Genome-wide identification and evolutionary analysis of RLKs involved in response to Aluminum stress in peanut

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Abstract

Background: As an important cash crop, the yield of peanut is influenced by soil acidification and pathogen infection. Receptor-like protein kinase plays important roles in plant growth, development and stress responses. However, little is known about the number, location, structure, molecular phylogenetic, and expression of the RLKs in peanut, and no comprehensive analysis of RLKs in Al stress response in peanut have been reported.

Results: A total of 1311 *AhRLK*s were identified from the peanut genome. The AhLRR-RLKs and AhLec-RLKs were further divided into 24 subfamilies and 35 subfamilies, respectively. The *AhRLK*s are randomly distributed across all 20 chromosomes in peanut. Among them, 67.8% and 0.6% of the *AhRLK*s originated from tandem duplications and segmental duplications, respectively. The ka/ks ratios of 94.9% (1290/1360) of *AhRLK*s were less than 1. Moreover, totally 90 Al-responsive *AhRLK*s were identified by mining transcriptome data, and they were divided into 7 groups. Most of Al responsive *AhRLK*s clustered together had similar motifs and evolutionarily conserved structures. The gene expression patterns of these genes in different tissues were further analyzed, and tissue specific expression genes, including 14 root-specific Al responsive *AhRLK*s were found. Besides, all of the 90 Al responsive *AhRLK*s which distributed unevenly in the subfamilies of *AhRLK*s have different expression pattern between two peanut varieties (Al-sensitive and Al-tolerant) under Al stress.

Conclusions: In this study, we analyzed the RLK gene family by the peanut genome. Tandem replication events were the main driving force for *AhRLK*s evolution, and most *AhRLK*s were selected for purification. A total of 90 genes were identified as Al responsive *AhRLK*s, and the classification, conservative motif, structure, tissue expression pattern and predicted function of Al responsive *AhRLK*s were further analyzed and discussed, revealing their putative roles. This study provides a better understanding of the structures and functions of *AhRLK*s as well as Al responsive *AhRLK*s.

Background

Al is one of the most harmful factors for plant growth in acidic soils, and it can cause a 25–80% yield loss depending on the crops [1, 2]. Al signal induces a series of physiological events in plant cells. The most obvious phenomena of Al toxicity are inhibition of cell elongation in the apical elongation region and induction of PCD [3–5]. PCD is an active, orderly, and genetically controlled form of cell death and it occurs in plant throughout development and in response to environmental stresses [6]. Early studies had found that Al-treatment can enhance Fe^{2+}-induced lipid peroxidation and PCD in tobacco cells [7]. For decades, Al-induced PCD had been proved in many plant species including: soybean (*Glycine max*) [8], maize (*Zea mays*) [9], barley (*Hordeum vulgare*) [10], tomato (*Lycopersicon esculentum*) [11]and peanut (*Arachis hypogaea*) [12]. It is known that Al-induced PCD is mediated through two cell signal transduction pathways: a mitochondrial-dependent pathway and a nuclear-dominated mitochondrial-independent pathway [5]. However, Al signal information and its transmembrane transduction are unknown. Both plants use plasma membrane and/or cell wall localized receptors to sense environment stimulus and

efficiently transduce signal between cells, which perceive and transduce signals to modulate gene expression and/or enzyme activity as well as motility [13]. RLKs play important roles in the process of cell signal transduction, involving in a variety of plant physiological processes including: self-incompatibility [14], environmental signal processing [15], organ shape and meristem activity [16], hormone signal transduction [17], Programed cell death (PCD) [18], and tolerance to oxidative stress [19]. RLKs sense and transduce signals through protein interaction and phosphorylation [20]. Based on the structure of extracellular domain, RLKs have been classified into several families such as S-RLK, LRR-RLK, EGF-RLK, LecRLK, TNFR-RLK and PR5K-RLK and so on [21].While many RLKs involved in environmental stress response have been found, few RLKs have been reported to be involved in Al stress response. *WAK1*, which mediates the interaction between cell wall and cytoplasm, and may participate in cell elongation and morphogenesis [22], was the first RLK that was found to involve in Al stress response. It was reported that overexpression of *WAK1* enhanced Al tolerance in Arabidopsis [23]. The results showed that *RLKs* play important role in Al-induced PCD, but the mechanism of *RLKs* in the regulation of Al-induced PCD is unknown.

Peanut is an important oil crop over the world. Al-dependent inhibition of growth causes reduction in peanut yield in acid soil. There is no comprehensive analysis of the RLK gene family in peanut. In the present study, the recently released peanut whole genome sequence data (http://peanutgr.fafu.edu.cn/index.php) was utilized to analyze the RLK gene family in peanut. A total of 1311 *AhRLK*s had been identified. The LRR-RLKs and LecRLKs were further divided into 24 subfamilies and 35 subfamilies based on a phylogenetic analysis, respectively. The evolution and collinearity of *AhRLK*s were investigated. The evolutionary patterns of the RLK gene family was tested by investigating gene duplication events in peanut. In addition, 90 *AhRLK*s in response to Al stress were identified by transcriptomic analysis and comprehensively determined the expression profiles of *AhRLK*s at different Al treatment time-point. These results will provide a basis for further research on the evolution and physiological functions of *AhRLK*s in response to Al stress in peanut.

Results

Identification of RLKs in peanut

In order to identify the members of RLKs in peanut, we downloaded the publicly available peanut genome sequence data and used the Arabidopsis RLK sequence as a query to perform a genome-wide similarity search. After filtration of the sequence, a total of 1311 RLKs that contain at least one kinase domain were initially identified, including 548 LRR-RLKs, 274 LecRLKs, 83 Cysteine-rich, 76 EGF, 49 Proline-rich, 46 s-domain, 22 TMK, 2 TNFR, 1 RRO-RICH, 28RLCK, 24 LysM domain, and 158 no obvious domain (Additional file 1 & 2). LRR-RLKs and LecRLKs were considered for further analyses.

Phylogenetic analysis of LRR-RLKs and LecRLKs in peanut

To explore the phylogenetic relationships within *AhRLK*s class, full-length amino acid sequences of LRR-RLKs and LecRLKs were analyzed separately. AhLRR-RLKs and AhLecRLKs were clustered with AtLRR-

RLKs (209) and AtLecRLKs (274) respectively. The RLKs classification in Arabidopsis were followed to analyze the phylogenetic relationship of peanut RLKs. AhLRR-RLKs were divided into 24 subclades in ML tree (Fig. 1). The largest subclade LRR-XI contains 74 members, while the smallest subclade LRR-V contains only 1 member. Meanwhile, followed the classification standard of LecRLKs as Marcella's [24] and Klass's [25] reports, peanut LecRLKs were classified into 35 subfamilies, and subdivided into 3 classes, C-type LecRKs (C-LecRKs), L-type LecRKs (L-LecRKs) and G-type LecRKs (G-LecRKs) (Fig. 2). The largest subclade G-LecRKs-XI and L-LecRKs-IX contains 37 and 28 members separately, while no member from G-LecRKs-VIb,G-LecRKs-VIII□G-LecRKs-VII□ G-LecRKs-X□G-LecRKs-III, L-LecRKs-VI, L-LecRKs-I, L-LecRKs-II, L-LecRKs-III, and L-LecRKs-V were found in peanut.

Chromosomal location and gene duplication of *AhRLK*s

Physical positions of *AhRLK*s obtained from the "Peanut Genome resource" (http://peanutgr.fafu.edu.cn/) [26] were used to map them onto peanut chromosomes. Chromosome location information demonstrated that all the *AhRLK*s were unevenly distributed among the 20 chromosomes of peanut (Fig. 3). Many *AhRLK*s were located on chromosome 14 (111, 8.47%) and chromosome 13 (106, 8.09%), while only 31 (2.36%) *AhRLK*s located on chromosome 6 and 15 (1.14%) *AhRLK*s were located on chloroplast versus mitochondria chromosomes. As far as LRR-RLKs be concerned, subfamily LRR-XI and LRR-□ were present in all chromosomes, while others were only found in some chromosomes. The majority of the LRR-RLKs (165, 60.2%) were located on chr 3, 13, 8 and 18. Similarly, most of the LecRLK*s* subfamily were also found mainly in chr 3, 13, 8 and 18 (Additional file 3), particularly, all members of the G-LecRKs-XVII and G-LecRKs-VIa subfamilies were distributed on chr 8 and 18 (Additional file 4).

Gene replication events play an important role in the evolution of new functions of proteins and the expansion of genomes. It is known that segmental duplication and tandem duplication are the main causes for the expansion of gene family in plant [27]. The position of two or more *AhRLKs* on the chromosome within 100 kb was considered as a tandem duplication cluster. The results showed that about 67.8% (889 out of 1311) genes were located in tandem duplications regions and constituted 397 clusters (Additional file 5). The largest tandem duplication cluster contained ten genes, while the smallest one contained only two. Up to 67.2% (368/548) AhLRR-RLKs and 70.1% (192/274) AhLecRLKs were located in regions with tandem duplications. Segmental duplications produced a total of 4 putatively related gene pairs (0.6% of the total genes) (Fig. 4). To investigate the selection forces acting upon individual *AhRLK*s, the ratio of the non-synonymous substitution rate to the synonymous substitution rate (Ka/Ks) was calculated. The AhRLKs in tandem duplication regions showed variable Ks ranging from 0 to 1 and most of them were between 0-0.06. The ka/ks ratios of 94.9% (1290/1360) of *AhRLK*s were less than 1, 5% (68/1360) of *AhRLK*s were more than 1, 6 pairs genes whose Ka/Ks ratios were greater than 1 (ka/ks > > 1) and two pairs of genes that cannot calculated Ka/Ks values (Fig. 5). In addition, we calculated the divergence time with the formula T = Ks/2r, in which r is the rate of divergence for nuclear genes from plants. The r of dicotyledonous plants was taken to be 1.5*10^8 synonymous substitutions

per site per year according to the methods of Koch [28], the results show that, the tandem duplication events appeared to have occurred during relatively recent key periods, 0–2 Mya (Additional file 6), illustrating that these *AhRLK*s were generated by recent gene duplication events in *Arachis hypogaea* L.

Phylogenetic analysis of Al-responsive *AhRLK*s

In previous study, we have performed a transcriptome analysis to identify differentially expression genes (DEGs) and pathways between two peanut cultivars under Al Stress (data unpublished). In this study, we scrutinized the transcriptome data to detect the *AhRLK*s involved in Al response. A total of 90 *AhRLK*s were found as Al-responsive genes, including 44 LRR-RLKs, 19 LecRLKs, 8 Cysteine-rich, 1 EGF, 2 Proline-rich ,4 s-domain, 1 TMK, 1 RLCK, 1 LysM domain, and 9 no obvious domain (Additional file 2). To reveal the evolutionary relationships of these proteins, a phylogenetic tree was constructed using the ML method (Fig. 6). Phylogenetic analysis of all the 90 *AhRLK*s revealed that the Al-responsive *AhRLK*s were further classified into 7 groups,including 48.9% LRR-RLKs, 21.1% LecRLKs and 8.9% CRKs, and so on. The phylogenetic tree showed that most of these genes belonged to LRR-RLKs and LecRLKs, covering the main subfamilies of LRR-RLKs and LecRLKs. Interestingly, these Al-responsive *AhRLK*s were evenly distributed across the LecRLKs family, but unevenly distributed across the LRR-RLKs families, focused on LRR-III, LRR-XI, LRR-XII, LRR-VIII-1, LRR-VIII-2.

Characterization of the amino acid sequences and gene structure of Al stress related *AhRLK*s

As shown in Fig. 7, 90 Al stress related *AhRLK*s were divided into 7 groups. It was reported that the diversification of exons/introns was an important reason for the evolution of certain gene family [29]. The distribution of exon/introns of *AhRLK*s was further analyzed. The results showed that 7.8% of Al stress related *AhRLK*s (7/90) had no intron. One, two and three introns were found in 30% (27/90), 15.6% (14/90) and 1.1% (1/90) Al stress related *AhRLK*s, respectively. Meanwhile, 45.6% (41/90) genes had more than three introns. All genes in subgroup I, II and VII contain more than there introns. The majority of genes in subgroup III, IV and VI, contain one or two introns respectively. Moreover, in order to analyze the diversity of the Al stress related *AhRLK*s, the MEME tool was used to predict putative motifs of these proteins. A total of 5 different motifs were detected in Al stress related *AhRLK*s and named as motif 1 to motif 5(Additional file 7). It was shown that 82.4% (14/17) of genes in subgroup I, 70% (7/10) of genes in subgroup II,50% of genes in subgroup III, 42.9% (6/14) of genes in subgroup IV, 88.9% (8/9) of genes in subgroup V, 75.8% (25/33) of genes in subgroups VI, and 33.3% (1/3) of genes in subgroup VII contain the same motif composition as motif 3-motif 4-motif 1-motif 2-motif 5.

Expression Profiles of Al-responsive *AhRLK*s in Different Tissues

To further understand the role of Al-responsive *AhRLKs* in peanut growth and development, the expression profiles of Al-responsive *AhRLK*s from different organs, including leaves, stems, florescence, roots and root tips, were tested in a cultivated variety (*A. hypogaea* L.) using transcriptomic data (Fig. 8). Among these Al-responsive *AhRLK*s, the majority of them (78/90, 86.7%) were expressed in all examined organs. It was exhibited that 6 (6.7% *AH16G41130.1*, *AH07G04000.1*, *AH07G24540.1*, *AH07G24580.1*, *AH08G04680.1*, and *AH16G09430.1*) genes expressed at a high level (value > 5) in leaves, 12 (13.3% *AH05G37250.1*, *AH04G28680.1*, *AH16G41130.1*, *AH01G21880.1*, *AH07G04000.1*, *AH07G24540.1*, *AH07G24580.1*, *AH03G13700.1*, *AH10G03910.1*, *AH08G04680.1*, *AH08G04640.1*, and *AH16G09430.1*) genes in stems, 6 (6.7%, *AH16G41130.1*, *AH01G21880.1*, *AH07G04000.1*, *AH07G24540.1*, *AH08G04640.1*, and *AH16G09430*) genes in florescences, and 14 (15.6%, *AH07G04000.1*, *AH03G13700.1*, *AH10G03910.1*, *AH08G04680.1*, *AH08G04640.1*, *AH16G09430.1*, *AH14G07810.1*, *AH03G21680.1 AH19G41030.1*, *AH13G57290.1*, *AH10G29990.1*, *AH08G20520.1*, *AH08G06390.1*, and *AH01G04120.1*) genes in roots or root tips.

Expression patterns of Al stress related *RLKs* under Al stress

To further investigate the putative functions of Al stress related *RLKs*, the RNA-Seq dataset that were generated from different Al treatment time-point were utilized to reveal the expression profiles of these genes under Al stress. The expression profiles of Al stress related *RLK*s were shown with histograms (Fig. 9). As shown in Fig. 9, 41.1% (37/90) *AhRLK*s exhibited > 2-fold up regulation under Al stress for 8 h in the 99-1507. 12.2% (11/90) and 8.9% (8/90) *AhRLK*s exhibited > 2-fold down regulation under Al stress for 8 h in the ZH2 and 99-1507, respectively. 3.3% (3/90) and 12.2% (11/90) *AhRLK*s exhibited > 2-fold up regulation in 24 h vs 0 h Al-treatment comparison, 6.7% (6/90) and 1.1% (1/90) *AhRLK*s exhibited > 2-fold down regulation in 24 h vs 0 h Al-treatment comparison in the ZH2 and 99-1507, respectively (Additional file 8).

Discussion

Tandem duplication events played an important role in *AhRLK*s family evolution

RLKs involve in a variety of plant physiological processes and various abiotic and biotic stress responses [30, 31]. In this study, a total of 1311 *AhRLK*s, including 548 LRR-RLKs, 274 LecRLKs, 83 Cysteine-rich, 76 EGF, 49 Proline-rich, 46 s-domain, 22 TMK, 2 TNFR, 1 RRO-RICH, 28RLCK, 24 LysM domain, and 158 no obvious domain RLKs, were identified from whole peanut genome sequences (Additional file 1).

It was shown that 548 LRR-RLKs were classified into 24 subfamilies (I to XXIV) based on the phylogenetic relationship with Arabidopsis, 2 times the number of Arabidopsis LRR-RLK genes (Fig. 1). In general, the number of LRR-RLK receptors for most of the subfamilis among the peanut was two times of

Arabidopsis, except LRR-XII, LRR-XIV, LRR-XV and LRR-XVI, which had more than three times the members of Arabidopsis. Only one subfamily, LRR-V, had fewer members than Arabidopsis. The number of LecRLKs was over 3 times the number of AtLecRLKs (Fig. 2). The subfamilies in peanut like L-LecRK-VII, L-LecRKs-IX and G-LecRKs-VIa were much larger than those of Arabidopsis, while some subfamilies, including G-LecRKs-VIb, G-LecRKs-VIII, G-LecRKs-VII, G-LecRKs-X, G-LecRKs-III, L-LecRKs-VI, L-LecRKs-I, L-LecRKs-II, L-LecRKs-III and L-LecRKs-V, were not found in peanut (Table 1&Table 2).

Table 1
Total number of receptors distributed in the different subfamilies of LRR-RLKs

Subfamilies	Plant species	
	Peanut	*A. thaliana*
LRR-Ⅰ	34	38
LRR-Ⅱ	27	13
LRR-Ⅲ	70	41
LRR-Ⅳ	10	4
LRR-Ⅴ	1	9
LRR-Ⅵ-1	17	6
LRR-Ⅵ-2	9	4
LRR-Ⅶ	9	8
LRR-Ⅷ-1	18	7
LRR-Ⅷ-2	32	12
LRR-Ⅸ	3	4
LRR-Ⅹ-a	11	4
LRR-Ⅹ-b	6	9
LRR-Ⅺ	74	29
LRR-Ⅻ	61	9
LRR-XIII-a	7	3
LRR-XIII-b	4	3
LRR-XIV	10	3
LRR-XV	6	2
LRR-XVI	5	1
LRR-XVII	65	0
LRR-XVIII	6	0
1RR-XIX	15	0
LRR-XX	32	0
LRR-XXI	2	0

Subfamilies	Plant species	
LRR-XXII	2	0
LRR-XXIII	2	0
LRR-XXIV	10	0
Total	548	209

Table 2
Total number of receptors distributed in the different subfamilies of LecRLKs

Subfamilies	Plant species	
	Peanut	*A. thaliana*
G-LecRKs-I	16	2
G-LecRKs-II	7	2
G-LecRKs-III	0	2
G-LecRKs-IV	2	2
G-LecRKs-V	18	3
G-LecRKs-VIa	29	2
G-LecRKs-VIb	0	3
G-LecRKs-VII	0	5
G-LecRKs-VIII	0	9
G-LecRKs-IX	2	1
G-LecRKs-X	0	1
G-LecRKs-XI	37	0
G-LecRKs-XII	2	0
G-LecRKs-XIII	16	0
G-LecRKs-XIV	6	0
G-LecRKs-XV	9	0
G-LecRKs-XVI	14	0
G-LecRKs-XVII	10	0
G-LecRKs-XVIII	9	0
G-LecRKs-XIX	1	0
G-LecRKs-XX	12	0
G-LecRKs-XXI	15	0
L-LecRKs-I	0	11
L-LecRKs-II	0	2
L-LecRKs-III	0	2

Subfamilies	Plant species	
L-LecRKs-IV	4	4
L-LecRKs-V	0	9
L-LecRKs-VI	0	4
L-LecRKs-VII	15	3
L-LecRKs-VIII	7	4
L-LecRKs-IX	28	2
L-LecRKs-X	4	1
L-LecRKs-XI	1	0
L-LecRKs-XII	6	1
L-LecRKs-XIII	2	0
C-LecRKs	2	1
Total	274	76

As gene duplication was the main mechanism for evolutionary events [32]. About 67.8% *AhRLK*s were located in regions with tandem duplications, revealing the presence of high tandem and low segmental duplications in *AhRLK*s (Additional file 5). Study in *LRR-RLKs* had shown that tandem replication has a greater contribution to the birth of new genes [33], which suggested that the expansion of the *LRR* subfamilies may be caused by tandem duplication. It was found that about 67.2% (368/548) LRR-RLKs and 70.1% (192/274) LecRLKs were located on the regions with tandem duplications. Segmental replication was also an important driven force for the amplification of gene family. However, our results revealed that only 0.6% (8 genes) of the *AhRLK*s originated from segmental duplication, which suggested that tandem replication events were the main driving force for *AhRLK*s evolution. Besides, the ka/ks ratios of 94.9% (1290/1360) of *AhRLK*s were less than 1, which suggested that most *AhRLK*s were selected for purification (Fig. 5). There were 6 pairs genes whose Ka/Ks ratios were greater than 1 (ka/ks > > 1), which indicated that these genes were in a state of positive selection in peanuts, evolving rapidly, and might be very important for the evolution of peanut.

Conservation of the *AhRLK*s in response to Al stress

In this study, a total of 90 *AhRLK*s were identified as Al responsive genes, which were divided into 7 groups (Fig. 7) [34, 35]. Most of the subgroup shows certain regularity of exon-intron structure. For instance, all genes in subgroup I, II and VII contain more than there introns. Members belonging to the same subgroup had similar exon/intron organizations. Furthermore, 5 conserved motifs were identified in these *AhRLK*s and the motif compositions among subgroups were consistent with the phylogenetic

classification. These results indicated that the members in the subgroups were more conservative in the evolution.

Diversity roles of Al-responsive *AhRLK*s in different subgroups

To further understand the Al-responsive RLKs in peanut, we investigated the potential functions of each subgroup (Table 3). In subgroup I, *PERK1* has been reported to regulate ABA signaling pathways and modulate the expression of genes related to cell elongation and ABA signaling during root growth [36], implying that the genes in Subgroup I was essential to plant signaling and growth. It is known that the inhibition of Al on root elongation is the primary symptom of Al toxicity, and the members of subgroup I maybe take part in Al response by influencing cell elongation. The function-known genes in subgroup II were reported to play a role in plant signaling transduction, plants growth and biotic stress response, for instance, *PXC1*, *CRCK1* played a role in signal transduction [37, 38], *PRK1* was essential for post meiotic development of pollen [39], *FLS2* involved in preinvasive immunity against bacterial infection [40], *RCH1* was critical to the resistance of hemibiotrophic fungal pathogen *Colletotrichum higginsinaum* [41]. In Subgroup III, *ANXUR1/ANXUR2* were involved in controlling pollen tube rupture during the fertilization process and regulating signal transduction [42], *FERONIA* was required for cell elongation during vegetative growth [43], suggested the genes in subgroup III might play an important role in plant morphology. In subgroup IV, *TMK1* was an essential enzyme for DNA synthesis in bacteria [44], it indicated that the genes of subgroup IV might play a critical role in cell expansion and proliferation regulation. Subgroup V gene *RLK1* were reported to increase the tolerance to salinity, heavy-metal stresses, and *Botrytis cinerea* infection [45], it is suggested the genes of subgroup V are implicated with biotic and abiotic stress response. In subgroup VI, *CRK5* were reported to response to drought and salt stresses [46], and *CRK45* was a potentially positive regulator of ABA signaling in early seedling growth [47], stomatal movement [48], it is indicated that the genes of subgroup VI are critical to abiotic stress response and related to plant morphology. The reported genes in subgroup VII, such as *GsSRK* was an positive regulator of plant tolerance to salt stress [49], *SD1-29* improved plant resistance to bacteria [50], it shown that the genes of subgroup VII have critical role in response to biotic and abiotic responses. In general, Al-responsive *AhRLK*s in different subgroups take part in Al response by different pathways. Subgroup I and II are related to signal transduction, subgroup II is implicated with biotic stress response, subgroup III and VI play an essential role in plant morphology, subgroup IV play an critical role in cell expansion and proliferation regulation, subgroup V and VII are critical to biotic stress and abiotic stress response (Table 3).

Table 3
The classification of subgroups for Al responsive *AhRLK*s

Subgroups	Gene ID	Gene Name	Reported	Function
I	*AH05G06780.1*	Proline-rich receptor-like protein kinase PERK4	PERK1	responses to wounding and treatment with salicylic acid and PERK1 mRNA accumulation in response to these treatments shows a role in plant defense signaling [36]
II	*AH09G18420.1*	Leucine-rich repeat receptor-like protein kinase PXC1	PXC1	a regulator of secondary wall formation correlated with the TDIF-PXY/TDR-WOX4 signaling pathway [37]
II	*AH01G04120.1*	Calmodulin-binding receptor-like cytoplasmic kinase 1	CRCK1	plays a role in stress signal transduction in plants [38]
II	*AH13G53400.1*	Probable LRR receptor-like serine/threonine-protein kinase RKF3	RKF1	regulates early flower primordia during stamen development [51]
II	*AH13G49190.2* *AH04G06840.1*	LRR receptor-like serine/threonine-protein kinase FLS2	FLS2	involves in preinvasive immunity against bacterial infection [52]
II	*AH02G15400.1*	Proline-rich receptor-like protein kinase PERK3	PERK1	responses to wounding and treatment with salicylic acid and PERK1 mRNA accumulation in response to these treatments shows a role in plant defense signaling [36]
II	*AH01G20770.1* *AH03G24540.1*	Pollen receptor-like kinase 3	PRK1	PRK1 is essential for postmeiotic development of pollen [39]
II	*AH09G25780.1*	LRR receptor-like serine/threonine-protein kinase ERL1	ERECTA	regulates elongation of above-ground organs [53]
II	*AH08G04970.1*	LRR receptor-like serine/threonine-protein kinase RCH1	RCH1	resistances to the hemibiotrophic fungal pathogen colletotrichum higginsianum [41]

Subgroups	Gene ID	Gene Name	Reported	Function
II	*AH09G16620.1*	Leucine-rich repeat receptor-like protein kinase PXL1	PXL1	regulates signal transduction pathways under temperature fluctuations [54]
II	*AH05G37250.1*	Leucine-rich repeat receptor-like tyrosine-protein kinase PXC3	PXC1	a regulator of secondary wall formation correlated with the TDIF-PXY/TDR-WOX4 signaling pathway [37]
II	*AH05G22210.1*	LRR receptor-like serine/threonine-protein kinase HSL2	HSL2	involves in Floral organ abscission and lateral root emergence [55]
II	*AH05G25480.1*	Receptor-like protein kinase HSL1	HSL1	participates in the Repression of Seed Maturation Genes in Arabidopsis Seedlings [56]
II	*AH02G27570.1*	Probable LRR receptor-like serine/threonine-protein kinase RKF3	RKF1	regulates early flower primordia during stamen development [51]
III	*AH01G26450.1*	Receptor-like protein kinase ANXUR1	ANXUR1/ANXUR2	control pollen tube rupture during the fertilization process in *A. thaliana* [42]
III	*AH10G26000.1* *AH14G43820.1* *AH05G20280.1*	Receptor-like protein kinase FERONIA	FERONIA	affects plant reproduction, development, and stress tolerance [43]
III	*AH05G06270.1*	LysM domain receptor-like kinase 4	RLK1	activates defense and Abiotic-Stress Responses [45]
III	*AH14G43630.1*	Receptor-like protein kinase ANXUR2	ANXUR1/ANXUR2	control pollen tube rupture during the fertilization process in Arabidopsis thaliana [42]
III	*AH11G35150.1*	LRR receptor-like serine/threonine-protein kinase HSL2	HSL2	involved in Floral organ abscission and lateral root emergence [55]

Subgroups	Gene ID	Gene Name	Reported	Function
IV	*AH02G03870.1*	Receptor protein kinase TMK1	TMK1	an essential enzyme for DNA synthesis in bacteria, phosphorylating deoxythymidine monophosphate (dTMP) to deoxythymidine diphosphate (dTDP), and thus is a potential new antibacterial drug target [44]
V	*AH01G31190.1* *AH01G31150.1*	G-type lectin S-receptor-like serine/threonine-protein kinase RLK1 isoform X2	RLK1	activates defense and Abiotic-Stress Responses [45]
VI	*AH09G27120.1* *AH19G41030.1*	Cysteine-rich receptor-like protein kinase 29	CRK45/CRK5	response to abscisic acid and abiotic stressesa potentially positive regulator of ABA signaling in early seedling growth, stomatal movement and plant drought tolerance [46, 47]
VI	*AH08G24070.1* *AH14G27090.1*	Cysteine-rich receptor-like protein kinase 25	CRK45/CRK5	response to abscisic acid and abiotic stresses, a potentially positive regulator of ABA signaling in early seedling growth, stomatal movement and plant drought tolerance[46, 47]
VI	*AH10G29990.1* *AH13G57290.1* *AH09G27070.1*	Cysteine-rich receptor-like protein kinase 10	CRK45/CRK5	response to abscisic acid and abiotic stresses, a potentially positive regulator of ABA signaling in early seedling growth, stomatal movement and plant drought tolerance[46, 47]
VI	*AH03G40310.1*	Cysteine-rich receptor-like protein kinase 2	CRK45/CRK5	response to abscisic acid and abiotic stresses, a potentially positive regulator of ABA signaling in early seedling growth, stomatal movement and plant drought tolerance[46, 47]

Subgroups	Gene ID	Gene Name	Reported	Function
VII	*AH10G03910.1*	G-type lectin S-receptor-like serine/threonine-protein kinase B120	GsSRK	a positive regulator of plant tolerance to salt stress [49]
VII	*AH20G01850.1* *AH10G04020.1* *AH06G22210.1*	Receptor-like serine/threonine-protein kinase SD1-8	SD1-29	resistances to bacteria in crop species [57]
VII	*AH01G24170.1*	G-type lectin S-receptor-like serine/threonine-protein kinase B120	GsSRK	a positive regulator of plant tolerance to salt stress [49]

Note: only the Al responsive *AhRLK*s with characterized homologs were listed in the table.

The *AtRLK* gene family plays a role in plant growth and development processes [58]. As shown in the histograms in Fig. 8, the expression pattern of the Al-responsive *AhRLK*s exhibited tissue specificity, about 2.2% (2/90, *AH07G04000.1* and *AH16G09430.1*) of Al-responsive *AhRLK*s were expressed in all four tested organs with high expression levels (value > 5) in peanut, implying that these genes might play essential roles in plant growth and development. About 2.2% (2/90, *AH16G41130.1* and *AH07G24540.1*) of Al-responsive *AhRLK*s were expres sed specifically and at a high level in aerial organs. About 8.8% (8/90, *AH14G07810.1, AH03G21680.1 AH19G41030.1 AH13G57290.1, AH10G29990.1, AH08G20520.1, AH08G06390.1*, and *AH01G04120.1*) of Al-responsive *AhRLK*s were expressed specifically and at a high level in root or root tips. The tissue specificity of these Al-responsive *AhRLK*s indicates their key roles in tissue development or tissue functions. Additionally, 6 tissue non-specific genes (*AH07G04000.1*, *AH03G13700.1*, *AH10G03910.1*, *AH08G04680.1*, *AH08G04640.1*, and *AH16G09430.1*) that expressed at a high level specifically in root were also worth concern. As shown in the histograms in Fig. 9, the majority of the Al responsive *RLK*s were up-regulated after 8 hours of Al treatment in 99-1507 while only moderate changes were detected in some Al responsive *RLK*s in ZH2, which suggested that Al responsive *RLK*s responded rapidly to Al stress in Al tolerant variety. Although the genes had different expression profiles under Al stress in different varieties, the expression changes of 12 (*AH04G28680.1*, *AH16G41130.1*, *AH01G21880.1*, *AH10G16100.1*, *AH08G24070.1*, *AH02G27570.1*, *AH07G04000.1*, *AH09G08540.1*, *AH13G57290.1*, *AH03G17500.1*, *AH05G06780.1*, and *AH08G04970.1*) and 9 (*AH04G23000.1*, *AH11G34340.1*, *AH06G07770.1*, *AH14G40110.1*, *AH10G26000.1*, *AH02G15400.1*, *AH11G35150.1*, *AH14G40170.1*, *AH08G04660.1*) genes reached their peak in 24 h vs 0 h Al-treatment comparison in *AH01G21880.1* and *AH04G28680.1* were expressed at a high level in stems, implying their potential roles in regulating the growth of stems. *AH13G57290.1* was expressed specifically and at a high level in root, implying its critical roles in mediating Al response in peanut. *AH07G04000.1* was expressed in all four tested organs with high expression levels, and itit might play essential roles in plant growth and

development under Al stress. Taken together, our results revealed that 13 genes (*AH11G35150.1*, *AH08G24070.1*, *AH13G57290.1*, *AH02G27570.1*, *AH05G06780.1*, *AH02G15400.1*, *AH01G35150.1, AH14G27090.1*, AH05G37250.1, AH10G03910.1, *AH19G41030.1*, *AH10G29990.1*, and *AH10G26000.1*), which homologs have been reported to be involved in early seedling growth regulation, early flower primordia and stamen development, lateral root emergence, abiotic stress responses and plant defense signaling in *Arabidopsis thaliana*, were important Al responsive genes that can be suitable candidates to interpret the mechanisms underlying Al response in peanuts in future work.

Conclusions

The soil affected by Al is widely distributed throughout the world, which poses a great threat to agricultural production, meanwhile there are few studies on RLK under Al stress, and therefore, the research on peanut RLK is of great significance. In this study, a total of 1311 RLKs were identified in the peanut genome, 2 times the number of Arabidopsis RLKs, including 548 LRR-RLKs and 274 LecRLKs. LRR-RLK represented the largest RLK gene family identified in plant. These *AhRLK*s were unevenly distributed among 20 chromosomes, Chloroplasts and mitochondria of peanut. Compared with segmental duplication, tandem duplication might play a more critical role in some *AhRLK*s. The tandem duplication events appeared to have occurred during relatively recent key periods, 0–2 Mya, illustrating that these *AhRLK*s were generated by recent gene duplication events in *Arachis hypogaea* L. Besides, Estimation of Ka/Ks ratios for 1360 *AhRLK*s revealed that most *AhRLK*s were selected for purification. Furthermore, we identified a total of 90 Al responsive *AhRLK*s by mining transcriptome database. These genes were divided into 7 groups. The exon/intron compositions and motif arrangements were considerably conserved among members in the same groups or subgroups. Analysis of transcriptome data revealed tissue expression patterns of the 90 Al responsive *AhRLK*s, and tissue specific expression genes were found. Among them, genes that were identified as root-specific genes might play a key role in Al sensing and response in peanut. The close phylogenetic relationship of Al responsive *AhRLK*s and characterized RLKs in the same subgroup provided insight into their putative functions. Overall, this systematic analysis provided valuable information to understanding the biological functions of the *AhRLK*s genes under Al stress in peanut.

Methods

1. The resources of peanut *AhRLK*s

The genome sequence, protein sequences and genome annotation of peanut were according to PEANUT GENOME RESOURSE (http://peanutgr.fafu.edu.cn/). The amino acid sequences of Arabidopsis RLKs were acquired from UniPort (https://www.uniprot.org/), then run a blast search to select *A. duranensis* RLKs in Peanut Base(https://www.peanutbase.org), directly filter out *A. hypogasa* L RLKs from the database after used the amino acid sequences of *A. duranensis* RLKs to run a blast in PEANUT GENOME RESOURCE (http://peanutgr.fafu.edu.cn/), the protein sequences obtained from BlastP search were

analyzed for the presence of at least one kinase domain using NCBI's Conserved Domains Database (CDD; http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), and finally obtained 1311 *AhRLK*s.

2. Multiple sequence alignments and phylogenetic tree construction of *AhRLK*s

The sequences of LRR-*AhRLK*s and 90 *AhRLK*s response to Al stress were aligned using ClustalX in MEGA 7 with default parameters [59]. The phylogenenic tree based on the multiple sequence alignments of peanut LRR-RLK*s* (Fig. 1), LecRLKs (Fig. 2) and 90 *AhRLK*s response to Al stress (Fig. 6) were performed by MEGA 7 using the ML method with bootstrap test replicated 1000 times, the Poisson model, uniform rates and Partial deletion. Based on the multiple sequence alignment and the previously reported classification of *Arabidopsis thaliana*, the peanut *RLKs* was assigned to different subfamilies and subgroups [60–62].

3. Chromosomal locations and duplication analysis for peanut *RLK*s

The physical location of *AhRLK*s on the chromosomes was obtained from the database of PEANUT GENOME RESOURSE (http://peanutgr.fafu.edu.cn/). All members of *AhRLK*s were mapped onto peanut chromosomes based on the physical positions of them, and the image of chromosomal location was produced with the online software Map Gene 2 Chromosome v2 (MG2C:http://mg2c.iask.in/mg2c_v2.0/). *RLKS* clustered together within 100 kb were regarded as tandem duplicated genes based on the criteria of other plants in previous reports. The duplication events and syntenic analysis of *AhRLK*s were determined using Circos software [63]. The non-synonymous (Ka) and synonymous (Ks) substitution ratios were calculated by KaKs_Calculator 2.0 software [64]. The divergence time were calculated with formula T = Ks/2r, the r of dicotyledonous plants was 1.5*10^8 synonymous substitutions per site per year [65].

4. The gene structure, motif analysis and heatmap of *AhRLK*s response to Al stress

The exon-intron structures of 90 *AhRLK*s response to Al stress were determined based on their coding sequence alignments and their respective genomics sequences, while diagrams were obtained from the online program Gene Structure Display Server (http://gsds.cbi.pku.edu.cn/) [66]. MEME (Multiple Em for Motif Elicitation) tool was used to predict putative motifs of these proteins (http://meme-suite.org/). The Heatmap generation and the combination of phylogenetic tree, gene and protein structures was generated using TBtools, The presence of signal peptides, kinase domains and transmembrane domains were predicted with SMART (http://smart.embl-heidelberg.de) [67]. The Amino acid residue base, Molecular weight were predicted with ExPaSy ProtParam tool (https://web.expasy.org/protparam/).

Abbreviations

Ah
Arachis hypogaea.L
Al
Aluminum
At
Arabidopsis thaliana
EGF-RLK
epidermal growth factor like RLK
LecRLK
Lectin-like RLK
LRR-RLK
Leucine-Rich Repeat RLK
PCD
Programmed Cell Death
PR5K-RLK
Pathogenesis related protein-5 like receptor kinases RLK
MEME
Multiple Em for Motif Elicitation
ML
Maxinum Likehood
RLK
Receptor-like protein Kinase
S-RLK
S-domain RLK
TNFR-RLK
Tumor-necrosis factor receptor-like RLK
WAK1
Cell wall-associated receptor kinase 1

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. The two peanut cultivars that had used to generate the RNA-seq data with Al treatment were kindly provided by Prof. Bo-shou Liao from the Oil Crop Research Institute, Chinese Academy of Agricultural Sciences (CAAS), and they were routinely planted on the farm of Guangxi University in Nanning, Guangxi Province, China and identified by Prof. He. In detail, ZhongHua No. 2 (ZH2) (85 – 007, CHINA PEANUNT DATA CENTEI, http://www.peanutdata.cn/variety/index.htm) has been used widely in agriculture practice while 99-1507 has not been approved for commercial use. The RNA-seq data of ZH2 and 99-1507 under Al treatment had been deposited in the database of the National Center for Biotechnology Information (NCBI) under accession number PRJNA525247 (https://www.ncbi.nlm.nih.gov/sra/PRJNA525247). The raw RNA-seq reads in different tissues and *AhRLKs* sequences are available at Peanut Genome Resource (http://peanutgr.fafu.edu.cn/).

Competing interests

The authors declare that they have no competing interests.

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Authors’ contribution

DX and LFH conceived and designed the experiments. XW, MHW and RLH performed the data analysis, XW, DX wrote the manuscript. DX, LFH AQW and J Z revised this manuscript. All authors read and approved the final manuscript.

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Supplementary Information

File 1-File 8. File 1. Complete list and classification of 1311 *AhRLK*s in peanut. File 2. Complete list and classification of Al response *AhRLK*s in peanut. File 3. Subfamily and chromosome distribution of AhLRR-RLKs in peanut. File 4. Subfamily and chromosome distribution of AhLec-RLKs in peanut. File 5. Tandem duplication clusters of *AhRLK*s. File 6. Devergence time among *AhRLK*s tandem duplication pairs. File 7. The motif of Al stress related *AhRLK*s. File 8. Expression Profiles of Al stress related *AhRLK*s under Al stress.

Figures

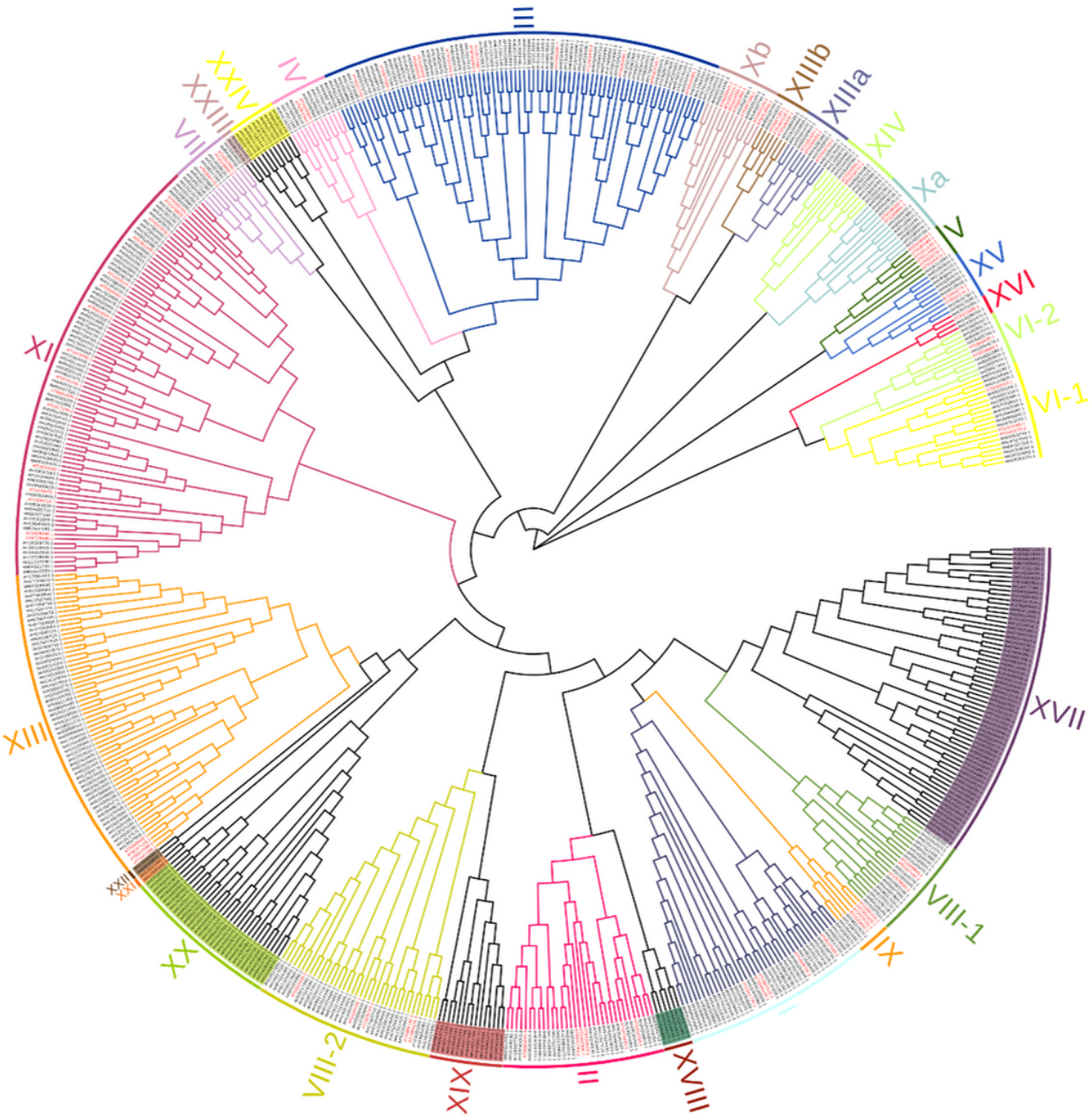

Figure 1

Phylogenetic analysis and classification of peanut and A. thaliana LRR-RLK proteins.

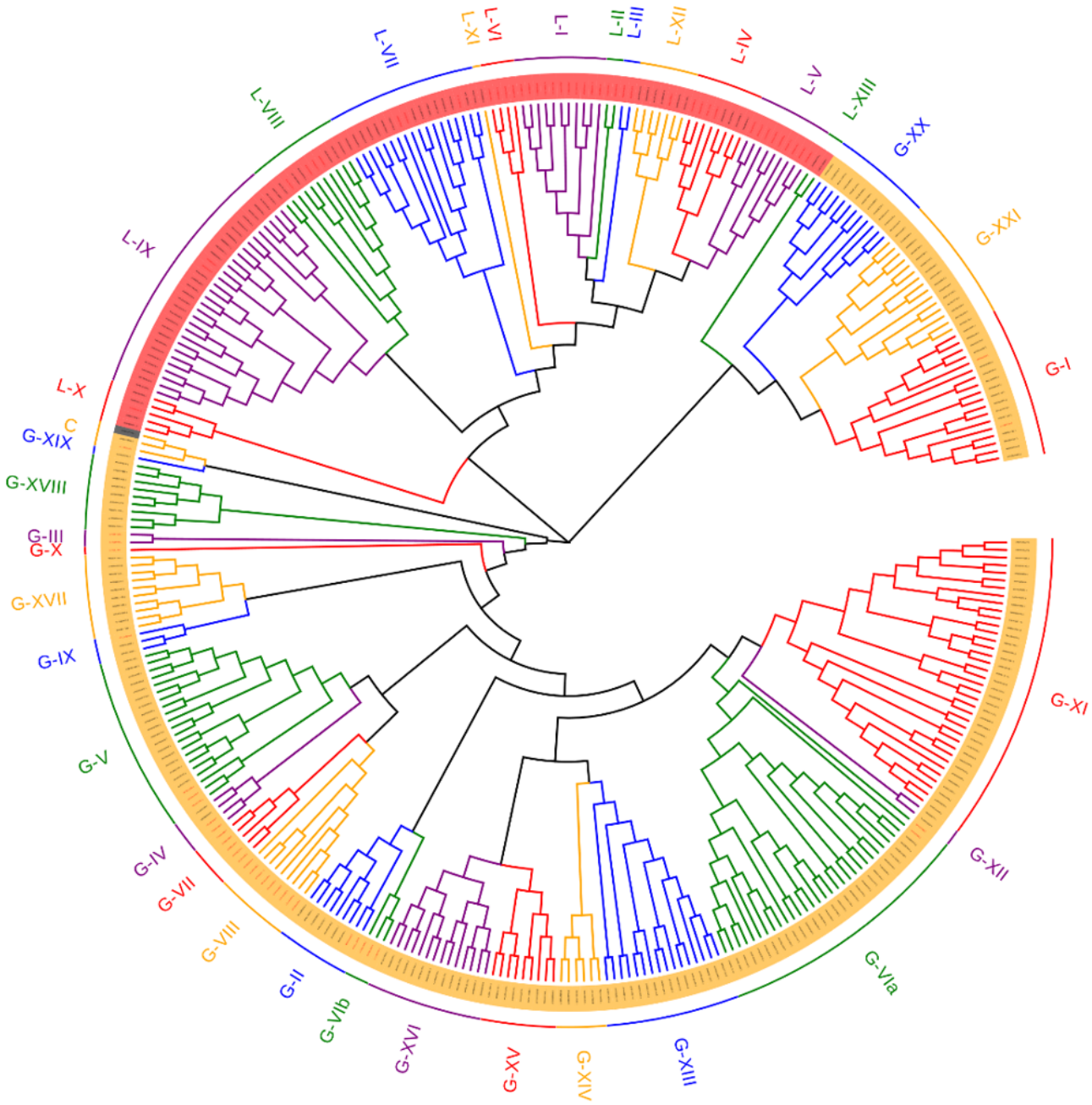

Figure 2

Phylogenetic analysis and classification of peanut and A. thaliana LecRKs proteins.

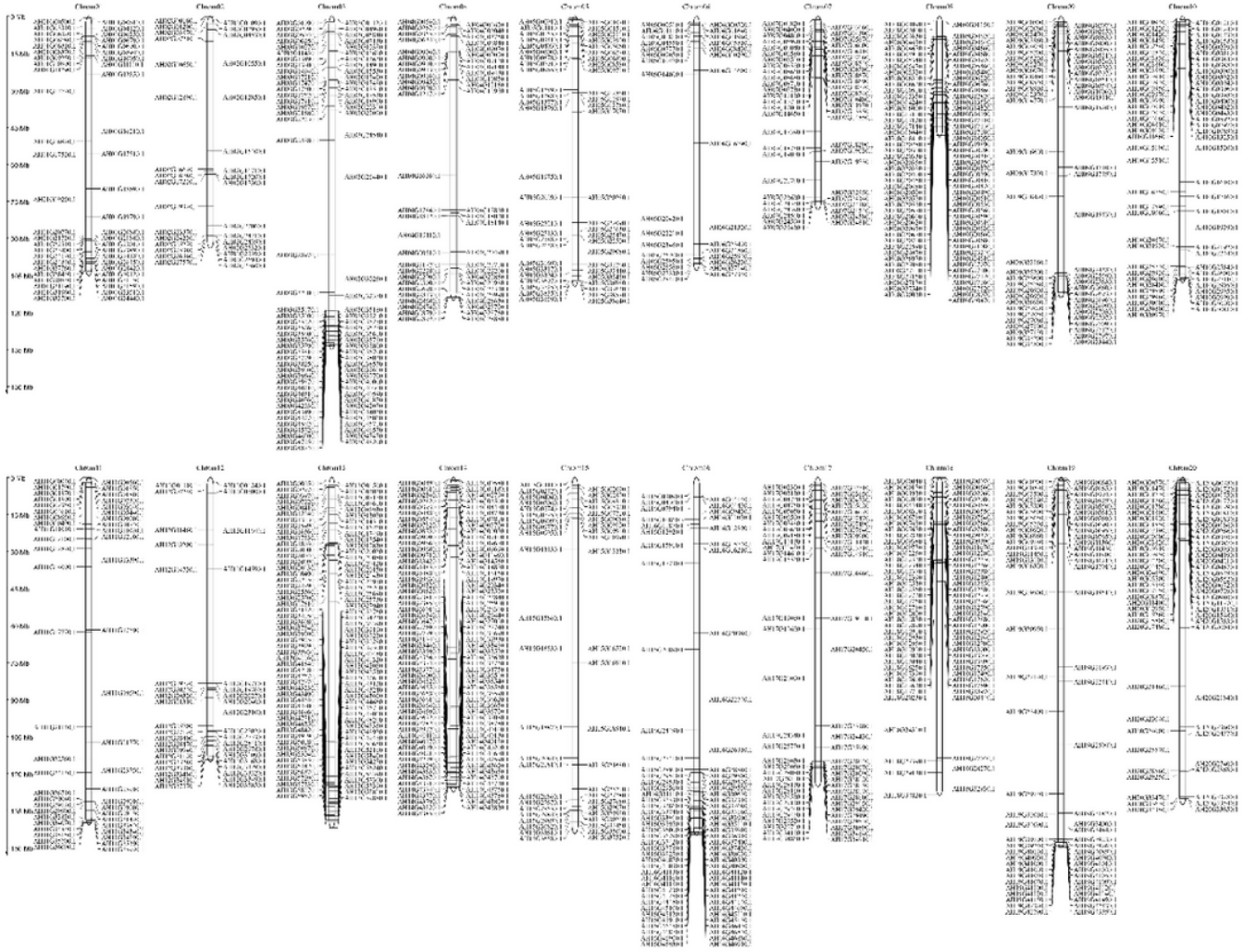

Figure 3

Genomic distribution of AhRLKs across peanut chromosomes.

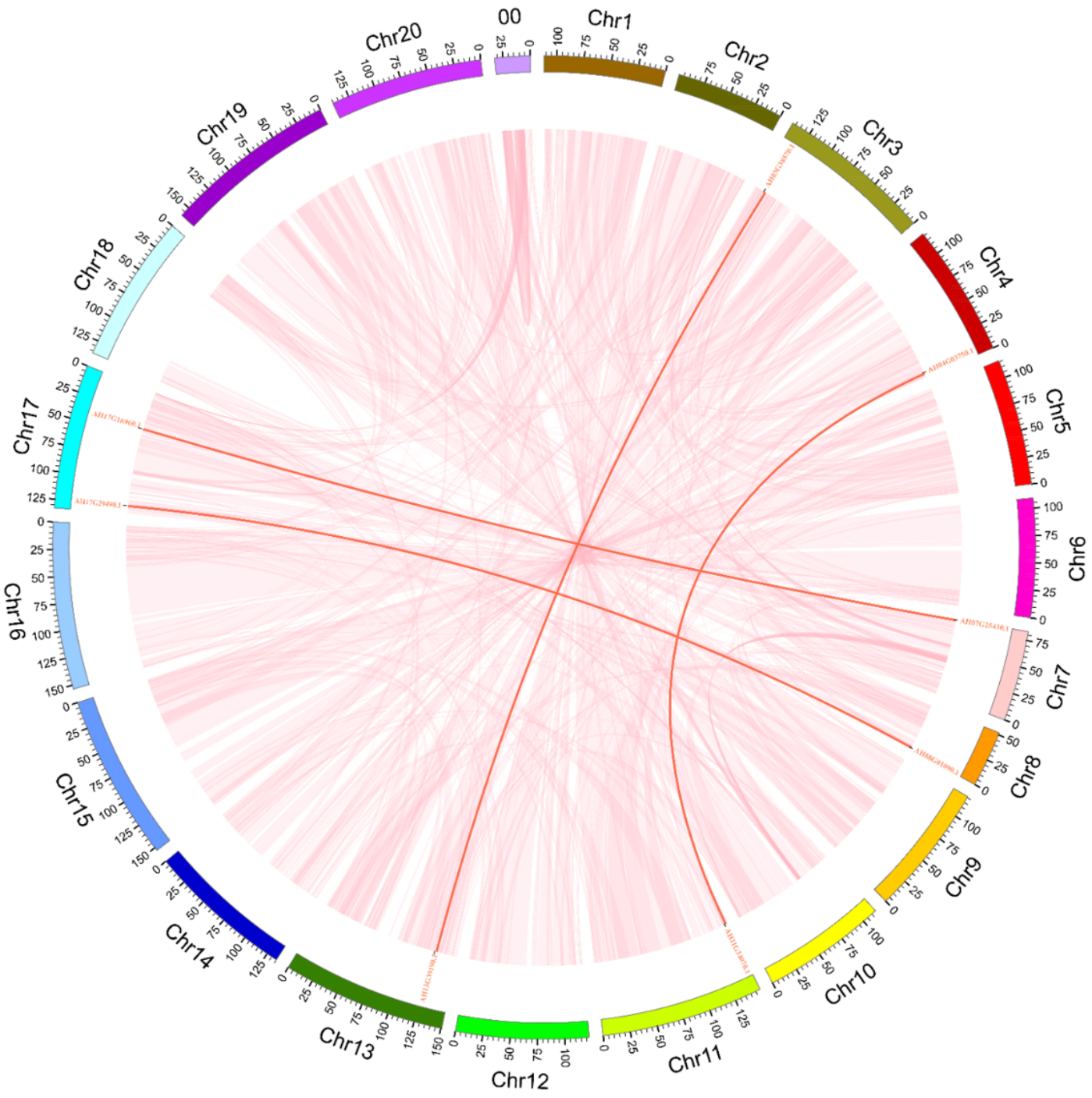

Figure 4

Genomic distribution of AhRLKs across peanut chromosomes.

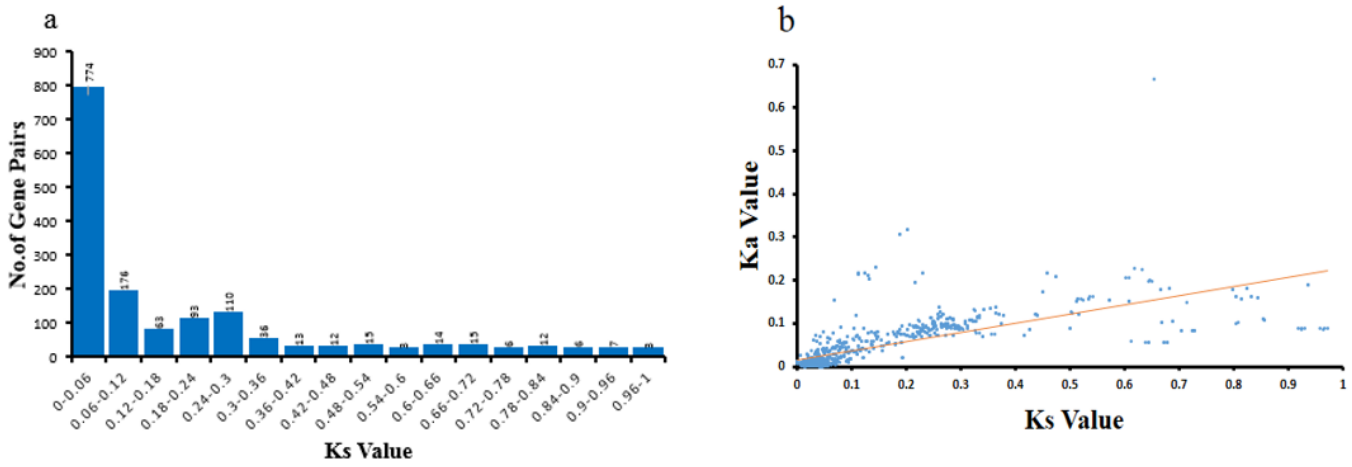

Figure 5

The distribution of Ka and Ks value in all tandem duplicated AhRLKs. a. The distribution of Ks values in all tandem duplicated AhRLKs. b. Ka, Ks values in all tandem duplication AhRLKs.

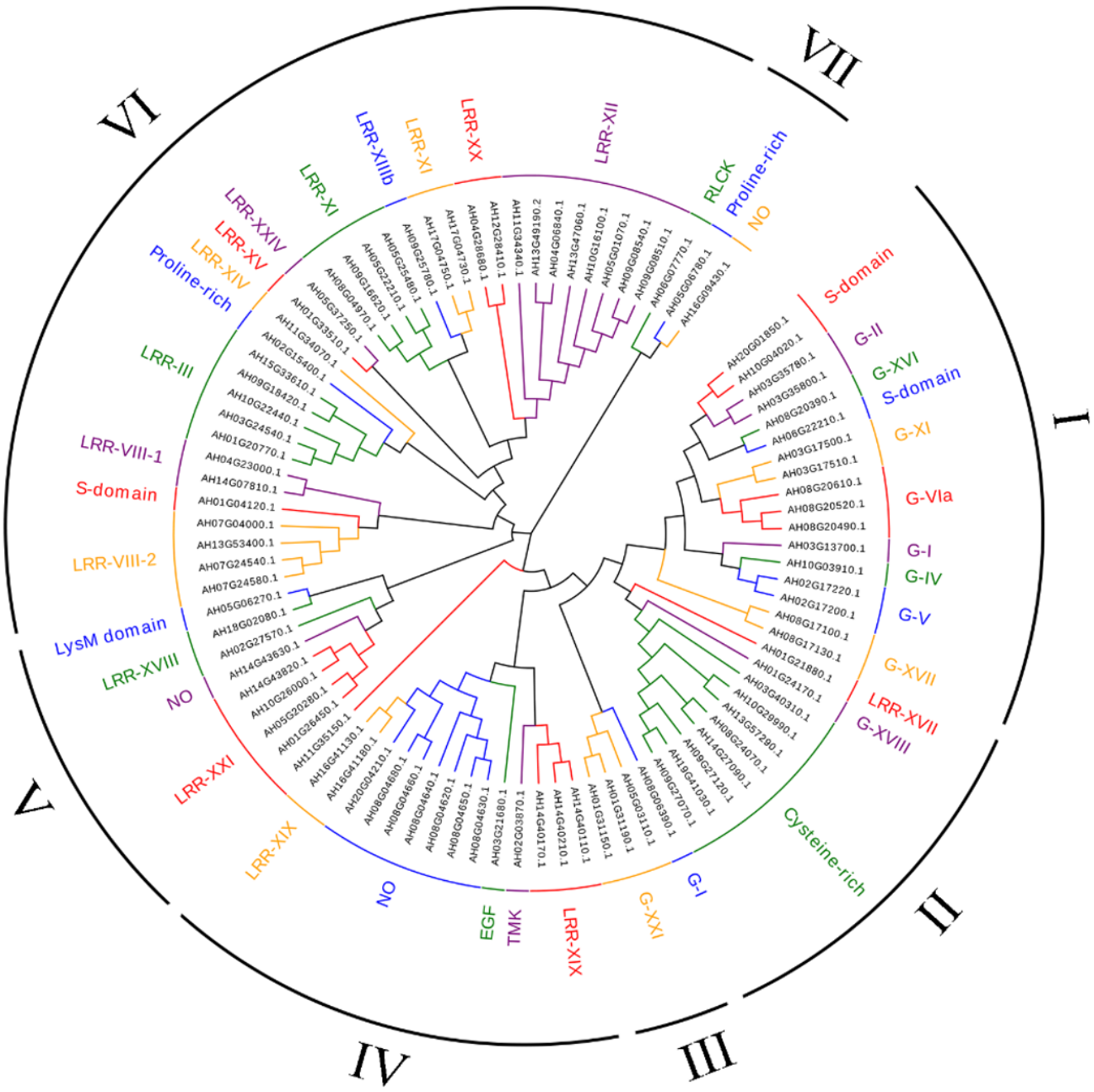

Figure 6

Phylogenetic analysis of Al-responsive AhRLKs.

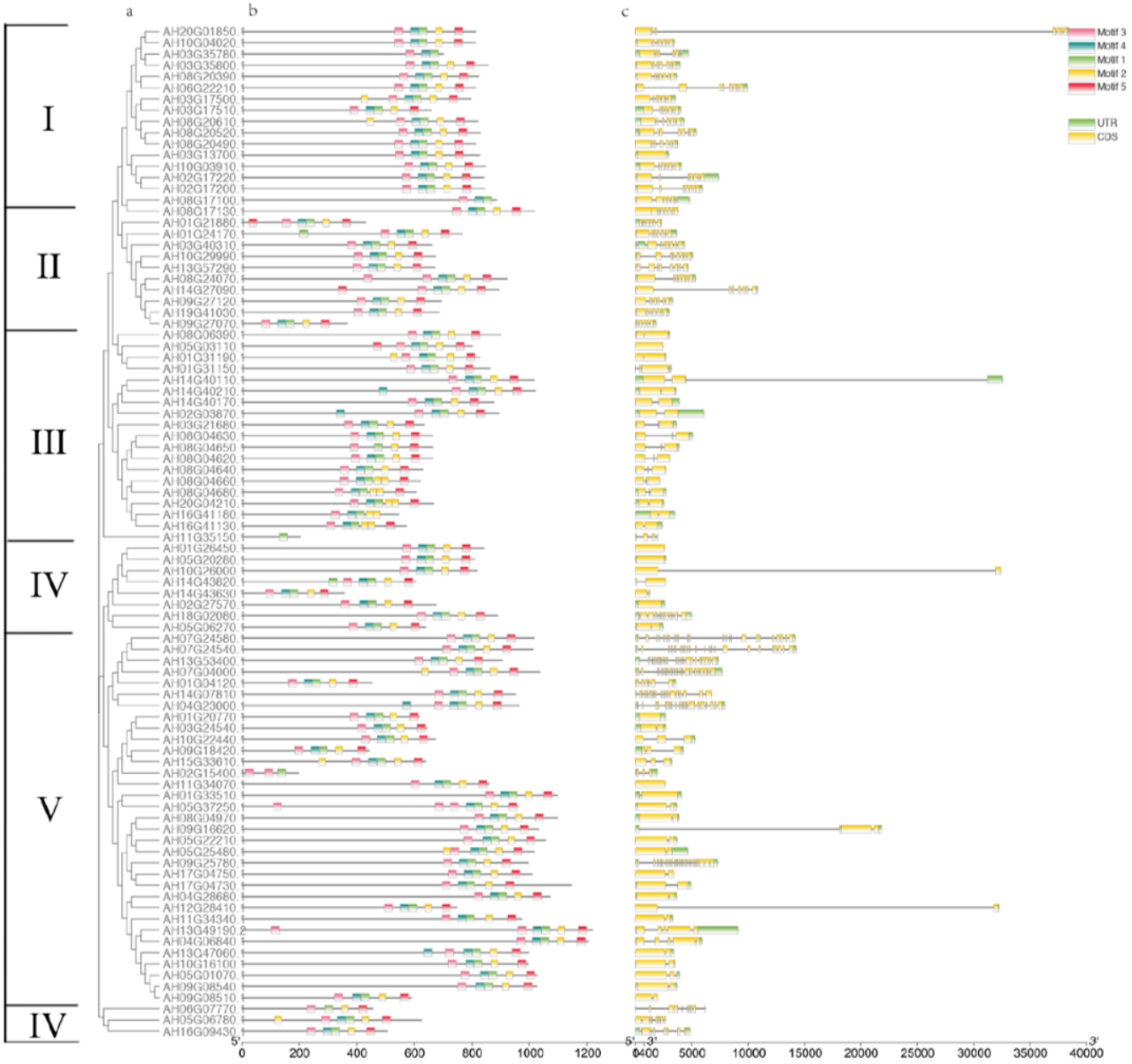

Figure 7

Phylogenetic relationships, gene structures and compositions of the conserved protein motifs of the Al-responsive AhRLKs.

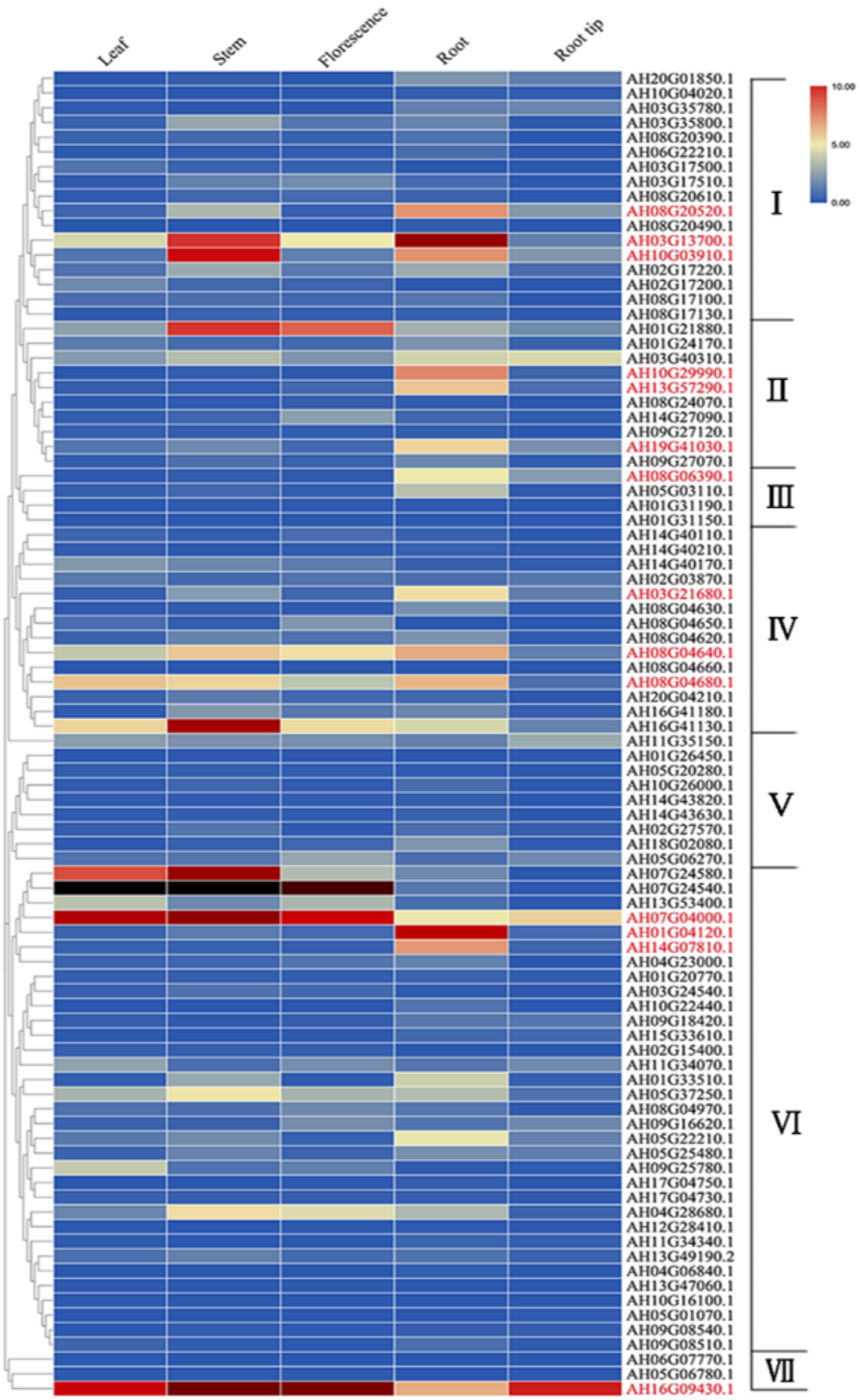

Figure 8

Expression profiles of Al-responsive AhRLKs in different tissues.

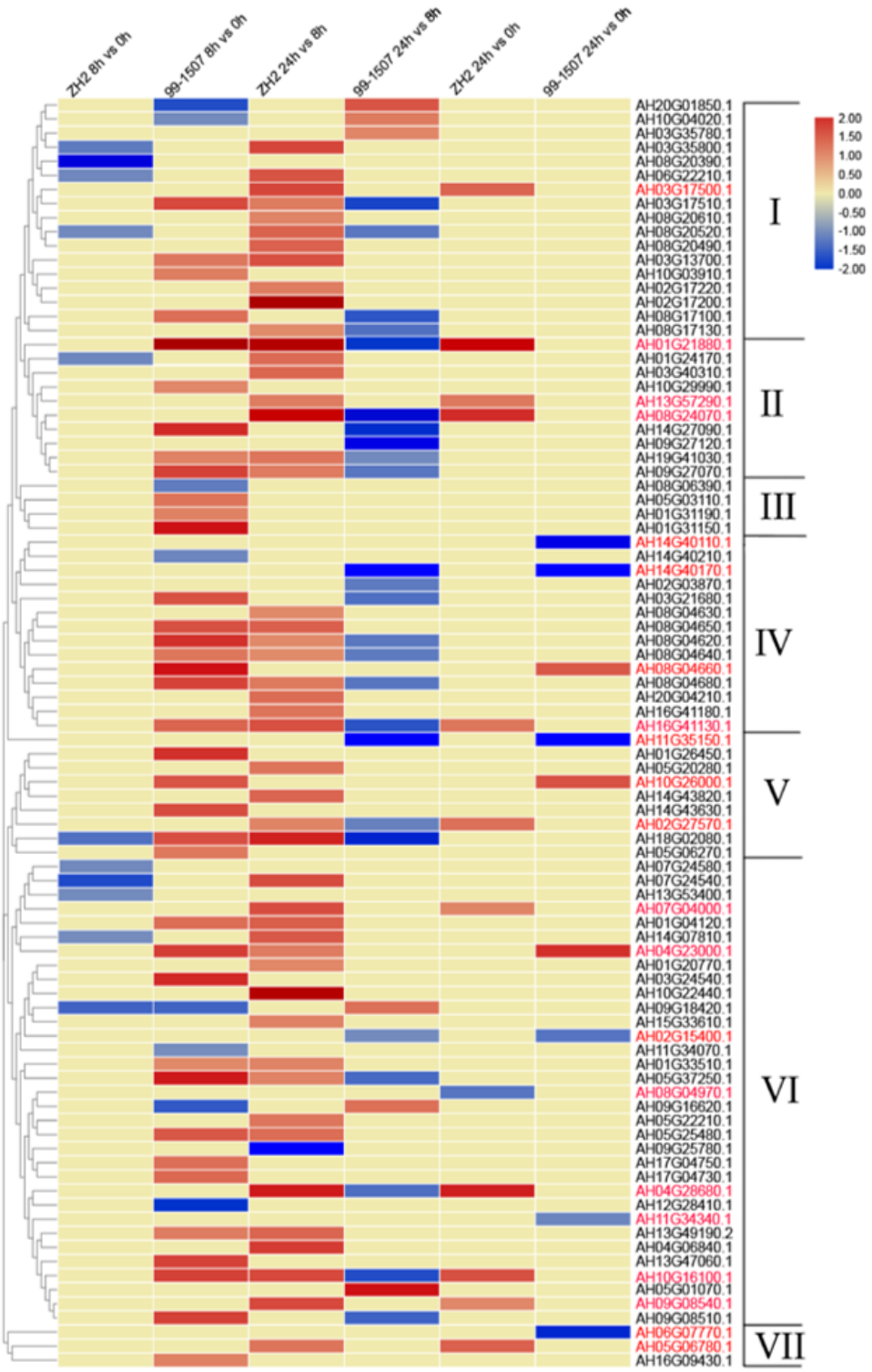

Figure 9

Expression profiles of Al-responsive AhRLKs in two varieties.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile8.xlsx
- Additionalfile7.docx
- Additionalfile6.xlsx
- Additionalfile5.xlsx
- Additionalfile4.xlsx
- Additionalfile3.xlsx
- Additionalfile2.xlsx
- Additionalfile1.xlsx