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ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2021; 26(5): 2936-2941
doi: 10.25083/rbl/26.5/2936.2941

Received for publication, April, 13, 2021
Accepted, July, 15, 2021

Original paper

Mapping QTLs controlling the biosynthesis of maltose in soybean

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Abstract The present study was carried out to identify genomic regions associated with maltose in 2 F_2 populations through assessment of sugars using HPLC and genotyping using SSR markers across the genome. SSR markers, Sat_216 (*chr* 12) and Satt681 (*chr* 6) in F_2 population I and Sat_105 (*chr* 20) in F_2 population II showed significant ($P< 0.5$) association with maltose content through single marker analysis (SMA) with LOD score of 3.18 (R^2=9.7), 2.54 (R^2=6.8), and 3.54 (R^2=10.4), respectively. Composite interval mapping analysis (CIM) let to identify different QTLs (other than SMA) for maltose content on *chr* 11, *chr* 13 and *chr* 17 in F_2 population I while *chr* 6 and *chr*15 in F_2 population II. QTLs identified for maltose content are in proximity of known functional genes responsible for degradation of starch into maltose. QTLs identified for maltose in the study may be deployed for improving efficiency of marker assisted breeding for development of soybean genotypes with high levels of this sugar.

Keywords Maltose, Quantitative trait loci, Soybean, SSR marker, HPLC (High performance liquid Chromatography)

To cite this article: JHA P, KUMAR V, RANI A, KUMAR A. Mapping QTLs controlling the biosynthesis of maltose in soybean. *Rom Biotechnol Lett*. 2021; 26(5): 2936-2941.
DOI: 10.25083/rbl/26.5/2936-2941

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Introduction

Soy-based food products are fast gaining the sobriquet of 'functional food of the century' across the world, due to the presence of basic nutrients, such as, protein (40%), essential amino acids, oil (18-20%), essential fatty acids, vitamins, minerals and several nutraceutical components like isoflavones, tocopherols, lecithin that stave off the killer diseases, namely, atherosclerosis, diabetes, breast cancer, osteoporosis at bay (Kumar et al, 2010a). It is also one of the limited plant-based protein sources available for vegetarians/vegans to meet the daily requirement of protein. Soybean seeds are utilised in the manufacturing of various kinds of processed foods, including *tofu* (soy bean curd), soymilk, *natto* (fermented soybean), tempeh, *miso* (soy bean paste) and soy sauce. However, organoleptic acceptance of soy food products depends upon the taste and flavour. Sweetness depends upon the presence of several soluble sugars, sucrose, maltose, sucrose derived monosaccharides viz. glucose and fructose in soybean seeds. Of the several soluble sugars, sucrose content in soybean seeds is very important component which is known to impart sweetness in the final product and improve its flavour as suggested in earlier studies (Taira et al, 1990; Kumar et al, 2011; Escamilla et al, 2019). However, maltose, a disaccharide composed of two glucose residues joined by α(1-4) glycosidic linkage, present in soybean seeds has been reported to possess 30% of the sweetness imparted by sucrose (Bhagavan, 2002). Therefore, besides high sucrose content soybean genotypes, high maltose content genotypes are also equally important to overcome the astringent/bland taste of soy products. For breeding high sucrose soybean genotypes, genetic variability for this trait (Hou et al, 2009; Kumar et al, 2007; Kumar et al, 2010b; Kumar et al, 2011) and QTLs contributing to it have been identified (Kim et al, 2005, 2006; Saghai Maroof and Buss, 2008; Skoneczka et al, 2009; Wang et al, 2014).

With regard to maltose in soybean, the organoleptic test of boiled R6-stage soybean seeds was carried out in relation to the maltose production in seven Japanese vegetable genotypes (Nomura, 2014). However, both genetic variability for maltose in soybean mature seeds and the genomic regions contributing to this sweetness imparting trait have not yet been reported. In the present investigation, 2 F_2 populations derived from 2 parental combinations of soybean were genotyped using polymorphic simple sequence repeat markers spanning across the genome to identify genomic regions associated with maltose content. These QTLs linked to genomic regions controlling the biosynthesis of maltose can be deployed to breed soybean genotypes with high maltose content which can serve as special raw material to process good quality soy products.

Materials and Methods

Development of mapping populations

All the 3 genotypes, namely, Dadachamame, NRC121 and NRC101, selected for developing mapping populations flower in 28-30 days. Dadachamame is a vegetable soybean genotype and early maturity. The country of origin of this genotype is Japan and the maltose content of its seeds is 0.8mg/g. The genotype was procured from World Vegetable Centre, Taiwan for research and development purpose. NRC121 is a germplasm accession with large seed size, early maturity and 6.2 mg/g maltose content. This genotype has the potential to be promoted as vegetable genotype. NRC101 is the Kunitz trypsin inhibitor free Indian soybean genotype developed from the cross Samrat × PI 542044 (Rani and Kumar, 2015), with 0.5mg/g maltose content. The genotype Samrat was a local selection by the farmers in the Malwa Plateau of Central India. PI 542044 is a germplasm accession developed from the cross William 82 × PI157440 at United States Department of Agriculture (USDA). This germplasm accession was procured from USDA by ICAR-National Bureau of Plant Genetic Resources, New Delhi, and delivered to ICAR-Institute of Soybean Research, Indore. Dadachamame gives white flowers while NRC121 and NRC101 bear purple flowers. Planting was done on staggered dates in the fields of ICAR-Indian Institute of Soybean Research, Indore, which have black cotton, to effect following crosses: Dadachamame × NRC101, NRC121 × NRC101. All the three parents and two F_2 populations were planted in 3 m plot, maintaining row-to-row and plant-to-plant distance of 45 and 5 cm, respectively, in the experimental fields of ICAR-Indian Institute of Soybean Research, Indore, M.P., India. . The F_2 population I (167 plant individuals) obtained from the cross Dadachamame × NRC101 and F_2 population II (159 plant individuals) from the cross NRC121 × NRC101 were raised in the field in the cropping season 2018 to obtain $F_{2:3}$ seeds. Standard recommended agronomic practices were followed for raising sowing till harvesting of the crop. The plants were harvested at maturity, and oven-dried (60°C) F_3 soybean seeds were used to determine the maltose content for mapping QTLs associated with this trait in soybean.

Determination of maltose through HPLC

Extraction of maltose from soybean seeds was carried out following the method of Liu and Markakis (1987). The extracted sugars were determined through HPLC as described elsewhere (Kumar et al. 2007). Peak of maltose in the sample was identified using the retention time of the peak of the corresponding external standard procured from Sigma Aldrich, which was obtained at 8.3 mins (Figure 1). Quantification of maltose content (per gram of the flour) was carried out by comparing the area of the peak in the sample chromatogram with that of the standard using software CSW 1.7.

Molecular markers, DNA isolation and PCR amplification

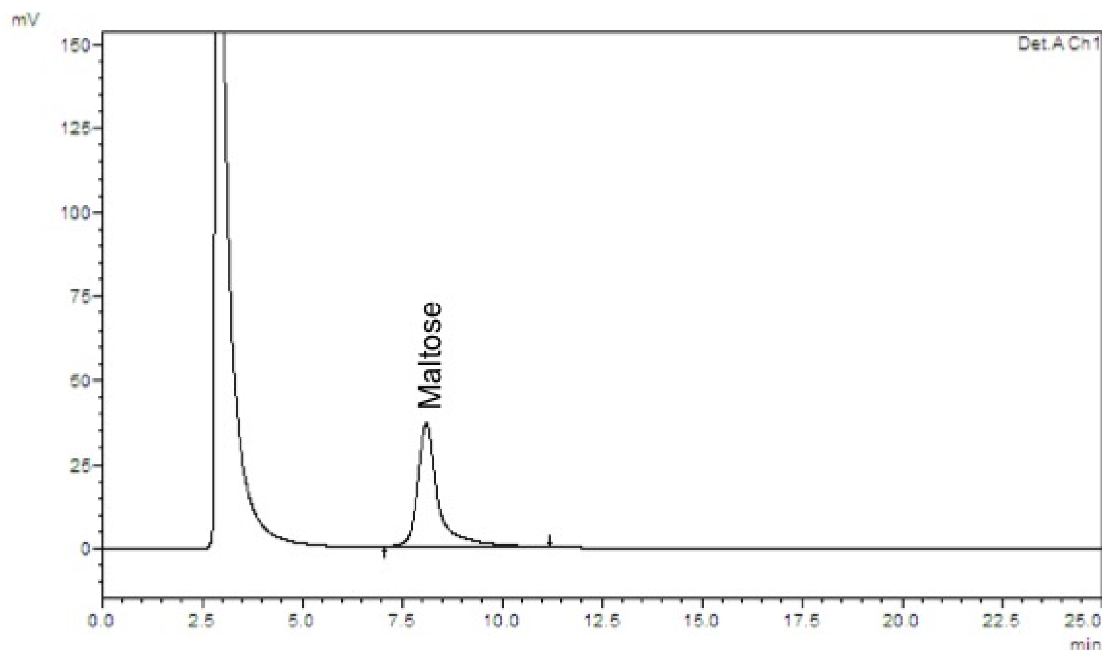
The sequences of SSR markers were taken from the list of soybean SSR loci mapped by Agricultural Research Services, United States Department of Agriculture and are available at http://bldg6.arsusda.gov/cregan/soymap.htm. The synthesis of the oligonucleotide sequences of SSR primer pairs was outsourced to Sigma Aldrich, Bangalore.

Genomic DNA was extracted from the young leaves of parents and F_2 individuals of both the crosses, Dadachamame×NRC101 and NRC121×NRC101, through cetyl-trimethyl ammonium bromide procedure (Doyle and Doyle, 1990). DNA was purified through phenol: chloroform: iso-amyl alcohol method. DNA quality was tested on 0.8% agarose, while the DNA concentration was estimated through nano-drop spectrophotometer (DeNovix, DS-11+Spectrophotometer). *Polymerase chain r*eaction (PCR) was carried out to amplify the genomic DNA by the method as described elsewhere (Rani et al, 2019). PCR products amplified through SSR markers were resolved on 3% metaphor agarose. DNA ladder (50 bp) was also loaded to assess the size of the amplicons generated through each SSR primer in both the F_2-popullations. The images were analysed in Gel Documentation Unit (Syngene, Cambridge, United Kingdom). Six hundred SSR markers across the genome, selecting minimum 25 SSR markers from each of the 20 chromosomes, were employed for parental polymorphism survey.

Statistical Analysis

All the statistical analysis was carried out through SAS 9.3 software. Association of genomic regions with maltose was assessed through single marker analysis (SMA) and composite interval mapping (CIM) using JMP genomics component of this software. For SMA, anno and geno files for marker variables and trait variables, respectively, were created, and analysis was performed by selecting numeric genotype and α value 0.05. For CIM, the threshold LOD score settings were set at 1000 permutations, 0.05 significant level, 2 cM QTL test step, Kosambi map function and control marker numbers of 5 was setup to run JMP genomics for QTL mapping. Analysis of quantitative data was performed in Microsoft excel 2019.

Figure 1. HPLC chromatogram depicting the resolution of maltose standard.



Results and Discussion

Result

Variability for maltose and transgressive seggregants

Maltose content of Dadachamame , NRC101, NRC121 was 0.8, 0.5 and 6.2 mg/g, respectively. Maltose content of NRC121 was about 12 and 8 times higher than NRC101 and Dadachamame, respectively. The range of maltose content was 0.2 - 2.5 mg/g and 2.0 - 8.3 mg/g for F_2 population I and II, respectively.

Genomic regions significantly associated with maltose across two different F_2 populations

Parental polymorphism survey using 600 SSR markers at an average distance of 9.2 cM spanning across 20 linkage groups of soybean genome revealed 130 and 119 polymorphic SSR markers for parental combination Dadachamame × NRC101 and NRC121 × NRC101, respectively. In case of parental combination Dadachamame × NRC101, number of polymorphic SSR markers on *chr* 1, *chr* 2, *chr* 3, *chr* 4, *chr* 5, *chr* 6, *chr* 7, *chr* 8, *chr* 9, *chr* 10, *chr* 11, *chr* 12, *chr* 13, *chr* 14, *chr* 15, *chr* 16, *chr* 17, *chr* 18, *chr* 19, and *chr* 20 were 7, 4, 12, 5, 4, 3, 11, 3, 5, 13, 3, 6, 7, 2, 10, 11, 2, 8, 9, and 5,

respectively. On the other hand, *chr* 1, *chr* 2, *chr* 3, *chr* 4, *chr* 5, *chr* 6, *chr* 7, *chr* 8, *chr* 9, *chr* 10, *chr* 11, *chr* 12, *chr* 13, *chr* 14, *chr* 15, *chr* 16, *chr* 17, *chr* 18, *chr* 19 and *chr* 20 exhibited 4, 2, 5, 2, 6, 7, 4, 4, 3, 3, 5, 7, 13, 2, 12, 6, 10, 11, 3, and 10 polymorphic SSR markers, respectively, for the parental combination NRC121× NRC101. These polymorphic SSR markers were deployed for genotyping 167 and 159 F_2 plant individuals in F_2 population I and II, respectively. SSR markers which showed significant ($P < 0.05$) association with maltose content in both the F_2 populations are given in Table 1, identified through SMA along with respective LOD (logarithm of the odds) score and R^2 value.

Maltose content

Four different SSR markers each, in both F_2 population I and II were identified for maltose content (Table 1). In F_2 populations I, Sat_407 (LG-A1/*chr* 5, 52.32 cM), Satt681 (LG-C2/*chr* 6, 3.15 cM), Satt720 (LG-E/*chr* 15, 20.8 cM) and Sat_216 (LG-H/ chr 12, 85.27cM) were found to have significant association with maltose content. In F_2 populations II, Satt522 (LG-F/*chr* 13, 119.19 cM), Satt469 (LG-H/*chr* 12, 58.91 cM), Sat_105 (LG-I/ chr 20, 49.34 cM), and Satt631(LG-N/*chr* 3,1.60 cM) showed significant association with this sugar. SSR marker, Sat_216 (LG-H/ chr 12) and Sat_105 (LG-I/ chr 20) exhibited the highest LOD score of 3.18 and 3.54 with R^2 value of 9.7 and 10.4 for this sugar in F_2 population I and II, respectively.

Genomic regions identified for maltose content through CIM are presented in Table 2. In F_2 population I, three QTLs were identified for maltose content on LGs: (B1/*chr11*), (D2/*chr*17*)*, (F/*chr*13), with LOD score of 2.51, 5.78 and 2.73, respectively. In F_2 population II, 3 QTLs were identified for maltose content on LGs: (C2/*chr*6), (E/*chr*15), and (I/*chr*20) with LOD score of 2.8, 3.2, and 2.5, respectively.

Discussion

There is no report on the range of maltose content in mature soybean seeds. However, Nomura et al, (2014) reported the range of 9.1- 15.2 mg/g for maltose content in immature (R6-stage) boiled soybean genotypes. Both F_2 population I and II showed transgressive segregates for this soluble sugar.

Till date no QTL has been reported to be associated with the synthesis of maltose in soybean seeds (Soybase, 2020). In the present study, QTLs were identified for maltose in 2 F_2 populations developed through bi-parental crossing. Table 1 summarizes the contribution of different QTLs found to be significantly ($P < 0.05$) associated with maltose content in F_2 population I and II, respectively. In F_2 population I, allele 0 (from Dadachamame) of Satt681(LG-C2) contributed positively whereas Sat_407 (LG-A1), Satt720 (LG-E) and Sat_216 (LG-H) showed negative contribution to maltose content. In F_2 population II, allele 0 (from NRC121) of SSR markers Satt522 (LG-F), Satt469 (LG-H) and Satt631 (LG-N) positively contributed to maltose content, while Sat_105 (LG-I) which exhibited strong association (LOD = 3.54) with this trait showed negative contribution to this sugar.

Maltose is synthesized by the breakdown of stored starch in seed by the combined action of several enzymes. In legumes, starch is stored in cotyledons and both alpha amylase and starch phosphorylase are responsible for its breakdown into maltose (Halmer, 1985). α-Amylase, an endohydrolase that acts on α (1-4) linkages, known to be the only seed produced enzyme that can attack an intact starch granule. The linear and branched oligosaccharides thus produced are hydrolysed by debranching enzyme (which acts on α-1-6 linkages) and β-amylase (which hydrolyses α-1-4 linkages), thereby releasing maltose (Bang and Huyen, 2015). Alpha-amylase is synthesized de novo in the aleurone layer, whereas β-amylase, which is present in an inactive form in the protein matrix in the mature seeds, is activated by proteases produced by aleurone layer. Sat_216 (LG-H/*chr* 12, 85.27cM, LOD score=3.18) identified for maltose content in F_2 population I through SMA in the present study was in the proximity of functional gene *Glyma12g32330* (Chr12: start 35830896-end 35834230) for β-amylase activity (Soybase, 2020). Similarly, Satt681 (LG-C2/*chr* 6, 3.15 cM, LOD score=2.54) identified for maltose content in F_2 population I through SMA was close to the functional gene *Glyma06g00680* (Chr06: start 376352 -end 379449) for glycoside hydrolase family 10 (hydrolyzing O-glycosyl compounds) (Soybase, 2020), which catalyses the hydrolysis of glycosidic bond in non-reducing sugar and breaks down polysaccharides into disaccharides and monosaccharides moieties which might be utilised in the biosynthesis of maltose in soybean seeds. Further, Satt720 (LG-E/ *chr* 15, 20.8cM, LOD score=2.10) identified for maltose content in F_2 population I through SMA was in the proximity of functional gene *Glyma15g05710* (Chr15: start 4023433 -end 4024990) responsible for UDP-glucuronosyl and UDP-glucosyl transferase activities, which was reported to be found among the genes located within the region containing the QTL associated with starch content in cassava (Sraphet et al, 2017) and the starch as mentioned above is degraded into maltose through multi-enzymes involving alpha- amylase, β-amylase and debranching enzymes.

CIM analysis revealed different QTLs (other than SMA) identified for maltose content on *chr* 11, *chr* 13 and *chr* 17 in F_2 population I with significantly high peak LOD score=5.78 for QTL on *chr* 17, resided between the marker intervals: Satt430 (81.92cM)-Satt359 (102.56 cM), Sat_133 (50.78cM)-Satt114 (63.69 cM) and Sat_092 (57.51cM)-Satt311 (84.62 cM), respectively (Table 2). Similarly, in F_2 population II two different QTLs were identified for this trait by CIM on *chr* 6 and *chr* 15 with significantly high peak LOD score=3.2 for QTL on *chr* 15, were in marker intervals : Satt227 (26.65cM)-Sat_062 (30.80 cM) and Sat_381 (64.18cM)-Sat_376 (69.23 cM), respectively.

Table 1. SSR markers significantly associated with maltose content and their allelic effect in F_2 population I and II through SMA.

SSR marker	LOD	R^2(%)	Average content (mg/g) of F_2-population with allele 0	Average content (mg/g) of F_2-population with allele 2	Difference [a]
F_2 population I (Dadachamame × NRC101)					
Sat_407 LG-A1 52.32 cM	2.08	6.2	0.7	0.9	0.2
Satt681 LG-C2 3.15 cM	2.54	6.8	1.0	0.8	0.2
Satt720 LG-E 20.8 cM	2.10	5.3	0.9	1.5	0.6
Sat_216 LG-H 85.27 cM	3.18	9.7	0.7	1.8	1.1
F_2 population II (NRC121 × NRC101)					
Satt522 LG-F 119.19 cM	2.44	6.6	4.5	3.5	1.0
Sat_105 LG-I 49.34 cM	3.54	10.4	3.7	4.9	1.2

[a] Difference in average content of maltose is attributed to allele 0 (Dadachamame/ NRC121) or 2 (NRC101) inherited from Dadachamame/NRC121 or NRC101, respectively, in F_2 population.

Table 2. Chromosomal locations and parameters associated with QTLs identified for maltose content in soybean seeds of F_2 population I and II using Composite Interval Mapping (CIM).

LG/*Chr*	Peak Position (cM)	Interval range (cM)	Marker Interval	Peak LOD	R^2 (%)
F_2 population I (Dadachamame × NRC101)					
B1/ *chr11*	92.0	81.92-102.56	Satt430-Satt359	2.51	6.8
D2/ *chr*17	68.53	57.51- 84.62	Sat_092-Satt311	5.78	15.0
F/ *chr*13	57.8	50.78- 63.69	Sat_133-Satt114	2.73	7.4
F_2 population II (NRC121 × NRC101)					
C2/ *chr*6	27.16	26.65-30.80	Satt227- Sat_062	2.8	7.5
E/ *chr*15	67.73	64.18-69.23	Sat_381-Sat_376	3.2	8.5
I/ *chr*20	49.34	49.34-65.62	Sat_105-Sat_104	2.5	6.8

Conclusion

Taste, which is the important determinant of organoleptic acceptance of soy-food, can be improved by enhancing the levels of sweetness-imparting soluble carbohydrate *viz.* maltose in soybean seed. In the present study, phenotyping for maltose, and genotyping using polymorphic SSR markers of 2 F_2 populations led to the identification of QTLs associated with this sugar in soybean. Some of these identified QTLs, namely, Sat_216 (LG-H/*chr* 12, 85.27cM, LOD score=3.18) , Satt681 (LG-C2/*chr* 6, 3.15 cM, LOD score=2.54) and Satt720 (LG-E/ *chr* 15, 20.8cM, LOD score=2.10) are in the proximity of functional genes involved for the biosynthesis of enzymes, such as β-amylase, glycoside hydrolase and UDP-glucosyl transferase which degrade starch into maltose. Allelic contribution of these genomic regions to maltose varied significantly ($P < 0.5$). These genomic regions can be potentially useful in marker assisted breeding for development of high maltose content soybean genotypes.

Acknowledgements

The authors thank Indian Council of Agricultural Research-Indian Institute of Soybean Research, Indore, for providing infrastructure to carry out this work.

Conflict of interest

The authors declared no conflict of interest.

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