

QTL Study to Reveal Soybean Response on Abiotic and Biotic Stresses

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ABSTRAK

Studi QTL untuk Mengungkap Respon Kedelai terhadap Cekaman Abiotik dan Biotik. Puji Lestari, Sutrisno, dan I Made Tasma. Sebagai tanaman kacang-kacangan yang penting, kedelai (*Glycine max* [L.] Merr.) yang adaptif terhadap perubahan lingkungan merupakan sumber daya genetik yang berharga. Strategi untuk meminimalkan dampak dari efek iklim harus digaribawahi pada produksi kedelai yang meliputi ilmu genomika terbaru dan ramalan iklim di masa depan secara tepat. Tanaman, termasuk kedelai memberikan respon pada perubahan iklim dalam aspek faktor lingkungan abiotik dan biotik. Untuk memprediksi respon kedelai terhadap cekaman abiotik dan biotik, kemajuan terkini *quantitative trait loci* (QTL) yang berhubungan dengan cekaman abiotik dan biotik, pembungaan dan sumber daya terkait genomik dapat diakses di SoyBase (<http://www.soybase.org>) dan Phytozome (<http://www.phytozome.net>). Karena cekaman abiotik dan biotik terlibat dalam modulasi pembungaan pada kedelai, gen terkait QTL untuk cekaman abiotik/biotik dan pembungaan/*maturity* juga potensial untuk menghadapi perubahan lingkungan. Dengan memetakan QTL untuk pembungaan menggunakan satu populasi di lokasi yang berlainan (Korea dan Cina) dengan garis bujur, lintang, dan ketinggian yang berbeda, korelasi *syntenic* antara dua QTL ini pada kromosom kedelai 6 dan 13 menunjukkan peran spesifik lingkungan daerah *syntenic*. Informasi QTL dan kandidat gen yang terkait dapat membantu pemuliaan dengan bantuan marka molekuler dan menjadikan kedelai sebagai model tanaman kacang-kacangan yang adaptif terhadap cekaman abiotik/biotik.

Kata kunci: Kedelai, cekaman abiotik, cekaman biotik, perubahan iklim, QTL.

ABSTRACT

QTL Study to Reveal Soybean Response on Abiotic and Biotic Stresses. Puji Lestari, Sutrisno, and I Made Tasma. As an important grain legume, the improved soybean (*Glycine max* [L.] Merr.) adaptive to environmental changes is a valuable genetic resource. Strategy to minimize the impact of climate effects should be underlined on soybean production encompassing advanced genomics and well predicted future climate. Crops including soybean respond to climate change in the aspect of abiotic and biotic environmental factors. To predict soybean response to

abiotic and biotic stresses, current progress of quantitative trait loci (QTL) for abiotic and biotic stresses and flowering and related genomic resources could be accessed at SoyBase (<http://www.soybase.org>) and Phytozome (<http://www.phytozome.net>). As the involvement of abiotic and biotic stresses modulating flowering in soybean, genes linked to QTL for abiotic/biotic stress and flowering/maturity were also potential for resisting the environmental changes. By mapping QTLs for flowering using one population in different locations (Korea and China) with distinctive longitude, latitude, and altitude, syntenic correlation between these two QTLs on soybean chromosomes 6 and 13 indicates the environmental specific role of syntenic regions. The information on QTL and related candidate genes may assist marker-assisted breeding and enact soybean as a model of adaptive legume crop under abiotic/biotic stress.

Keywords: Abiotic stress, biotic stress, climate change, QTL, soybean.

INTRODUCTION

Global climate change has been debated for its causes and potential impacts to many sectors including agriculture and natural resources in the recent years (IPCC, 2009). Incorporated the climate change and population growth convergence frighten worldwide food security. Approaches involving stress-tolerant germplasm in parallel with sustainable crops expectedly could deal with food demand under less favorable conditions (Reynolds and Ortiz, 2010). In terms of climate change, plants should respond to not only abiotic (temperature, precipitation, soil moisture/water stress), but also biotic (pests and diseases) environmental factors. Since abiotic and biotic stresses could influence the production of many crops, growth, yield, and quality of soybean (*Glycine max* [L.] Merr.) are also subjected to the changes of climatic conditions (Southworth *et al.*, 2002).

Soybean is widely used for human food consumption and animal feed because its seeds are rich in protein and oil along with other nutritional values. Additionally, inexpensive and high quality of protein and edible oil make soybean more valuable regarding industrial uses. Unlike other crops, biological

symbiotic interaction between *Rhizobium* and soybean for fixing nitrogen from atmosphere contribute to improve soil fertility. Because of the economic and environmental importance of soybean, high efforts have been undertaken for its sustainable production. As the most important grain crop in the world, this legume is well grown in wide range of climates. The major productions are made in the region between 25° and 45° latitude at low to medium altitude in which temperature is favorable (Fageria *et al.*, 2011). More than 251 million metric tons of soybeans were produced worldwide in 2011 and about 261 and 151 million metric tons are consumed as protein meal and vegetable oil, respectively (Soy Stats, 2012). However, reduced soybean yields under future climates scenario were suggested, influencing food security (Southworth *et al.*, 2002).

Plant responses to stress coordinated by adjusting several activities, such as, physiological, cellular, molecular, growth, development, and these various changes could be detected by analyses of transcriptome, proteome, and metabolome (Ahuja *et al.*, 2010). In complement to molecular genetic approach, genomics strategy will be the excellent choice for studying plant yield stability under stress conditions and allow to combine physiological and phenotypic data with the information of gene complement, transcription, protein complexes and pathway, evolutionary adaptive diversification, and mutated characters (Bohnert *et al.*, 2006). The presence of the recent duplicated soybean genome at 13 million years ago offers the better understanding of molecular and physiological basis of any trait. Cannon and Shoemaker (2012) suggested that quantitative trait loci (QTLs) of plants, whose whole genome have been sequenced like soybean, provide superior source for candidate genes of interests, including abiotic and biotic stresses.

Soybean varieties adaptive to environmental stress could be developed by selection of germplasm or breeding. Recently, breeding program using marker assisted selection (MAS) becomes more efficient due to the identified QTL for numerous traits including resistance to abiotic and biotic stresses. Hundreds of QTLs for fungal disease resistance have been detected, such as sudden death syndrome, phomopsis seed decay, *Sclerotinia* stem rot, soybean rust, and *Phytophthora* root rot. A number of QTLs related to insect and cyst nematode resistances have been identified using various soybean populations under multiple environments (Terry *et al.*, 2000; Xing *et al.*, 2012). Studies on QTL and MAS have also been focused on mitigating negative effects of abiotic stress.

Many studies report major QTLs controlling aluminum tolerance, phosphorus use efficiency, salinity, drought, and lodging (Abdel-Haleem *et al.*, 2012; Bianchi-Hall *et al.*, 2000; Du *et al.*, 2009; Hamwieh *et al.*, 2011; Li *et al.*, 2005). All these identified major QTL are very valuable to identify the underlying candidate genes for abiotic and biotic stresses for increasing soybean tolerance. These QTL informations might help to give insights how soybean plants respond to environmental changes.

The availability of publicly accessed and detailed genomic information can contribute on further studies for soybean improvement. Soybean genomic information and related sources were retrieved from Phytozome (<http://www.phytozome.net/soybean>) and SoyBase (<http://soybase.org>). QTL related to abiotic stress (such as aluminum tolerance, drought, flooding tolerance, lodging, water use efficiency, reactions to alkalinity and salinity, etc.) and biotic stresses (diseases and pests) and flowering/maturity in soybean were obtained from SoyBase. This article aims to introduce valuable sources of QTLs in relation with soybean responses to abiotic and biotic stresses and related researches and to describe a case study of syntenic soybean QTLs for flowering identified at different latitudes.

QTLs FOR TOLERANCE TO ABIOTIC AND BIOTIC STRESSES IN SOYBEAN

Current progress of soybean genomic resources including the whole genome of approximately 975 Mb in 20 chromosomes has led a rapid evolvement of genomics and genetic studies. The recent duplication in soybean genome was confirmed according to co-linearity between the genome of soybean and *Lotus japonicus*. Integrated genetic and physical maps were reported using various kinds of DNA markers. These databases present the most current genetic, physical, and genomic sequence maps integrated with qualitative and quantitative traits. The QTLs represent more than 18 years of QTL mapping of various traits including abiotic and biotic stresses and flowering/maturity were deposited in SoyBase. More than 60,000 protein-coding loci in soybean genome and 1,100 QTLs could assist to identify stress-responsive genes. QTLs associated with complex abiotic and biotic stresses tolerance traits have been targeted and elucidated their relations with other traits by several approaches (www.phytozome.net/soybean; <http://soybase.org>). A number of QTLs for abiotic and biotic stresses as well as flowering/maturity have been identified using several populations across environments (Abdel-

Haleem *et al.*, 2012; Cheng *et al.*, 2011; Hamwieh *et al.*, 2011; Liu *et al.*, 2011; Xing *et al.*, 2012).

At least 103 QTLs related to abiotic stress were identified with high variations. Along with a high number of QTL for iron efficiency and flooding tolerance, the identified QTL related to aluminum tolerance, drought susceptibility, water use efficiency, and tolerance to alkalinity and salinity were also important because these traits are related with climate change. Among more than 100 soybean diseases in terms of biotic stress, the diseases caused by infections of fungi and cyst nematodes showed significant effects on soybean yield. The soybean cyst nematode (SCN) (*Heterodera glycines*) is the most severe disease regarding yield reduction. New QTL for disease resistance were discovered, such as stem rot (*Sclerotinia sclerotiorum*), root and stem rots (*Phytophthora sojae*), sudden death syndrome (*Fusarium solani* f.sp. *glycines*), root knot nematode (*Meloidogyne incognita* [Kofoed and White] Chitwood), and more others. Among 340 QTLs related to pests and diseases deposited in SoyBase, 124 QTLs for flowering/maturity are found to be linked with QTLs for abiotic and biotic stresses.

SOYBEAN RESPONSE TO ENVIRONMENTAL CHANGES

Earth surface temperature will increase rapidly in the future and carbondioxide is projected to around 700 ppm by 2100 due to climate change, especially double carbondioxide may decrease soil moisture in summer that affect plants adaptation (IPCC, 2009). To respond climate change, soybean plant may sustain growth and development only in narrow range of environmental fluctuation, while others reveal flexibility under a wide range of conditions. Current ozone concentration is estimated to cost of 5–20 metric tons/km² of potential soybean yields (Van Dingen *et al.*, 2009). In many cases, soybean yields are negatively correlated with temperature, in which high temperature and drought may partially trigger early soybean flowering (Wolf, 2002). Every additional degree (°C) of future global warming during summer could decrease soybean yield by 16% (Kucharik and Serbin, 2008).

Various mechanisms are shown by soybean to respond adaptive changes. Some soybean cultivars demonstrate that elevated carbondioxide (550 ppm) increase 20% light saturated photosynthesis and decrease 20% stomatal conductance (Morgan *et al.*, 2005) and enhance expression of transcripts for sugar metabolism and respiration (Leakey *et al.*, 2009).

Soybean metabolites such as hexose, sucrose, starch, ureides, and amino acids increase for metabolic adaption to climate change catastrophes (Ainsworth *et al.*, 2007). Interestingly, overexpression of soybean *GmWRKY13*, *GmWRKY21*, and *GmWRKY54* confers differential tolerance to abiotic stresses in *Arabidopsis* plants (Zhou *et al.*, 2008). Some regulators of genes associated with defense and abiotic/biotic stress are found to be induced by sodium, drought, some hormones, and soybean mosaic virus (Zhang *et al.*, 2009).

Improved soybean varieties with high tolerance to abiotic stresses and resistance to diseases are thought to be better adaption to environments changes, such as by increasing the efficiency of photosynthesis and respiration, and antioxidant capacity (Betzberger *et al.*, 2010; Zhu *et al.*, 2007). However, the multigenic nature of improved tolerance in addition to the complexity of soybean genome leads to conduct long-term breeding program or identify new materials from biodiversity. In this regard, a selective manipulation of genetic components is needed along with the estimates of the magnitude of phenotypic effect of different QTLs. Variation caused by environments interacted with genotype and by interaction of genes at different loci offer a way to predict a QTL possibly useful for adapting in diverse environments (McCouch and Doerge, 1995).

QTLs FOR ABIOTIC AND BIOTIC STRESSES IN RESPECT OF ADAPTIVE CHANGES

According to the role of regulatory factors in the environmental adaptation mechanisms, some common QTLs/genes influencing more than one adaptation process have been identified in some plant species. Wheat QTLs for frost tolerance (*Fr-A1*, *Fr-A2*) and vernalization requirement (*Vm-A1*) were positioned on chromosome (Chr) 5A. QTLs for copper tolerance were colocalized as the *Vm-A1* gene, in other case, heat and drought adaptive QTLs were identified in one wheat population (Pinto *et al.*, 2010). The developmental-stage specificity of QTLs for abiotic stress tolerance was also shown by evaluating drought tolerance in barley (Balint *et al.*, 2008).

Many QTLs tolerant to abiotic stress and pest and diseases introduced in previous studies were focused to complement soybean breeding program and some QTLs demonstrated their relations with yield in different environments. Major QTLs for both alkalinity and salinity (Tuyen *et al.*, 2010) and consistent major QTL for iron tolerance in different fields could be good candidates (Lin *et al.*, 2000). QTL with consistent yield

was identified across different water regimes (Du *et al.*, 2009) and some markers significant for flooding tolerance were linked to disease resistance (*Rps*) genes against *Phytophthora sojae* (Cornelious *et al.*, 2005). Consistent QTLs for flowering were colocalized with QTLs across different water regimes and seasons (Githiri *et al.*, 2006). Major QTLs associated with tolerance to abiotic and biotic stresses and their linked markers may encompass genes implicated in tolerance and resistance mechanisms, which could be associated with flowering/maturity genes in soybean.

A CASE STUDY: SYNTENIC QTL FOR FLOWERING AT DIFFERENT LATITUDES

Coordinated flowering/maturity responses of soybean as a short day crop are often related to environmental parameters changes. To investigate how soybean responds to different environmental conditions (Table 1), recombinant inbred lines (RILs) from a cross between late-flowering “Jinpumkong 2” and early-flowering “SS2-2” were used for the QTL analysis for flowering time at different latitudes (Table 2). Phenotypic evaluations for days to flowering were conducted at two areas with different latitudes, Suwon, South Korea (37°15'N) and Longjing, China (42°46'N), and a strong correlation of days to flowering was observed between Korea and China (Liu *et al.*, 2011).

These two different latitudinal and altitudinal conditions with different photoperiod showed environment-sensitive and less sensitive QTLs

modulating flowering time in soybean, indicating soybean response to environmental changes (Table 2). Significant QTLs for flowering were detected with high phenotypic variations in both Korea and China and in either Korea or China. Based on interval mapping analysis, QTLs for flowering were found to be specific only in Korea, in both Korea and China but not found in China. A flowering QTL in the Satt202-Satt371 (Chr 6) was for both Korea and China. The environmentally less sensitive QTL with higher phenotypic variation and maximum additive affect in China was shown than that in Korea (Liu *et al.*, 2011). Different photoperiod and temperature on these two environments may contribute mainly for the distinctive flowering variations.

Syntenic QTL regions were identified between Chrs 6 and 13, indicating an association of the QTL with homeologous chromosomal regions in soybean genome. About 60 kb in Chr 6 in Satt202-Satt371 was co-linear with a 317 kb region flanked by Satt206 and Satt595 in Chr 13. Six duplicated gene pairs were clustered in homeologous QTL for flowering on Chrs 6 and 13 with high homology around 80–90%. The Korea-specific QTL for flowering on Chr 13 might be associated with the recent duplication of soybean genome (Liu *et al.*, 2011). This case suggests that these syntenic regions might be involved in environmental specific regulation.

In fact, soybean developmental stages are greatly influenced by variety-specific day length initialing flower development. It is not easy to predict soybean maturity accurately because of combination between

Table 1. Geographical locations and soybean planting date of two different environments.

Region condition	Suwon, South Korea	Longjin, China
Latitude (N)	37°15'	42°46'
Longitude (E)	126°59'	129°26'
Altitude (m)	31	242
Planting date (year-month-date)	09-5-27	09-5-10

Source: Liu *et al.* (2011).

Table 2. SSR markers associated with QTLs for flowering in the RILs derived Jinpumkong 2 and SS2-2 ($P < 0.01$) which were observed in different location with different environment, a case study.

Marker	Chr.	Map position (cM) ^a	P value	R ² (%)	P value	R ² (%)
Satt041	2	84.0	0.0066	8.56	0.0085	8.34
Satt591	6	31.1	-	-	0.0099	10.90
Satt202	6	126.2	0.0017	14.7	<0.0001	22.30
Satt055	9	33.0	0.0052	9.0	-	-
Satt417	9	46.2	0.0047	12.1	-	-
Satt633	10	56.9	0.0012	11.9	-	-
Satt146	13	1.9	0.0050	13.2	-	-
Satt206	13	27.0	0.0002	15.3	-	-

Source: Liu *et al.* (2011).

different particular photoperiod demand and other environmental variations such as abiotic/biotic stress. Since rapid climate change may effect on pathogenicity in different environment and stability of resistant variety, soybean disease epidemics may cause the disease survival in production with new pathogen as a consequence of shifting climates. Understanding soybean responses to abiotic and biotic stresses with their interactions is the key to increasing soybean yield in the future. Well-predicted QTLs and candidate genes for stress tolerance may reveal novel mechanism of adaptation in soybean.

CONCLUSIONS

The unpredictable climates need couple efforts against abiotic and biotic stresses to develop adapted soybean lines that minimize the risk of yield loss. Advanced and complete soybean databases facilitate to explore and freely access a large number of QTLs related to abiotic and biotic stresses which can be useful information to support many studies on soybean response to environmental stresses. Syntenic QTL regions identified between Chrs 6 and 13 indicates an association of the QTL with homeologous chromosomal regions in soybean genome. Well-predicted QTLs and candidate genes for stress tolerance may reveal novel mechanism of adaptation in soybean. These QTLs are valuable for improving tolerant/resistant soybean varieties via molecular breeding program. For this goal, an integrated research effort among soybean breeders and plant geneticists, plant physiologists, plant pathologists, and agronomists is needed for the impact of future environmental changes to sustainable soybean grain security.

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