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Analysis of NBS-LRR Gene Family in Adzuki Bean Evolution and Disease Resistance Potential

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Abstract Adzuki beans (*Vigna angularis*), as one of the important legume crops in China, are widely used in food, health care and agricultural production. However, they are often threatened by various diseases during their growth process. The *NS-LRR* (nucleotide-binding site-leucine-rich repeat) gene family is a core gene population in the study of plant disease resistance and plays an important role in pathogen recognition and signal transduction. To systematically analyze the structural characteristics, evolutionary relationships and disease resistance potential of the *NBS-LRR* gene in adzuki beans, this study conducted the identification and analysis of the NBS-LRR gene family based on whole-genome data. In this study, several *NS-LRR* genes in the adzuki bean genome were identified and classified into subtypes such as TNL type and CNL type according to domain characteristics. Through phylogenetic tree construction, gene structure analysis and conserved motif comparison, the diversity and evolutionary dynamics of the NB-LRR gene family of adzuki beans were revealed. The expression patterns at different tissues and growth stages were further analyzed. Combined with RNA-Seq and qRT-PCR data, it was found that multiple genes were induced to express in disease-resistant strains and had potential disease-resistant functions. Meanwhile, some key genes also demonstrated correlations with hormone signaling pathways, such as salicylic acid (SA) and jasmonic acid (JA) responses. This research not only enriches the understanding of the disease-resistant genes related to adzuki beans, but also provides a theoretical basis and candidate gene resources for subsequent functional verification and molecular breeding.

Keywords Adzuki beans; *NBS-LRR*; Disease resistance; Evolutionary analysis; Expression spectrum

1 Introduction

Adzuki beans are highly nutritious, and there is no dispute about this. They contain protein, dietary fiber, and some essential trace elements for the human body. As a result, they are receiving increasing global attention. However, in actual cultivation, it also faces considerable problems. Diseases such as anthracnose and bacterial wilt occur from time to time. In mild cases, they affect the quality; in severe cases, even the yield cannot be maintained (Wu et al., 2017). To stabilize planting and ensure food security, ultimately, it still depends on the cultivation of disease-resistant varieties as a safety net.

When it comes to disease resistance, genes like *NS-LRR* are almost the "number one players" in plants. The proteins they encode can recognize the effector molecules of pathogens and trigger defense responses, playing a key role in the plant immune system (Marone et al., 2013). However, such genes are not "well-behaved". They have many types, change rapidly, are often distributed in clusters, and the evolution pace is not slow. They almost emerged to cope with the constantly changing pathogen pressure (Shao et al., 2019). The quantity and the degree of variation are actually closely related to the disease resistance of plants. Therefore, if one wants to understand the disease resistance mechanism of adzuki beans or find useful genetic resources for breeding, systematically analyzing its NBS-LRR gene family is an inevitable step (Wu et al., 2017).

This study will systematically analyze the NB-LRR gene family of adzuki beans, with a focus on exploring its evolutionary pattern, genomic structure and potential role in disease resistance. By integrating whole-genome identification, phylogenetic analysis and expression profiling analysis, this study aims to reveal key disease-resistant loci and provide molecular markers for disease-resistant breeding. This study supports the breeding of disease-resistant adzuki bean varieties and aims to promote sustainable agriculture and food security.

2 Genome-Wide Identification of *NBS-LRR* Genes in Adzuki Bean

2.1 Genome data sources and analytical methods overview

In fact, to identify a complete NBS-LRR gene family, the most crucial prerequisite is still to have a reliable draft genome. Without high-quality assembly, the subsequent annotations are basically useless. The common practice nowadays is basically to use tools like NLGenomeSweeper, along with homology alignment, to find sequences containing NB-ARC domains (Toda et al., 2020; Andolfo et al., 2022). The process is generally divided into two steps. First, Pfam (such as PF00931) is used to pull out a batch of candidates, and then a hidden Markov model trained for a specific species is used for further screening. Finally, annotation tools like InterProScan will come into play to do some manual organization and classification. Although this method involves many steps, its advantage is that it can also extract the functional *NB-LRR* genes hidden in the repetitive regions.

2.2 Identification criteria and classification of NBS, LRR, and associated domains

Many people get a headache when they hear the term "domain", but in fact, the core structure of genes like NS-LRR is quite regular. In the middle is NB-ARC, followed by a leucine repeat LRR region (Andolfo et al., 2022). As for the front-end domains, different types have different configurations: those with the CC prefix are called CNL, those with the TIR prefix are called TNL, and another type with the RPW8 domain is classified as RNL (Toda et al., 2020). If any N-terminal domain is missing, there is also a classification - that is the NL class. When classifying, two lines are basically followed: one is that the NB-ARC must be complete, and the other is that at least one LRR motif should be included. Other domains are supplemented with annotations by tools such as InterProScan. This set of standards is not only for classification but also to provide a reference for subsequent analysis of their differences in evolutionary paths and functions.

2.3 Chromosomal distribution and genomic features of *NBS-LRR* genes in adzuki bean

If we refer to the research on kidney beans or soybeans, we can roughly guess that the situation of adzuki beans won't be much different. The *NS-LRR* gene is generally not scattered but prefers to "cluster" - either in clusters or simply in tandem (Afzal et al., 2021). Take kidney beans as an example. The research found that a total of 178 complete *NB-LRR* genes and 145 partial genes were located on its 11 chromosomes, and they were all classified into the two major categories of TNL and CNL (Wu et al., 2017). The locations of these genes often overlap with the known disease-resistant QTL regions, indicating that they are likely related to resistance (Kang et al., 2012). As for the characteristic of cluster distribution, it is more the result of the continuous replication and expansion of *NS-LRR* during the evolutionary process. Precisely because of this, they are also more adaptable to the variable pathogen environment. Therefore, conducting a comprehensive genomic mapping of adzuki beans is not only fundamental research but may also provide direct clues for disease-resistant breeding.

3 Phylogenetic Analysis and Classification Characteristics

3.1 Construction of phylogenetic tree for *NBS-LRR* genes in adzuki bean

Building a phylogenetic tree is a very standard thing: usually, it involves comparing the NB-ARC conserved domains in the *NB-LRR* gene, applying the adjacency method, running an evolutionary analysis program, and finally creating a tree with supporting values. But although it seems standardized, in fact, every step may affect the details of classification. In leguminous plants like kidney beans, this method can indeed divide *NBS-LRR* into two main categories: TNL (TIR type) and CNL (CC type), with clear classification and corresponding structures (Wu et al., 2017). But don't forget that such an analysis mainly provides a framework. To understand the function later, more levels of verification are still needed.

3.2 Evolutionary comparison with *NBS-LRR* genes in other legume species

Not all plants have the same *NS-LRR* gene; the differences among leguminous plants are quite significant. For example, there are 178 full-length *NBS-LRR* genes in kidney beans, among which the CNL type is significantly more, while the TNL type is relatively less. A similar phenomenon also exists in plants such as chili peppers (Wu et al., 2017; Liu et al., 2025). But looking at *Arabidopsis thaliana* in reverse, the proportion of TNL is much higher on its side. This indicates that different species actually have their own evolutionary rhythms (Shao et al.,

2016; Zhang et al., 2016). Most of these differences are due to the fact that species face different types of pathogens, and the directions of their evolutionary choices are naturally different as well.

3.3 Gene family expansion, duplication events, and selection pressure analysis

Ultimately, why are there so many genes like *NS-LRR*? In fact, the expansion mode is quite clear. Most of them are achieved through tandem replication or fragment repetition, and over time, a bunch of gene clusters are formed (Liu et al., 2025). More interestingly, genes of the CNL class often expand gradually, while TNL shrinks in some lineages (Zhang et al., 2016). Some studies have pointed out that these changes are closely related to past genome-wide repeat events, especially during the period from the Cretaceous to the Paleogene, which might be the node of the massive explosion of the *NS-LRR* gene (Shao et al., 2016). Coupled with the continuous pressure from pathogens, these gene families have become increasingly diverse and "specialized" in terms of immune function.

4 Gene Structure and Conserved Motif Analysis

4.1 Comparative Analysis of exon–intron Structures

If one examines the structure of *NS-LRR* genes in leguminous plants, especially in close relatives of red beans like common kidney beans and soybeans, it is not difficult to notice a characteristic - the structural types are highly inconsistent. Some genes have a considerable number of exons and introns, but there are exceptions. Simple structures also exist (Kang et al., 2012; Wu et al., 2017). Among them, the TNL type usually has several more exons and a slightly longer intron length than the CNL type. Some people think that this might be related to their functional differentiation and the complication of regulatory mechanisms (Bezerra-Neto et al., 2020). However, such structural diversity actually indicates from another perspective that this type of genetic system might be a kind of "flexibility" evolved to cope with the constantly changing pathogenic environment.

4.2 Conserved amino acid sequences and functional motif identification

When it comes to NS-LRR proteins, the key to their function in immunity lies in those several classic conserved motifs within them. In the NB-ARC region, sequences such as the P-ring, kinase 2, and GLPL are almost standard, used to assist in binding and hydrolyzing ATP or GTP (Bezerra-Neto et al., 2020). Although the LRR region varies greatly, the recurring leucine-rich motif remains the core part as it is responsible for interacting with other proteins or recognizing pathogens (Bezerra-Neto et al., 2020). Also, don't forget the N-terminal domain, TIR or CC. Different types of genes also have differences here, corresponding to different functional characteristics and signaling pathways (Wu et al., 2017). So although there are many types of sequences, those conservative segments are basically the basis for maintaining the immune function of NS-LRR.

4.3 Structural variation and potential functional implications among genes

Is there any significance in structural differences at all? From the current perspective, there indeed is. There are significant differences among *NBS-LRR* genes in exon-intron structure, the number of domains and even motif composition (Zhang et al., 2016; Bezerra-Neto et al., 2020). Behind these differences, events such as gene replication, recombination and motif rearrangement often play a role, which may generate some "new versions" of resistance genes, giving plants more room to deal with different pathogens. Interestingly, these changes are not only at the individual level; in many cases, they can also contribute to the formation of gene clusters, thereby enabling an entire set of related resistance traits to be regulated and co-evolve together.

5 Expression Patterns and Stress Response Characteristics

5.1 Expression profiles in different tissues and developmental stages

Not all *NS-LRR* genes are expressed "actively" in plants. In kidney beans, some genes prefer to emerge at specific sites, such as roots, flowers, flower buds and young pods, while others either "quietly" maintain low expression levels or remain online throughout the year with little fluctuation (Wu et al., 2017). In fact, this situation is not uncommon among leguminous plants, and soybeans are no exception. This organizational specificity makes one have to consider that they may each undertake different "job responsibilities", some may be more inclined towards developmental control, while others are like "defensive posts" on standby at all times.

5.2 Expression responses under biotic stresses (pathogens, viruses, etc.)

However, once the pathogen intervenes, the state of the *NS-LRR* gene changes. Like in common kidney beans, the study found that the expression levels of 67 such genes changed significantly when responding to anthracnose and common bacterial blight. Some were activated rapidly, while others showed almost no response (Wu et al., 2017). The expression patterns between resistant and susceptible varieties are also inconsistent. On the soybean side, genes like *GmTNL16* are also up-regulated in expression when encountering diseases such as Phytophthora root rot (Figure 1) (Afzal et al., 2021; Zhou et al., 2022). These differences illustrate a point: not all *NS-LRR* genes step up at critical moments, but the part that plays a role is indeed the main force of the immune system.

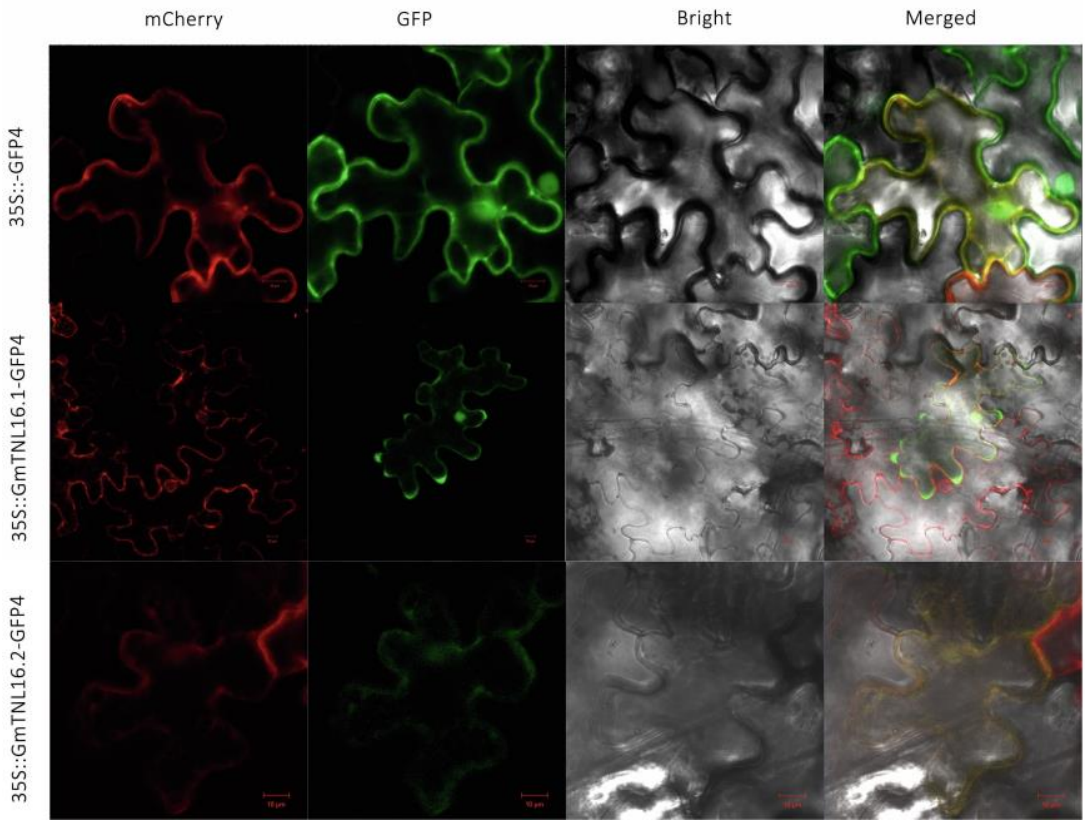

Figure 1 Subcellular localization of GmTNL16. *35S:GmTNL16.1-GFP4*, *35S::GmTNL16.2-GFP4* and *35S::GFP4* (control) constructs were transformed into tobacco leaves. mCherry was used as an endoplasmic reticulum marker. Scale bars, 10 μm (Adopted from Zhou et al., 2022)

5.3 Transcriptional regulation related to signaling pathways (e.g., SA, JA, ET)

When it comes to regulation, the expression of *NB-LRR* is not merely a simple on and off; it is actually a multi-level complex network behind it. In addition to the cis elements in the promoter region, trans regulatory factors, mirnas, and even alternative splicing will also be involved (Marone et al., 2013). This system is also closely related to the hormone signaling pathways of plants, especially the SA (salicylic acid) and JA (jasmonic acid) pathways. In soybeans, the *GmTNL16* gene is regulated by miR1510 and further activates downstream response factors such as JAZ, COI1, TGA and PR through JA and SA signaling pathways (Zhou et al., 2022). Judging from the results, *NBS-LRR* is not fighting alone but is "mobilized" by the entire hormone network to make a coordinated and clearly divided response to external attacks.

6 Case Study: Disease Resistance Potential of Specific *NBS-LRR* Genes

6.1 Differential expression analysis of *NBS-LRR* genes in resistant lines using RNA-Seq

After the invasion of pathogenic bacteria, the expression of *NS-LRR* genes is actually not constant. They often fluctuate sharply as the infection progresses. Through RNA sequencing, we can capture this dynamic regulatory process. In common kidney beans, some studies using qRT-PCR in combination with expression analysis have

found that when diseases such as anthracnose and bacterial blight occur, the *NB-LRR* gene in resistant strains is significantly upregulated, while the response in susceptible strains is much weaker or even inconsistent. For example, the gene *Phvul.001G134500* has a strong induced expression in the context of disease resistance (Figure 2) (Wu et al., 2017). These results once again demonstrate that using transcriptome methods to screen candidate disease-resistant genes is indeed of great reference value.

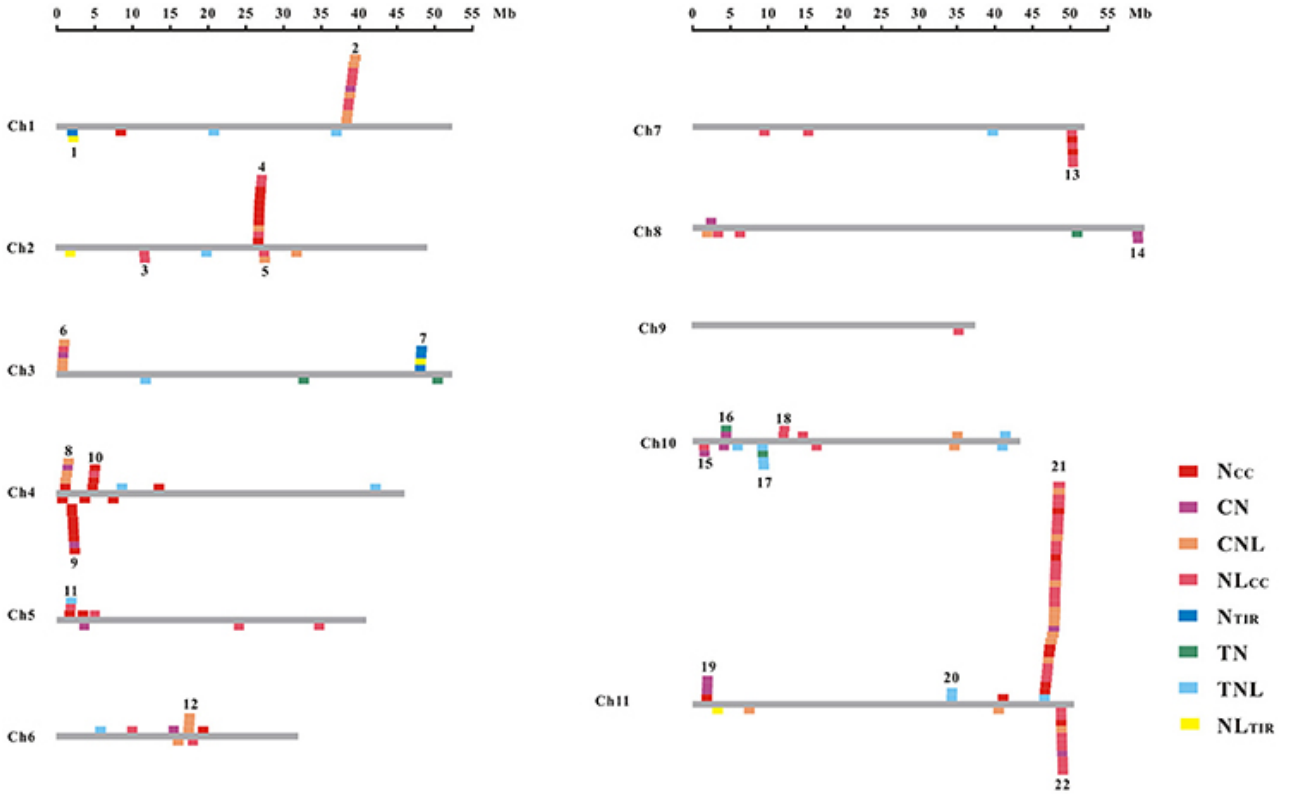

Figure 2 Chromosomal distribution of the *NBS-LRR* genes in the common bean. The gray bars represent all 11 chromosomes in the common bean. Boxes across each bar designate the location of each gene. The cluster number is shown at the top of each cluster (Adopted from Wu et al., 2017)

6.2 qRT-PCR validation and tissue-specific expression of candidate resistance genes

RNA-Seq alone is not enough. Subsequent qRT-PCR verification is crucial, especially for confirming the expression patterns of *NB-LRR* genes that are sensitive to pathogen responses. Experiments on kidney beans have shown that some genes are rapidly upregulated in resistant materials when exposed to disease stimuli, and this is not a systemic response but rather concentrated in certain tissues, such as "frontline" sites like leaves and pods. This tissue-specific expression indicates that they are likely to directly participate in the local defense response, and also indirectly emphasizes that the results of RNA-Seq cannot be directly used and need to be further verified by quantitative means (Wu et al., 2017).

6.3 Functional annotation and protein structure modeling for mechanism prediction

Of course, identifying candidate genes is just the beginning. To understand exactly what they do, we still need to look at the results of functional annotations and protein modeling. In the research of common kidney beans, the *NS-LRR* genes associated with known resistance loci (such as NSSR24, NSSR65, NSSR73, NSSR260, NSSR265) have been clearly located, and the protein domains have also been analyzed in depth (Wu et al., 2017). Through these models, combined with phylogenetic relationships and conserved motifs, it is possible to infer how they might recognize the effector proteins of pathogens and initiate immune pathways. This type of analysis has great guiding significance for subsequent functional verification and selection of breeding target genes (Marone et al., 2013).

7 Conclusions and Perspectives

Among leguminous plants, the *NS-LRR* gene is the most numerous and has the closest relationship with disease resistance, and adzuki beans are no exception. However, if you look at related species like the kidney bean, many whole-genome studies have long sorted out these gene families very clearly. They can be roughly divided into two categories: *TIR-NB-LRR* (TNL) and *CC-NB-LRR* (CNL), and they are basically present on every chromosome. These genes often appear near key sites that combat major pathogens such as anthracnose and common bacterial blight. It is precisely because of the assistance of expression profiling analysis and some molecular markers (such as NBS-SSR markers) that researchers can accurately identify candidate disease-resistant genes and verify them, laying the foundation for subsequent functional research and breeding.

But then again, this road is actually not that smooth. Although some progress has been remarkable, most of the data we currently have at hand is still focused on model plants or other leguminous crops. As for the small beans themselves, the direct research on them is not yet comprehensive and in-depth enough. Not to mention figuring out exactly how a specific *NBS-LRR* gene functions, how it is regulated, and how it participates in the disease resistance mechanism. These issues remain undetermined at present. Moreover, don't forget that the evolutionary changes among such genes are rapid and there may be redundancy, all of which make the search for key resistance factors even more challenging. Future research will probably have to be more focused, such as mapping out more detailed genetic maps, verifying functions (like gene editing and transgenic), and combining data from different omics to gradually understand the complex mechanisms behind it.

The relevant research on the *NS-LRR* gene has indeed opened a door for molecular breeding of adzuki beans. For instance, NBS-SSR markers, when used in marker-assisted selection, can significantly accelerate the breeding speed of disease-resistant varieties. Each time a new resistance locus or allele is discovered, the breeder's toolbox becomes one more item, and there is greater hope of developing varieties that can both have a broad spectrum of disease resistance and are less likely to "fail". In the long run, deeply exploring the value of the *NS-LRR* gene family is not only for the benefit of small beans, but also can enhance the stress resistance of the entire leguminous crop family, improve yield stability, and even have certain significance for food security.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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