

Gene Duplication to Reveal Adaptation Clue of Plant to Environmental Stress: A Case Study of *NBS-LRR* Genes in Soybean

(Duplikasi Gen Mengungkap Petunjuk Adaptasi Tanaman terhadap Cekaman Lingkungan: Studi Kasus Gen *NBS-LRR* pada Kedelai)

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Submitted: 4 August 2016; Revised: 30 September 2016; Accepted: 15 November 2016

ABSTRAK

Gen responsif terhadap cekaman lingkungan yang bertahan setelah mengalami duplikasi genom skala kecil merupakan bagian dari duplikasi genom. Namun demikian, informasi duplikasi gen yang berpengaruh terhadap sifat adaptif tanaman terhadap perubahan lingkungan masih terbatas. Ulasan ini menyajikan gambaran peristiwa duplikasi dalam genom tanaman yang berdampak terhadap duplikasi gen yang berkaitan dengan perubahan lingkungan, informasi duplikasi gen yang merupakan bagian dari mekanisme adaptasi, kasus duplikasi gen *nucleotide-binding site-leucine-rich repeat (NBS-LRR)* pada kedelai, dan implementasi informasi duplikasi gen terhadap pemuliaan kedelai di Indonesia. Peristiwa duplikasi genom menghasilkan duplikasi gen dan berperan terhadap evolusi adaptasi pada perubahan lingkungan. Informasi umum tentang tanaman yang beradaptasi dengan cekaman lingkungan memungkinkan untuk meningkatkan pemahaman kita tentang duplikasi gen sebagai mekanisme adaptasi. Gen-gen *NBS-LRR* yang terduplikasi diketahui berasosiasi dengan QTL terkait ketahanan penyakit dan ekspresi diferensialnya membuktikan kontribusi gen tersebut pada ketahanan kedelai terhadap cekaman biotik. Model yang diusulkan dari proses gen duplikasi *NBS-LRR* dapat membantu dalam memahami respons gen tersebut terhadap perubahan lingkungan. Duplikasi gen ketahanan terhadap hama/penyakit, *NBS-LRR* khususnya, memberikan informasi penting dalam pemilihan tetua pemuliaan dan pengembangan marka molekuler terkait ketahanan penyakit untuk memperbaiki kedelai di Indonesia secara genetik. Karena itu, sangat mungkin untuk meningkatkan program pemuliaan yang menargetkan gen ketahanan terhadap cekaman biotik/abiotik dan memberikan dasar molekuler sebagai strategi perlindungan tanaman terhadap cekaman dan pengembangan kultivar kedelai khusus untuk lingkungan ekstrim.

Kata kunci: Cekaman lingkungan, duplikasi gen, hama/penyakit, kedelai, *NBS-LRR*.

ABSTRACT

Genes responsive to the environmental stresses which are retained after small scale duplication are part of plant genome duplication. However, information of duplicated genes that could be adaptive to environmental changes in plant is limited. This review presents an overview of duplication events in plant genomes which impact to gene duplication in relation to environmental changes, gene duplication as an adaptation mechanism, a case of duplicated *nucleotide-binding site-leucine-rich repeat (NBS-LRR)* genes in soybean, and the gene duplication implementation for soybean plant breeding in Indonesia. Notably, genome duplication events generate gene duplication and contribute to adaptive evolution against environmental changes. Generalization of plants adaptation to the stressful conditions also probably improves our understanding of gene duplication as a mechanism of adaptation. Several recently duplicated *NBS-LRR* genes in soybean retain disease resistance QTL and the differential expression convince their contribution to biotic stress resistance in soybean. Proposed models of *NBS-LRR* genes duplication process may help to understand these genes response to the environmental changes. The duplication of genes resistant to pest/disease, particularly *NBS-LRR*, provides important information for breeding parents selection and developing molecular markers related to disease resistance to genetically improve soybean in Indonesia. Overall, it may therefore be possible to enhance breeding programs which target on genes tolerant/resistant to biotic/abiotic stresses and provide a molecular basis for crop-stress protection strategy and improve soybean cultivars specified for harsh environment.

Keywords: Environmental stress, gene duplication, soybean, pest/disease, *NBS-LRR*.

INTRODUCTION

Climate change affects many aspects of plant architecture and represents a serious obstacle for developing sustainable agriculture. To cope with climate change, plants have evolved various molecular programs for adaptation. Consequently, plant adaptive strategies to environmental stresses have been a subject to great interest. Recent physiological and molecular programs have been identified in plants which could be relevant to global changes (Meehl and Stocker, 2007; Winning *et al.*, 2009). However, more efforts are still needed due to less ability to encounter the stress challenges, especially to breed crops with enhanced resistance to multiple environmental stresses. The broad range of genes associated with resistance to the environmental stresses in many plants species has been progressed and may assist to develop new plant cultivars (Ahuja *et al.*, 2010). The interlinked genetic and genomic features would make the improved crops achievable to encounter environmental stresses (Mochida and Shinozaki, 2010).

Advanced genomics and molecular biology have impacted to speed the identified genetic regions associated with quantitative trait loci (QTL) that increase opportunities for extensive breeding programs. Since the entire genome of many plant species has been published, the detailed genomic resources facilitate to easily identify desired traits. Whole genome duplication (WGD) forming polyploidy and small-scale duplication (SSD) provide a crucial understanding of genome relationship among plant species with close ancestry. Moreover, these duplication events which impact to genome structure and duplicated genes could be useful as a basis information for the interspecific breeding of plant with at least diploid or higher ploidy level that usually results in the appearance of new ploidy allele, such as allotetraploid in *Brassica*, wheat, maize, cotton, etc. (Renny-byfield and Wendel, 2014; Ziolkowski *et al.*, 2012). Such genome structure with its impact to genes also is beneficial resource for breeding strategy in the corresponding crops, especially challenging with unpredictable climate. In addition to substantial study of the gene divergence and evolution associated with cycles of polyploidization, deeper information of agricultural useful genes and QTLs are able to be explored, including those related to resistance to biotic and abiotic stresses. Evolution of resistance genes has been reported to be implication of genome duplication and gene duplication events (Guo *et al.*, 2004; Hanada *et al.*, 2008).

Gene duplication is considered the main source of functional diversity on genotypic level. After duplication, each copy could evolve independently and diversify the effects, leading to functional novelty. Gene copies of various degree of divergence exist in genomes. Since any mutation, a duplication event may result on organism's fitness. Thus, gene duplication affects gene dosage which is in turn to affect fitness. The fitness effects of a gene duplication that lies mainly in the arisen copy number cause an increase in protein dosage (Kondrashov, 2012), while other mechanisms can lead to adaptive response (Innan and Kondrashov, 2010). Particularly, an environmental adaptation can be the driving force in the fixation of gene duplication. Even some genes are clearly fixed during evolution by positive selection which plays an important role in the fixing of fraction of gene duplication. Genomic approach may identify the action of selection on specific gene duplication including adaptive gene duplications (Kondrashov, 2012).

Unlike the rich data shown by numerous publications from microbiological community suggesting adaptive impact of gene duplication under certain environmental conditions (Kondrashov, 2012), the information of genome and gene duplications in plants are still limited to support the adaptation to the environmental changes. Reports showed that some stress-responsive genes in plants were as tandem array, which might have been duplicated (Hanada *et al.*, 2008; Zhang, 2003). Also, genes related to biotic stress response in *A. thaliana* (Maere *et al.*, 2005) and disease resistance genes containing domain of a nucleotide-binding site and a leucine-rich repeat in soybean (*Glycine max*) (Kang *et al.*, 2012; Shin *et al.*, 2008) were well retained after the SSD and large scale duplication (LSD), suggesting their roles of adaptive evolution against environmental conditions.

We highlighted an overview based on genome resources focusing on plant genome duplication which impacted to gene duplication with their related aspects to environmental changes. This review also presented an information of gene duplication as an adaptation mechanism, a case of duplicated *nucleotide-binding site-leucine-rich repeat (NBS-LRR)* genes in soybean to support a view of these gene relation to biotic stress, and the prospect and challenges of gene duplication implemented on soybean breeding for resistance/tolerance to biotic/abiotic stress in Indonesia.

GENOME AND GENE DUPLICATIONS IN PLANTS

In the lineage of plants, genome duplications involving entire genome duplication (WGD) and SSD have occurred. WGD and SSD are more frequent in plants in comparison to other eukaryotes, as demonstrated in angiosperms as an outstanding model for genome and gene duplications elucidation. Many angiosperms have undergone multiple WGDs with certain mechanisms (Carretero-Paulet and Fares, 2012; Wang *et al.*, 2012). The first angiosperm that has its genome sequenced, *A. thaliana*, evidenced at least three duplication rounds during its evolutionary history, of which two were recent WGDs. Moreover, cereals and legumes appear to experience different numbers of WGDs and reveal their shared duplications with other relative species and their ancient (Freeling, 2009). In the case of legume crops (e.g. soybean), in general they have undergone two rounds of duplications. Mungbean genome, however, demonstrated the exception of legume duplication phenomena that its genome has experienced only one round of duplication event (Kang *et al.*, 2014). Unlike WGD that involves large scale duplication (LSD) leading to a dramatic risen in duplicated genes number, SSD is more restricted only involving one or few genes. Both WGD and SSD contribute significantly to the genome composition and gene duplication (Guo *et al.*, 2004).

Considering its potency to result in novel functions, gene duplication is believed to relate with diverse evolutionary importance. Consequently, new genes and gene function play a role in performing phenotypic diversity of plants (Guo *et al.*, 2004). One duplicated gene is predicted to be retained the ancestral function and another one evolves neutrally and free from selective constrains, indicating the divergence of the duplicated genes. For example, as a result of genome and gene duplications, two main branches of angiosperms, namely eudicots and monocots were estimated to diverge between 125 to 140 million years ago (Mya) and 170 to 235 Mya, respectively (Davis *et al.*, 2004). Million years ago red clover, *Medicago truncatula*, soybean, and common bean were diverged from each other and from *A. thaliana*. Between two legume members, *M. truncatula* and alfalfa, syntenic and chromosome relationships of both genomes were also indicated, supporting their ancient duplication histories (Kim *et al.*, 2015).

Genetic mechanism underlying duplication events can be different to result in duplicated genes. Several modes of gene duplication are distinguishable such as WGD/segmental, tandem, DNA-based

transposed duplications, etc. Gene families expansion possibly is enriched with particular modes of gene duplication events. Recent genome duplication also revealed homologous retention and chromosomal rearrangement (Schmuts *et al.*, 2010). Homeologous blocks such as QTLs related to protein and oil content along with the corresponding genes have been identified in duplicated segments on the twenty chromosomes of soybean genome as depicted in Figure 1A (Lestari *et al.*, 2013). DNA-based transposed duplications are enriched in disease resistance gene homologs in *A. thaliana* (Figure 1B) and WGS duplications are enriched in the C₂H₂ zinc finger protein in rice (Figure 1C).

Orthologous and paralogous relationships were also found in the genomic regions harboring soybean nematode resistance genes in the genomes of soybean and *M. truncatula* (Wang *et al.*, 2013). These phenomena suggest that gene family member is likely to have common pattern of origin in different evolutionary lineages. Thus, duplicated genomes and genes can facilitate materials for evolutionary and functional divergence. The gene duplication is attributable to increase functional diversity and the increased expression divergence in duplicated genes can substantially contribute to phenotypic diversifications (Wang *et al.*, 2012).

PLANT GENOME DUPLICATION IN RELATION TO ENVIRONMENTAL CHANGES

Duplicated genes, performing a key role in generating phenotypic variation and speciation to distinct species, are less preserved and remain unbalance via the SSD than LSD (Lynch, 2007). Notably, a region of older sister paralogs suggests that ancient SSD may occur before WGD in some crops. The SSDs are thought to be undergone continuously after WGD. Thus, the footprint of most recently duplicated genes should be left behind in the SSD in addition to LSD during its evolution (Guo *et al.*, 2004).

Many non-overlapping duplicated regions show a conserved gene order and orientation in plants (Guo *et al.*, 2004; Peterson *et al.*, 2003). Genes are created by LSD to dramatic increase the duplicated genes numbers, however, these genes are easily decayed after SSD (Severin *et al.*, 2011). Interestingly, most of genes in genome belonging to a gene family are found through continual SSD events. The SSD event, tandem gene duplication accounts for a significant contribution of the increased gene family size, in relative to the LSD depending on plant species (AGI, 2000; Goff *et al.*, 2002).

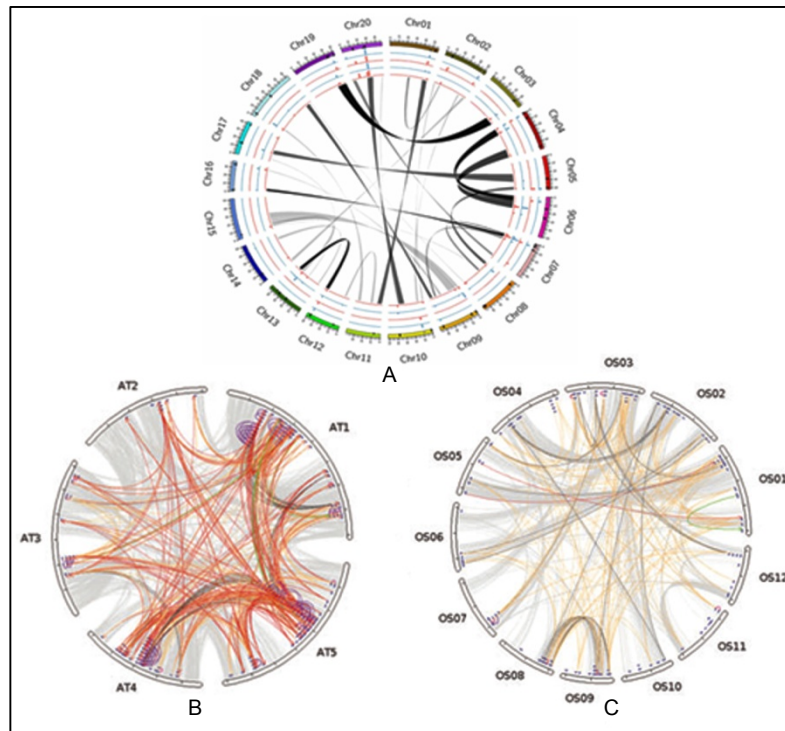


Figure 1. Circular maps showing homologous relationships among chromosomes after genome and gene duplications in the genomes of soybean, *A. thaliana*, and rice. A = QTLs associated with protein and oil content in recently duplicated regions on twenty chromosomes of soybean (Lestari *et al.*, 2013), B = disease resistance gene homologs in *A. thaliana* (Wang *et al.*, 2011), C = C₂H₂ zinc finger gene family in rice (Wang *et al.*, 2011). Chr1–Chr20, AT1–AT5, and OS1–OS12 in each circus denote the shown chromosome number of the corresponding plant genomes. Several colored ribbons represent duplicated regions which are grouped as bundles.

LSD like “polyploidy episode” is thought to be one of the survival mechanisms of plants that may often coincide with the occurrence of extreme environments (Fawcett *et al.*, 2009). That adaptive selection is suggested to possibly play a role in fixing pale-polyploidy in a plant species. Not only polyploidy event, the SSDs in plant genome might be also as a response to environmental changes which is likely to associate with environmental selection (Guo, 2013; Guo *et al.*, 2004). The continuous mode of the SSD results in high rate of gene birth and decay in comparison to LSD. The duplication events may have consequences on the plant fitness, thus any mutation and/or selection could be undergone on the redundant gene copies against environmental changes for providing adaptation (Kandrashov, 2012).

Gene duplication leading to genetic redundancy would implicate to functional buffering. Partial redundancy by knocking out of either duplicate generates in more mitigated phenotypic changes than acting of both copies. Furthermore, functional compensation by duplicated genes for severe phenotypic effects is likely to be preserved longer than for a less severe effect by natural selection (Wang *et al.*, 2013). Thus, differential gene

duplication which contributes to on-going process in genome evolution in geographically isolated plant populations also could cause reproductive isolation and particular adaptation (Magadum *et al.*, 2013).

GENE DUPLICATION AS AN ADAPTATION MECHANISM

The duplicated genes may evolutionary acquire new functions, but how these changes occur and can adapt to different environments are still under investigation as many scientists suspects. Gene duplication as a form of adaptation to various environmental conditions is not a rare mechanism. Adaptive duplication seems to involve the genes which their products interact with molecules associated with variable environments and how rapidly/constantly they are produced at high level (Kondrashov, 2012). Furthermore, some logic hypothesis propose that fixed gene duplication playing an adaptive role in dosage response to environmental stresses are thought to be the functions of gene duplication with characterized adaptive roles (Flagel and Wendel, 2009). It is also instructive to predict the gene categories/types

possibly conferring an adaptation in certain or various environments based on the functional repertoire of the gene duplication in genome (Kang *et al.*, 2012; Kondrashov, 2012). Current accumulated studies demonstrated that new gene copy number may affect function. Many copy number variations (CNVs), including their association with diseases, are selected in the genome, which has to increase in gene dosage (Kondrashov and Kondrashov, 2006; Lupski, 2007). It appears that a gene duplication which is adaptive under a stressful environment comes as fitness cost in a benign environment (Kondrashov and Kondrashov, 2006).

Adaptive gene duplications could be categorized according to organism responses, such as nutrients transport, protection to heat, cold, and salt, heavy metals, adaptation to domestication, etc. A clear example of a gene duplication conferring an adaptation to nutrient limitation was demonstrated by the yeast hexose transporter genes. Under low glucose, the appearance of a new hybrid copy from two very closely related gene paralogues, namely HXT6 and HXT7, arose the expression level of hexose transporter gene and the glucose transport rate into the cells (Brown *et al.*, 1998). Another example, adaptation to heat stress was shown via gene duplication of several stress-related genes in *Escherichia coli* (Riehle *et al.*, 2001). Then, similar observation was also performed in yeast, revealing an increase propensity for chromosomal segmental duplications (James *et al.*, 2008). Other examples included a study of the genome-wide expression and the corresponding copy numbers convinced an evidence for cold adaptations in Antarctic cod (James *et al.*, 2008).

Polyploidy events were observed to play an important role in adaptation in yeast and plant. A consistent polyploidy of yeast strains was observed to play an important role in adaptation. Similarly in plant, polyploidy has been linked to resistance to high salt concentrations in citrus (Saleh *et al.*, 2008) and

sorghum (Ceccarelli *et al.*, 2006), suggesting that polyploidy is likely a general physiological adaptive response to osmotic stress (Dhar *et al.*, 2011). Duplication-induced metal resistance in different plant species might be related to export of the cations outside cell (Kondrashov and Kondrashov, 2006). At present, duplicated genes are associated with recent domestication-related phenotypic characters such as characters controlling milk proteins and the proteins themselves (Liu *et al.*, 2009; Bickhart *et al.*, 2012).

Even though studies of adaptive gene duplication in microorganism seem more than those in plants species, some adaptive gene duplication studies have been reported in plants. A number of adaptive gene duplications in plant species to encounter various environmental conditions are presented in Table 1. A gene encoding 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in common bean, soybean, and tobacco (*Nicotiana tabacum*) was identified to function in the herbicide glyphosate induction (Shaner *et al.*, 2012; Widholm *et al.*, 2001). Some gene copies resulting from duplication events in *A. thaliana* were found related to tolerance to high temperature (DeBolt, 2010). Duplication of genes involved in pathogens resistance suggest the role of gene duplication involved in rapid coevolution between the host and the symbiont or pathogens (Hanada *et al.*, 2008). Six duplicated gene pairs were clustered in specific regions on chromosome 6 and 13 with high homology level of around 80–90%. Syntenic QTL regions identified between the two chromosomes of soybean indicate an association of the QTL with homeologous chromosomal regions in the genome. Well predicted QTLs and candidate genes for stress tolerance may reveal novel mechanism of adaptation in soybean (Lestari *et al.*, 2014). As a result of recent genome duplication, gene duplication could contribute not only to environmental adaptation but also affect other agronomical important traits in soybean such as seed protein/oil content (Lestari and Lee, 2014; Lestari *et al.*, 2013).

Table 1. Few examples of adaptive gene duplication events found in several plant families/species.

Gene	Function	Plant family/species	Environmental condition	Evidence	References
Prolamine-coding genes	Storage proteins	Poaceae	Nutrient storage in seeds	Indirect inference from large number of copies	Xu and Messing (2009)
IRI-like gene family	Ice recrystallisation inhibition	Pooideae	Low temperature	Gene family expansion in cold-tolerant lineage	Sandve <i>et al.</i> (2008)
COR 15	Cold tolerance-related protein	Brassicaceae	Low temperature	Several independent duplications	Zhou <i>et al.</i> (2009)
EPSPS	5-enolpyruvyl shikimate-3-phosphate synthase	<i>Amaranthus palmeri</i> , <i>Medicago sativa</i> , <i>Glycine max</i> , <i>Nicotiana tabacum</i> , other species	Glyphosate	Field studies, experimental evidence	Gaines <i>et al.</i> (2010, 2011); Shaner <i>et al.</i> (2012)

The developed gene families or clusters with related functions are still uncertain whether chromosome duplication itself has adaptive values. Even though the evolutionary forces involve in adaptation to a changing environments, both positive selection and the relaxation of the selective pressures maintaining an old function are equally important. In addition to the chromosome number in the genome, gene duplication events can function not only as an evolutionary mechanism, but also as a form of genetic variation to preserve plants (Kondrashov, 2012; Ramsey, 2011).

AN EXAMPLE: STUDY OF ASSOCIATION BETWEEN *NBS-LRR* GENES AND RESISTANCE TO BIOTIC STRESS IN SOYBEAN

Resistance (*R*) genes with their functional sources against biotic and abiotic stresses could be used to gain improved crops with adaptation (Ahuja *et al.*, 2010). Even though lack studies of *R* genes in diverse diseases in soybean, some promising *NBS-LRR* genes are reported to be associated with important traits related to disease resistance and tolerance to abiotic stresses. *CC-NBS-LRR* especially *ADRI* along with *EDSI* and *ABII* is involved in drought tolerance enhancement (Chini *et al.*, 2004). LRR protein was found only in the aluminum treated library suggesting that the TIR-*NBS-LRR* protein probably takes part in a regulating pathway involved in the recognition of biotic and abiotic stresses. The cleavage of the TIR-*NBS-LRR* protein under aluminum stress might result in the disruption of the immune system, which might increase the susceptibility of the plant to other stresses (Zeng *et al.*, 2012). *NBS-LRR* genes show a high association with resistance to various diseases such as disease caused by *Pseudomonas syringae* pv. *glycinea*, sudden death syndrome and rust diseases, and nodulation in soybean (Ashfield *et al.*, 2003; Zhu *et al.*, 2010). We described an example of correlation between the *NBS-LRR* and disease resistance QTL, functional redundancy of disease resistance in soybean on recently duplicated regions harboring these genes as reported by Kang *et al.* (2012).

A total of 314 *NBS-LRR* genes was located across twenty soybean chromosomes (Table 2) and five *NBS-LRR* were in scaffolds. Of the total *NBS-LRR* genes detected based on their N-terminal domains using homology analyses to the *A. thaliana* *NBS-LRR* genes, 116 *TIR-NBS-LRRs*, 20 *CC-NBS-LRRs*, and 183 other *NBS-LRR* were classified (Kang *et al.*, 2012). A significant correlation was shown between the

numbers of *NBS-LRR* genes and the disease resistance QTLs within the *NBS-LRR*-flanked 2 Mb region supporting the common feature of association between the *NBS-LRRs* and the loci/QTL for biotic stress in plants. Disease resistance QTL for multiple pathogens tend to be positioned near the highly cluster regions harboring *NBS-LRRs*. The cluster was to be associated with duplication events during evolution with possibly driving forces of tandem duplication and mobile DNA-like transposase and integrase (Ream and Neidle, 2004; Roth *et al.*, 1996).

To prove duplication events, it was notable that the distribution of *NBS-LRR* genes in the soybean was biased and clustered on certain chromosomes (Chr 3, 6, 13, 15, 16, and 18), and more than half were existed on these six chromosomes. The clusters were a result of duplication events on the chromosomal location of the *NBS-LRR* genes as was depicted on a circle genome map (Figure 2) (Kang *et al.*, 2012). A number of *NBS-LRR* genes resides within recently duplicated genomic regions where the duplicated disease resistance QTLs also exist, suggesting specific copy number variations of *NBS-LRR* genes in the duplications. Kang *et al.* (2012) identified that most of *NBS-LRR* genes on the recently duplicated regions seem to be in the position of QTLs related to Sclerotinia stem rot, sudden death syndrome, brown stem rot, bacterial leaf pustule (BLP), *Phytophthora sojae*, and soybean cyst nematode infection. Duplicated regions containing the QTLs showed distinctive specificities for divergent diseases. Some duplicated *NBS-LRR* genes in a homeologous pair indicate the probability of the *NBS-LRR* genes to retain their biotic resistance functions and/or acquiring their novel specificities. However, recently duplicated regions contain biased number of *NBS-LRR* genes including tandem duplicated genes and several disease resistance QTLs located on several chromosome locations segregated (Table 2). The functional redundancy between duplicated regions was reported for several traits in soybean including the BLP resistance (Kim *et al.*, 2009) and the recently *NBS-LRR* duplicated regions controlling several disease resistance phenotypes (Kang *et al.*, 2012).

R genes contain abundant copies throughout genomes, possibly produced by unequal crossing over (Meyers *et al.*, 2003). Three models were proposed explaining the duplication process of *NBS-LRR* genes (Kang *et al.*, 2012). Model 1 explained the tandem duplication occurred prior to the recent duplication (e.g. ID 10176678) and Model 2, the recent duplication copied genes/regions to another chromosome, then the tandem duplication occurred

Table 2. The *NBS-LRR* genes, disease resistance QTLs and disease resistance QTLs located within the 2-Mb flanking regions of *NBS-LRR* genes selected in recently duplicated regions in soybean chromosomes (Kang *et al.*, 2012).

Chr	No. <i>NBS-LRR</i> genes	No. QTLs	No. QTLs within 2-Mb flanking region of <i>NBS-LRR</i> genes ^{a)}	No. disease resistance QTLs in recently duplicated regions ^{b)}		No. <i>NBS-LRR</i> loci in recently duplicated regions	
				Chr A	Chr A'	Chr A	Chr A'
1	20	8	4	SCN (4), Sclero (4)	Sclero (2), SDL (1), Phytoph (1), Bra (1)	7 loci	7 loci
2	10	9	5	-	-	-	-
3	36	7	7	Sclero (1), SDS (1)	Sclero (4), Bra (1), OCS (2), LMG (1)	2 loci	5 loc
5	5	3	3	-	-	-	-
6	23	8	7	-	-	-	-
8	15	10	9	Sclero (4), OCS (1), SCN (5)	Sclero (3), LMG (1)	1 locus	1 locus
9	11	9	9	Sclero (5)	BSR (13), SCN (6), Sclero (5), Mi (5)	2 loci	17 loci
12	14	3	2	-	-	-	-
13	22	15	12	-	-	-	-
14	11	5	3	-	-	-	-
15	25	4	4	Ma (1)	Sclero (5), Ma (1), Mj (1)	13 loci	14 loci
17	6	9	4	Sclero (3)	SCN (2)	2 loci	2 loci
20	13	2	1	OCS (1), SDL (1), SDS (1)	SCN (2), Bra (1), Sclero (14)	3 loci	3 loci

^{a)}Regression analyses were done between the number of *NBS-LRR* genes and the number of QTLs within the 2-Mb flanking region of *NBS-LRR* genes.

^{b)}Fungal resistance QTLs: Sclerotinia stem rot (Sclero), sudden death syndrome (SDS), brown stem rot (BSR), *Phytophthora sojae* infection (Phytoph); Nematode resistance QTLs: soybean cyst nematode (SCN), peanut root-knot nematode (Ma), southern root-knot nematode (Mi), Javanese root-knot nematode (Mj); bacterial leaf pustule (BLP) resistance QTLs: OCS-G, SDL2178, 8ra, OCS-F, LMG7403.

More than half of the disease resistance QTLs colocalized with *NBS-LRR* genes within the 2-Mb flanking region. Chromosome A (origin) and A' (duplicate) represent the recently duplicated regions.

independently (e.g. ID 18934088). The mixed model described that the tandem duplication events occurred prior to the recent duplication and the independent tandem duplications underwent after the recent duplication as they can be seen on chromosomes 13 and 15 (e.g. ID 18159398). Based on the possibility of a combination of tandem and inter-chromosomal duplications among the proposed model scenarios, the duplication history of *NBS-LRR* genes can be one of the factors involved in the diversifying QTLs for disease resistance phenotypes and in a more general, QTLs related to biotic stress.

Transcriptome analysis (Kang *et al.*, 2012) demonstrates a significant fold-change in these *NBS-LRR* genes that seem to work either strain-specific or broad spectrum of pathogens, in line with the previous studies which observed against various diseases invasions and cyst nematode damage (Brechenmacher *et al.*, 2008; Calla *et al.*, 2009). Thus, it is likely that the duplicated *NBS-LRR* genes associated with disease resistance could contribute to the adaptation to cope with biotic stresses in soybean. Further investigation of these duplicated genes in genome in relation to abiotic stress will support the broad adaptation of soybean against environmental changes.

PROSPECTS AND CHALLENGES ON THE APPLICATION OF GENE DUPLICATION PHENOMENON ON SOYBEAN BREEDING FOR PEST/DISEASE RESISTANCE IN INDONESIA

Comparative and collinear analyses between soybean and other legumes on their respective duplicated regions have been performed to assist soybean improvement for pest/disease resistance. Orthologous and paralogous relationships of the genomic regions between soybean and *M. truncatula* harbored soybean nematode resistance genes, *rhg1* and *rhg4* (Mudge *et al.*, 2005). A total of 1,273 tandem duplicated genes with categories of defense response in adzuki bean (Kang *et al.*, 2014) could also be useful in soybean breeding program. Higher significant QTLs related to 114 traits including the ones for pest/disease resistance were detected to have high phenotypic variation in soybean than those in mungbean, mainly as a result of two rounds of genome duplication events which have occurred in soybean. Synteny blocks consisting of QTLs responsible for bruchid resistance matched soybean synteny blocks locating SSR markers associated with nematode resistance QTLs (Kim *et al.*, 2015). More than 1100 QTLs and 60,000 protein-coding loci are beneficial to reveal stress-response genes, which

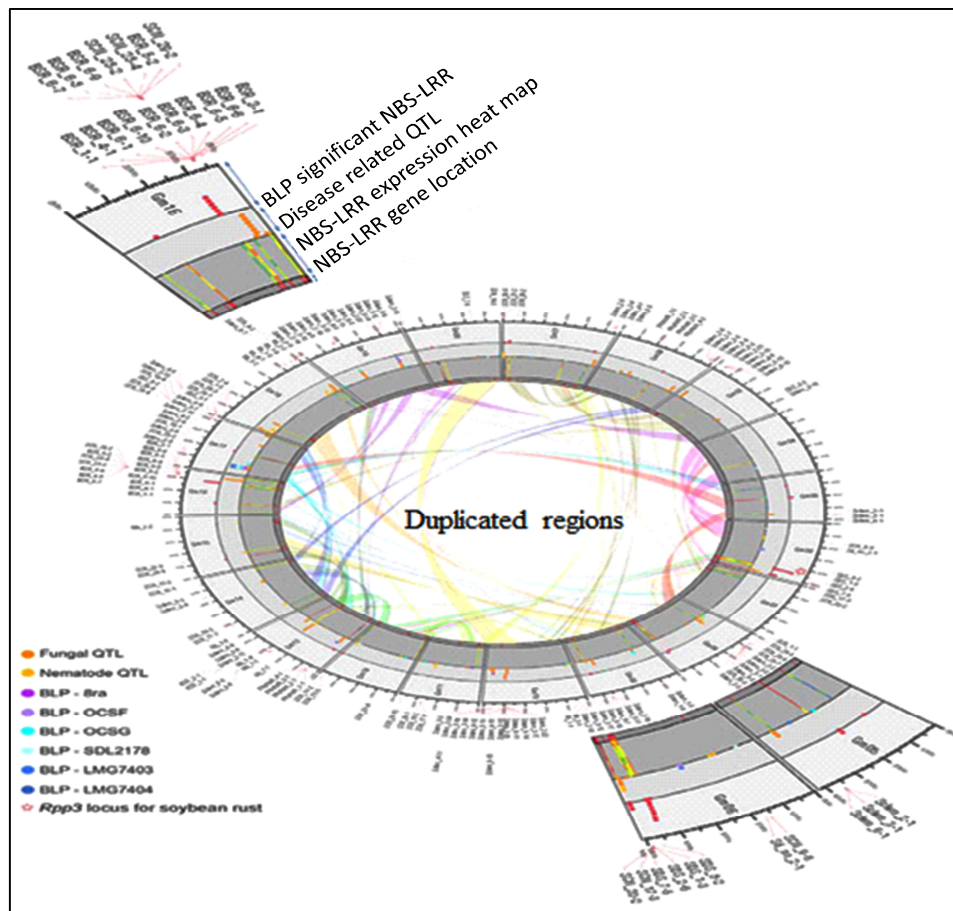


Figure 2. The circular genome map depicting the locations of recently duplicated regions with *NBS-LRR* genes, transcription levels of *NBS-LRR* genes after BLP treatment, locations of disease resistance QTL, and significantly expressed *NBS-LRR* genes. Minimum values to maximum values of expression are denoted with black, grey, red, orange, yellow, lime, green, blue, and purple (Kang *et al.*, 2012).

have been built into soybean databases (www.phytozome.net/soybean; <http://soybase.org>) and elucidated for their synteny within duplication regions in soybean and with other legumes (Kang *et al.*, 2012, 2014, 2015, 2016; Kim *et al.*, 2015; Lestari *et al.*, 2013). Syntenic QTL regions have been detected between chromosome pairs in soybean genome, showing duplicated genes clustered in homeologous QTLs to be possibly associated with environmental specific regulation as demonstrated in chromosomes 6 and 13 (Lestari *et al.*, 2014). QTLs and candidate *NBS-LRR* genes corresponding to pest/disease resistance in duplication regions were also well predicted (Kang *et al.*, 2012).

Eight Indonesian soybean genotypes with distinctive genetic background were resequenced and provided unique genome resources (Lestari *et al.*, 2016; Satyawati *et al.*, 2014) containing a number of SNPs with their descriptive genes related to defense systems (<http://genom.litbang.pertanian.go.id>). To date, however, studies on QTLs and

predicted genes in duplication regions in soybean genome are very limited in Indonesia. Therefore, this information provides a valuable starting point to identify genes controlling pest/disease resistance and develop their corresponding markers in soybean.

Breeding program of soybean, the third important food crop in Indonesia just after rice and maize, is still challenging to face pests and diseases that can significantly decline its quality and yield. In addition to abiotic stress, biotic stress (pest and disease) influences the production of soybean subjected to the changes of unpredictable climate. Prominent pests/diseases in soybean have been found in several areas in Indonesia and cause significant economic losses. As observed in Java, eleven important pests, two viral diseases vectors, and sixteen important diseases were distributed according to location as a result of cropping system, local weather, and host existence. According to disease intensity and area distribution, leaf rust, downy mildew, leaf blight, *Sclerotium rolfsii*, and BLP

have been considered to be important diseases and put in a high priority to be controlled (www.balitkabi.litbang.pertanian.go.id). Thus, the genetically improved soybean cultivars are preferred to be highly adaptive to biotic/abiotic stress, high yielding, and high grain quality in Indonesia. Since, conventional breeding of soybean in Indonesia has long been a common way, with the advantages of genome and gene duplications clue implementing to marker-assisted breeding, improving soybean cultivars resistant to pests and diseases could be accelerated.

Special interest would be addressed on duplicated *R* genes such as *NBS-LRR* genes that have expectedly led to make more efficient soybean breeding using marker-assisted selection (MAS). As an initial clue, there is a number of duplicated *NBS-LRR* genes (Kang *et al.*, 2012) to be known to control several soybean diseases existing in Indonesia including bacterial leaf rust, leaf pustule, Sclerotinia stem rot, etc. Given that the developed near isogenic lines (NILs) showed contrast expression between resistance and susceptible for BLP caused by *Xanthomonas axonopodis* pv. *glycines* (*Xag*) (Kang *et al.*, 2012), similar model of NILs could be developed to identify recently duplicated *NBS-LRR* gene expression, more convincing the functional relevance of the disease resistance in Indonesia.

Molecular markers for *NBS-LRR* domains should be developed possibly by integrating several disease resistance genes to generate elite Indonesian soybean cultivars which are capable of resisting diverse range of pathogens. In addition, deeper analysis of gene duplication among Indonesian soybean genotypes could give clearer information of *NBS-LRR*. Most importantly, this gene duplication that implicates genetic resources with different mechanism is important for breeding materials. Taken together, these pest and disease obstacles in Indonesia should be controlled and managed using integrated technologies and interdisciplinary fields related to genomics, computational biology, and related-omics into useful and systematic soybean breeding programs. This, at the end, should in part help the national food self-sufficiency and food security programs in Indonesia.

CONCLUSIONS

WGD and SSD contribute significantly to gene duplication events in plants. Gene duplication leads to genetic redundancy that would implicate to functional buffering and is a form of adaptation to

various environmental conditions. To understand the adaptation of plant species to environmental stresses, it would be advisable to consider taking a closer look at the gene duplication from the recently duplicated regions. Gene duplication phenomenon which affects phenotypes, would prospectively direct researchers and breeders to select candidate genes and informative genetic markers for traits of interest. Developing biotic and abiotic stress resistance crops could be performed in cultivated gene pool for *R* genes. As an alternative, molecular markers corresponding to *NBS-LRR* domains are integrated with biotic stress against broad range of pests/diseases to generate improved and well-adapted crop cultivars. The integrating knowledge of gene duplication phenomenon should promote research and is prospectively implemented in breeding programs to develop new soybean cultivars capable of encountering various environmental stresses in Indonesia.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. Yang Jae Kang and Dr. Sutrisno for their constructive comments on this manuscript. We also thank the reviewers for their thorough reviews and useful comments.

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