR-144-1, performance of different generations based on mean PDI exhibited a similar trend as described in the first cross (Table 2). The F_1, F_2, BC_1 and BC_2 generations in both the crosses fell within the parental range. This suggests resistance/susceptibility to powdery mildew is an inherited character.

The AUDPC ranged from 0.31 to 3960.82 and 0.31 to 4354.33 in different generations of the cross Arka Harit × IIHR-144-1 and Sel-5-8 × IIHR-144-1, respectively. Among the progenies, BC_1 possessed the highest AUDPC value and BC_2 exhibited the lowest value in both the crosses. The results of apparent infection rate (AIR) indicated that, there was positive increase in the disease infection over the period of time in susceptible generation's *viz.*, P_1 and BC_1 of both the crosses. The mean disease rating of BC_1 was nearer to susceptible parent and BC_2 was nearer to resistant parent. Such slow disease build-up as observed is practically important, which can hold up during an epidemic without being affected by disease for a longer time, thus providing longer window for adoption of other disease control strategies.

Genetic analysis of powdery mildew resistance

The segregation in F_2 and backcross progeny of the two crosses was subjected to chi-square analysis for assessing the goodness of fit to various classical Mendelian ratios (Table 3). For the cross Arka Harit × IIHR-144-1, the number of segregants into resistant and susceptible classes in the F_2 generation was 250 and 50, respectively. Out of the various Mendelian ratios tested, chi-square values were significant for all the ratios except 13 (resistant): 3 (susceptible) with a chi square value of 0.85 and probability of 0.30 - 0.40, indicating the involvement of two pairs of genes for resistance in the resistant parent (IIHR-144-1). In the

Table 1 : Mean PDI, AUDPC and apparent infection rate of six generations of the cross Arka Harit x IIHR-144-1

Treatment	Mean PDI	AUDPC	Disease reaction	Apparent infection rate (r) per unit per day							
				1 to 2 week	2 to 3 week	3 to 4 week	4 to 5 week	5 to 6 week	6 to 7 week	7 to 8 week	Average
P_1	77.92	3960.82	HS	1.13	1.21	1.25	1.30	1.31	1.31	1.31	1.26
P_2	0.09	0.31	R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F_1	0.14	0.49	R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F_2	6.81	340.05	R	0.00	0.47	0.50	0.50	0.57	0.63	0.66	0.47
BC_1	40.36	2041.05	S	0.76	0.98	1.06	1.12	1.14	1.15	1.16	1.05
BC_2	0.12	0.43	R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 2 : Mean PDI, AUDPC and apparent infection rate of six generations of the cross IIHR Sel-5-8 × IIHR-144-1

Treatment	Mean PDI	AUDPC	Disease reaction	Apparent infection rate (r) per unit per day							
				1 to 2 week	2 to 3 week	3 to 4 week	4 to 5 week	5 to 6 week	6 to 7 week	7 to 8 week	Average
P_1	89.97	4354.33	HS	1.19	1.25	1.27	1.28	1.31	1.31	1.32	1.29
P_2	0.09	0.31	R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F_1	0.47	3.80	R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F_2	8.72	321.35	R	0	0.02	0.41	0.49	0.57	0.63	0.66	0.39
BC_1	48.81	2489.49	S	0.86	1.09	1.09	1.13	1.16	1.21	1.27	1.12
BC_2	0.66	5.06	R	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

test cross with susceptible parent, segregation ratio had the best fit with 1:1 (χ^2= 1.11; P = 0.20 – 0.30) where as in the test cross with resistant parent, the segregation ratio had goodness of fit with 1:0 (χ2 = 0.00; *p* = 1.00) *ie*., all the plants were resistant. This was further confirmed in the second cross IIHR Sel-5-8 × IIHR-144-1. These results showed that the genetic control of the resistance to powdery mildew in IIHR-144-1 is a dominant-recessive epistasis. The detailed dominant-recessive epistasis gene interaction model for powdery mildew inheritance was given by Lisbona et al. (2010) in melon 'TGR-1551'. Dhillon et al. (2016) reported that the powdery mildew resistance in bitter gourd was controlled by recessive polygenes. Dhillon et al. (2019) reported the involvement of at least two independent, recessive genes to bitter gourd powdery mildew. Dominant-recessive epistasis for powdery mildew resistance was also reported by Tetteh et al. (2013) in water melon.

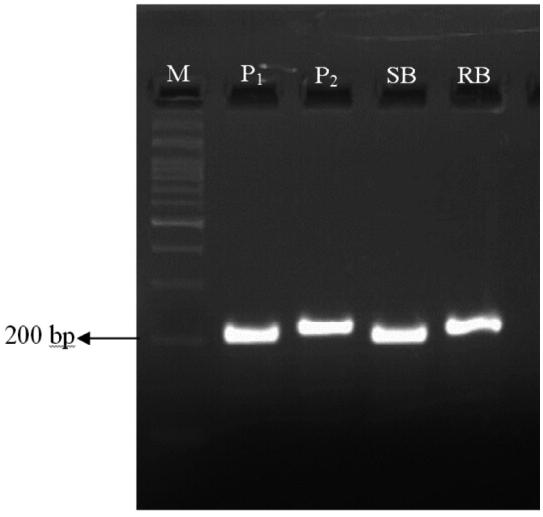

Fig.1: SSR marker McSSR 57 showing polymorphism in BSA

M- 100 bp DNA Ladder, P1- susceptible parent (Arka Harit), P2- resistant parent (IIHR-144-1), SB- susceptible bulk and RB - resistant bulk

Molecular tagging of powdery mildew resistance

Identification of markers linked to powdery mildew resistance genes would help breeders to introgress the new source of resistance into elite backgrounds. The genomic DNA of Arka Harit and IIHR-144-1 were used for identification of polymorphism with 300 SSR markers. Most of the SSR markers were monomorphic among parents; only 32 primers produced polymorphism between resistant and susceptible parents. These polymorphic primers were used for BSA. The results revealed that the primer McSSR 57 (repeat motif: $(GAA)_4$, $(AATA)_2$, $(AAATT)_2$) produced a polymorphic DNA fragment that co-segregated with disease reaction. The sizes of these fragments were approximately 200 kb (Fig. 1). McSSR 57 showed smaller fragment size in all the plants of susceptible bulk while larger fragment size was found in the plants of resistant bulk.

The F_2 genotyping of 193 F_2 plants using the McSSR 57 indicated that 44 plants were resistant (A), 97 plants were heterozygous resistant (AB) and 52 plants were susceptible (B). The segregation of marker was 1 dominant homozygous resistant: 2 heterozygous resistant: 1 homozygous susceptible pattern (1:2:1) in chi square analysis for goodness of fit and the F value was significant in single marker analysis indicating a linkage between the marker McSSR 57 and disease resistance gene (Table 4 & 5). PCR amplification of 193 DNA samples from F_2 resulted in amplification of a 220 bp fragment in 52 F_2 plants (designated as R) heterozygous bands were present in 89 F_2 plants and 200 bp fragment was present in 52 F_2 plants (designated as S) indicating co-segregation of marker with resistant gene. During single plant analysis few recombinants with a recombination frequency of

Table 3 : Estimates of chi square values and their probability for different classical Mendalian ratios for powdery mildew intensity in F_2 and test cross generations of bitter gourd

Cross		F_2				Test cross with susceptible parent		Test cross with resistant parent	
		3:1	9:7	13:3	15:1	1:1	3:1	1:1	3:1
ArkaHarit × IIHR 144-1	χ^2	11.11**	89.41**	0.85	55.55**	1.11	18.14**	90**	30**
	P	< 0.001	< 0.001	0.30-0.40	< 0.001	0.20-0.30	< 0.001	< 0.001	< 0.001
IIHR Sel-5-8 × IIHR 144-1	χ^2	9.40**	85.07**	0.39	62.89**	0.40	22.53**	90**	30**
	P	0.002	< 0.001	0.50-0.60	< 0.001	0.50-0.60	<0.001	< 0.001	< 0.001

Table 4 : Segregation pattern of McSSR 57 marker locus using 193 F_2 plants derived from the cross of Arka Harit × IIHR-144-1

Marker	Observed F_2 genotype			Total number of recombinants	Recombination frequency (%)	Expected ratio	$\chi 2$ value	P valueat 5%
	A	AB	B			A: AB: B		
McSSR 57	52	89	52	35	18.13	1:2:1	1.16	0.50 - 0.60

Table 5 : Phenotypic segregation pattern of McSSR 57 marker locus using 193 F_2 plants derived from the cross of Arka Harit × IIHR-144-1

Marker	Observed F_2 phenotype		Expected ratio	$\chi 2$ value	P value at 5%
	R	S			
McSSR 57	150	43	13:3	1.57	0.20-0.30

18.13% (18.13 cM) were observed with McSSR 57. The identified SSR marker distance is likely to be too large which require a larger screening population for a single SSR marker to discriminate genotypes. Comparing with other cucurbits such as melon, watermelon and cucumber, use of molecular markers in bitter gourd breeding is in its early stage and has been limited to diversity analysis (Dey et al., 2006; Behera et al., 2008a; Behera et al., 2008b).

CONCLUSION

The existence of the genetic system described, which controls resistance to *P. xanthii*, means that IIHR-144-1 is a good genetic source for the development of breeding lines with resistance to powdery mildew. This is the first report on genetics of resistance governed by two independent epistatic gens (one dominant and one recessive) to powdery mildew in bitter gourd. Likewise, SSR marker identified will benefit MAS for powdery mildew resistance in future bitter gourd resistance breeding programme. Further studies should be carried out to fine map the location for identify a tightly linked marker associated with resistant gene.

REFERENCES

Behera, T. K., Gaikwad, A. B., Singh, A. K., & Staub, J. E. (2008a). Comparative analysis of genetic diversity in Indian bitter gourd (*Momordica charantia* L.) using RAPD and ISSR markers for developing crop improvement strategies. *Scientia Horticulturae*, *115*, 209-217. https://doi.org/10.1016/j.scienta.2007.08.013

Behera, T. K., Gaikwad, A. B., Singh, A. K., & Staub. J. E. (2008b). Relative efficiency of DNA markers (RAPD, ISSR and AFLP) in detecting genetic diversity of bitter gourd (*Momordica charantia* L.). *Journal of the Science of Food and Agriculture*, *88*, 733-737. https://api.semanticscholar.org/CorpusID:27588497

Campbell, C. L., & Madden, L. V. (1990). Introduction to plant epidemiology. Wiley. p 531.

Dey, S. S., Singh, A. K., Chandel, D., & Behera. T. K. (2006). Genetic diversity of bitter gourd (*Momordica charantia* L.) genotypes revealed by RAPD markers and agronomic traits. *Scientia Horticulturae*, *109*, 21-28. https://doi.org/10.1016/j.scienta.2006.03.006

Dhillon, N. P. S., Sanguansil, S., Srimat, S., Cheng, H., Lin, C., Srinivasan, R., Kenyon, L., Schafleitner, R., Yang, R., & Hanson, P. (2016). Status of cucurbit breeding at AVRDC-The world vegetable center. In: Kozik E. U., Paris H. S. (Eds.). The XIth Eucarpia meeting on cucurbit genetics and breeding 2016, Warsaw, Poland, 21-25.

Dhillon, N. P. S., Sanguansil, S., Srimat, S., & Laenoi, S. (2019). Inheritance of resistance to cucurbit powdery mildew in bitter gourd. *Hortscience*, *54*(6), 1013–1016. https://doi.org/10.21273/HORTSCI13906-19

Doyle, J. J., & Doyle, J. J. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, *19*, 11-15.

Fanourakis, N. E. (1990). Screening procedure for powdery mildew resistance in cucumber. *Acta Horticulture*, *287*, 147-154. https://doi.org/10.17660/ActaHortic.1991.287.14

Giovanni, C. D., Dell'Orco, P., Bruno, A., Ciccarese, F., Lotti, C., & Ricciardi, L. (2004). Identification of PCR-based marker (RAPD, AFLP) linked to a novel powdery mildew resistance gene (ol-2) in tomato. *Plant Science*, *166*, 41-48. https://doi.org/10.1016/j.plantsci.2003.07.005

Guo, D. L., Zhang, J. P., Xue, Y. M., & Hou, X. G. (2012). Isolation and characterization of 10 SSR markers of *Momordica charantia* (Cucurbitaceae). *American Journal of Botany*, e182-e183. doi: 10.3732/ajb.1100277

Huang, X., & Roder, M. (2004). Molecular mapping of powdery mildew resistance genes in wheat: A review. *Euphytica*, *137*, 203-223. https://doi.org/10.1023B:EUPH.0000041576.74566.d7

Jenkins, S. F., & Wehner, T. C. (1983). A system for the measurement of foliar diseases of cucumber. *Cucurbit Genetic Cooperative Report*, *6*, 10-12.

Ji, Y., Luo, Y., Hou, B., Wang, W., Zhao, J., Yang, L., Xue, Q., & Ding, X. (2012). Development of polymorphic microsatellite loci in *Momordica charantia* (Cucurbitaceae) and their transferability to other cucurbit species. *Scientia Horticulturae*, *140*, 115-118. https://doi.org/10.1016/j.scienta.2012.03.024

Lisbona, F. J. Y., Sese, A. I. L., & Guillamon, M. L. G. (2010). Inheritance of resistance to races 1, 2 and 5 of powdery mildew in the melon TGR 1551. *Plant Breeding*, *129*, 72-75. https://doi.org/10.1111/j.1439-0523.2009.01655.x

Michelmore, R. W., Paran, I., & Kesseli, R. V. (1991). Identification of markers linked to disease resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of National Academy of Science*, USA, *88*, 9828-9832.

Panse, V. G., & Sukhatme, P. V. (1985). Statistical methods for agricultural workers (4th ed.). ICAR, New Delhi.

Prasanth, K., Varalakshmi, B., Venugopalan, R., & Sriram, S. (2019). Screening of bitter gourd germplasm and advanced breeding lines against powdery mildew. *Indian Phytopathology*, *72*(1), 15-22. https://doi.org/10.1007/s42360-018-0070-7

Saxena, M., Rathore, R. P. S., Gupta, R. P., Bhargav, H., & Thakur, B. (2017). Horticultural Statistics at a Glance 2017. Horticulture Statistics Division, Department of Agriculture, Cooperation & Farmers Welfare, Ministry of Agriculture & Farmers Welfare, Government of India.

Saxena, S., Singh, A., Archak, S., Behera, T. K., John, J. K., Meshram, S. U., & 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. *Applied Biochemistry and Biotechnology*, *175*, 93-118. https://doi.org/10.1007/s12010-014-1249-8

Shukla, A., Singh, V. K., Bharadwaj, D. R., Kumar, R., Rai, A. K., Mugasimangalam, R., Parameswaran, S., Singh, M., & Naik, P. S. (2015). *De novo* assembly of bitter gourd transcriptomes: Gene expression and sequence variations in gynoecious and monoecious lines. *Plos One*, *10*, e0128331. https://doi.org/10.1371/journal.pone.0128331

Tan, S. P., Kha, T. C., Parks, S. E., & Roach, P. D. (2016). Bitter melon (*Momordica charantia* L.) bioactive composition and health benefits: A review. *Food Reviews International*, *32*, 181–202. https://doi.org/10.1080/87559129.2015.1057843

Tetteh, A. Y., Wehner, T. C., & Davis, A. R. (2013). Inheritance of resistance to the new race of powdery mildew in watermelon. *Crop Science*, *53*, 880-887. https://doi.org/10.2135/cropsci2012.07.0453

Vanderplank, J. E. (1963). Plant diseases: Epidemics and control (p. 349). NY: Academic Press.

Wang, Z., & Xiang, C. (2013). Genetic mapping of QTLs for horticulture traits in an F_{2-3} population of bitter gourd (*Momordica charantia* L.). *Euphytica, 193*, 235-250. doi:10.1007/s10681-013-0932-0

Yang, B., Li, X., Zhang, C., Yan, S., Wei, W., Wang, X., Deng, X., Qian, H., Lin, H., & Huang, W. (2015). Design, synthesis and biological evaluation of novel peptide MC2 analogues from *Momordica charantia* as potential anti-diabetic agents. *Organic and Biomolecular Chemistry, 13*, 4551-4561. doi:10.1039/C5OB00333D

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