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Identification of SSR markers linked to activity of lipoxygenase in soybean *Glycine max* (L.) Merr.

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Abstract

Lipoxygenase (LOX) activity in soybean seeds contributes to aroma and flavor of soybean, including the disease resistant traits. The research was aimed to determine SSR markers for this enzyme activity in the seeds by conducting on 146 RILs population, obtained from a cross between 'Nakhon Sawan1' x 'AGS129'. The RILs and their parents were planted in the field experiment in Phayao, a province in the north of Thailand, using a randomized complete block design (RCBD) with two replications in dry seasons of 2017. A total of 43 SSR markers segregated in RILs population were distributed over 15 linkage groups (LGs), covering 352.76 cM of the soybean genome with the average distance between adjacent markers of 8.2 cM. These linked markers were used to locate quantitative trait loci (QTL) by composite interval mapping (CIM). QTL, identified for LOX activity, is located between Satt236 – Satt258 on LG A1, explaining 7.7 % phenotypic variation. Our results will be useful for LOX activity trait selection in soybean breeding programs.

Introduction

Most of soybeans are used for soybean oil extraction, food processing and feed rations. However, the off-flavor happened during the processing of soy-food products is limitations of consumption. The off-flavor has been attributed to the release of hexanal compounds during the catalytic oxidation of polyunsaturated fatty acids viz. linoleic and α-linolenic acid by lipoxygenase (LOX) in soybean seed. This enzyme presents in the three isozymic forms i.e. LOX-1, LOX-2 and LOX-3.[1] Heat can inactivate the LOX activity but the heat affects the protein functions.[2] Therefore, the key of off-flavor elimination is the development of LOX-free genotypes in soybean cultivars. Recently, using SSR makers can identity QTL that are associated with locus on chromosomes which are genes controlling the interesting quantitative traits.[5] Importantly, soybean contains over 700 SSR marker loci on 20 linkage groups in the whole genome.[6] Moreover, SSR markers are used for the marker-assisted selection (MAS) that can lead to accuracy selection in breeding program for producing new cultivars. In previous studies, SSR markers were used to identify QTL of yield components traits [7] and LOX activity in soybean.[3,8] SSR markers, Sat_074 and Satt522 from LG F, have been reported to be linked with *LOX-2* locus by conducted on F_2 population of India's soybean. However, there is no reports about QTL of this trait in soybean cultivars of Thailand.

Therefore, this research was aimed to determine SSR markers for LOX activity in the seeds by conducting on 146 RILs population, obtained from a cross between 'Nakhon Sawan1' (NS1) x 'AVRDC Glycine Selection No. 129' (AGS129).

Methodology

Plant materials

'NS1' and 'AGS129' are parents for recombinant inbred lines (RILs) population in this study. The F_1 seed was self-fertilized and upped by single-seed descent method. Finally, 146 F_6 RILs were used as a materials in this study. 'NS1' is popular soybean cultivar due to its large and good quality seed with high oil content.[9] 'AGS129' is vegetable soybean cultivar from Japan. Srinives et al. (1988)[10] reported that the line 'AGS129' was resistant to downy mildew disease. One hundred and forty six of RILs and their parents were planted at Phayao, a province in the northern of Thailand, using a randomized complete block design (RCBD) with two replications during the dry seasons (January – April, 2017). In each block, seeds were sown in row with 8 plants per row (12.5 cm plant spacing) and 50 cm row spacing. The 'NS1' cultivar was grown as border rows.

Evaluation for LOX activity

For determination of LOX isozymes, the 0.1 g of seeds were ground in a liquid nitrogen. The ground soy flour was mixed with 5 ml of 0.2 M sodium phosphate buffer solution pH 6.8. The solution was centrifuged at 10,000 RPM for 15 min at 4 ◦C. The supernatant was collected as a crude enzyme extract using for LOX activity and protein determination.[11]

LOX activity was analyzed using the protocol of Sirikesorn *et al*. (2015)[12] with a minor modification. The reaction mixture consisted of 10 µl crude enzyme extract as enzyme source, 180 µl of 0.2 M sodium phosphate buffer pH 6.8 and 10 µl of 10 mM linoleic acid solution as a substrate. Absorbance of reaction mixture was measured at 234 nm in a microplate reader (SpectraMax M3) every 15 seconds for 3 minutes at 25 ◦C. An increase in absorbance at 234 nm was due to happening of hydroperoxides from linoleic acid as a substrate in the catalytic oxidation reaction.

Quantity of protein in crude enzyme extract was assayed according to the standard Bradford method with bovine serum albumin as using for a standard curve.[13] LOX activity (U/mg) were calculated using the formulas:

$$\text{Enzyme activity (U/mg)} = \frac{\Delta\text{A (milli–unit/min)} \times \text{Volum of reaction (ml)}}{\varepsilon\left(\text{M}^{-1}\text{cm}^{-1}\right) \times \text{Volum of crude enzyme (ml)} \times \text{protein conc. (mg/ml)}}$$

Where ΔA is change in absorbance (milli-unit/min),

ε is molar extinction coefficient; 25,000 $M^{-1}cm^{-1}$

SSR marker analysis

Genomic DNA was extracted from young soybean leaves of parents and RILs population according to the CTAB method[14] with minor modifications. DNA was qualified and quantified using spectrophotometer at 260 and 280 nm.

A total of 61 SSR primer pairs were used to detect polymorphism between the parents. Polymerase chain reaction (PCR) was performed in a 10 µl reaction volumes, consisting of 2 µl of 50 ng/µl genomic DNA, 1 µl of 10X reaction buffer, 0.4 µl of 50 mM $MgCl_2$, 0.2 µl of 10 mM dNTPs, 2 µl of 2.5 nmol each forward and reverse primers, 0.2 µl of 5U *Taq* DNA polymerase (Biotechrabbit) and 2.2 µl of nuclease-free water. The PCR reaction was amplified in a Biometra® PCR thermocycler. Thermal cycles for SSR primer was programmed as following: 94 °C for 5 min followed by 35 cycles of 94 °C for denaturation 30 s, 55 °C for annealing 40 s, 72 °C for primer extension 1 min, and 72 °C for final extension 5 min. Then, 5 µl of the final PCR product was detected onto 3% agarose gels electrophoresis and stained with ethidium bromide.

Linkage map and QTL analyses

The genetic linkage map was build and drawn by QTL IciMapping version 4.1.[15] A minimum LOD threshold of 3.0 were used to requirement for building of linkage groups. The map distances in centimorgan (cM) values were estimated from recombination values using Kosambi mapping function.[16]

For QTL analysis, The QTLs associated with the LOX activity were identified by composite interval mapping (CIM) method using the software program QTL IciMapping version 4.1. Walking speed was determined at 1 cM. Significant threshold for appearing of QTL (P = 0.05) was calculated to compute LOD thresholds for LOX activity. Permutation test indicated LOD score of 2.0 was suitable threshold for this data.

Results and Discussion

LOX activity variation in the RIL population

Figure 1 showed that the LOX activity of RILs, varied from 0.026 - 0.159 units/mg protein with the mean of 0.097 ± 0.026 units/mg protein. The average of LOX activity in 'NS1' and 'AGS129' were 0.069 ± 0.013 and 0.040 ± 0.016 units/mg protein, respectively. Significant difference was detected between the two parents. The mean of RILs population exhibited higher than their parents. This result showed that transgressive segregation was observed for the LOX activity traits in this population. Furthermore, frequency distribution of LOX activity was continuous, showing that the LOX activity was quantitatively inherited trait.

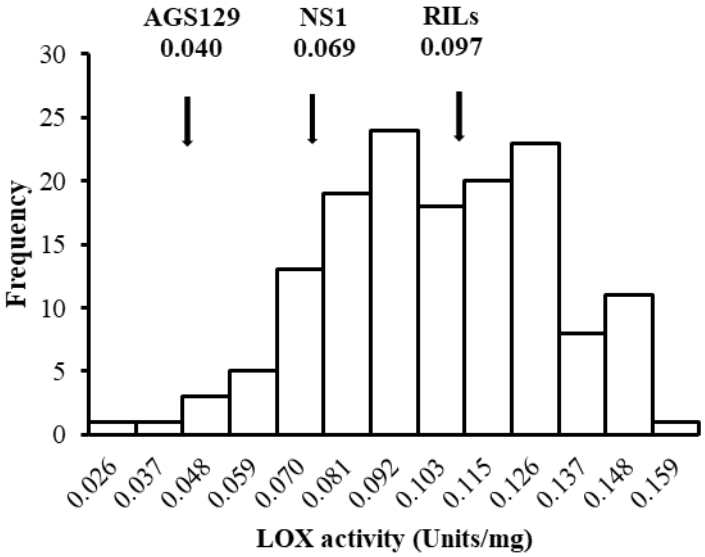

Figure 1 Frequency distribution of LOX activity among 146 RILs individuals derived from the cross between 'NS1' x 'AGS129'.

Linkage map and QTL mapping

Sixty one of the SSR markers were used to construct a partial genetic map. Forty three SSR markers could be defined in the 15 linkage groups (LG) and 18 markers were unlinked. The SSR markers mapping in our study was consistent with recorded in the genetic linkage map by Williams 82 Physical Map (2008).[17] However, the haploid chromosome number of soybean is 20 but amount of linkage groups building by our study was 15 LG and many unlinked markers. The total distance of the map was 352.76 cM. The largest LG was O, with 7 SSR markers and a length of 125.56 cM. The smallest LG was C1, with 2 SSR markers. The mean chromosome length was 8.2 cM (Figure 2.) Identification for QTL controlling LOX activity by CIM showed in 1 marker on LG A1with significant LOD 2.49. The QTL was located on LG A1 between markers Satt236 and Satt258 at the position of 65.00 cM. The left-side

marker of the identified QTL is Satt236 at the position of 60.50 cM and the right-side marker of the identified QTL is Satt258 at the position of 71.50 cM (Figure 2.) The percentage of phenotypic variance explained (PVE) was 7.7 %. Previous studies showed that Satt258 locus was located between Satt225 and Sat_217 in LG A1.[17] Bachlava *et al*. (2008)[18] reported that Satt225 and Sat_217 was linked with seed linoleic acid content locus by mapping on F_5 population of soybean ('N97-3363-3' x 'PI 423893'). In addition, Satt258 was associated with QTL of seed oil content trait in the mapping population of F_2 derived from 'Pak Chong 2' x 'Laos 7122'.[19] Also, Satt236 associated with several traits such as seed protein content,[20] leaflet shape,[21] and whitefly resistance.[22] Thus, the same QTL could influence several different characteristics. In this study, discovering QTL of LOX activity trait was located on the same area with seed linoleic acid content trait while linoleic acid is used as a substrate of LOX activity. Satt236 and Satt258 were new markers linking to LOX activity trait. There were no markers on LG A1 associated with this trait in previous studies.

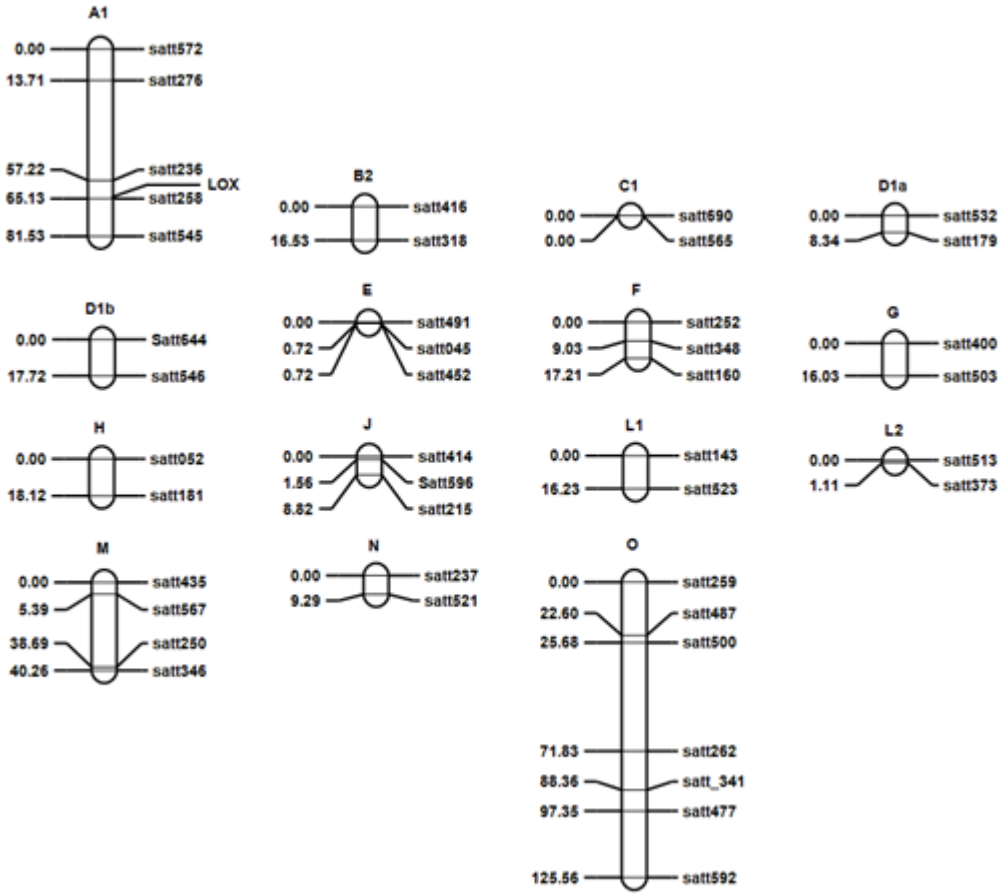

Figure 2. Linkage map based on the RILs population derived from the cross between 'NS1' x 'AGS129'.

LOX in soybean seeds is present in the form of three isozymes i.e. Lox1, Lox2 and Lox3.[1] Locus of *Lox1* and *Lox2* have been provided to be strongly linked to each other in previous studies.[23,24] *Lox2* gene was located at the termination of LG F between Satt522 and Sat_074. Satt522 was recorded to be connected with *Lox2* locus in the earlier study.[8] In our result, 61 SSR markers was included Satt522 but it could not be assigned in any linkage groups. We also used single marker analysis to determine the association between Satt522 and Lox activity. However, there was no significant difference between them. This result could be explained that Satt522 was unlinked with *Lox* locus in our mapping population. Moreover, the amount of SSR markers in this study was partial genome. This may be since the minor coverage

of genetic linkage map of whole genome was reported in this study. There is a possibility that the other QTLs with major, moderate and minor effects linked to the LOX activity.

Conclusion

QTL, identified for LOX activity, was located between Satt236 – Satt258 on LG A1, explaining 7.7% phenotypic variation. Our results will be useful for LOX activity trait selection in soybean breeding programs.

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