Transcriptome analysis reveals the mechanism of improving erect-plant-type peanut yield by single-seeding precision sowing

Sha Yang [Equal first author, 1, 2] , **Jialei Zhang** [Equal first author, 1, 2] , **Yun Geng** [1, 2] , **Zhaohui Tang** [1, 2] , **Jianguo Wang** [1, 2] , **Feng Guo** [1, 2] , **Jingjing Meng** [1, 2] , **Quan Wang** [1, 3] , **Shubo Wan** [Corresp., 4] , **Xinguo Li** [Corresp. 1, 2]

[1] Biotechnology Research Center, Shandong Academy of Agricultural Sciences, Ji'nan, China

[2] Scientific observation and experiment station of crop cultivation in east China, Ministry of Agriculture and Rural Affairs, Dongying, China

[3] College of Life Sciences, Shandong Normal University, Ji'nan, China

[4] Shandong Academy of Agricultural Sciences/Shandong Provincial Key Laboratory of Crop Genetic Improvement, Ecology and Physiology, Ji'nan, China

Corresponding Authors: Shubo Wan, Xinguo Li
Email address: wansb@saas.ac.cn, xinguol@163.com

Background: In China, double-seed sowing (i.e., sowing two seeds per hole) has been conventionally performed towards the erect-plant-type peanuts to increase the low germination rate due to poor seed preservation conditions. However, the corresponding within-hole plant competition usually limits the subsequent plant growth and the final yield. We developed a high-yield cultivation system of single-seed precision sowing to solve this paradox, saving 20% of seeds and increasing yields by more than 10% relative to the conventional double-seed sowing. Methods: To explore the mechanisms of these two different cropping patterns in peanut yields, we conducted transcriptomic and physiological comparisons in the seeding plant leaf and root tissues between single-seed precision sowing and standard double-seed sowing treatments. **Results:** After assembly, each library contained an average of 43 million reads and generated a total of 523,800, 338 clean reads. After GO and KEGG pathway analysis, we found the key genes for biotic and abiotic stress showed higher expression in roots of plants grown under the single-seed precision sowing treatment, including genes encoding disease resistance, oxidation-reduction, hormone related, and stress response transcription factors and signaling regulation proteins. In particular, the resveratrol synthesis genes related to stress and disease resistance appeared induced in roots under the single-seed sowing treatment. **Conclusion:** These data indicated that *Aspergillus flavus* resistance and stress tolerance in roots under single-seed precision sowing were enhanced compared with roots under the double-seed sowing treatment. This work benefits the development of underground pods and thus increasing peanut yields.

Title: Transcriptome analysis reveals the mechanism of improving erect-plant-type peanut yield by single-seeding precision sowing

Authors: Sha Yang[1,2]†, Jialei Zhang[1,2]†, Yun Geng[1,2], Zhaohui Tang[1,2], Jianguo Wang[1,2], Feng Guo[1,2], Jingjing Meng[1,2], Quan Wang[1,3], Shubo Wan[4]*, Xinguo Li[1,2]*

Co-authors Sha Yang[1,2]†, Jialei Zhang[1,2]†

These authors contributed equally to this work.

Authors' addresses

[1]Biotechnology Research Center, Shandong Academy of Agricultural Sciences, Ji'nan, 250100, PR China

[2]Scientific observation and experiment station of crop cultivation in east China, Ministry of Agriculture and Rural Affairs, Dongying, 257000, PR China

[3]College of Life Sciences, Shandong Normal University, Ji'nan, 250014, PR China

[4]Shandong Academy of Agricultural Sciences/Shandong Provincial Key Laboratory of Crop Genetic Improvement, Ecology and Physiology, Ji'nan, 250100, China

* Corresponding author Shubo Wan[4]*, Xinguo Li[1,2]*

[1]Biotechnology Research Center, Shandong Academy of Agricultural Sciences, Ji'nan, 250100, PR China

[2]Scientific observation and experiment station of crop cultivation in east China, Ministry of Agriculture and Rural Affairs, Dongying, 257000, PR China

[4]Shandong Academy of Agricultural Sciences/Shandong Provincial Key Laboratory of Crop Genetic Improvement, Ecology and Physiology, Ji'nan, 250100, China

Tel: +86 531 66659047;

Fax: +86 531 66658156;

E-mail: wansb@saas.ac.cn, lixinguo@tom.com

List of all author and e-mail:

Sha Yang yangsha0904@126.com; Jialei Zhang zhangjialei19@163.com; Yun Geng 1397623123@qq.com; Zhaohui Tang tangzhaohui1116@163.com; Jianguo Wang

28   wang_jianguo2020@163.com;   Feng   Guo   gf123456gh@126.com;   Jingjing   Meng

29   mengj80@163.com; Quan Wang quanwang0120@163.com; Shubo Wan wansb@saas.ac.cn;

30   Xinguo Li lixinguo@tom.com

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60 **Abstract**

61 **Background:** In China, double-seed sowing (i.e., sowing two seeds per hole) has been

62 conventionally performed towards the erect-plant-type peanuts to increase the low germination

63 rate due to poor seed preservation conditions. However, the corresponding within-hole plant

64 competition usually limits the subsequent plant growth and the final yield. We developed a high-

65 yield cultivation system of single-seed precision sowing to solve this paradox, saving 20% of

66 seeds and increasing yields by more than 10% relative to the conventional double-seed sowing.

67 **Methods:** To explore the mechanisms of these two different cropping patterns in peanut yields,

68 we conducted transcriptomic and physiological comparisons in the seeding plant leaf and root

69 tissues between single-seed precision sowing and standard double-seed sowing treatments.

70 **Results:** After assembly, each library contained an average of 43 million reads and generated a

71 total of 523,800,338 clean reads.   After GO and KEGG pathway analysis, we found the key

72 genes for biotic and abiotic stress showed higher expression in roots of plants grown under the

73 single-seed precision sowing treatment, including genes encoding disease resistance, oxidation-

74 reduction, hormone related, and stress response transcription factors and signaling regulation

75 proteins. In particular, the resveratrol synthesis genes related to stress and disease resistance

76 appeared induced in roots under the single-seed sowing treatment.

77 **Conclusion:** These data indicated that *Aspergillus flavus* resistance and stress tolerance in roots

78 under single-seed precision sowing were enhanced compared with roots under the double-seed

79 sowing treatment. This work benefits the development of underground pods and thus increasing

80 peanut yields.

81 **Keywords:** Peanut (*Arachis hypogaea* L.), RNA-Seq, Stress tolerance, Resveratrol synthase,

82 Yield

83

84

85

86

Introduction

88 Before the 1960s, agricultural production in China was labor-intensive due to the lack of modern

89 farming conditions. Thus, erect-plant-type peanuts, which are easier for manual labor relative to

90 prostrate-plant-type peanuts, were successfully bred and cultivated widely in China. Because the

91 seed germination rate of peanut is usually unable to meet the necessary high field emergence

92 percentage owing to uneven seed quality, conventionally, the sowing patterns with double-seed

93 (DS) and multi-seed in one hole were widely adopted by farmers as the main planting patterns,

94 which generally leads to two issues: a requirement of a large number of seeds and poor

95 population quality. The total amount of peanut seeds per year is 1.5 million tons, accounting for

96 about 10% of the country's total peanut yield. Poor population quality is mainly attributed to the

97 increased competition under double-seed sowing, which hinders the increases of peanut yield

98 (*Zhang et al., 2020*).

99 Our team introduced the principle of competitive exclusion, proposed the technical idea of

100 "single-seed (SS) precision sowing of erect-plant-type peanuts, robust individual, optimized

101 population", and created a high-yield cultivation technique of single-seed precision sowing. SS

102 precision sowing can alleviate inter-plant competition, save 20% of seeds used for planting, and

103 increase yields by more than 10% in common fields relative to DS precision sowing (*Liang et al.,*

104 *2019*). However, the molecular mechanisms regulating individual development under SS are still

105 unknown.

106 Crop yield is usually affected by biotic and abiotic stress. In recent years, biotic stress research

107 has mainly focused on disease resistance in wheat, rice, maize, and soybean. It has been reported

108 that *Fhb1* was associated with resistance to wheat scab and has important breeding potential

109 (*Rawat et al., 2016*). Two grape stilbene synthase (STS) genes, *VST1* and *VST2*, were

110 transformed into tobacco for the first time, which improved tobacco resistance to *Botrytis*

111 *cinerea* (*Hain et al., 1993*). "MutRenSeq," proposed by a British research team, is an efficient

112 method to discover disease-resistant genes in crops (*Steuernagel et al., 2016*). These advances

113 lay a solid foundation for the further study of crop disease resistance.

114 Aflatoxin contamination caused by *Aspergillus flavus* is a major obstacle to the development

115 of peanuts (*Fajardo et al., 1994*). It was indicated that the resveratrol content could be

116 significantly induced when *Aspergillus flavus* infects the peanuts seeds; and resistance of peanut

117 to *Aspergillus flavus* infection can be conferred by increasing the content of resveratrol in peanut

118 seeds as well as the speed of its synthesis (*Tian et al., 2008*). Resveratrol (Res), known as 3,4,5-

119 trihydroxy-stilbene, was first isolated from *Veratrum grandiflorum* root in 1940 (*Takaoka 1940*).

120 At present, Res has been identified in many different plant species, and recent research is mainly

121 focusing on grapes (*Wang et al., 2010*), peanuts (*Tang et al., 2010*), and a few other species.

122 Grape pericarps are the main grape tissue in which the compound is synthesized. The content of

123 Res in peanut pericarps is higher than that in peanut kernels (*Sanders et al., 2000*). Accumulated

124 of Res, a non-flavonoid polyphenol, could improve abiotic stress resistance. Although there has

125 remained a query of the specific mechanisms of Res's anti-stress abilities, a recent report showed

126 that Res resistance to ROS is one aspect involved in the stress-resistant processes (*Zheng et al.*,

127 *2011*).

128 Abiotic stresses, including drought, salt, and extreme temperature, remain the most

129 challenging frontier in the field of plant abiotic interaction research. Research on abiotic stress

130 focuses on identifying primary sensors and essential stress-resistant genes that respond to abiotic

131 stresses (*Zhu, 2016*). Significant progress has been made in the past few years. The cold-tolerant

132 rice protein COLD1 has been identified to mediate extracellular Ca^{2+} influx and net cytosolic

133 Ca^{2+} concentration in response to chilling stress (*Ma et al., 2015*). The basic leucine zipper bZIP

134 and NAC transcription factors may sense or contribute tolerance to salt stress through their

135 interaction with corresponding proteins (*Liu et al., 2016*). The chloroplast is the main site of the

136   production of reactive oxygen species (ROS), including hydrogen peroxide, superoxide anions,

137   singlet oxygen, and hydroxyl radicals (*Mignolet-Spruyt et al., 2016*). Various abiotic stresses,

138   particularly high light stress, aggravate ROS production, destroying ROS-scavenging systems

139   and generating various secondary messengers. The protective mechanisms that scavenge ROS in

140   plants can be divided into two categories: enzymatic (e.g., superoxide dismutase [SOD], catalase

141   [CAT], and peroxidase [POD]) mechanisms and non-enzymatic (e.g., glutathione, mannitol, and

142   flavonoids) mechanisms. Improving the content of these substances in plants by genetic

143   engineering can effectively remove excessive ROS in plants and thus improve stress tolerance.

144   Whether the yield advantage of SS precision sowing is related to the stress resistance genes

145   needs to be studied.

146   With the development of high-throughput sequencing technologies, more attention is being

147   paid to combining genomic methods such as genome sequencing with transcriptome, proteome,

148   and metabolome analyses in order to reveal the molecular mechanism of life science research.

149   This integrative research offers synergies that can uncover the molecular mechanism of different

150   phenomena in crop production. The publication of reference transcripts for *Arachis_duranensis*

151   (*Chopra et al., 2014*) facilitated a better understanding of agronomically important phenomena

152   and genetic improvement of peanuts. This study used these techniques mentioned above to reveal

153   the mechanism attributing to the higher peanuts yield by SS precision sowing.

154   **Materials & Methods**

155   **Plant materials and growth conditions**

156   Peanut (*Arachis hypogaea* L.) cultivar 'Huayu 22' (provided by the Shandong Peanut Research

157   Institute, China), a large-grain peanut cultivar, was used in this study. Peanut seeds were

158   cultivated under field pot conditions at the Shandong Academy of Agricultural Sciences Station

159   (117°5′ E, 36°43′ N), Ji'nan, China. The soil is sandy loam, containing 1.1% organic matter

160   (W/W), 82.7 mg kg^{-1} alkali-hydrolyzed nitrogen, 36.2 mg kg^{-1} available phosphorus, 94.5 mg kg^{-1}

161   available potassium, and 14.9 g kg^{-1} exchangeable calcium. The single-seed (SS) sowing

162   treatment consisted of 237,000 holes per hectare with 1 grain per hole, while the double-seed

163 (DS) sowing consisted of 138,000 holes per hectare with two grains per hole as the control; the

164 distances between two adjacent holes were 10.5 cm and 18 cm in the SS and DS treatments,

165 respectively. Three biological replicates were used in this study. The mixed tissues were used in

166 transcriptome sequencing.

167

168 **RNA extraction and cDNA library construction**

169 When the peanut plants reached the seedling stage, the top third leaf and the whole root systems

170 were collected for RNA extraction and library construction. Total RNA was isolated from the

171 leaves and roots respectively, using the total RNAiso Reagent (TaKaRa, Dalian, China)

172 according to the manufacturer's instructions. The quality and purity of RNA samples were

173 detected using the Agilent 2100 Bioanalyzer platform and Agilent RNA 6000 Nano Kit (Agilent,

174 Santa Clara, CA, USA). Qualified RNA samples were used to isolate mRNA with the oligo (dT)

175 method, and the mRNAs were fragmented. Then, first-strand cDNA and second strand cDNA

176 were synthesized. Next, the purified cDNA fragments were linked with adapters, and sequencing

177 was performed by a commercial service provider (BGI Tech, Shenzhen, China). The sequenced

178 reads that contained adaptor sequences, low-quality bases, and high contents of unknown bases

179 (i.e., N calls) were removed before downstream analyses. After read filtering, clean reads were

180 mapped to the *Arachis_duranensis* reference genome (BioSample: SAMN02982871, BioProject:

181 PRJNA258023) using Hierarchical Indexing for Spliced Alignment of Transcripts (HISAT). The

182 sequence   data   can   be   located   from

183 https://www.ncbi.nlm.nih.gov/assembly/GCF_000817695.2/.Then, Genome Analysis Toolkit

184 (GATK) was used to call single SNPs and INDELs (*McKenna et al., 2010*), and RNA-Seq by

185 Expectation-Maximization (RSEM) was used to calculate gene expression levels for each sample,

186 which were then determined using the fragments per kb per million (FPKM) mapped fragments

187 method developed by Li et al. (2011) (*Li et al., 2011*). DEGseq algorithms were used to assess

188 the DEGs. Based on the gene expression level, DEG was identified between samples or groups.

189 Gene Ontology (GO) was performed classification and Kyoto Encyclopedia of Genes and

190 Genomes (KEGG) pathway classification based on these DEGs. The GO framework includes

191 three ontologies: molecular biological function, cellular component, and biological process.

192 KEGG is a database resource for understanding high-level functions and utilities of the

193 biological system, such as the cell, the organism, and the ecosystem, from molecular-level

194 information, especially large-scale molecular datasets generated by genome sequencing and

195 other high-throughput experimental technologies. Co-expression analysis was performed using

196 the WGCNA R package according to the methods detailed by previously published studies (*Gao*

197 *et al., 2018; Song et al., 2018*).

198

199 **Yield composition per plant and measurement**

200 At maturity, ten representative plants were selected to investigate the number of pods, full pods,

201 and double kernels, as well as pod weight per plant. The pods and plants were dried to constant

202 weights, and the economic coefficient was calculated as follows: economic coefficient = pod dry

203 weight / (plant dry weight + pod dry weight).

204

205 **Antioxidant enzyme activities and root activity**

206 Leaf tissues (0.5 g) were ground up with phosphate buffer (pH 7.8) containing 0.1 mM EDTA

207 and 1% (g ml^{-1}) PVP and centrifuged at 4°C for 10 min. Then, the supernatants were used as

208 enzyme extracts. The nitroblue tetrazole (NBT) method was used to detect SOD activity. The

209 absorbance value was determined at a 560 mm wavelength after the reaction; 50% inhibition of

210 NBT reduction was regarded as the enzyme activity unit (U), expressed as U g^{-1} FW. The

211 guaiacol method was used to determine the POD activity; the increase in OD_{470} per min was used

212 as the enzyme activity unit (U), expressed as $\triangle 470$ g^{-1}. CAT activity was also determined by the

213 guaiacol method. The decreased in OD_{240} per min was used as the unit of enzyme activity (U)

214 and expressed as mg·g^{-1}·min^{-1} (*Giannopolitis et al., 1977; Aebi et al., 1984; Jimenez et al., 1977*).

215 Root activity was measured by the TTC method; 0.5 g of root tip samples were revolved in a

216 mixture of 0.4% TTC and phosphoric acid buffer solution and kept in the dark for 1–3 h at 37°C.

217   After that, 2 ml of 1 mol L^{-1} sulfuric acid was added to stop the reaction. Simultaneously, a blank

218   experiment was conducted. The root samples and sulfuric acid were added first, and 10 min later,

219   the other reagents were added. The operation was the same as that described above. The roots

220   were removed, dried, and ground together with 3–4 ml of quartz sand and ethyl acetate in a

221   mortar. The supernatants were transferred to a test tube, and the residue was washed with a small

222   amount of ethyl acetate two or three times, with all ethyl acetate collected into the test tube.

223   Finally, ethyl acetate was added to bring the total volume up to 10 ml. Blank experiments were

224   used as the control, and the OD value of the reaction solution was measured at λ = 485 nm.

225

226   **qRT-PCR verification analysis**

227   Leaves and roots of plants grown under the SS and DS treatments, as in the RNA-seq experiment,

228   were selected randomly and analyzed using qRT-PCR. The qRT-PCR amplification instrument

229   (ABI 7500 fast; Applied Biosystems, Foster City, CA, USA) was used to amplify the related

230   genes with SYBR Premix Ex Taq^{TM} (TaKaRa) following the manufacturer's instructions (*Yang*

231   *et al., 2013*). The relative gene expression was calculated using the $2^{-\triangle\triangle CT}$ method described by

232   Livak and Schmittgen (2001) (*Livak & Schmittgen, 2011*). For log$_2$-transformed FPKM values,

233   each selected gene's maximum expression level was considered to be 100, and the expression

234   levels of the other genes were transformed accordingly (*Zhang et al., 2016*).

235

236   **Determination of resveratrol content**

237   The Res extraction processes were carried out according to the method detailed by Tang et al.

238   (*Tang et al., 2010*) with minor modification; 0.25 g of leaves or roots were collected and ground

239   into a fine powder, which was dissolved with 20 ml of 95% ethanol in a flask and oscillated at

240   room temperature overnight. After centrifugation at 8000 rpm for 10 min, the supernatants were

241   subjected to HPLC analysis. The extracts were filtered through a 0.45-μm membrane and then

242   tested with the Rigol L3000 HPLC system (Rigol Technologies, Beaverton, OR, USA) using a

243   Kromasil C18 reversed phase column (4 μm, 250 mm × 4.6 mm; Kromasil, Bohus, Sweden) at

244   room temperature. Mobile phase A consisted of 0.1% phosphoric acid–H_2O, while phase B

245   consisted of acetonitrile (A: B = 70:30, flow rate 1 ml min^{-1}); the determination wavelength was

246   280 nm.

247   **Results**

248   **Transcriptome sequencing and gene analysis**

249   The peanut plants grown under different planting patterns differed little in their above-ground

250   portions, while great differences were observed between their root systems. The basis of high

251   and stable crop yields is high biological yields, which depends largely on the development of

252   root systems. The capillary lateral root is the main type of root exhibited under SS. Thus, the

253   capillary lateral root length and its proportion within the root system were promoted accordingly,

254   thereby increasing the total root length and root absorption area of peanut plants. This

255   phenomenon was verified by the mean absorbing area and dry weight of root tissue observed

256   under SS (Fig. 1).

257   Twelve cDNA libraries were constructed from leaf and root tissues of peanut plants grown

258   under SS and DS sowing treatments. All 12 samples were sequenced on the Illumina HiSeq

259   Platform in total, generating about 6.55 Gb per sample. The reads that contained adaptor

260   sequences of low-quality or had high contents of unknown base (i.e., N) calls were removed,

261   resulting in a total of 523,800,338 clean reads that were acquired, with an average of 43 million

262   reads per libraries (Table 1). Approximately 77% and 80% of reads from leaf and root tissues

263   were mapped to the reference genome, and 36,778 genes were identified, of which 34,529 are

264   known genes and 2,322 are novel genes. The RNA-seq sequencing data for the present work has

265   been uploaded in NCBI Sequence Read Archive under BioProjects, PRJNA497502 (SRA:

266   SRP166140).

267   Single nucleotide polymorphisms (SNP), which include transitions or transversions of single

268   bases, refer to the difference of a single nucleotide (A, T, C, or G) between homologous DNA

269   sequences, a key element of the diversity of genomes among species or individuals. There were

270   more transitions than transversions among all the samples. Among SNPs, transition, transversion,

271   A-G, and C-T were the most abundant terms (Fig. S1). Insertion-Deletion (INDEL) refers to the

272   insertion or deletion of the small fragments (one or more, less than 50 bp) that occur in a sample

273   relative to the reference genome. Most INDELs occurred in exonic and intronic sequences, with

274   the proportion differing among samples (Fig. S2). Based on the results of SNP, INDEL, and gene

275   expression, our study was presented in the form of a ring diagram with Circos software (Fig. S3).

276   **Differentially expressed gene detection**

277   A total of 2,567 and 2,706 differentially expressed genes (DEGs) were identified from leaves and

278   roots, respectively. Under SS sowing treatment, 544 and 1,771 genes were expressed at a higher

279   level, while 2,023 and 935 genes were expressed at lower levels compared with the DS sowing

280   treatment in leaves and roots, respectively (Fig. 2). The expression patterns of the majority of

281   DEGs differed between roots and leaves. For example, only 83 (15.2%) of the 1,771 genes up-

282   regulated in roots were also up-regulated in leaves (Fig. 3). Notably, some genes in roots and

283   leaves even showed opposite expression patterns; for example, forty DEGs were up-regulated in

284   leaves but down-regulated in roots. However, 245 DEGs in roots and leaves had the same

285   expression trends, including 83 up-regulated DEGs and 162 down-regulated DEGs (Fig. 3).

286

287   **qRT-PCR verification of RNA-Seq results**

288   To verify the RNA-seq data, quantitative real-time PCR (qRT-PCR) was used to test the

289   expression of 24 genes with different functional assignments (Fig. 4a, 4b). Among them, six and

290   five genes were up-regulated under the SS sowing treatment in leaves and roots, respectively.

291   UDP-glycosyltransferase, chalcone synthase, and GPI mannosyltransferase were each encoded

292   by one of these genes, while two genes encoding hypothetical proteins were also used for qRT-

293   PCR detection. The other eleven selected genes were downregulated both in roots and leaves.

294   These genes included one phospholipase, one seed linoleate 9S-lipoxygenase, one zinc finger

295   SWIM domain-containing protein, and one pectinesterase. The RNA-seq data were consistent

296   with the qRT-PCR results for these 24 genes. The correlation between the relative expression

297   ($\log_2$ SS/DS) estimated by RNA-seq and the qRT-PCR results were rather high in leaves (R^2 =

298   0.9772) and a little lower in roots ($R^2 = 0.8884$; Fig. 4c,4d).

299

300   **Functional analysis of DEGs**

301   Blast2GO was used to perform Gene Ontology (GO) classification and functional enrichment

302   (*Conesa et al., 2005*) on the identified DEGs. The sequences were categorized into 45 functional

303   groups according to sequence homology. The main categories of biological process, molecular

304   function, and cellular component were visualized employing WEGO (Fig. 5). In the biological

305   process category, "metabolic process", "single-organism process", and "cellular process"

306   terms were enriched, suggesting the metabolic activity is higher under SS sowing. In the cellular

307   component category, "cell," "cell part," and "membrane" were the most abundant terms. The

308   most enriched category was molecular function, with enrichment for "binding," "catalytic

309   activity," and "transporter activity" in particular, suggesting a high level of metabolic activity

310   changes under SS sowing (Fig. 5).

311   KEGG pathway classification was performed to acquire biological information for

312   understanding the regulatory networks and molecular mechanisms associated with the SS sowing

313   treatment. DEGs were mainly enriched in the MAPK signaling pathway, glycerolipid

314   metabolism, phenylalanine metabolism, sphingolipid metabolism, isoflavonoid biosynthesis,

315   flavonoid biosynthesis, and tryptophan metabolism in leaves. Meanwhile, phenylpropanoid

316   biosynthesis, biosynthesis of secondary metabolites, MAPK signaling pathway, flavonoid

317   biosynthesis, zeatin biosynthesis, and flavone and flavonol biosynthesis were mainly enriched

318   among DEGs in roots (Fig. S4). Interestingly, all of these pathways participated in biosynthesis

319   associated with particular metabolic processes, suggesting that these processes were activated.

320   Weighted gene correlation network analysis (WGCNA) is a systematic biological method for

321   describing gene association pattern among different samples. Information about nearly 10,000

322   genes, corresponding to the genes with the greatest changes in expression, was used to identify

323   gene sets of interest and analyze associations with phenotypes. In our study, the genes from all

324   the samples were divided into modules for analysis, with twelve colors representing each of the

325   different modules in Fig. 6.

326

327   **Changes of resveratrol synthesis related pathway between cultivation techniques**

328   In our study, a total of 20 resveratrol synthesis-related genes were dramatically induced, while no

329   resveratrol synthesis-related genes were down-regulated in the roots under the SS sowing

330   treatment. In contrast, a total of 10 DEGs related to resveratrol synthesis were down-regulated in

331   the leaves (Table 2). We also measured the resveratrol content, and the results were in agreement

332   with the gene expression levels in leaf and root tissues (Fig. 7). Accordingly, compared with the

333   roots of plants grown under DS sowing, the higher resveratrol content under SS precision sowing

334   appeared to support the initiation of the defense response against toxins produced by *Aspergillus*

335   *flavus*; pathogenesis-related proteins, transcription factors (e.g., WRKY, bZIP, ERF), and 4CL

336   were up-regulated (Table 3).

337

338   **Key genes related to stress tolerance under SS**

339   Transcription factors (TFs), which bind to the *cis*-elements upstream of promoters, have been

340   reported to orchestrate abiotic stress responses (*Joshi et al., 2016*). Under the SS precision

341   sowing treatment, the higher expression of these TF genes (WRKY, MYB, bZIP, ERF) may

342   improve stress tolerance compared with that observed in the DS treatment. In plants, redox

343   processes also play an important role in stress tolerance. Several oxidoreductase genes encoding

344   ascorbate oxidase, glutaredoxin, and cytochrome P450 monooxygenases (CYP) exhibited

345   increased expression in roots under SS relative to DS. Specifically, compared with DS, about

346   thirty genes encoding CYP were up-regulated under SS (Table 3). In addition, four genes

347   encoding L-ascorbate oxidase homologs, which take part in ascorbate recycling, were enriched in

348   the SS sowing treatment. In plants, ascorbate contributes to improving tolerance against various

349   stresses by regulating the levels of cellular H_2O_2 (*Ishikawa et al., 2008*). Higher enzyme

350   activities of POD, SOD, and CAT were also observed in roots under SS compared to that under

351   DS (Table S1). Four genes encoding glutaredoxin, which might promote reducing disulfide

352   bridges, were slightly induced under SS compared with DS (Table 3).

353

354   **Peanut pod yields and associated genes under the SS sowing pattern**

355   The number of pods and pod weight per plant is the direct factors affecting peanut yield. In this

356   study, yield estimation indicated that the number of pods, full pods, and double kernels per plant

357   under SS was higher than under DS. The theoretical pod yield of SS reached 1.225 kg, which

358   was 12.9% higher than that of DS (1.085) (Table 4). Hormones, such as indole-3-acetic acid

359   (IAA), abscisic acid (ABA), gibberellin (GA), and brassinosteroid (BR), play important roles in

360   the regulation of growth and development in plants (*Tian et al., 2017*). Auxin response factor

361   (ARF), a transcription factor involved in auxin and regulating plant root growth and seed

362   development (*Salmon et al., 2008*), showed increased expression levels under SS. Nine genes

363   that participate in auxin and BR biosynthesis were abundantly expressed under SS compared

364   with under DS (Table 3). Two lipoxygenase family genes also had increased expression levels

365   under SS.

366

367   **Discussion**

368   In recent years, with the rapid development of sequencing technology, genomes and

369   transcriptomes have been analyzed to explore the mechanisms mediating various problems in

370   agricultural production. However, few studies have focused on the mechanisms by which single-

371   seed precision sowing improves yields in erect-plant-type peanut cultivars. Aerial flowering

372   combined with underground fruit development are special characteristics of peanut plants; thus,

373   the growth and development of root tissues are particularly important for peanut yields. Robust

374   roots are the basis of crop growth and high yields (*Zheng et al., 2013*).

375   Various biotic and abiotic stresses under adverse environmental conditions severely reduce

376   global crop production and food security (*Mengiste et al., 2003*). Peanut is one of the most

377   susceptible crops to *Aspergillus flavus*. Abiotic stresses such as salinity, heat, mechanical

378   damage, and drought seriously affect peanut crops' growth and development (*Amin et al., 2019*).

379   In our study, families of TFs, including Zinc finger protein, MYB, bZIP, ERF, and WRKY, were

380   increased in the SS sowing treatment compared with the DS sowing treatment. Many studies

381   have identified these TFs as being involved in abiotic stress responses (*Wei et al., 2017; Wang et*

382   *al., 2016; Banerjee et al., 2015*). In addition, secondary messengers, like Ca^{2+} and ROS, trigger

383   signaling proteins downstream, such as calcium-dependent protein kinases (CDPKs), calmodulin

384   (CaM), calcineurin-B-like proteins (CBLs), mitogen-activated protein kinase (MAPK) cascades,

385   and ROS-modulated protein kinases (PKs), and these can also respond to numerous plant

386   development and environmental challenges (*Yang et al., 2019*). Genes encoded calcium-binding

387   protein CML, calcium-binding protein KIC-like, and MAPKKK, which is part of the Ca^{2+}

388   signaling pathway, were expressed at higher levels under SS (Table 3). Genes involved in the

389   MAPK cascade include interlinked MAPK, MAPKK, and MAPKKK, and such cascades play

390   important roles in signal transduction of plant hormones, biotic stresses, and abiotic stresses

391   (*Wang et al., 2017*).

392   Resveratrol (Res), an important phytoalexin, can be induced by pathogenic bacteria or other

393   stimuli and has strong bactericidal and defensive activity in plants. So far, the heterogeneous

394   transformation of Res genes from rice, barley, wheat, tomato, tobacco, and other crops has been

395   reported. All these transgenic crops exhibit improved resistance to diseases such as rice blast

396   (*Stark et al., 1997*), powdery mildew in barley and wheat (*Fettig et al., 1999*), and gray mold in

397   tomato and tobacco (*Hain et al., 1990*). The rate of Res induction in peanut seeds has also been

398   linked to resistance to *Aspergillus flavus* infection (*Fajardo et al., 1994*). Res is synthesized by

399   the phenylalanine metabolic pathway in plants, which includes four key enzymes: cinnamate-4-

400   hydroxylase (C4H), phenylalanine ammonia lyase (PAL), 4-coumarate-CoA ligase (4CL), and

401   STS. STS genes can be divided into two types, one of which is resveratrol synthase (RS), which

402   synthesizes Res using malonyl-CoA and coumaric acid-CoA as substrates, and this type mainly

403   exists in *Arachis hypogaea* and *Vitis vinifera*. The other is pinosylvin synthase (PS), which

404   utilizes malonyl-CoA and cinnamyl-CoA as substrates, and has mainly been identified in *Pinus*

405   *sylvestris* and *Pinus strobus*. RS is the major rate-limiting enzyme for resveratrol synthesis,

406 which is reported to participate in ROS resistance and is a key element in stress resistance

407 processes (*Chang et al., 2011*). Endogenous or exogenous Res can also reduce ROS content in

408 plants (*Zheng et al., 2015*). These Res's effects are likely to promote disease resistance and

409 antioxidant ability in peanut roots, thus supporting high-quality harvests and high yields of

410 peanuts.

411   In this study, high activities of the enzymes POD, SOD, and CAT in the ROS scavenging

412 pathway were observed in roots of plants grown under SS (Table S1). SOD, as a key element of

413 enzymatic defense systems, catalyzes the disproportionation of radicals $O_2^{\bullet-}$ to O_2 and H_2O_2, the

414 latter of which can then be scavenged by POD or CAT. The elevated activity of antioxidant

415 enzymes can be regarded as an effective mechanism for resisting oxidative stress. The balance of

416 the redox state of plant cells contributes to improving plant resistance to abiotic stress (*Duan et*

417 *al., 2012*). It has been reported that overexpression of glutaredoxin in tomatoes can confer

418 drought, oxidation, and salt resistance (*Guo et al., 2010*). These results suggested

419 oxidoreductases are involved in protecting plants from various abiotic stresses through

420 maintaining oxidation-reduction homeostasis and scavenging surplus ROS. CYP catalyzes the

421 biological oxidation of various substrates through molecular oxygen activation and acts as a

422 pivotal part of stress responses and metabolic processes, exhibited as differential transcription

423 levels under SS and DS treatments (*Xiong et al., 2017*).

424   Based on previous studies, auxin and BR are known to play important roles in root

425 development. Still, the root tip phenotypes of auxin mutants differ from those of BR mutants

426 (*González-García et al., 2011*), indicating that the auxin and BR pathways acting on the root are

427 not the same. In *Arabidopsis*, the BR encoding gene *AtDWF4* was found to regulate leaf growth

428 by promoting cell expansion (*Hur et al., 2015*). Its overexpression in *Brassica napus* can

429 increase seed production (*Sahni et al., 2016*). Many unigenes encoding auxins and BR

430 biosynthesis-related genes are expressed at a high level under SS. Lipoxygenase, which exhibited

431 different expression levels between SS and DS treatments, is an enzyme that is important to the

432 synthesis of jasmonic acid, which plays a vital role in stored lipid migration during seed

433 germination (*Rahimi et al., 2016*). The up-regulated expression of lipoxygenases under SS is

434 likely to provide sufficient nutrients essential for pod development. ABA also plays crucial roles

435 in seed dormancy and development (*Chauffour et al., 2019*). These results strongly suggested

436 that the biotic and abiotic stress tolerance in roots of peanut plants grown from single-seed

437 precision sowing were enhanced compared with those under the double-seed sowing treatment.

438 These mechanisms may explain the higher yields achieved under SS precision sowing based on

439 patterns at the transcriptional level.

440 **Conclusions**

441 This study performed a comparative transcriptomic analysis of peanut leaves and roots between

442 precision SS sowing and standard DS sowing treatments. Genes involved in resveratrol synthesis

443 were found to be dramatically induced in roots under the SS treatment. Accordingly, a higher

444 content of resveratrol was detected under SS precision sowing. In addition, genes involved in

445 calcium signaling transduction and phytohormone metabolism were identified to be differentially

446 expressed in SS and DS roots. Redox process genes and transcription factors, including WRKY,

447 MYB, MADS-box, and zinc finger proteins, were up-regulated in roots of plants grown under

448 the SS precision sowing treatment. These genes participate in resistance to biotic and abiotic

449 stresses, thus protecting plants from disease and drought, salinity, and chilling stress. These

450 results may inform breeding to enhance roots' activity and growth, providing a basis for

451 achieving higher yields in peanuts. To the best of our knowledge, we have provided a novel

452 mechanism by which SS precision sowing improves erect-plant-type peanut yields.

453 **Funding**

454 The following grants supported this work: National Key R&D Program of China

455 (2018YFD1000900); Major Basic Research Project of Natural Science Foundation of Shandong

456 Province (2018GHZ007); Science and Technology Innovation Project of Shandong Academy of

457 Agricultural Sciences (CXGC2018D04); Agricultural scientific and technological innovation

458 project of Shandong Academy of Agricultural Sciences (CXGC2018E13).

459 **List of abbreviations**

ABA, Abscisic acid; BR, Brassinosteroid; ARF, Auxin response factor; CAT, Catalase; CBLs, Calcineurin-B-like proteins; CDPKs, Calcium-dependent protein kinases; C4H, Cinnamate-4-hydroxylase; CIPK, CBL-interacting protein kinase; 4CL, 4-coumarate-CoA ligase; CYP, Cytochrome P450; DEGs, Differentially expressed genes; FPKM, Fragments per kb per million; GA, Gibberellin; GATK, Genome analysis toolkit; GO, Gene ontology; HISAT, Hierarchical indexing for spliced alignment of transcripts; IAA, Indole-3-acetic acid; INDEL, Insertion-Deletion; MAPK, Mitogen-activated protein kinase; NBT, Nitroblue tetrazole; PAL, Phenylalanine ammonia-lyase; POD, Peroxidase; PS, Pinosylvin synthase; qRT-PCR, Quantitative real-time PCR; Res, Resveratrol; RSEM, RNA-Seq by expectation-maximization; SNP, Single nucleotide polymorphisms; SOD, Superoxide dismutase; STS, Stilbene synthase; WGCNA, Weighted gene correlation network analysis.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

SW and XL designed the study, SY and JZ carried out most of the experiments and data analysis and wrote part of the manuscript. YG wrote part of the manuscript and finalized the figures and tables. ZT, FG, and JW performed part of the experiments. JM and QW tended the plants. All authors have read and approved the manuscript.

References

Aebi H. 1984. Catalase in vitro. *Methods Enzymol* 105:121-126.

Amin AB, Rathnayake KN, Yim WC, Garcia TM, Wone B, Cushman JC, Wone BWM. 2019. Crassulacean acid metabolism abiotic stress-responsive transcription factors: a potential genetic engineering approach for improving crop tolerance to abiotic stress. *Frontiers in Plant Science* 10:129.

Banerjee A, Roychoudhury A. 2015. WRKY proteins: signaling and regulation of expression during abiotic stress responses. *The Scientific World Journal* 2015:807560.

Chang X, Heene E, Qiao F, Nick P. 2011. The phytoalexin resveratrol regulates the initiation of

487   hypersensitive cell death in *Vitis* cell. *Plos One* 6: e26405.

488   Chauffour F, Bailly M, Perreau F, Cueff G, Suzuki H, Collet B, Frey A, Clément G, Soubigou-

489   Taconnat L, Balliau T, Krieger-Liszkay A, Rajjou L, Marion-Poll A. 2019. Multi-omics

490   analysis reveals sequential roles for ABA during seed maturation. *Plant*

491   *Physiology* pp.00338.

492   Chopra R, Burow G, Farmer A, Mudge J, Simpson CE, Burow MD. 2014. Comparisons of de

493   novo transcriptome assemblers in diploid and polyploid species using peanut (Arachis spp.)

494   RNA-Seq data. *PLoS One* 9(12):e115055.

495   Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M. 2005. Blast2GO: a universal

496   tool for annotation, visualization and analysis in functional genomics research.

497   *Bioinformatics* 21(18):3674-3676.

498   Duan M, Feng HL, Wang LY, Li D, Meng QW. 2012. Overexpression of thylakoidal ascorbate

499   peroxidase shows enhanced resistance to chilling stress in tomato. *Journal of Plant*

500   *Physiology* 169(9):867-877.

501   Fajardo JE, Waniska RD, Cuero RG, Pettit RE. 1994. Phenolic compounds in peanut seed

502   enhanced elicitation by chitosan and effects of growth and aflatoxin B1 producing by

503   *Aspergillus flavus*. *Food Biotechnology* 8:191-211.

504   Fettig S, Hess D. 1999. Expression of a chimeric stilbene synthase gene in transgenic wheat lines.

505   *Transgenic Research* 8:179-189.

506   Gao HH, Wang Y, Li W, Gu YZ, Lai YC, Bi YD, He CY. 2018. Transcriptomic comparison

507   reveals genetic variation potentially underlying seed developmental evolution of soybeans.

508   *Journal of Experimental Botany* 69:5089-5104.

509   Giannopolitis CN, Ries SK. 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant*

510   *Physiology* 59(2):309-314.

511   González-García MP, Vilarrasa-Blasi J, Zhiponova M, Divol F, Mora-García S, Russinova

512   E, Caño-Delgado AI. 2011. Brassinosteroids control meristem size by promoting cell cycle

513   progression in Arabidopsis roots. *Development* 138(5):849-59.

514 Guo YS, Huang CJ, Xie Y, Song FM, Zhou XP. 2010. A tomato glutaredoxin gene SlGRX1
515   regulates plant responses to oxidative, drought and salt stresses. *Planta* 232:1499-1509.

516 Hain G, Reif HJ, Krause E, Langebartels R, Kindl H, Vornam B, Wiese W, Schmelzer E,
517   Schreier PH, StÖcker RH, Stenzel K. 1993. Disease resistance results from foreign
518   phytoalexin expression in a novel plant. *Nature* 361:153-156.

519 Hain R, Bieseler B, Kindl H. 1990. Expression of a stilbene gene in *Nicotiana tabacum* results in
520   synthesis of the phytoalexin resveratrol. *Plant Molecular Biology* 15:325-335.

521 Hur YS, Um JH, Kim S, Kim K, Park HJ, Lim JS, Kim WY, Jun SE, Yoon EK, Lim J, Ohme-
522   Takagi M, Kim D, Park J, Kim GT, Cheon CI. 2015. *Arabidopsis thaliana* homeobox 12
523   (ATHB12), a homeodomain-leucine zipper protein, regulates leaf growth by promoting cell
524   expansion and endoreduplication. *New Phytologist* 205:316-328.

525 Ishikawa T, Shigeoka S. 2008. Recent advances in ascorbate biosynthesis and the physiological
526   significance of ascorbate peroxidase in photosynthesizing organisms. *Bioscience*
527   *Biotechnology Biochemistry* 72:1143-1154.

528 Jimenez A, Hernandez JA, Del Rio LA, Sevilla F. 1997. Evidence for the presence of the
529   ascorbate–glutathione cycle in mitochondria and peroxisomes of pea leaves. *Plant*
530   *Physiology* 114(1):275-284.

531 Joshi R, Wani SH, Singh B, Bohra A, Dar ZA, Lone AA, Pareek A, Singla-Pareek SL. 2016.
532   Transcription factors and plants response to drought stress: current understanding and future
533   directions. *Frontiers in Plant Science* 17:1029.

534 Li B, Dewey CN. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or
535   without a reference genome. *BMC Bioinformatics* 12:323.

536 Liang XY, Guo F, Feng Y, Zhang JL, Yang S, Meng JJ, Li XG, Wan SB. 2019. Increased pod
537   yield by single-seed sowing at a reduced seeding rate in peanut. *Journal of Integrative*
538   *Agriculture* doi: 10.1016/S2095-3119(19)62712-7.

539 Liu JX, Howell SH. 2016. Managing the protein folding demands in the endoplasmic reticulum
540   of plants. *New Phytologist* 211:418-428.

541 Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time
542   quantitative PCR and the 2(−Delta Delta C(T)) Method. *Methods* 25(4):402-408.

543 Ma Y, Dai X, Xu Y, Luo W, Zheng X, Zeng D, Pan Y, Lin X, Liu H, Zhang D, Xiao J, Guo X,
544   Xu S, Niu Y, Jin J, Zhang H, Xu X, Li L, Wang W, Qian Q, Ge S, Chong K. 2015. COLD1
545   confers chilling tolerance in rice. *Cell* 160(6):1209-1221.

546 McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K,
547   Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The genome analysis toolkit: a
548   MapReduce framework for analyzing next-generation DNA sequencing data. *Genome*
549   *Research* 20:1297-1303.

550 Mengiste   T, Chen   X, Salmeron   J, Dietrich   R.   2003.
551   The *BOTRYTIS SUSCEPTIBLE1* gene encodes an R2R3MYB transcription factor protein th
552   at   is required for biotic and abiotic stress responses in Arabidopsis.   *The*   *Plant*
553   *Cell* 15(11):2551-2565.

554 Mignolet-Spruyt L, Xu E, Idanheimo N, Hoeberichts FA, Muhlenbock P, Brosche M, Van
555   Breusegem F, Kangasjarvi J. 2016. Spreading the news: subcellular and organellar reactive
556   oxygen species production and signalling. *Journal of Experimental Botany* 67:3831-3844.

557 Rahimi S, Kim YJ, Sukweenadhi J, Zhang D, Yang DC. 2016. PgLOX6 encoding a lipoxygenase
558   contributes to jasmonic acid biosynthesis and ginsenoside production in *Panax ginseng*.
559   *Journal of Experimental Botany* 67(21):6007-6019.

560 Rawat N, Pumphrey MO, Liu S, Zhang X, Tiwari VK, Ando K, Trick HN, Bockus WW,
561   Akhunov E, Anderson JA, Gill BS. 2016. Wheat *Fhb1* encodes a chimeric lectin with
562   agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium
563   head blight. *Nature Genetics* 48 (12):1576-1580.

564 Sahni S, Prasad BD, Liu Q, Grbic V, Sharpe A, Singh SP, Krishna P. 2016. Overexpression of
565   the brassinosteroid biosynthetic gene DWF4 in *Brassica napus* simultaneously increases
566   seed yield and stress tolerance. *Scientific Reports* 6:28298.

567 Salmon J, Ramos J, Callis J. 2008. Degradation of the auxin response factor ARF1. *The Plant*

568   *Journal* 54:118-128.

569   Sanders TH, McMichael RW, Hendrix KW. 2000. Occurrence of Resveratrol in Edible Peanuts.

570   *Journal of agricultural and food chemistry* 48(4):1243-1246

571   Song H, Guo ZL, Chen T, Sun J, Yang GF. 2018. Genome-wide identification of LRR-

572   containing sequences and the response of these sequences to nematode infection in *Arachis*

573   *duranensis. BMC Plant Biology* 18:279.

574   Stark L, Nelke B, Hanbler G. 1997. Transfer of a grapevine stilbene synthase gene to rice (*Oryza*

575   *sativa* L.). *Plant Cell Reports* 16:668-673.

576   Steuernagel B, Periyannan SK, Hernández-Pinzón I, Witek K, Rouse MN, Yu G, Hatta A,

577   Ayliffe M, Bariana H, Jones JD Lagudah ES, Wulff BB. 2016. Rapid cloning of disease-

578   resistance genes in plants using mutagenesis and sequence capture. *Nature Biotechnology*.

579   34:652-655.

580   Takaoka MJ. 1940. Of the Phenolic Substances of White Hellebore (*Veratrum grandiflorum*

581   Loes. fil.). *Journal of the Faculty of Science* Hokkaido Imperial University 3:1-16.

582   Tang K, Zhan JC, Yang HR, Huang WD. 2010. Changes of resveratrol and antioxidant enzymes

583   during UV induced plant defense response in peanut seedlings. *Journal of Plant Physiology*

584   167:95-102.

585   Tian HY, Lv BS, Ding TT, Bai MY, Ding ZJ. 2017. Auxin-BR interaction regulates plant growth

586   and development. *Frontiers in Plant Science* 8: 2256.

587   Tian LR, Liao BS, Huang JQ, Wang SY, Lei Y, Yan LY, Li D. 2008. Research progress on

588   resveratrol in peanut. *Chinese journal of oil crop sciences* 30(4):522-528.

589   Wang H, Wang H, Shao H, Tang X. 2016. Recent advances in utilizing transcription factors to

590   improve plant abiotic stress tolerance by transgenic technology. *Frontiers in Plant Science*

591   7:67.

592   Wang L, Hu W, Tie W, Ding Z, Ding X, Liu Y, Yan Y, Wu C, Peng M, Xu B, Jin Z. 2017. The

593   MAPKKK and MAPKK gene families in banana: identification, phylogeny and expression

594   during development, ripening and abiotic stress. *Scientific Reports* 7(1):1159.

595   Wang W, Tang K, Yang HR, Wen PF, Zhang P, Wang HL. 2010. Distribution of resveratrol and
596   stilbene synthase in young grape plants (*Vitis vinifera* L. cv. *Cabernet Sauvignon*) and the
597   effect of UV-C on its accumulation. *Plant Physiology and Biochemistry* 48:142-152.

598   Wei H, Zhao H, Su T, Bausewein A, Greiner S, Harms K, Rausch T. 2017. Chicory R2R3-MYB
599   transcription factors CiMYB5 and CiMYB3 regulate fructan 1-exohydrolase expression in
600   response to abiotic stress and hormonal cues. *Journal of Experimental Botany* 68(15):4323-
601   4338.

602   Xiong HC, Guo HJ, Xie YD, Zhao LS, Gu JY, Zhao SR, Li JH, Liu LX. 2017. RNAseq analysis
603   reveals pathways and candidate genes associated with salinity tolerance in a spaceflight-
604   induced wheat mutant. *Scientific Reports* 7(1):2731.

605   Yang S, Wang F, Wang F, Meng JJ, Li XG, Dong ST, Wan SB. 2013. Exogenous calcium
606   alleviates photoinhibition of PSII by improving the xanthophyll cycle in peanut (*Arachis
607   hypogaea*) leaves during heat stress under high irradiance. *Plos one* 8:e71214.

608   Yang Z, Wang C, Xue Y, Liu X, Chen S, Song C, Yang Y, Guo Y. 2019. Calcium-activated 14-
609   3-3 proteins as a molecular switch in salt stress tolerance. *Nature Commun* 10(1):1199.

610   Zhang JL, Geng Y, Guo F, Li XG, Wan SB. 2020. Research progress on the mechanism of
611   improving peanut yield by single-seed precision sowing. *Journal of Integrative Agriculture*
612   doi: 10.1016/S2095-3119(19)62763-2.

613   Zhang Y, Wang PF, Xia H, Zhao CZ, Hou L, Li CS, Gao C, Zhao SZ, Wang XJ. 2016.
614   Comparative transcriptome analysis of basal and zygote-located tip regions of peanut
615   ovaries provides insight into the mechanism of light regulation in peanut embryo and pod
616   development. *BMC Genomics* 17:606.

617   Zheng SG, Zhao SC, Li Z, Wang QG, Yao FY, Yang LQ, Pan JW, Liu W. 2015. Evaluating the
618   effect of expressing a peanut resveratrol synthase gene in rice. *PLos One* 10(8):e0136013.

619   Zheng YP, Xin CY, Wang CB, Sun XS, Yang WQ, Wan SB, Zheng YM, Feng H, Chen DX, Sun
620   XW, Wu ZF. 2013. Effects of phosphorus fertilizer on root morphology, physiological
621   characteristics and yield in peanut (*Arachis hypogaea*). *Chinese Journal of Plant Ecology*

622   37(8):777-785.

623 Zhu JK. 2016. Abiotic stress signaling and responses in plants. *Cell* 167:313-324.

624 **Figure Legends**

625 Fig. 1 Phenotypical comparisons of peanut in SS and DS sowing treatment. (a) The phenotypes,

626 (b) absorbing area of the root in SS and DS, and (c) Dry weight of root in SS and DS.

627 Fig. 2 Scatter plot of DEGs in leaf and root between SS and DS treatments.

628 Fig. 3 Differentially expressed genes analysis in roots and leaves. (a) Venn diagram

629 demonstrated the common and specific differentially expressed genes (DEGs), (b) Heat map

630 demonstrated the expression profile DEGs.

631 Fig. 4 qRT-PCR verification of randomly selected genes. Heatmaps represent the expression of

632 the 22 DEGs in (a) leaves and (b) roots between SS and DS treatments. For each heatmap, the

633 FPKM values increased from green to red. The correlation of the fold change in (c) leaves and (d)

634 roots analyzed by RNA-Seq (x-axis) with data obtained using qRT-PCR (y-axis).

635 Fig. 5 GO annotation among DEGs identified in the leaf of SS treatment compared with DS

636 treatment and root from the two different treatments. The GO ID was listed alongside the textual

637 annotations.

638 Fig. 6 Co-expression of DEGs between SS and DS treatments. (a) Hierarchical of 10,000 genes

639 based on topological overlap and visualization of gene modules with assigned colors. Branches

640 in the hierarchical clustering dendrograms correspond to modules. Color-coded module

641 membership is displayed in the colored bars below and to the right of the dendrograms. (b) The

642 network of co-expressed modules. The colored clustering bars directly correspond to the module

643 (color) designation for the clusters of genes.

644 Fig. 7 Determination of resveratrol contents according to the method detailed by Tang (*Tang et*

645 *al., 2010*) in leaf and root between SS and DS treatments.

646

647 **Additional files**

648 **Supplementary Information**

649 **Fig. S1** The SNP variants were summarized for each sample. A-G: The amount of A-G variant

650 type; C-T: The amount of C-T variant type; Transition: The amount of A-G and C-T variant type;

651 A-C: The amount of A-C variant type; A-T: The amount of A-T variant type; C-G: The amount

652 of C-G variant type; G-T: The amount of G-T variant type; Transversion: The amount of A-C,

653 A-T, C-G, and G-T variant type.

654 **Fig. S2** The distributions of INDEL location for each sample were analyzed. Up2k means

655 upstream 2,000 bp area of a gene. Down2k means downstream 2,000 bp area of a gene.

656 **Fig. S3** Based on the SNP, INDEL, and gene expression result, Circos were used to perform the

657 analysis.

658 **Fig. S4** Pathway functional enrichment of DEGs. X axis represents the enrichment factor. Y axis

659 represents the pathway name. The color indicates the q-value (high: white, low: blue); the lower

660 q-value indicates the more significant enrichment. Point size indicates DEG number (The bigger

661 dots refer to a larger amount). Rich Factor refers to the value of enrichment factor, which is the

662 quotient of foreground value (the number of DEGs) and background value (total Gene amount).

663 **Table S1** Variety of the activities of SOD, POD, CAT, and TTC in SS and DS sowing treatments,

664 respectively. The data presented are the mean values ± SD of three individual experiments.

665

666

667

668

Figure 1

Phenotypical comparisons of peanut in SS and DS sowing treatment.

(a) The phenotypes, (b) absorbing area of root in SS and DS, and (c) Dry weight of root in SS and DS. Fig. 2 Scatter plot of DEGs in leaf and root between SS and DS treatments.
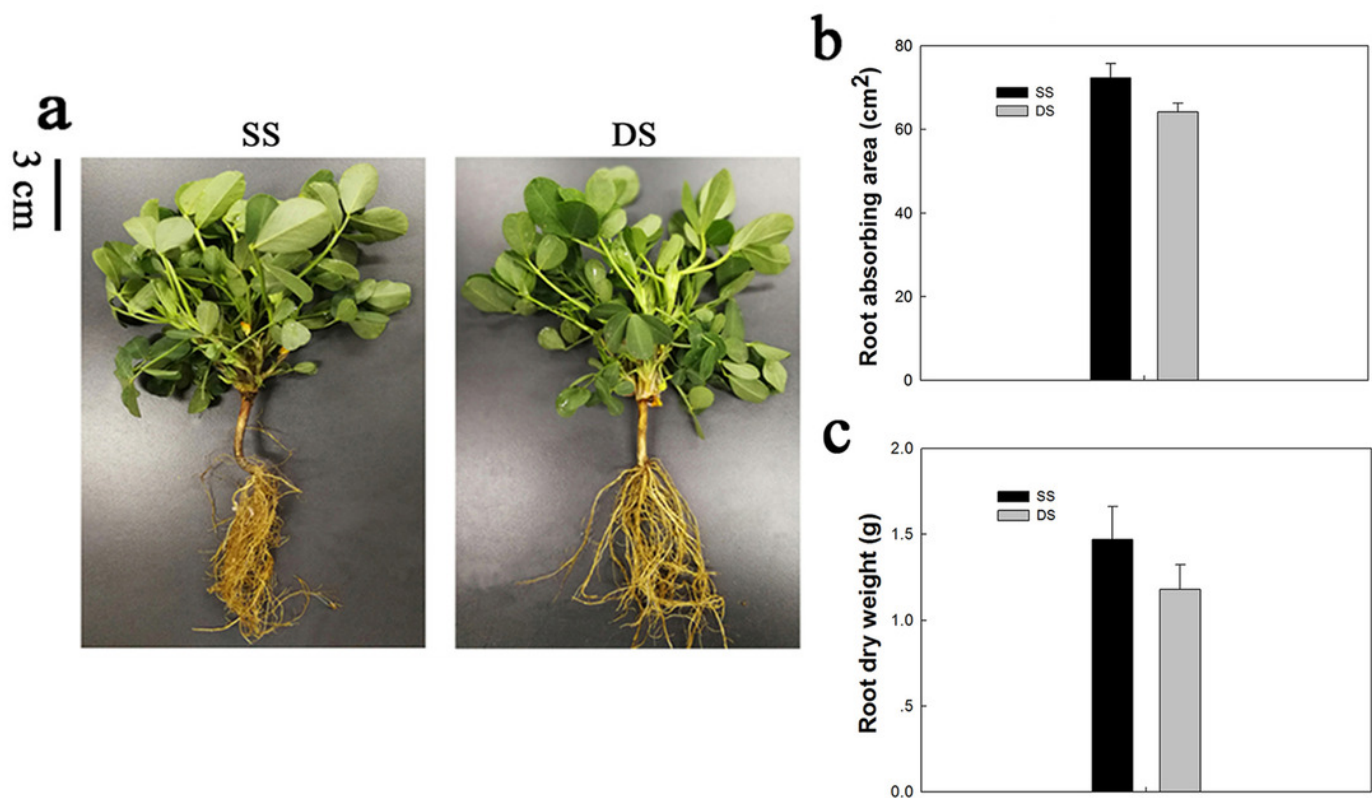
Figure 2

Fig. 2 Scatter plot of DEGs in leaf and root between SS and DS treatments.
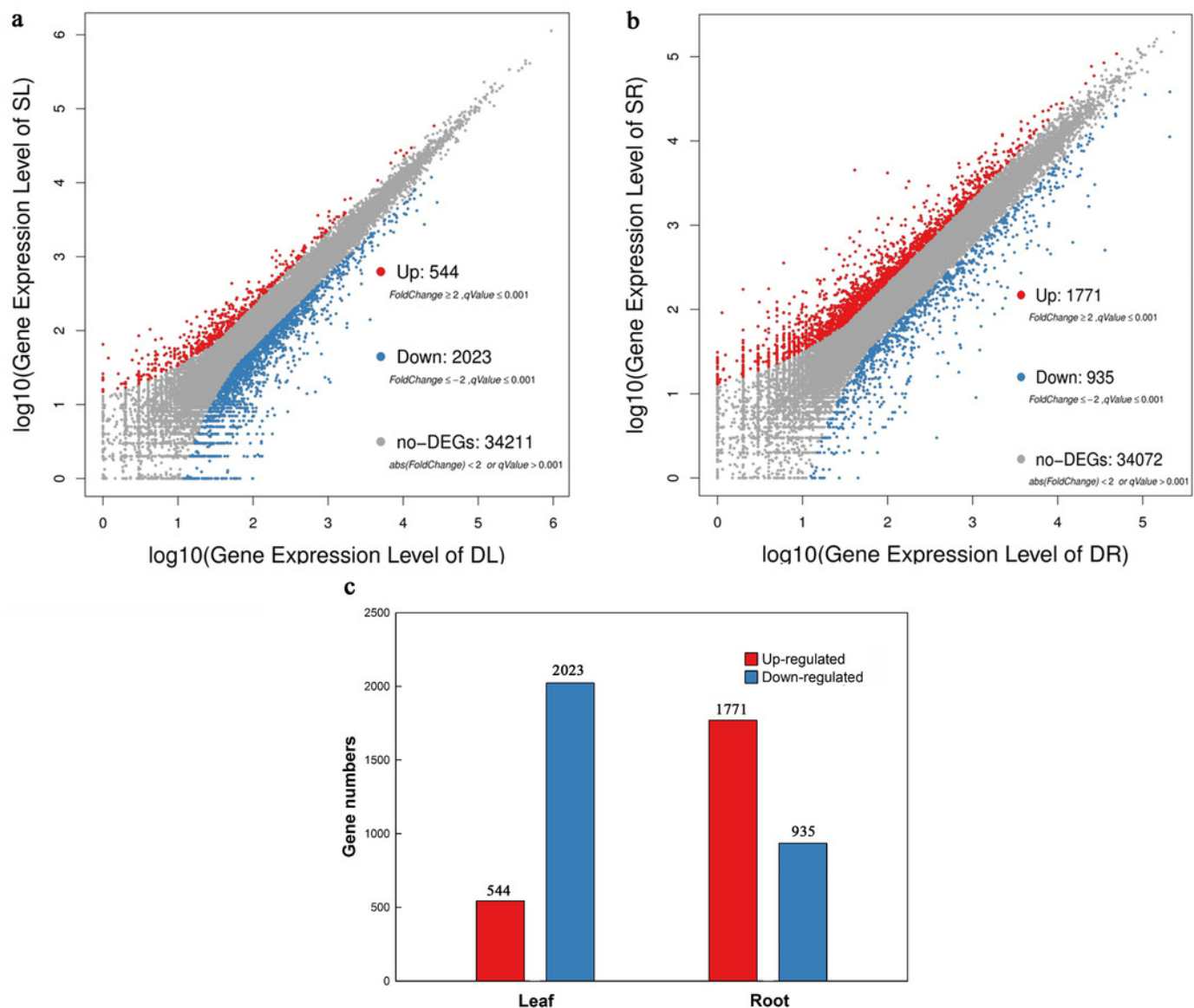
Figure 3

Fig. 3 Differentially expressed genes analysis in roots and leaves.

(a) Venn diagram demonstrated the common and specific differentially expressed genes (DEGs), (b) Heat map demonstrated the expression profile DEGs.
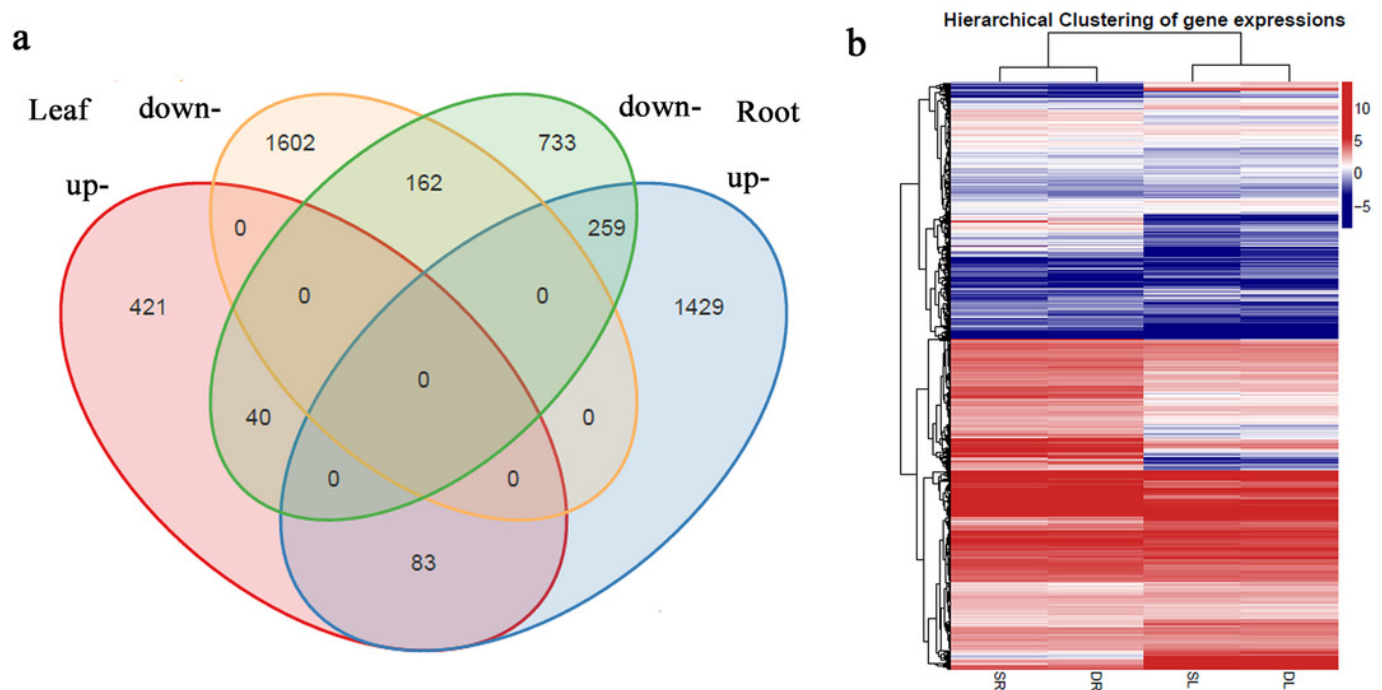
Figure 4

Fig. 4 qRT-PCR verification of randomly selected genes.

Heatmaps represent the expression of the 22 DEGs in (a) leaves and (b) roots between SS and DS treatments. For each heatmap, the FPKM values increased from green to red. The correlation of the fold change in (c) leaves and (d) roots analyzed by RNA-Seq (x-axis) with data obtained using qRT-PCR (y-axis).
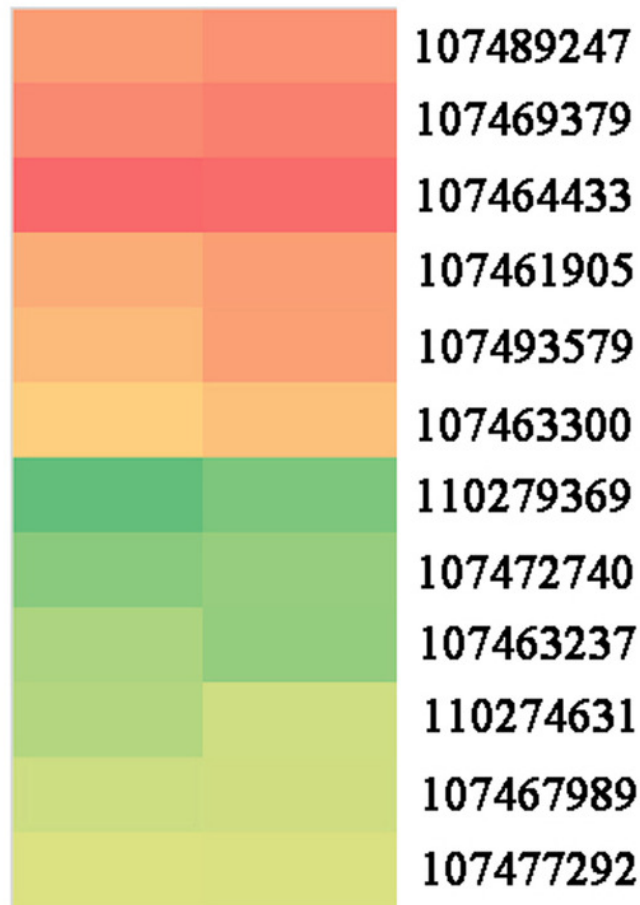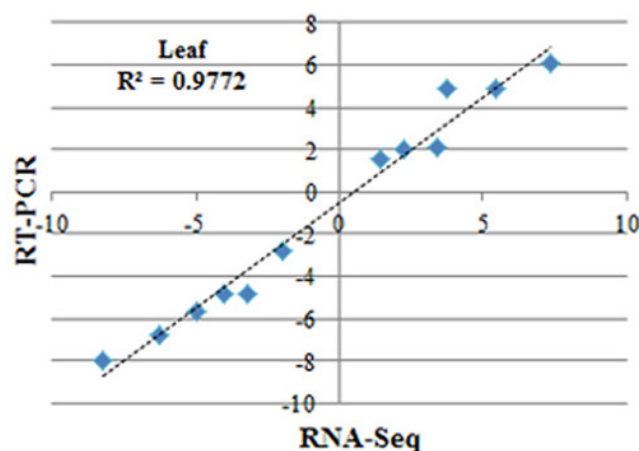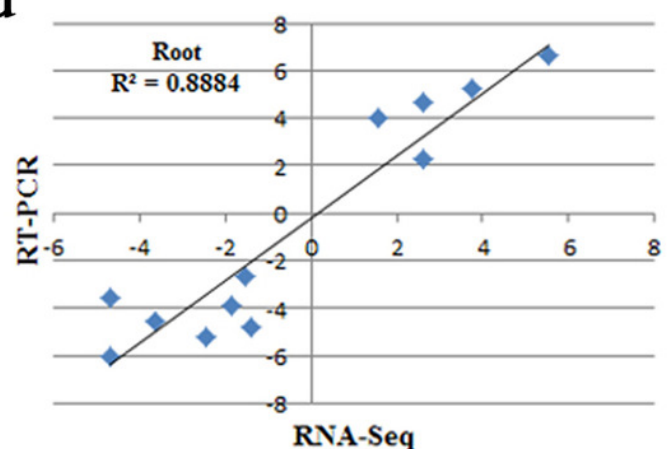
Figure 5

Fig. 5 GO annotation among DEGs identified in the leaf of SS treatment compared with DS treatment and root from the two different treatments.

The GO ID was listed alongside the textual annotations.
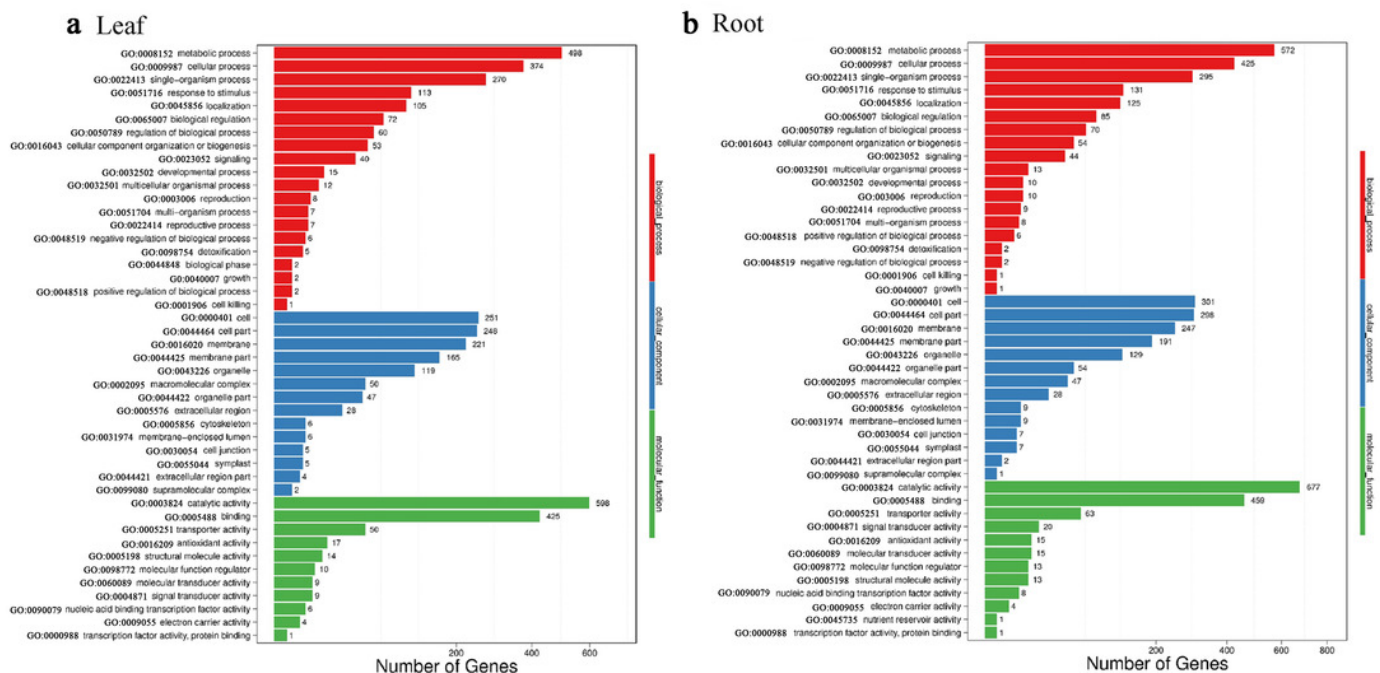
Figure 6

Fig. 6 Co-expression of DEGs between SS and DS treatments.

(a) Hierarchical of 10,000 genes based on topological overlap and visualization of gene modules with assigned colors. Branches in the hierarchical clustering dendrograms correspond to modules. Color-coded module membership is displayed in the colored bars below and to the right of the dendrograms. (b) Network of co-expressed modules. The colored clustering bars directly correspond to the module (color) designation for the clusters of genes.
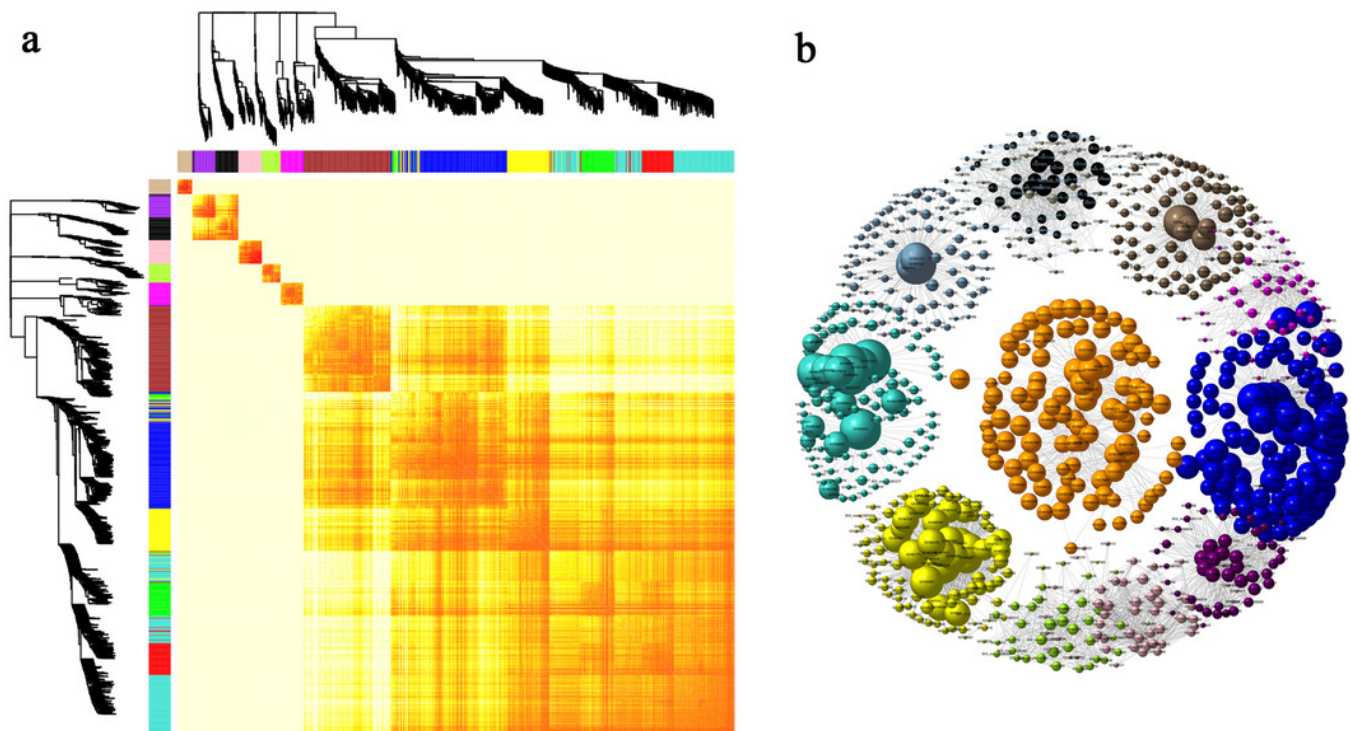
Figure 7

Fig. 7 Determination of resveratrol contents according to the method detailed by Tang (*Tang et al., 2010*) in leaf and root between SS and DS treatments.
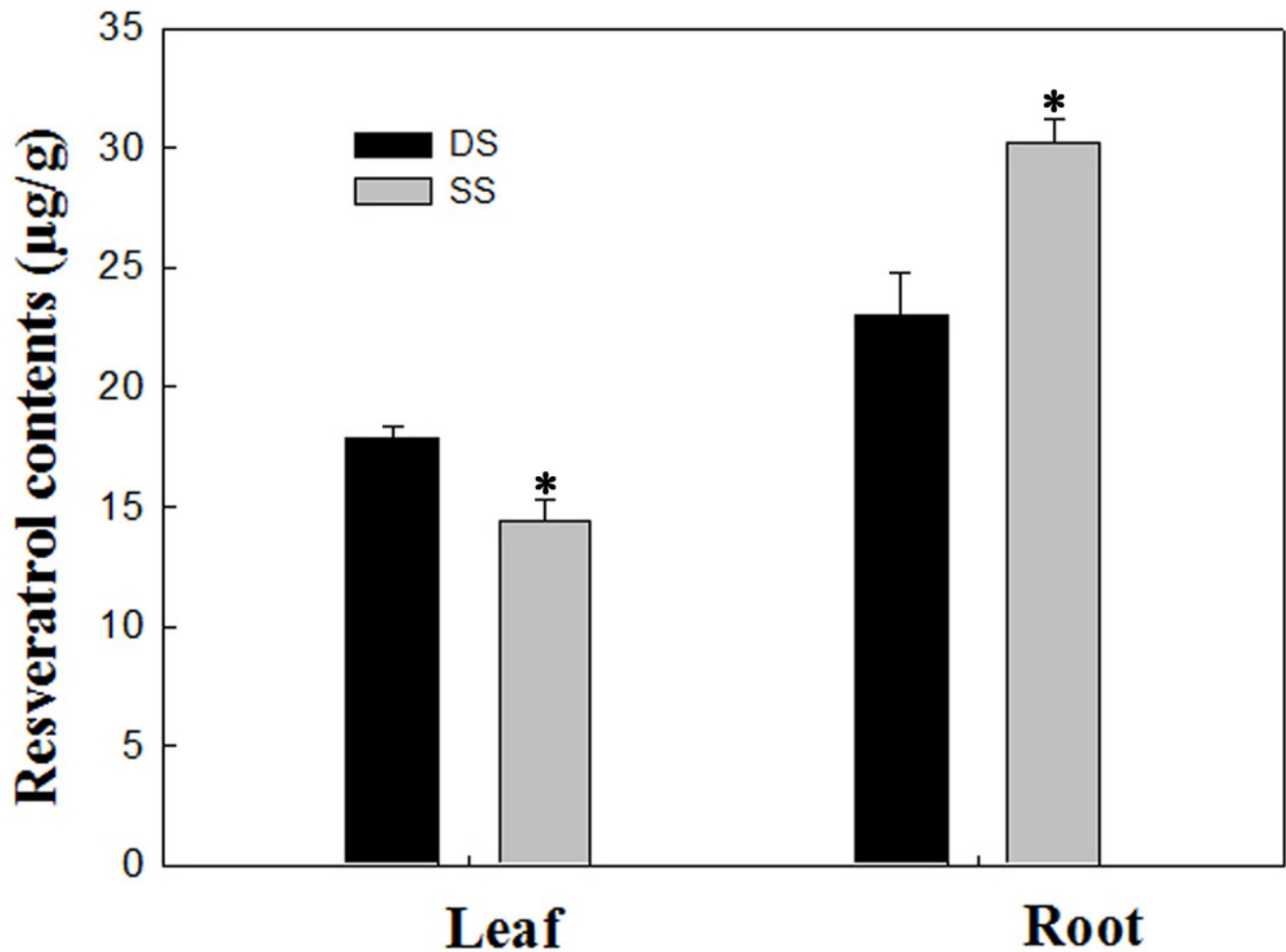
Table 1 Summary of read numbers from leaves and roots.

SL: Leaf in single-seed (SS) precision sowing; DL: Leaf in double-seed (DS) sowing; SR: Root in single-seed (SS) precision sowing; DR: Root in double-seed (DS) sowing.

1 Table 1 Summary of read numbers from leaves and roots. SL: Leaf in single-seed (SS) precision sowing; DL: Leaf in double-seed (DS)
2 sowing; SR: Root in single-seed (SS) precision sowing; DR: Root in double-seed (DS) sowing.
3

Samples	Reads in leaf						Reads in Root					
	SL1	SL2	SL3	DL1	DL2	DL3	SR1	SR2	SR3	DR1	DR2	DR3
Total clean reads	45,001,686	44,475,256	44,262,552	42,022,054	42,294,220	42,570,982	42,569,308	42,180,566	44,533,150	44,784,894	44,608,344	44,497,326
Total Mapping Ratio	75.27%	74.87%	74.88%	78.26%	80.52%	80.60%	80.73%	80.88%	82.71%	78.44%	78.15%	80.95%
Unique match Ratio	55.15%	53.51%	54.34%	49.41%	51.29%	54.53%	51.93%	52.18%	53.61%	46.73%	43.23%	52.30%
Unmapped reads	24.73%	25.13%	25.12%	21.74%	19.48%	19.40%	19.27%	19.12%	17.29%	21.56%	21.85%	19.05%
Novel transcript number	13,152	12,615	13,149	13,218	13,030	12,895	13,139	13,136	13,316	13,021	12,911	12,909

6
7
8
9
10
11
12
13
14
15
16
17
18
19

Table 2 Analysis of resveratrol synthesis related genes in leaves and roots.

The ratio was obtained from the transcriptome results. SL: Leaf in single-seed (SS) precision sowing; DL: Leaf in double-seed (DS) sowing; SR: Root in single-seed (SS) precision sowing; DR: Root in double-seed (DS) sowing.

1 Table 2 Analysis of resveratrol synthesis related genes in leaves and roots. The ratio was obtained from the transcriptome results. SL:
2 Leaf in single-seed (SS) precision sowing; DL: Leaf in double-seed (DS) sowing; SR: Root in single-seed (SS) precision sowing; DR:
3 Root in double-seed (DS) sowing.

	Annotation	$\log_2^{(SL/DL)}$	Gene expression change
	resveratrol synthase	-1.43	Down-regulated
	resveratrol synthase	-1.92	Down-regulated
	resveratrol synthase	-2.03	Down-regulated
	resveratrol synthase	-2.31	Down-regulated
Leaf	resveratrol synthase	-2.66	Down-regulated
	resveratrol synthase	-2.77	Down-regulated
	resveratrol synthase	-3.36	Down-regulated
	resveratrol synthase	-6.66	Down-regulated
	stilbene synthase	-1.18	Down-regulated
	stilbene synthase	-1.38	Down-regulated
	Annotation	$\log_2^{(SR/DR)}$	Gene expression change
	resveratrol synthase	2.67	Up-regulated
	resveratrol synthase	2.59	Up-regulated
	resveratrol synthase	2.37	Up-regulated
	resveratrol synthase	2.27	Up-regulated
	resveratrol synthase	2.24	Up-regulated
	resveratrol synthase	2.13	Up-regulated
	resveratrol synthase	2.12	Up-regulated
	resveratrol synthase	2.05	Up-regulated
	resveratrol synthase	1.86	Up-regulated
	resveratrol synthase	1.80	Up-regulated
	resveratrol synthase	1.68	Up-regulated
Root	resveratrol synthase	1.67	Up-regulated
	resveratrol synthase	1.61	Up-regulated

resveratrol synthase	1.56	Up-regulated
resveratrol synthase	1.55	Up-regulated
resveratrol synthase	1.47	Up-regulated
resveratrol synthase	1.34	Up-regulated
resveratrol synthase	1.27	Up-regulated
stilbene synthase	2.47	Up-regulated
stilbene synthase	2.08	Up-regulated
stilbene synthase	1.19	Up-regulated

Table 3 List of putative candidate genes for high yields in SS sowing treatment.

These candidate genes were up-regulated in SR vs DR comparison groups with the corrected *p* value < 0.05 and are here classified according to predicted gene function. The values in the columns are the log2 Fold Change values for the SR vs DR comparison groups obtained from the transcriptome results. SR: Root in single-seed (SS) precision sowing; DR: Root in double-seed (DS) sowing.

1 Table 3 List of putative candidate genes for high yields in SS sowing treatment. These candidate
2 genes were up-regulated in SR vs DR comparison groups with the corrected *p* value < 0.05 and
3 are here classified according to predicted gene function. The values in the columns are the log2
4 Fold Change values for the SR vs DR comparison groups obtained from the transcriptome results.
5 SR: Root in single-seed (SS) precision sowing; DR: Root in double-seed (DS) sowing.

Gene_id	Gene description	SR vs DR
Oxidation-reduction		
107460187	L-ascorbate oxidase homolog	1.4
110279516	L-ascorbate oxidase	1.4
107482474	L-ascorbate oxidase	1.2
107480398	L-ascorbate oxidase	1.1
107471312	glutaredoxin	2.8
107475918	glutaredoxin 3	2.4
107494759	glutaredoxin-C1-like	1.5
107461150	glutaredoxin 3	1.1
107475477	cytochrome P450 83B1-like	2.2
107491506	cytochrome P450 71A1-like	2.1
107475482	cytochrome P450 83B1-like	2.0
107475708	cytochrome P450 83B1-like	3.5
107478833	cytochrome P450 84A1-like	1.1
107475710	cytochrome P450 71A1-like	1.2
107487896	cytochrome P450 71D8-like	1.2
107492914	cytochrome P450 93A3	1.3
Hormone related		
107491535	Protein brassinosteroid	1.7
107486286	gibberellin-regulated protein	2.4
BGI_novel_G000490	gibberellin receptor GID1	2.0
107495262	gibberellin receptor GID1	2.6
107476131	gibberellin receptor GID1	1.9
107464975	gibberellin 2-beta-dioxygenase 2-like	1.7
107477445	DELLA protein	1.7
107491113	DELLA protein	1.1
107476118	auxin responsive GH3 gene family	1.9
107465048	auxin-responsive protein IAA	1.8
107492413	auxin response factor	1.9
107463844	auxin-responsive protein IAA	1.4
107462192	auxin responsive GH3 gene family	1.8
107459619	auxin-responsive protein IAA	1.5
107478268	auxin responsive GH3 gene family	1.0
107464096	auxin-responsive protein IAA	1.2

107495645	lipoxygenase	2.4
107464479	linoleate 9S-lipoxygenase	1.1
Transcription factor and signaling regulation		
107491013	MADS-box transcription factor	3.0
107476111	MADS-box transcription factor	5.7
107458618	zinc finger protein CONSTANS-LIKE 4-like	2.8
107492404	Zinc finger protein	2.4
110279598	zinc finger protein 3-like	2.3
107493757	zinc finger protein-like protein	1.8
107481180	Zinc finger protein 6	1.5
107460097	C2H2-like zinc finger protein	1.3
107483222	MYB family transcription factor	4.8
107461398	MYB86 Transcription factor	1.5
107475194	MYB transcription factor MYB51	1.2
107491849	R2R3 MYB protein 2	1.2
107459300	WRKY transcription factor 70	2.4
107472118	WRKY transcription factor	1.8
107472768	WRKY transcription factor 22-like	1.5
107481590	WRKY transcription factor 14	1.3
107463444	ERF114-like	2.4
107488356	ERF022	1.6
107464842	ERF13	1.5
107471058	ERF WRI1	1.0
107486141	ERF 1-like	1.0
BGI_novel_G002321	bZIP	7.3
107487341	bZIP	1.5
107466101	Calcium-binding protein CML	1.4
107491924	Calcium-binding protein KIC-like	1.3
107460313	Calcium-binding protein CML	1.2
107479434	MAPKKK	1.5
Disease resistance		
107468687	pathogenesis-related protein 1	2.2
107468499	pathogenesis-related protein 1	1.8
107468493	pathogenesis-related protein 1	1.4
107474846	pathogenesis-related protein 1	1.0
107460041	4-coumarate--CoA ligase	4.2
107472893	4-coumarate--CoA ligase	3.1
107480465	4-coumarate--CoA ligase	2.6
107458085	4-coumarate--CoA ligase	2.0
107466660	4-coumarate--CoA ligase-like 5	1.7

| 107481001 | 4-coumarate--CoA ligase | 1.6 |

Table 4 Pod per plant at maturity stage of peanut under different planting patterns

1   Table 4 Pod per plant at maturity stage of peanut under different planting patterns

Treatment	Pods number per plant	Full pods number per plant	Double kernel number per plant	Pod weight per plant (g)	Economic coefficient
SS	39.1a	22.6a	24.4a	51.7a	0.49a
DS	31.8b	16.3b	16.9b	39.3b	0.45b