Comprehensive genomic characterization of NAC transcription factor family and their response to salt and drought stress in peanut

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Abstract

**Background:** Peanut is one of the most important oil crop species worldwide. NAC transcription factor (TF) genes play important roles in the salt and drought stress responses of plants by activating or repressing target gene expression. However, little is known about NAC genes in peanut.

**Results:** We performed a genome-wide characterization of NAC genes from the diploid wild peanut species *Arachis duranensis* and *Arachis ipaensis*, which included analyses of chromosomal locations, gene structures, conserved motifs, expression patterns, and *cis*-acting elements within their promoter regions. In total, 81 and 79 NAC genes were identified from *A. duranensis* and *A. ipaensis* genomes. Phylogenetic analysis of peanut NACs along with their *Arabidopsis* and rice counterparts categorized these proteins into 18 distinct subgroups. Fifty-one orthologous gene pairs were identified, and 46 orthologues were found to be highly syntenic on the chromosomes of both *A. duranensis* and *A. ipaensis*. Comparative RNA sequencing (RNA-seq)-based analysis revealed that the expression of 43 NAC genes was up- or downregulated under salt stress and under drought stress. Among these genes, the expression of 17 genes in cultivated peanut (*Arachis hypogaea*) was up- or downregulated under both stresses. Moreover, quantitative reverse transcription PCR (RT-qPCR)-based analysis revealed that the expression of most of the randomly selected NAC genes tended to be consistent with the comparative RNA-seq results.

**Conclusion:** Our results facilitated the functional characterization of peanut NAC genes, and the genes involved in salt and drought stress responses identified in this study could be potential genes for peanut improvement.

**Keywords:** Peanut, NAC gene family, Genome-wide characterization, RNA-seq, RT-qPCR, Salt stress, Drought stress

Background

Cultivated peanut (*Arachis hypogaea*) is an important economic oil crop species worldwide and used to provide vegetable oil and proteins for human nutrition [1]. During the growth period of peanut plants, their yield is adversely affected by several environmental factors, such as salt and drought stresses, which prevent plants from realizing their full genetic potential [2]. Screening stress-resistant varieties is an important guarantee for achieving targets crop yields [3], and the identification and utilization of resistant genes is fundamental for the production of new varieties. Transcription factors (TFs), which play roles in activating or repressing gene expression by binding to specific *cis*-acting elements within the promoters of target functional genes, regulate many biological processes [4, 5]. As members of one of the largest...
plant-specific TF families, NAC [no apical meristem (NAM), *Arabidopsis thaliana* transcription activation factor (ATAF1/2) and cup-shaped cotyledon (CUC2)] proteins have been shown to regulate several biological processes, including responses to salt and drought stresses [6–8]. Remarkably, NAC TFs are considered to be very important for plant adaptations to land [9]. NAC proteins typically have a conserved NAM domain at the N-terminus and a highly variable domain at the C-terminus, the latter of which is related to specific biological functions. NAC family genes have been studied extensively in a variety of plant species, including gymnosperms and embryophytes [10–19]. However, until recently, comprehensive analyses of peanut NAC family genes and their response patterns to salt and drought stresses have been limited.

Increasing evidences have indicated that NAC proteins are involved in plant biotic and abiotic responses. For example, the poplar NAC13 gene plays a vital role in the salt stress response [20]. Over-expression of a wheat NAC (TaNaCl-D1) enhances resistance to Fusarium head blight disease [21], TaNaC30 negatively regulates the resistance of wheat to stripe rust [22], and TaNaC29 can provide salt stress tolerance by enhancing the antioxidant systems [23]. Over-expression of *TsNaC1* from the halophyte *Thallungiella halophila* was shown to improve abiotic stress resistance, especially salt stress tolerance [24]. *SINAC35* from *Solanum lycopersicum* can promote root growth and development under salt and drought stresses [25], and rice *ONAC033* is induced by drought and can provide strong resistance to both salt and drought stresses in transgenic plants [26]. In peanut, NAC TFs are known to be involved in responses to abiotic stresses. For example, *AhNAC2* and *AhNAC3* can improve salt and drought tolerance in transgenic *Arabidopsis* and tobacco [27, 28], and *AhNAC4* confers enhanced drought tolerance to transgenic tobacco [29]. In addition, over-expression of the *MuNaC4* transgene from horsegram was shown to confer enhanced drought tolerance to transgenic peanut [30].

The genomes of allotetraploid *A. hypogaea* (AABB) and its two wild diploid ancestors *Arachis duranensis* (AA) and *Arachis ipaensis* (BB) were recently sequenced [1, 31–35]. The A and B genomes of the two diploid peanut species are similar to the A and B sub-genomes of cultivated peanut and could be used to identify candidate resistance genes [32, 35]. The availability of genomic information provides opportunities to perform genome-wide analyses of NAC genes and to explore the potential genes involved in peanut biotic and abiotic responses. With the decreasing cost of RNA sequencing (RNA-seq), transcriptome sequencing has become a powerful high-throughput sensitive technique for the analyses of differentially expressed genes. Several peanut RNA-seq datasets containing information on different tissues or responses to different treatments have been published [36–39]. For example, RNA-seq data generated from 22 different tissues and from the development stage of the diploid peanut species *A. duranensis* and *A. ipaensis* have made it convenient to analyse peanut NAC homologue expression profiles [36]. Differential gene expression in response to salt and drought stress has also been analysed, which can help in the identification of NAC genes involved in salt and drought responses [37, 39].

In this paper, we present the results of a genome-wide identification and characterization of NAC genes from wild peanut genomes and their orthologous genes in response to salt and drought stresses in cultivated peanut. We analysed their phylogenetic relationships, structural characteristics, chromosomal locations and gene orthologous gene pairs. We also determined their expression characteristics in different tissues and in response to salt and drought stresses on the basis of RNA-seq data [36, 37, 39]. Seventeen genes were identified as being involved in the response to both salt and drought stresses in cultivated peanut, and these results were confirmed by quantitative reverse transcription PCR (RT-qPCR). The objectives of this study were to provide a theoretical basis for further functional analysis of NAC proteins in peanut and to explore orthologous NAC genes involved in the response to salt and/or drought stresses in cultivated peanut.

**Results**

**Identification of NAC proteins from *A. duranensis* and *A. ipaensis***

In total, 81 and 79 NAC genes (Table 1, Additional files 1 and 2) were identified from the diploids *A. duranensis* (~1.25 Gb) and *A. ipaensis* (~1.56 Gb), respectively, which were less than the totals identified in *Arabidopsis* (105) [40] and rice (141) [41]. However, 164 NAC proteins (Additional files 3 and 4) were identified in the cultivated allotetraploid *A. hypogaea* (~2.54 Gb). The number was close to the sum of gene numbers from *A. duranensis* and *A. ipaensis*. The density of NAC genes in *A. duranensis* (0.07/Mb) was greater than that (0.05/Mb) in *A. ipaensis*. The density of NAC genes in *A. hypogaea* was 0.06/Mb, which was approximately the average number between *A. duranensis* and *A. ipaensis*.

Owing to the lack of a designated standard annotation for NAC genes in *Arachis*, we named these genes *AdNaC1-AdNaC81* and *AiNaC1-AiNaC79*. The NAC genes identified in *A. duranensis* and *A. ipaensis* encoded proteins ranging from 95 to 681 amino acid (aa) residues in length, with an average of 345 aa, and the molecular weights (MWs) varied from 11 kDa to 77.4 kDa. The isoelectric points (pIs) of the predicted proteins ranged...
| Gene symbol | Gene model name | Gene location | Length (aa) | MW (kDa) | Theoretical pI | Putative Arabidopsis orthologues | Closest genes | E-value | Othologous genes with known function |
|-------------|----------------|---------------|-------------|----------|----------------|---------------------------------|---------------|---------|-----------------------------------|
| AdNAC1      | Aradu.08GFU.1   | Chr7: 4217194..4220440 | 367         | 42.7     | 6.19           | ANAC42                          |               | 4e-85   |                                   |
| AdNAC2      | Aradu.08TAH.1   | Chr10: 5997735..5999148 | 229         | 26.7     | 5.56           | ANAC104/XND1                     |               | 2e-90   |                                   |
| AdNAC3      | Aradu.0MI0X.1   | Chr3: 11724103..11725980 | 384         | 44.0     | 7.45           | ANAC70                          |               | 3e-157  |                                   |
| AdNAC4      | Aradu.13D06.1   | Chr1: 100229649..100231496 | 396         | 45.3     | 6.86           | ANAC35                          |               | 1e-119  |                                   |
| AdNAC5      | Aradu.15JI0.1   | Chr8: 28519479..28520527 | 150         | 16.7     | 8.69           | ANAC90                          |               | 1e-28   |                                   |
| AdNAC6      | Aradu.15QQT.1   | Chr1: 17654260..17657374 | 135         | 32.8     | 9.35           | ANAC14                          |               | 3e-28   |                                   |
| AdNAC7      | Aradu.1A4F.1    | Chr7: 46474022..46478416 | 350         | 40.4     | 6.93           | ANAC33                          |               | 5e-123  |                                   |
| AdNAC8      | Aradu.21DG.1    | Chr10: 2443477..2446668 | 322         | 36.8     | 8.14           | ANAC73                          |               | 1e-114  |                                   |
| AdNAC9      | Aradu.22647.1   | Chr10: 106757870..106759333 | 274        | 31.6     | 6.00           | ANAC87                          |               | 4e-100  |                                   |
| AdNAC10     | Aradu.3058W.1   | Chr1: 42645387..42650347 | 288         | 33.6     | 6.94           | ANAC7/VND4                      |               | 2e-103  |                                   |
| AdNAC11     | Aradu.3R7A3.1   | Chr6: 99554879..99559186 | 481         | 53.7     | 5.05           | ANAC44                          |               | 3e-92   |                                   |
| AdNAC12     | Aradu.46U1T.1   | Chr6: 8759633..8760991  | 251         | 28.0     | 6.37           | ANAC28/ TIP                     |               | 5e-13   |                                   |
| AdNAC13     | Aradu.47JQU.1   | Chr8: 49202066..49203551 | 321         | 36.3     | 8.99           | ANAC100                         |               | 3e-124  |                                   |
| AdNAC14     | Aradu.4RJO.1    | Chr6: 90892652..90894340 | 355         | 40.2     | 9.35           | ANAC47                          |               | 7e-104  |                                   |
| AdNAC15     | Aradu.58D1A.1   | Chr8: 48242228..48244188 | 193         | 22.8     | 10.13          | ANAC83                          |               | 4e-63   |                                   |
| AdNAC16     | Aradu.5D5JN.1   | Chr10: 66508689..66512014 | 592         | 67.0     | 5.52           | ANAC9                           |               | 1e-94   |                                   |
| AdNAC17     | Aradu.60U13.1   | Chr10: 95255502..95259031 | 374         | 41.6     | 8.64           | ANAC38                          |               | 3e-111  |                                   |
| AdNAC18     | Aradu.66XR.1    | Chr3: 118432883..118434275 | 318     | 34.9     | 7.79           | ANAC25                          |               | 1e-107  |                                   |
| AdNAC19     | Aradu.6H4PP.1   | Chr10: 84012608..84013897 | 230         | 26.1     | 5.23           | ANAC104/ XND1                   |               |         |                                   |
| AdNAC20     | Aradu.79PL2.1   | Chr3: 106298423..106299692 | 211         | 23.6     | 9.45           | ANAC41                          |               | 4e-64   |                                   |
| AdNAC21     | Aradu.7NI41.1   | Chr3: 20188210..20192587 | 286         | 32.6     | 8.19           | ANAC73                          |               | 2e-110  |                                   |
| AdNAC22     | Aradu.7X5EV.1   | Chr8: 36760639..36761970 | 328         | 36.3     | 8.67           | ANAC2                           |               | 9e-121  |                                   |
| AdNAC23     | Aradu.7T2TE.1   | Chr5: 108980829..108983109 | 341         | 39.3     | 6.30           | ANAC7/VND5                      |               | 2e-114  |                                   |
| AdNAC24     | Aradu.8Q7DY.1   | Chr10: 100727698..100729562 | 313         | 36.1     | 8.50           | ANAC94                          |               | 3e-87   |                                   |
| AdNAC25     | Aradu.9FF24.1   | Chr9: 104552010..104554828 | 583         | 65.2     | 4.72           | ANAC53                          |               | 2e-88   |                                   |
| AdNAC26     | Aradu.9T4H8.1   | Chr3: 129693427..129694223 | 228         | 25.8     | 4.95           | ANAC104/XND1                    |               | 3e-77   |                                   |
Table 1 NAC TF gene family members in wild *Arachis* (Continued)

| Gene symbol | Gene model name | Gene location | Length (aa) | MW (kDa) | Theoretical pl | Putative *Arabidopsis* orthologues | Closest orthologous genes with known function | E-value |
|-------------|-----------------|---------------|-------------|----------|----------------|-----------------------------------|-----------------------------------------------|---------|
| AdNAC27     | Aradu.9Y6NH.1   | Chr3: 126203898..126205566 | 432         | 48.2     | 6.86           | ANAC94                           | ANAC94                                       | 7e-96   |
| AdNAC28     | Aradu.AF9FZ.1   | Chr3: 11453820..11456527    | 373         | 43.2     | 5.89           | ANAC7/VND5                       | ANAC7/VND5                                    | 6e-118  |
| AdNAC29     | Aradu.BSXXL.1   | Chr5: 89010001..89014271    | 405         | 50.5     | 6.97           | ANAC75                           | ANAC75                                       | 2e-149  |
| AdNAC30     | Aradu.BPK98.1   | Chr2: 5418916..5424040      | 463         | 52.5     | 5.81           |                                   | ANAC9                                         | 2e-50   |
| AdNAC31     | Aradu.BS3JU.1   | Chr8: 32409380..32411511    | 300         | 34.1     | 8.55           | ANAC73                           | ANAC73                                       | 2e-116  |
| AdNAC32     | Aradu.C1QOA.1   | Chr8: 28596303..28598519    | 372         | 41.9     | 7.53           | ANAC40/NTL8                      | ANAC40/NTL8                                   | 4e-99   |
| AdNAC33     | Aradu.DII8L.1   | Chr4: 123542703..123544293  | 245         | 27.8     | 9.00           | ANAC83                           | ANAC83                                       | 1e-102  |
| AdNAC34     | Aradu.DQR3M.1   | Chr10: 3280639..3282098     | 347         | 39.5     | 6.81           | ANAC25                           | ANAC25                                       | 6e-96   |
| AdNAC35     | Aradu.EP425.1   | Chr10: 83305166..83307784   | 367         | 40.4     | 4.75           | ANAC82                           | ANAC82                                       | 7e-91   |
| AdNAC36     | Aradu.ETZ8K.1   | Chr5: 5429577..5431170      | 356         | 40.3     | 5.20           | ANAC71                           | ANAC71                                       | 2e-109  |
| AdNAC37     | Aradu.F2DT2.1   | Chr8: 26149830..26151706    | 360         | 41.0     | 5.95           | ANAC25                           | ANAC25                                       | 1e-78   |
| AdNAC38     | Aradu.F48KW.1   | Chr9: 118016952..118020856  | 557         | 63.0     | 4.58           | ANAC16                           | ANAC16                                       | 1e-131  |
| AdNAC39     | Aradu.F6Z4G.1   | Chr1: 105899702..105901040  | 330         | 37.2     | 8.16           | ANAC100                          | ANAC100                                      | 7e-139  |
| AdNAC40     | Aradu.F8VRL.1   | Chr3: 30065645..30068198    | 382         | 43.0     | 7.69           | ANAC75                           | ANAC75                                       | 2e-123  |
| AdNAC41     | Aradu.H2YS3.1   | Chr6: 113116..1132912       | 226         | 26.1     | 7.22           | ANAC74                           | ANAC74                                       | 2e-61   |
| AdNAC42     | Aradu.H5KV7.1   | Chr10: 101199649..10120008  | 317         | 36.6     | 4.96           | ANAC79/ANAC80/ATNAC4             | ANAC79/ANAC80/ATNAC4                           | 4e-85   |
| AdNAC43     | Aradu.JV7AK.1   | Chr5: 103968982..103973619  | 451         | 50.9     | 5.21           | ANAC8                           | ANAC8                                        | 4e-150  |
| AdNAC44     | Aradu.JZK15.1   | Chr7: 10652666..10652977    | 95          | 11.0     | 9.22           | ANAC14                           | ANAC14                                       | 5e-06   |
| AdNAC45     | Aradu.K2UJH.1   | Chr7: 28089666..28093564    | 681         | 75.4     | 4.57           | ANAC14                           | ANAC14                                       | 7e-58   |
| AdNAC46     | Aradu.KF8UQ.1   | Chr3: 111110289..1111111452 | 184         | 21.1     | 5.66           | ANAC104/XND1                     | ANAC104/XND1                                 | 3e-36   |
| AdNAC47     | Aradu.L3QY1.1   | Chr1: 27306711..27309985    | 331         | 37.2     | 4.82           | ANAC71                           | ANAC71                                       | 2e-97   |
| AdNAC48     | Aradu.L657Y.1   | Chr2: 14425633..14431626    | 246         | 27.8     | 5.89           | ANAC74                           | ANAC74                                       | 1e-81   |
| AdNAC49     | Aradu.L85VN.1   | Chr3: 12654444..126545867   | 286         | 32.8     | 8.32           | ANAC2                            | ANAC2                                        | 2e-109  |
| AdNAC50     | Aradu.LG4RX.1   | Chr6: 95430437..95431819    | 218         | 25.2     | 9.18           | ANAC83                           | ANAC83                                       | 3e-21   |
| AdNAC51     | Aradu.LZ0DB.1   | Chr7: 70232857..70235212    | 351         | 40.3     | 8.49           | ANAC42                           | ANAC42                                       | 2e-86   |
| AdNAC52     | Aradu.M7213.1   | Chr5: 11521321..11523020    | 324         | 37.4     | 5.38           | ANAC1                            | ANAC1                                        | 2e-140  |
| Gene symbol | Gene model name | Gene location | Length (aa) | MW (kDa) | Theoretical pI | Putative Arabidopsis orthologues | Closest genes | E-value | Orthologous genes with known function |
|-------------|----------------|---------------|-------------|----------|---------------|--------------------------------|---------------|---------|-------------------------------------|
| AdNAC53     | Aradu.M8PFR.1  | Chr9: 104514608..104520535 | 425         | 47.9     | 8.63          | ANAC52                        | 1e-86         |         |                                     |
| AdNAC54     | Aradu.M9GL4.1  | Chr5: 50856073..50857364    | 308         | 35.3     | 7.68          | ANAC2                         | 8e-118        |         |                                     |
| AdNAC55     | Aradu.N8F6V.1  | Chr5: 82433539..82435658     | 363         | 40.2     | 9.35          | ANAC040                       | 3e-81         |         |                                     |
| AdNAC56     | Aradu.N8MU8.1  | Chr5: 93368562..93371821     | 362         | 41.0     | 7.21          | ANAC58                        | 2e-125        |         |                                     |
| AdNAC57     | Aradu.NEU1C.1  | Chr2: 5363506..5368149       | 255         | 29.6     | 5.41          | ANAC14                        | 3e-39         |         |                                     |
| AdNAC58     | Aradu.R9F07.1  | Chr2: 4630145..4632702       | 463         | 51.1     | 6.05          | ANAC66                        | 1e-104        |         |                                     |
| AdNAC59     | Aradu.RP61F.1  | Chr6: 110760391..110763962   | 306         | 34.5     | 5.60          | ANAC7                         | 1e-19         |         |                                     |
| AdNAC60     | Aradu.RRT20.1  | Chr5: 13469204..13471956     | 394         | 45.6     | 6.98          | ANAC7                         | 3e-116        |         |                                     |
| AdNAC61     | Aradu.S13QQ.1  | Chr6: 25318703..25322833     | 344         | 39.3     | 6.33          | ANAC25                        | 2e-83         |         |                                     |
| AdNAC62     | Aradu.TGA11.1  | Chr3: 7357966..7359851       | 315         | 36.3     | 6.62          | ANAC36                        | 2e-117        |         |                                     |
| AdNAC63     | Aradu.TI02Z.1  | Chr7: 34924555..34930380     | 322         | 36.4     | 7.57          | ANAC1                         | 1e-124        |         |                                     |
| AdNAC64     | Aradu.U974Q.1  | Chr3: 12275474..122758369    | 633         | 71.7     | 6.34          | ANAC28                        | 2e-141        |         |                                     |
| AdNAC65     | Aradu.UH959.1  | Chr8: 38011875..38013744     | 369         | 40.7     | 7.22          | ANAC100                       | 4e-90         |         |                                     |
| AdNAC66     | Aradu.UXN6T.1  | Chr8: 46083445..46085088     | 304         | 35.1     | 6.42          | ANAC32                        | 3e-96         |         |                                     |
| AdNAC67     | Aradu.VUC67.1  | Chr9: 118496634..118500190   | 321         | 37.5     | 6.76          | ANAC7                         | 2e-113        |         |                                     |
| AdNAC68     | Aradu.W3GLH.1  | Chr7: 15758174..15764123     | 679         | 77.4     | 5.43          | ANAC28                        | 8e-167        |         |                                     |
| AdNAC69     | Aradu.WT00W.1  | Chr7: 44224604..44246557     | 346         | 39.5     | 5.05          | ANAC20                        | 2e-96         |         |                                     |
| AdNAC70     | Aradu.WS3DN.1  | Chr6: 71790233..71791501     | 213         | 24.4     | 5.39          | ANAC90                        | 1e-45         |         |                                     |
| AdNAC71     | Aradu.XE8WZ.1  | Chr3: 111523591..111525548   | 300         | 33.0     | 5.09          | ANAC103                       | 2e-50         |         |                                     |
| AdNAC72     | Aradu.XJFO9.1  | Chr5: 86074509..86078409     | 396         | 43.6     | 6.47          | ANAC44                        | 2e-87         |         |                                     |
| AdNAC73     | Aradu.XQ4Vp.1  | Chr5: 98563335..98567633     | 167         | 19.5     | 8.87          | ANAC57                        | 4e-90         |         |                                     |
| AdNAC74     | Aradu.Y1DM8.1  | Chr6: 90691784..90693411     | 396         | 44.5     | 6.21          | ANAC46                        | 5e-111        |         |                                     |
| AdNAC75     | Aradu.YJNS.1   | Chr8: 4371901..4373364       | 369         | 41.6     | 7.84          | ANAC100                       | 5e-75         |         |                                     |
| AdNAC76     | Aradu.YFQ3P.1  | Chr3: 110319004..110321231   | 260         | 29.8     | 7.71          | ANAC102                       | 7e-113        |         |                                     |
| AdNAC77     | Aradu.YIQ80.1  | Chr8: 36879860..36881784     | 349         | 39.1     | 8.20          | ANAC19                        | 4e-120        |         | AhNAC4 (HM776131) [29]               |
| dNAC78      | Aradu.YXW0Z.1  | Chr3: 119828022..119831252   | 342         | 38.6     | 8.66          | ANAC10/5ND3                   | 1e-120        |         |                                     |

Table 1 NAC TF gene family members in wild *Arachis* (Continued)
| Gene symbol | Gene model name | Gene location | Length (aa) | MW (kDa) | Theoretical pI | Putative Arabidopsis orthologues | Closest genes | E-value | Othologous genes with known function |
|------------|----------------|---------------|------------|----------|---------------|---------------------------------|---------------|---------|-------------------------------------|
| AdNAC79    | Aradu.Z4K97.1 | Chr9:120442436..120446530 | 403         | 55.0     | 5.11          | ANAC8                           | 3e-127        |         |                                     |
| AdNAC80    | Aradu.Z5H58.1 | Chr3:25915995..25917258 | 335         | 37.7     | 6.61          | ANAC3                           | 3e-110        |         |                                     |
| AdNAC81    | Aradu.Z9Y3J.1 | Chr4:117994993..117996740 | 330         | 38.0     | 5.72          | ANAC100                         | 6e-89         |         |                                     |
| AINAC1     | Araip.0550R.1 | Chr3:197325..198893 | 330         | 37.5     | 9.04          | ANAC100                         | 3e-123        |         |                                     |
| AINAC2     | Araip.053J1.1 | Chr5:139720050..139724356 | 358         | 40.3     | 6.40          | ANAC75                          | 1e-143        |         |                                     |
| AINAC3     | Araip.1N7I.1 | Chr10:4025791..4028779 | 324         | 36.9     | 8.41          | ANAC73                          | 8e-115        |         |                                     |
| AINAC4     | Araip.120SD.1 | Chr3:33051241..33054714 | 381         | 42.7     | 7.33          | ANAC75                          | 1e-123        |         |                                     |
| AINAC5     | Araip.2BL8E.1 | Chr8:5815169..5817042 | 320         | 36.2     | 9.08          | ANAC40                          | 9e-84         |         |                                     |
| AINAC6     | Araip.2W5R5.1 | Chr10:93270958..93274136 | 592         | 67.0     | 5.46          | ANAC14                          | 2e-91         |         |                                     |
| AINAC7     | Araip.310T2.1 | Chr7:11782789..117885019 | 301         | 33.7     | 6.54          | ANAC32                          | 2e-108        |         |                                     |
| AINAC8     | Araip.31EFM.1 | Chr5:142199068..142203628 | 410         | 45.4     | 5.76          | ANAC85                          | 2e-85         |         |                                     |
| AINAC9     | Araip.333QY.1 | Chr3:28574038..28575297 | 335         | 37.7     | 6.61          | ANAC19                          | 3e-109        |         | AhNAC3 (EU755022)[28]                |
| AINAC10    | Araip.4A49L.1 | Chr5:144854742..144856654 | 285         | 32.5     | 6.97          | ANAC40                          | 9e-74         |         |                                     |
| AINAC11    | Araip.6C1F    | Chr10:127061923..127064519 | 391         | 45.5     | 5.07          | ANAC79                          | 5e-73         |         |                                     |
| AINAC12    | Araip.609WS.1 | Chr5:134972759..134976087 | 363         | 41.1     | 7.21          | ANAC58                          | 2e-123        |         |                                     |
| AINAC13    | Araip.64GCN.1 |Chr5:45189298..45190788 | 308         | 35.4     | 7.69          | ANAC25                          | 1e-82         |         |                                     |
| AINAC14    | Araip.67R8V   |Chr6:123068423..123072685 | 471         | 52.6     | 5.11          | ANAC44                          | 3e-93         |         |                                     |
| AINAC15    | Araip.6YGY    |Chr7:4085598..4089132 | 280         | 32.7     | 5.49          | ANAC042                         | 2e-27         |         |                                     |
| AINAC16    | Araip.714GL   |Chr9:138000623..138005210 | 559         | 63.3     | 4.57          | ANAC16                          | 8e-134        |         |                                     |
| AINAC17    | Araip.71CS3   |Chr10:107295010..107298155 | 367         | 40.4     | 4.72          | ANAC103                         | 6e-87         |         |                                     |
| AINAC18    | Araip.77ISR   |Chr2:17820382..17822891 | 257         | 28.9     | 8.49          | ANAC74                          | 1e-63         |         |                                     |
| AINAC19    | Araip.78PTT   |Chr8:24667634..24669998 | 366         | 40.4     | 7.22          | ANAC100                         | 8e-90         |         |                                     |
| AINAC20    | Araip.79TDF   |Chr7:16494343..16499499 | 678         | 77.3     | 5.51          | ANAC086                         | 1e-115        |         |                                     |
| AINAC21    | Araip.7L9YW   |Chr5:110392178..110396183 | 497         | 56.8     | 5.05          | ANAC8                          | 6e-121        |         |                                     |
| AINAC22    | Araip.8NR3H   |Chr3:111878869..111880200 | 260         | 29.9     | 7.09          | ANAC032                         | 8e-98         |         |                                     |
| AINAC23    | Araip.92BTQ   |Chr10:108502499..108503880 | 213         | 24.3     | 5.25          | ANAC104                         | 3e-55         |         |                                     |
| Gene symbol | Gene model name | Gene location | Length (aa) | MW (kDa) | Theoretical pI | Putative *Arabidopsis* orthologues | Closest gen | E-value | Othologous genes with known function |
|-------------|-----------------|----------------|-------------|-----------|---------------|-----------------------------------|-------------|---------|-----------------------------------|
| AiNAC24     | Araip.98R1Z     | Chr3: 112774516..112775399 | 202         | 23.2      | 5.19          | ANAC104                           |             | 3e-47   |                                   |
| AiNAC25     | Araip.9MG9F     | Chr3: 22778530..22786094 | 321         | 36.6      | 7.37          | ANAC75                            |             | 1e-95   |                                   |
| AiNAC26     | Araip.9NS54     | Chr3: 14030155..14031366 | 338         | 39.0      | 5.91          | ANAC7                             |             | 4e-116  |                                   |
| AiNAC27     | Araip.9W65R     | Chr3: 123433544..123436914 | 633         | 71.6      | 6.09          | ANAC86                            |             | 2e-113  |                                   |
| AiNAC28     | Araip.A6QWC     | Chr2: 6650205..6654171 | 481         | 54.6      | 5.61          | ANAC14                            |             | 3e-51   |                                   |
| AiNAC29     | Araip.AVV74     | Chr4: 127912708..127914469 | 330         | 38.1      | 5.82          | ANAC100                           |             | 3e-88   |                                   |
| AiNAC30     | Araip.AWF0A     | Chr7: 95692641..95694174 | 354         | 39.7      | 7.39          | ANAC100                           |             | 6e-64   |                                   |
| AiNAC31     | Araip.CC7W1     | Chr2: 593755..5956019 | 461         | 51.0      | 6.05          | ANAC33                            |             | 1e-90   |                                   |
| AiNAC32     | Araip.D25H8     | Chr8: 70326624..70335981 | 390         | 44.0      | 6.27          | ANAC58                            |             | 3e-66   |                                   |
| AiNAC33     | Araip.D7N1Q     | Chr6: 34266288..34267077 | 142         | 16.1      | 9.78          | ANAC25                            |             | 2e-60   |                                   |
| AiNAC34     | Araip.DEH65     | Chr5: 14567806..14570484 | 343         | 39.8      | 6.24          | ANAC7                             |             | 7e-117  |                                   |
| AiNAC35     | Araip.DL86S     | Chr6: 21485570..21487407 | 349         | 39.1      | 8.20          | ANAC19                            |             | 2e-120  | AhNAC2 (EU755023) [27]            |
| AiNAC36     | Araip.DR280     | Chr10: 126350971..126352623 | 304        | 34.7      | 6.66          | ANAC94                            |             | 3e-65   |                                   |
| AiNAC37     | Araip.E0NQ0     | Chr9: 131326018..131330122 | 494         | 55.1      | 5.05          | ANAC8                             |             | 5e-132  |                                   |
| AiNAC38     | Araip.FSAGL     | Chr1: 114413161..114414326 | 330         | 37.2      | 8.16          | ANAC100                           |             | 3e-137  |                                   |
| AiNAC39     | Araip.F862      | Chr9: 127070762..127076419 | 425         | 47.9      | 8.63          | ANAC51                            |             | 1e-87   |                                   |
| AiNAC40     | Araip.FR0NA     | Chr8: 128783925..128786313 | 116         | 13.5      | 10.25         | ANAC83                            |             | 1e-24   |                                   |
| AiNAC41     | Araip.FRS32     | Chr6: 22593548..22597918 | 259         | 29.9      | 6.70          | ANAC74                            |             | 4e-92   |                                   |
| AiNAC42     | Araip.G3ZLR     | Chr5: 12372525..12374201 | 321         | 36.7      | 5.21          | ANAC7                             |             | 2e-97   |                                   |
| AiNAC43     | Araip.G88UP     | Chr3: 120758733..120762390 | 330         | 37.3      | 8.47          | ANAC75                            |             | 4e-97   |                                   |
| AiNAC44     | Araip.HU9F      | Chr6: 113252685..113254364 | 418         | 47.1      | 9.44          | ANAC47                            |             | 8e-98   |                                   |
| AiNAC45     | Araip.HYMB8     | Chr6: 113117048..113118424 | 395         | 44.5      | 6.21          | ANAC46                            |             | 4e-111  |                                   |
| AiNAC46     | Araip.I60BC     | Chr8: 5732262..5733688 | 234         | 26.3      | 6.09          | ANAC90                            |             | 3e-64   |                                   |
| AiNAC47     | Araip.I6LH9     | Chr8: 126952889..126954575 | 318         | 36.7      | 6.01          | ANAC32                            |             | 2e-96   |                                   |
| AiNAC48     | Araip.J93F1     | Chr8: 10780467..10782598 | 294         | 33.5      | 8.82          | ANAC75                            |             | 2e-98   |                                   |
| AiNAC49     | Araip.J9WHS     | Chr3: 14391485..14393315 | 375         | 42.5      | 7.28          | ANAC70                            |             | 2e-159  |                                   |
| Gene symbol | Gene model name | Gene location | Length (aa) | MW (kDa) | Theoretical pI | Putative Arabidopsis orthologues | Closest genes | E-value | Orthologous genes with known function |
|-------------|----------------|---------------|-------------|----------|---------------|---------------------------------|---------------|---------|-------------------------------------|
| AiNAC50     | Araip.KI83M    | Chr5: 144883533..144885223 | 306 | 34.4 | 5.48 | ANAC40 | 6e-83 |
| AiNAC51     | Araip.KM02ZG   | Chr3: 130519333..130520361 | 143 | 16.6 | 7.84 | ANAC104 | 1e-57 |
| AiNAC52     | Araip.KP5QZ    | Chr3: 119580802..119582423 | 362 | 40.1 | 8.83 | ANAC25 | 2e-111 |
| AiNAC53     | Araip.ZU60G    | Chr8: 105359001..105363289 | 350 | 40.5 | 6.73 | ANAC33 | 4e-125 |
| AiNAC54     | Araip.ZS75D    | Chr9: 127100454..127103707 | 593 | 66.4 | 4.66 | ANAC2 | 6e-82 |
| AiNAC55     | Araip.L221I    | Chr3: 127136414..127138105 | 423 | 47.3 | 6.49 | ANAC94 | 8e-97 |
| AiNAC56     | Araip.MQD55    | Chr2: 6609921..6613742 | 289 | 33.1 | 5.65 | ANAC14 | 1e-39 |
| AiNAC57     | Araip.NB7HU    | Chr8: 21185319..21187122 | 332 | 36.9 | 8.67 | ANAC25 | 2e-102 |
| AiNAC58     | Araip.NL359    | Chr5: 126310932..126316255 | 255 | 29.4 | 5.16 | ANAC86 | 1e-78 |
| AiNAC59     | Araip.PNX61    | Chr6: 135601578..135605205 | 308 | 34.8 | 5.61 | ANAC96 | 8e-19 |
| AiNAC60     | Araip.PT231    | Chr3: 113538059..11354219 | 343 | 38.0 | 5.85 | ANAC103 | 1e-58 |
| AiNAC61     | Araip.PW8UQ    | Chr3: 10649536..10655226 | 256 | 29.8 | 5.83 | ANAC36 | 5e-98 |
| AiNAC62     | Araip.PXQQP    | Chr7: 29146174..29149324 | 573 | 63.3 | 4.59 | ANAC14 | 1e-28 |
| AiNAC63     | Araip.Q1JTI    | Chr1: 50655721..50661455 | 377 | 43.5 | 6.24 | ANAC7 | 2e-133 |
| AiNAC64     | Araip.Q3R6H    | Chr8: 3508743..3510623 | 360 | 41.0 | 5.95 | ANAC25 | 2e-78 |
| AiNAC65     | Araip.QS7JY    | Chr9: 136867581..136871254 | 291 | 34.1 | 6.97 | ANAC7 | 1e-83 |
| AiNAC66     | Araip.R0657    | Chr3: 107857753..107858791 | 211 | 23.7 | 9.45 | ANAC83 | 4e-63 |
| AiNAC67     | Araip.TG61C    | Chr3: 127434557..127435546 | 286 | 32.8 | 8.04 | ANAC25 | 7e-78 |
| AiNAC68     | Araip.TL0B5    | Chr5: 15820769..15822869 | 234 | 26.7 | 5.45 | ANAC62 | 7e-20 |
| AiNAC69     | Araip.U9RGH    | Chr1: 124432208..124434079 | 403 | 46.1 | 6.86 | ANAC35 | 1e-116 |
| AiNAC70     | Araip.U1A0W9   | Chr10: 133594504..133595933 | 277 | 31.8 | 6.32 | ANAC87 | 9e-100 |
| AiNAC71     | Araip.WV14F    | Chr8: 79712569..79714789 | 342 | 39.4 | 8.70 | ANAC42 | 4e-87 |
| AiNAC72     | Araip.X2Kc1    | Chr5: 5593161..5595535 | 352 | 39.9 | 5.26 | ANAC71 | 3e-108 |
| AiNAC73     | Araip.X3T4     | Chr10: 118962456..118966247 | 342 | 37.6 | 7.70 | ANAC38 | 4e-82 |
| AiNAC74     | Araip.XJX11    | Chr8: 97499988..97504182 | 319 | 36.9 | 5.64 | ANAC20 | 3e-96 |
| AiNAC75     | Araip.XK9AB    | Chr1: 33384496..33387453 | 277 | 31.3 | 5.37 | ANAC71 | 5e-99 |
The phylogenetic tree divided NACs from wild peanut into 18 distinct subgroups (NAC-a to NAC-r) along with their *Arabidopsis* and rice homologues (Fig. 2). In general, the *Arabidopsis*, rice and peanut NAC proteins were distributed uniformly in all subgroups. However, the NAC-o and NAC-r subgroups contained only *Arabidopsis* and rice NACs and no peanut NACs. Remarkably, the NAC-p subfamily included 36 rice NACs but only 1 *AdNAC* and 1 *Arabidopsis* NAC, while no rice NAC was found in the NAC-n subgroup. Another phylogenetic tree based on the conserved NAM domain is shown in Additional file 8.

To investigate the structural diversity of NAC genes, the exon/intron structure among the peanut NAC genes was analysed accompanying with their phylogenetic similarities (Fig. 3). All the NAC genes from *A. duranensis* and *A. ipaensis* were classified into twelve subfamilies (Fig. 3a). Commonly, orthologous genes from *A. duranensis* and *A. ipaensis* shared similar exon/intron structures including intron number and exon length, for example, *AdNAC80* and *AiNAC9* in subfamily I, *AdNAC59* and *AiNAC59* in subfamily III, while *AdNAC81* and *AiNAC29* in subfamily IV (Additional file 9). Gene structural analysis indicated that the intron distribution within the peanut NAC genes was diverse and varied from 1 to 9 (Fig. 3b). In general, most of the NACs contained 2–3 introns; for instance, 77 genes contained 2 introns, and 43 genes contained 3 introns.

To determine the diversification of NAC genes further, putative motifs were predicted, and ten conserved motifs within the *Arachis* NAC proteins were analysed (Additional file 10). As expected, the motif compositions among the closely related members were common. For instance, the majority of NAC proteins in subfamily XII contained 8 motifs. Notably, most of the predicted motifs were located in the N-terminal region of the NAC domain, which indicated that the N-terminal region was critical for the function of NAC genes (Fig. 3c).

### Cis-acting elements in the promoter region of *Arachis* NAC genes

NAC genes play critical roles in the response to numerous stresses. The putative cis-acting elements involved in

| Gene symbol | Gene model name | Gene location | Length (aa) | MW (kDa) | Theoretical pl | Putative *Arabidopsis* orthologues | Closest genes | E-value | Orthologous genes with known function |
|-------------|-----------------|---------------|-------------|----------|---------------|-----------------------------------|--------------|--------|--------------------------------------|
| AINAC76     | Arapi.XQA0A     | Chr5: 14948871,149490936 | 339         | 39.1     | 6.30          | ANAC7                             | 1e-113       |        |                                      |
| AINAC77     | Arapi.XT8UZ     | Chr10: 4890767,4892438 | 365         | 41.6     | 6.43          | ANAC25                            | 2e-93        |        |                                      |
| AINAC78     | Arapi.ZX5IX     | Chr6: 7136977,7138554 | 259         | 29.1     | 6.27          | ANAC62                            | 3e-10        |        |                                      |
| AINAC79     | Arapi.YS3WM     | Chr10: 10330806,10332162 | 229         | 26.7     | 5.69          | ANAC104                           | 1e-90        |        |                                      |
the response to biotic or abiotic stresses within the 2.5-kb sequence upstream of the start codon (ATG) (Additional file 11) were analysed. As shown in Additional files 12, 14 known stress-related cis-acting elements within the promoters of these NAC genes were identified. The numbers of cis-acting factors ranged from 0 to 10, and there were 10 different types of cis-acting elements within the promoter region of AdNAC34, AdNAC30, and AiNAC30. Only promoters of 4 genes (AdNAC7, AdNAC15, AdNAC44, and AiNAC15) contained the TC-rich motif, which is involved in defence and stress responses [42]. Of the 160 promoters, 133 had 1–9 copies of AREs, which are essential for anaerobic induction [43]. The CGTCA motif, which is involved in stress responses mediated by the hormone methyl jasmonate (MeJA) [44], was present within 93 genes. Several other elements related to abiotic and biotic stress responses, such as TGA, W1, HSE, and LTR elements, were also found in these 2.5-kb promoter regions. These results indicated that NAC genes were

Fig. 1 Chromosome location of NAC genes on each chromosomes of A. duranensis and A. ipaensis. a Diagrammatic sketch of distribution of NAC genes on each chromosome (black bars). The approximate location of each NAC gene are shown at the left side of each chromosome. b-c The NAC genes' distribution on each chromosome. The number of NAC genes on each chromosome is shown in brackets.
transcriptionally regulated in response to biotic and abiotic stresses.

Expression profile of NAC genes in different tissues of *A. duranensis* and *A. ipaensis*

To investigate the tissue-specific expression profile of NAC genes, we utilized transcriptome data from Clevenger et al. [36]. The examined 22 tissues encompassed nearly all tissues and developmental stages. As shown in Fig. 4, there was no detection of *AdNAC44* expression in any of the 22 tissues. Twenty-three NAC genes were expressed at a relatively high level in the 22 tissues. Among these 23 genes, *AiNAC7* exhibited relatively high expression levels in all 22 tissues, while its homologue *AdNAC12* was expressed only in reproductive shoot tip tissue. The genes with the same expression patterns, for example, *AdNAC16* and *AiNAC6*, were classified into the same group (group V, Fig. 3). Moreover, some NAC genes displayed tissue-specific or preferential expression patterns. For example, *AdNAC58* was not expressed in the seeds, pistils or stamens. This tissue-specific expression data analysis could ultimately help determine the locations of the regulatory function of NAC genes.

Mining NAC genes involved in the response to salt and drought stresses

Many NAC genes are considered to be abiotic stress-responsive genes. To explore NAC genes involved in the response to salt and/or drought stresses, we analysed the published transcriptome sequencing results of cultivated peanut under salt [39] and drought [37] treatments. Under salt treatment, the expression level of 28 genes was upregulated by 2-fold, whereas the expression of 15 genes was downregulated more than 2-fold. The expression of 8 genes was significantly upregulated more than 5-fold, and the greatest expression reached 17-fold, and the expression of 6 genes was downregulated more than 5-fold (Fig. 5, Additional file 13). Under drought treatment, the expression of 30 genes was up-regulated more than 2-fold, the expression of 9 genes was up-regulated
### Table 2: Putative orthologous gene pairs in *A. duranensis* and *A. ipaensis*

| Gene pairs      | Groups | Chromosome | CDS identity (%) | Protein identity (%) |
|-----------------|--------|------------|------------------|----------------------|
| AdNAC1-AiNAC15  | IX-IX  | 7–7        | 62.33            | 73.17                |
| AdNAC2-AiNAC79  | IX-IX  | 10–10      | 96.73            | 99.13                |
| AdNAC3-AiNAC49  | VIII-VIII | 3–3       | 96.09            | 96.35                |
| AdNAC4-AiNAC69  | IX-IX  | 1–1        | 95.51            | 96.30                |
| AdNAC7-AiNAC53  | VI-VI  | 7–8        | 97.68            | 97.43                |
| AdNAC10-AiNAC63 | X-X    | 1–1        | 79.17            | 73.47                |
| AdNAC12-AiNAC78 | VIII-VIII | 6–6       | 93.89            | 85.71                |
| AdNAC16-AiNAC6  | V-V    | 10–10      | 98.67            | 99.16                |
| AdNAC17-AiNAC73 | III-III | 10–10     | 90.02            | 88.27                |
| AdNAC18-AiNAC52 | V-V    | 3–3        | 84.50            | 86.74                |
| AdNAC20-AiNAC66 | I-I    | 3–3        | 96.68            | 98.58                |
| AdNAC21-AiNAC25 | IX-IX  | 3–3        | 89.14            | 84.78                |
| AdNAC22-AiNAC57 | I-I    | 8–8        | 95.67            | 94.58                |
| AdNAC23-AiNAC76 | X-X    | 5–5        | 93.51            | 95.07                |
| AdNAC24-AiNAC36 | III-III | 10–10     | 86.73            | 84.76                |
| AdNAC25-AiNAC54 | II-II  | 9–9        | 94.42            | 94.01                |
| AdNAC28-AiNAC26 | VI-VII | 3–3        | 87.92            | 87.77                |
| AdNAC29-AiNAC2  | VIII-VIII | 5–5      | 76.79            | 74.10                |
| AdNAC34-AiNAC77 | XI-XI  | 10–10      | 91.48            | 88.28                |
| AdNAC35-AiNAC17 | XII-XII | 10–10     | 98.64            | 98.91                |
| AdNAC36-AiNAC72 | XII-XII | 5–5        | 97.76            | 97.19                |
| AdNAC37-AiNAC64 | III-III | 8–8        | 98.89            | 99.17                |
| AdNAC39-AiNAC38 | III-III | 1–1        | 98.39            | 99.39                |
| AdNAC40-AiNAC4  | VI-VI  | 3–3        | 97.46            | 96.60                |
| AdNAC47-AiNAC75 | XII-XII | 1–1       | 82.03            | 80.36                |
| AdNAC48-AiNAC41 | VI-VI  | 2–6        | 61.05            | 55.21                |
| AdNAC49-AiNAC67 | XII-XII | 3–3        | 95.76            | 98.60                |
| AdNAC52-AiNAC42 | XII-XII | 5–5        | 94.43            | 92.97                |
| AdNAC53-AiNAC39 | II-II  | 9–9        | 98.54            | 98.82                |
| AdNAC54-AiNAC13 | VIII-VIII | 5–5      | 97.08            | 99.35                |
| AdNAC55-AiNAC10 | III-III | 5–5        | 69.45            | 43.90                |
| AdNAC56-AiNAC12 | II-II  | 5–5        | 96.01            | 95.91                |
| AdNAC57-AiNAC56 | XI-XI  | 2–2        | 85.78            | 80.97                |
| AdNAC58-AiNAC31 | I-I    | 2–2        | 97.00            | 97.85                |
| AdNAC59-AiNAC59 | III-III | 6–6        | 97.70            | 97.08                |
| AdNAC62-AiNAC61 | I-I    | 3–3        | 79.21            | 79.05                |
| AdNAC63-AiNAC32 | XII-XII | 7–8        | 76.35            | 68.29                |
| AdNAC64-AiNAC27 | XII-XII | 3–3        | 97.04            | 97.79                |
| AdNAC65-AiNAC19 | IV-IV  | 8–8        | 97.82            | 97.57                |
| AdNAC67-AiNAC65 | X-X    | 9–9        | 83.21            | 84.80                |
| AdNAC68-AiNAC20 | XII-XII | 7–7        | 98.61            | 97.94                |
| AdNAC69-AiNAC74 | XII-XII | 7–8        | 80.24            | 76.57                |
| AdNAC73-AiNAC58 | XII-XII | 5–5        | 65.10            | 58.43                |
| AdNAC74-AiNAC45 | I-I    | 6–6        | 98.99            | 98.74                |
Table 2  Putative orthologous gene pairs in *A. duranensis* and *A. ipaensis* (Continued)

| Gene pairs         | Groups | Chromosome | CDS identity (%) | Protein identity (%) |
|--------------------|--------|------------|------------------|----------------------|
| AdNAC75-AiNAC30    | IV-IV  | 8–7        | 89.36            | 85.87                |
| AdNAC76-AiNAC22    | X-X    | 3–3        | 98.19            | 99.23                |
| AdNAC77-AiNAC35    | I-I    | 8–8        | 97.32            | 99.71                |
| AdNAC78-AiNAC43    | IV-IV  | 3–3        | 93.50            | 94.07                |
| AdNAC79-AiNAC37    | V-V    | 9–9        | 97.04            | 95.98                |
| AdNAC80-AiNAC9     | I-I    | 3–3        | 98.80            | 99.40                |
| AdNAC81-AiNAC29    | IV-IV  | 4–4        | 97.32            | 96.97                |

Fig. 3  Gene structure and motif compositions of NAC genes from *A. duranensis* and *A. ipaensis*. a NAC proteins from two wild peanut were divided into twelve phylogenetic subgroups via MEGA 6.0 using NJ method with 1000 bootstrap replicates, designated as I to XII in different colour backgrounds. b Gene structure of peanut NAC genes was analysed using the online GSDS tool. The exons and introns are indicated by yellow boxes and black lines, respectively. The scale at bottom represents the sizes of exons and introns. c The distribution of conserved motifs within peanut NAC proteins was explored by MEME. Each motif is distinguished by a number in the coloured box. The black lines show the non-conserved sequences. Detailed information of each motif is listed in Additional file 10.
more than 10-fold, and the greatest expression reached 38-fold. The expression of 13 genes was down-regulated more than 2-fold, and the greatest expression reached 15-fold (Fig. 5, Additional file 14). The expression of 17 genes was found to be responsive to both salt and drought stresses. Four genes (AhNAC1, AhNAC37, AhNAC83 and AhNAC156) displayed the opposite response to salt and drought stresses (Fig. 5).
Fig. 5 Expression patterns of AhNAC genes under drought and salt stresses based on RNA-seq data. The Y-axis indicates the relative expression level. The X-axis represents the genes whose expression was upregulated or downregulated more than 2-fold under both salt and drought treatments in cultivated peanut.

Fig. 6 Expression profiling of AhNAC genes under salt stress. The Y-axis indicates the relative expression level. The X-axis represents hours (0, 6, 12, 18, 24, 36, and 48) after salt treatment in cultivated peanut. The actin gene was used as an internal control. The error bars were obtained from three biological replicates, and asterisks represent the genes whose expression was significantly up- or downregulated under salt stress, according to t-tests (*, \( p < 0.05 \); **, \( P < 0.01 \)).
concerning these NAC genes from cultivated *A. hypogaea* is listed in Additional file 3. These observations indicated that some of the NAC proteins may function in multiple stress responses.

**RT-qPCR of NAC genes under salt and drought stresses in cultivated peanut**

To confirm which genes respond to stress for further genetic engineering of cultivated peanut with improved stress resistance, we performed RT-qPCR expression analysis of the root. Several genes were randomly selected from the 17 NAC genes that were involved in both salt and drought stress responses. Under salt stress (51.33 mM) treatment, the expression trends of most of the detected NACs in roots (except the trends of AhNAC73) were identical to the RNA-seq results. For example, the expression of AhNAC1, AhNAC37, AhNAC103, and AhNAC156 was downregulated under salt stress at all detected time points, while the expression levels of AhNAC10, AhNAC18, AhNAC22, AhNAC27, AhNAC65, AhNAC87, AhNAC102, and AhNAC117 were upregulated. Notably, the expression of AhNAC10, AhNAC18, AhNAC22, AhNAC27, AhNAC65, and AhNAC117 peaked at 48 h after salt stress treatment, and the increase in expression of AhNAC65 reached more than 200-fold (Fig. 6). Under 20% PEG6000 treatment, the expression levels of AhNAC10, AhNAC18, AhNAC65, AhNAC73, AhNAC87, and AhNAC102 increased at all subsequent time points after treatment, and the expression level of AhNAC65 increased by nearly 30-fold after treatment for 24 h (Fig. 7).

These results were consistent with the RNA-seq results (Fig. 5). Overall, these results indicated that the response of these genes to salt and drought treatment could potentially improve peanut.

**Discussion**

**Characterization of Arachis NAC genes**

NAC genes are members of one of the largest plant TF families and play critical roles in numerous stress responses [4, 5]. The NAC gene family has been characterized from several plant genomes [10–19, 40, 41]. However, little is known about NAC genes in *Arachis* species. Cultivated peanut *A. hypogaea* originated via hybridization of two diploid wild peanut. The A and B genomes of wild peanut *A. duranensis* (AA) and *A. ipaensis* (BB) are highly identical to the A and B sub-genomes of cultivated peanut (AABB) [32]. The diploid wild peanuts are more convenient for gene cloning than the allotetraploid cultivated peanut (which contains A and B sub-genomes) because the diploids contain only one genome set (AA or BB). The available RNA-seq data of 22 distinct tissue types of the wild peanut *A.duranensis* and *A.ipaensis* made it convenient for gene expression profiling analysis [36]. Therefore, in this study, we performed a genome-wide analysis of NAC TFs from wild peanut and explored their orthologous genes’ potential functions in response to salt and drought stress in cultivated peanut. Information (for example, chromosomal location, gene structure, tissue expression profiles) of NAC genes from cultivated peanut could be deduced...
from the orthologous genes of wild peanut from this study.

In total, 81, 79 and 164 NAC TFs were identified from the wild peanut species *A. duranensis*, *A. ipaensis* and cultivated peanut *A. hypogaea*, respectively. Two or more peanut NAC genes were found for every orthologue in *Arabidopsis*. Detailed information on the *Arachis* NAC gene family, including model name, location, nucleotide acid length, molecular weight and theoretical pl, as well as *Arabidopsis* orthologues is listed in Table 1 and Additional file 3. A previous study showed that the number of nucleotide-binding site (NBS) domains characteristic of biotic stress resistance genes in tetraploid peanut was less than the sum of them between *A. duranensis* and *A. ipaensis* and caused some resistance abilities lost in cultivated peanut [32]. However, in our study, the number (164) of NACs in *A. hypogaea* was nearly the sum of those between wild *A. duranensis* (81) and *A. ipaensis* (79). This expansion might arise from multiple gene duplication events, including whole-genome duplication in the *Arachis* lineage followed by multiple segmental and tandem duplication events [27, 32]. These results were identical to those NAC from cultivated cotton *Gossypium barbadense* and two diploid cotton species, *Gossypium raimondii* and *Gossypium arboreum* [45]. Previous studies revealed that the involvement of NAC genes performed major functions in transcription regulation [45]. Thus, we speculated that NACs might perform functions through regulating stress-resistant-related genes or proteins, while not performing functions like a “on-off” switch. The number of NAC genes in cultivated peanut (164) was larger than that in other plant species (for example, 105 in *Arabidopsis* [40], 141 in rice [41], and 101 in soybean [46]), which was approximately 1.56-fold than that in *Arabidopsis*, and a similar result was found in *Populus* [10]. The NAC gene density in *A. duranensis*, *A. ipaensis* and *A. hypogaea* (0.07/Mb, 0.05/Mb, 0.06/Mb) was lower than that in *Arabidopsis* (0.87/Mb) and rice (0.37/Mb) [11]. This may be attributed to *Arachis* large genome sizes, which suggested that the genome size and number of NAC family members were not always correlated. These NAC genes were unevenly distributed on each *Arachis* chromosome (Fig. 1). The numbers on each chromosome ranged from 1 to 17, which indicated that there was no positive correlation between chromosome length and the number of NAC genes. Some NAC genes, such as *AdNAC58*, *AdNAC57* and *AdNAC30*, tended to be located in clusters on the chromosome, these gene therefore might function cooperatively [47].

Tissue-specific expression profiling were useful because it identified the genes that were involved in defining the precise nature of individual tissues [48]. In this study, we utilized the published available RNA-seq data of 22 tissue types to examine the specific expression patterns of *Arachis* NAC genes [36]. Twenty-three NAC genes were ubiquitously expressed, which could serve as a platform to regulate a broad set of genes that were subsequently fine tuned by specific regulators. Notably, we found that *AdNAC58* was not expressed in seeds, pistils or stamens, which indicated that its promoter could be used for non-seed genetic engineering.

**Phylogenetic analysis and expression profiling of *Arachis* NAC genes under salt and drought stress**

We performed phylogenetic analysis of *Arachis* NAC with monocot (rice) and dicot (*Arabidopsis*) model plant species to investigate the evolutionary relationships and predict drought- or salt-responsive genes. In the present study, these NACs were classified into 18 subgroups, which was largely consistent with the results of previous analyses [10, 40, 41]. Remarkably, the subfamily NAC-p included 36 rice NACs but only 1 *AdNAC* and 1 *Arabidopsis* NAC (Fig. 2), which suggested that they might have been either acquired in the rice or lost in *Arabidopsis* and *Arachis* when they split from their common ancestor. In contrast, there was no rice NAC gene in the subfamily NAC-n (Fig. 2), suggesting that diversification and expansion of this subgroup occurred after the monocot-dicot divergence. This phenomenon has also been found in radish, *Populus* and other species [10, 11].

If the *AdNAC* and *AiNAC* genes were clustered in pairs in phylogenetic tree, the gene pairs were considered as orthologous genes [49, 50]. In this study, 51 orthologous genes were identified from two wild peanut according to the phylogenetic relationship of the *AdNAC* and *AiNAC* genes (Fig. 2, Table 2), which accounted for more than 57% of the entire family, with sequence identities ranging from 61 to 99% (Table 2). Forty-six genes were located at syntenic loci and exhibited high collinearity on the *A. duranensis* and *A. ipaensis* chromosomes (Table 2, Fig. 1). Several putative orthologous gene pairs exhibited low coding DNA sequence (CDS) or low protein identity, which could be attributed to wrong exon-intron splicing originating from genome sequencing mistakes (for example, *AdNAC55* and its orthologous *AiNAC10*). Several NAC genes from both wild peanut species were not located in the corresponding chromosome regions, suggesting the occurrence of large chromosomal rearrangement in the diploid genomes. Orthologous genes usually exhibit similar characteristics and expression patterns [49, 51]. The functions of orthologous NAC genes of cultivated species which derived from two wild species may be redundant. For example, *AdNAC54* and *AiNAC13* from subfamily VIII have 3 exons and shared the same conserved motif. Both were highly expressed in nodule roots and flowers, but expression at a relatively low levels of in
the other organs, which was similar to the results of its corresponding *Arabidopsis* orthologues *NAC2* which expressed in roots and flowers with respect to regulating the salt stress response and lateral root development [52]. Additionally, *ANAC2* can also be induced by abscisic acid (ABA), 1-aminocyclopropane-1-carboxylic acid (ACC) and 1-naphthylacetic acid (NAA) [52]. Their corresponding orthologous genes in cultivated peanut may function together. Orthologous genes from different plant species showed a tendency to fall into one subgroup and shared similar functions. Many NAC genes have been functionally characterized in *Arabidopsis*, and their orthologous genes in *Arachis* were identified in this study (Table 1). Together with the phylogenetic results, it was possible to predict the functions of peanut NAC genes on the basis of the functions of their *Arabidopsis* and rice orthologues, which could also be potentially utilized for further functional studies. For example, *AdNAC77*, *AiNAC9*, and *AiNAC35*, together with their *Arabidopsis* orthologous gene, *ANAC19* (At1g52890) gene were clustered into the same NAC-g subfamily (Fig 2). The expression of *ANAC19* was induced by drought, high salinity, and abscisic acid (ABA). In the same subfamily, the expression of *Arabidopsis ANAC55* (At3g15500) and *ANAC72* (At4g27410) was also induced by drought and high salinity [8]. Therefore, we speculated that *AdNAC77*, *AiNAC9*, and *AiNAC35* are drought- and high salinity-responsive genes that regulate peanut survival under adverse growth conditions. Not surprisingly, *AhNAC87* (the orthologous gene of *AdNAC77* and *AiNAC35* in cultivated peanut) was induced under both salt and drought treatments based on RNA-seq analysis (Fig. 5), and the RT-qPCR-based results confirmed that, in cultivated peanut, the expression of *AhNAC87* was upregulated under both salt and drought stress treatments (Figs. 6 and 7). Additionally, *Arabidopsis ANAC2* (At1g01720, also known as AATAF1), which is orthologous to *AdNAC2*, was induced by drought stress [53]. The expression of their orthologue *AhNAC37*, was upregulated approximately 27.5-fold under drought stress according to the comparative RNA-seq analysis (Fig. 5). These findings strongly supported that the functions of *Arachis* NAC genes could be deduced from these orthologous genes from *Arabidopsis* and rice.

Previous reports have provided strong evidence for phylogenetic analysis based prediction of the stress-related function of several gene family members. The dehydration-induced gene *AhNAC3* (EU755022, *AhNAC117* in our study) provided hyper-resistance to dehydration and drought stresses [27]. In our study, the expression of *AhNAC117* was induced under salt treatment based on the comparative RNA-seq data (Fig. 5), and was confirmed by RT-qPCR (Figs. 6 and 7). Similar results were found for *AhNAC4* (HM776131, the orthologue of *AhNAC87* in our study, and orthologous to *AdNAC77* and *AiNAC35*) and *AhNAC2* (EU755023) [28, 29]. These two genes shared 97.78% similarity, were highly induced by drought and salt stresses, and conferred drought and salt tolerance to transgenic plants.

### Methods

#### Sequence database searches

The sequences of all NAC genes in this study were retrieved from the PeanutBase database (www.peanutbase.org) using the NAM domain (PF02365) as a search query. We verified the putative candidate proteins manually using the NCBI database (https://www.ncbi.nlm.nih.gov/) to confirm the presence of NAM domain. Each protein sequence was examined via the Simple Modular Architecture Research Tool (SMART; http://smart.embl-heidelberg.de/) domain analysis program and the Pfam (Protein family; http://pfam.xfam.org/) database to confirm the reliability of the search results. Only the sequences containing these domains were retained. The MWs and pls of each protein were predicted by proteomic and sequence analysis tools on the ExPASy Proteomics Server (http://web.expasy.org/compute_pi/). The putative *Arabidopsis* orthologues of peanut NACs were identified via BLASTp searches.

#### Sequence alignment and phylogenetic analysis

To study the phylogenetic relationships between NAC proteins from peanut and those from dicot *Arabidopsis* and monocot rice, the *Arabidopsis* NAC protein sequences were downloaded from The Arabidopsis Information Resource (TAIR; https://www.arabidopsis.org/) and the rice NAC protein sequences were downloaded from the Rice Genome Annotation Project (RGAP; http://rice.plantbiology.msu.edu/). Full length amino acid sequence multiple alignments were performed by the ClustalW program. Unrooted phylogenetic trees were constructed using the neighbour-joining (NJ) method by MEGA 6.0 software, and the bootstrap test was carried out with 1000 iterations.

#### Chromosomal locations, gene structure and conserved motif analysis

The chromosomal location information of NAC genes was retrieved from the PeanutBase website (www.peanutbase.org). These genes were mapped onto the chromosomes via the MapInspect program (http://mapinspect.software.informer.com). Information concerning both the mRNA and gDNA of the peanut NAC genes was obtained from the PeanutBase database (www.peanutbase.org). We used the GSDS (http://gsds.cbi.pku.edu.cn) online program to explore the exon/intron organization of the NAC genes. The MEME (http://
meme-suit.org) program was used to investigate the motifs within the NAC protein sequences. The domains in all the protein sequences were analysed via Pfam 31.0 (http://pfam.xfam.org/) based on the hidden Markov model.

Prediction of cis-acting elements within promoters
Promoter sequences (2.5 kb in length) were downloaded from the PeanutBase website (www.peanutbase.org) for cis-acting element analyses. The numbers of several elements related to biotic and abiotic stress responses were identified via New PLACE (https://sogo.dna.affrc.go.jp/cgi-bin/sogo.cgi?lang=en&pj=640&action=page&page=newplace) [54].

RNA-seq-based expression profiling of NAC genes in peanut
The average fragments per kilobase per million reads mapped (FPKM) values of 22 distinct tissue types and developmental stages were obtained from the study by Clevenger et al. [36]. The FPKM values of each NAC gene were log2 transformed and displayed in the form of heatmaps via HemI [55].

To investigate the expression patterns of NAC genes under salt and drought stress treatments, the average FPKM values of each gene under salt [37] and drought [39] treatments were obtained from our previous work. The average FPKM values of these NAC genes whose expression changed by more than twofold were compared via Excel software, log2 transformed and displayed in the form of heatmaps using HemI [55].

Plant materials, growth conditions and stress treatments
‘Huayu 9303’, a cultivated peanut bred by our team, was grown in a temperature-controlled chamber at 20 °C with a photoperiod of 16 h of light and 8 h of darkness unless stated otherwise. After approximately 1 month, the plants were treated with 51.33 mM NaCl (for salt treatment) or 20% polyethylene glycol (PEG) 6000 (for drought treatment). The roots were collected after 0, 6, 12, 18, 24, 36, and 48 h of treatment, immediately frozen in liquid nitrogen and stored at −80 °C.

RNA extraction and RT-qPCR based analysis
Total RNA was extracted with a MiniBEST Plant RNA Extraction Kit (Takara, Dalian, China). First-strand cDNAs were synthesized using a PrimeScript RT-PCR Kit (Takara), and qPCR was carried to check the expression levels of AhNAC genes under salt and drought treatments. The reactions mixtures consisted of 2 μL of cDNA (10.3 ng/μL), forward and reverse primers (400 nM each), 10 μL of TB Green Premix Ex Taq II (Takara), and added sterile water to bring total volume to 20 μL. Amplification was performed on an ABI 7500 Fast Real-Time System (Applied Biosystems, CA, USA) as follows: 50 °C for 2 min; 95 °C for 2 min; and 40 cycles of 95 °C for 15 s and 60 °C for 34 s. The specificity of the reactions was verified by melting curve analysis. Gene specific primers for each detected NAC gene for RT-qPCR were designed based on the basis of the difference between othologous genes and were listed in Additional file 15. Each gene was performed with three biological replicates. Gene transcript levels were calculated using ΔΔCt method [56]. Student’s t-test was performed to calculate the P values using SPSS software. When P was < 0.05, we considered the NAC genes were differentially expressed genes. To normalize the expression level of the selected NAC genes, actin gene was used as an internal control [47].

Conclusion
In the present study, a comprehensive analysis including phylogeny, chromosomal location, gene structure, conserved motif, cis-acting elements within promoter regions, and expression profiling of NAC gene family members in two diploid Arachis species was performed. These results provide a useful foundation for future research on Arachis NAC genes. On the basis of comparative RNA-seq and RT-qPCR-based analysis, we also identified NAC genes involved in drought and/or salt stress responses, which could be potentially used for peanut improvement.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12870-020-02678-9.

Additional file 1. mRNA sequence of NAC genes from two wild peanuts.
Additional file 2. gDNA sequence of NAC genes from two wild peanuts.
Additional file 3. NAC TF gene family members in cultivated peanut.
Additional file 4. mRNA sequence of NAC genes from cultivated peanut.
Additional file 5. NAC proteins of two wild peanuts.
Additional file 6. Arabidopsis NAC proteins.
Additional file 7. Rice NAC proteins.
Additional file 8. Phylogenetic tree analysis of NAC proteins among Arachis, Arabidopsis and rice based on conserved NAM domains.
Additional file 9. Exon-intron structure comparison between AdNAC59, AdNAC80, AdNAC87 and their orthologues AiNAC59, AiNAC9, AiNAC29.
Additional file 10. Sequence logos for the conserved motifs within NAC proteins.
Additional file 11. 2500 bp promoter region of NAC genes from two wild peanuts.
Additional file 12. Number of different cis-acting elements present within the promoter of NAC genes.
Additional file 13. Genes involved in the salt response based on comparative RNA-seq data. The Y-axis represents the fold change compared with the level in un-treated plants. The X-axis shows the genes.
whose expression was upregulated and downregulated more than 2-fold under drought treatment in cultivated peanut.

**Additional file 14.** Genes involved in the drought response based on comparative RNA-seq data. The Y-axis represents the fold change compared with the level in untreated plants. The X-axis shows the genes whose expression was upregulated or downregulated more than 2-fold under drought treatment in cultivated peanut.

**Additional file 15.** Primers used in this study.

Abbreviations

NAC: NAM, ATAF1/2, and CUC2; NAM: No apical meristem; ATAF1/2: *Arabidopsis thaliana* transcription activation factor; CUC2: Cup-shaped cotyledon; TF: Transcription factor; RNA-seq: RNA sequencing; RT-qPCR: Real time quantitative polymerase chain reaction; Gb: Giga-base pair; Mb: Million base pair; aa: Amino acid; KDa: Kilo Dalton; MeUA: Methyl jasmonate; ABA: Abscisic acid; ACC: 1-aminocyclopropane-1-carboxylic acid; NAA: 1-naphthylacetic acid; CDS: Coding DNA sequence; MW: Molecular weights; PI: Isoelectric point; RGAP: Rice Genome Annotation Project; TAIR: The *Arabidopsis* Information Resource; NJ: Neighbor-joining; FPKM: Fragments per kilobase of transcript per million mapped reads; PEG: Polyethylene glycol

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Authors’ contributions

QXS and SHS conceived and designed the research. CLY and CJL performed the bioinformatic analysis. CLY and CIL performed the experiments and drafted the manuscript. XDL and XZB participated in the molecular analysis. CIL, CYJ, JW and QXS contributed to revisions of the manuscript. All the authors have read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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