Evaluation and GWAS of radicle gravitropic response in a core rice germplasms population

qiaojun lou
  Shanghai Agrobiological Gene Center

Qingsong Li
  Shanghai Agrobiological Gene Center

Fangjun Feng
  Shanghai Agrobiological Gene Center

Ryan Joynson
  Earlham Institute

Yunan Yang
  Shanghai Agrobiological Gene Center

Lijun Luo
  Shanghai Agrobiological Gene Center

Liang Chen

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Abstract

Gravitropism is one of the primary determinants of root development, facilitating root penetration into soil and subsequent absorption of water and nutrients. In this study, the gravitropism of 226 Chinese rice micro-core accessions and drought-resistant core accessions were assessed. The average value of gravitropic response speed of radicle roots was 41.05°/h in the population ranging from 16.77°/h to 62.83°/h. We observed a highly significant difference in gravity response speed between Indica (42.49°/h) and Japonica (39.71°/h) subspecies with p-value < 0.002. The correlation analysis showed that the gravitational response speed of radicle roots was significantly positively correlated with the number of deep roots (correlation coefficient = 0.16), the growth speed of radicle roots (correlation coefficient = 0.21) and the drought resistance coefficient (correlation coefficient = 0.14). Using genome-wide association analysis (GWAS), 4 QTLs(quantitative traits) associating with gravitropic response speed were identified on chromosome 4,11 and 12. From within these intervals 5 candidate genes were screened for qPCR verification in 6 extreme rice varieties, demonstrating that gene LOC_Os12g29350 may regulate gravitropism negatively and confirming it's candidacy for further study.

Introduction

Scientists have been trying to understand gravitropism for more than 120 years (Darwin and Darwin 1880). Gravitropism is the orientation of growth in response to gravity, which is necessary for roots to grow into soil, to acquire water and nutrients and to anchor plants, providing stability and preventing lodging.

The starch–statolith hypothesis and the Cholodny–Went theory attempt to explain some aspects of gravitropism (Haberlandt 1900; Němec 1900; Went 1926; Cholodny N 1927). The starch–statolith hypothesis proposes that the starch-filled amyloplasts of gravity-sensing cells act as statoliths, signalling the direction of gravity by their sedimentation. The Cholodny–Went theory indicated, gravi-bending is the result of differential accumulation of auxin on opposite sides of the elongation zone, resulting in differential growth and tip curvature. In addition, mechanosensitive ion channel hypothesis also could explain some parts of the gravitropism(Ding and Pickard 1993).

A lot of hormones and genes have been found to be involved in gravitropism previously (Blancaflor and Masson 2003; Baldwin et al. 2013; Mai et al. 2014; Ge and Chen 2016). Auxin and its related transporters(e.g. AUX1 and AtPIN2)have been observed to regulate gravitropism (Bennett et al. 1996; Müller et al. 1998; Rigó et al. 2013). Cytokinin functions as an anti-gravitropic signaller in lateral roots(Waidmann et al. 2019). Additionally, brassinosteroid also play important role in the root gravitropic response(Kim et al. 2000; Chang et al. 2004; Amzallag and Vaisman 2006). *AGR1* gene involved in root gravitropism could increase root-growth sensitivity to auxin and decrease sensitivity to ethylene in Arabidopsis (Chen et al. 1998). *NPY* genes play an essential role in root gravitropic responses in Arabidopsis(Li et al. 2011). Although the vast majority of research to date has been conducted in Arabidopsis thaliana, few examples of genes affecting gravitropism have been identified in crop species. The organization of the actin cytoskeleton influence the gravitropic response of primary roots of maize (Blancaflor and Hasenstein 1997). Maize *LAZY1* mediates shoot gravitropism through regulating auxin transport(Dong et al. 2013).

Rice is a staple food for nearly half of the world's population. It has a typical fibrous root system. In rice, some mutants and genes related to gravitropism have been identified. *LAZY1* gene controls rice shoot gravitropism through regulating polar auxin transport(Li et al. 2007), but the primary roots of *lazy1* mutants show normal gravitropism and circumnutation (Yoshihara et al. 2013). *Aem1* mutant causes defects in root development and gravity response (Debi et al. 2005a). Overexpression of *OsRAA1* effects root development and root response to gravity(Ge et al. 2004).

The mechanism of gravity sensing in plants is one of the most fascinating questions in molecular biology, and because of the new availability of high-throughput sequencing and phenotyping technology, we can expand our knowledge of this trait through association analysis. For example, in the common bean, Liao et al mapped the QTLs controlling basal root gravitropism (Liao et al. 2004). Using a mapping population derived from a Bala × Azucena, two main QTLs for gravitropic response have been mapped to chromosome 6 and 11(Norton and Price 2009). Measurement of gravitropism related traits with a throughput lending itself to the sample size required for association analysis is now more feasible with tools such as the ROTATO with an automated camera, that could help researchers to dissect the gravi-response(Mullen et al. 2000).

Despite progress made in the past decades, processes involved in positive root gravitropic response in the root tip still remains largely unclear in rice. Because root gravitropism is widely believed to be regulated by a tipping-point mechanism (Band et al. 2012), the gravitropic response speed could be represented by the bending angle of the seminal in agar-filled Perspex chambers after rotation as Uga and Price et al did (Norton and Price 2009; Uga et al. 2013). Here, we measured gravitropic response speed in an association mapping population (Lou et al. 2015), and identified several QTLs related to gravitropic response that can be deployed into marker assisted selection programmes.

Results

Gravitropic response speed of radicle roots

In this study, we detected the bending angle of radicle roots of 266 core rice accessions representing their gravitropic response speed. Among these accessions, the average value of gravitropic response speed of radicle roots was 41.05°/h. The fastest speed was 62.83°/h, meanwhile the slowest speed was 16.77°/h. The standard deviation and coefficient of variation were 6.42°/h and 15.63%, respectively. As shown in Fig. 1, the gravitropic response speed of radicle roots generally presented a normal distribution and was mostly distributed between 31°/h to 51°/h, accounting for 89.4% of the total accessions. This indicated that this set of data was suitable for association mapping analysis.

Comparison of gravitropic response speed between *Indica* and *Japonica* rice

Notable differences of gravitropic response speed were found between *Indica* and *Japonica* rice (Table 1). The gravitropic response speed of *Indica* rice was mostly distributed between 33.5°/h to 53.5°/h, accounting for 94.7% of the *Indica* accessions. As for the *Japonica* rice, it was mostly distributed at 33.5°/h to 48.5°/h, accounting for 90.3% of the *Japonica* accessions. The gravitropic response speed of *Indica* (42.49°/h) was faster than that of *Japonica* (39.71°/h). Likewise, the range of variation of *Indica* (46.06°/h) was larger than that of *Japonica* (39.91°/h). The standard deviation and coefficient of variation of *Indica* and *Japonica* accessions were 6.41°/h and 6.30°/h, 15.32% and 15.77%, respectively. Compared the gravitropic response speed of *Indica* and *Japonica* rice by student's (t) test resulted in a p value of inequality at 0.002. This result suggested that there was significant difference in the gravitropic response speed of *Indica* and *Japonica* subspecies, and the gravity response speed of *Indica* radicle roots was significantly faster than that of *Japonica* rice.

Table 1
Gravitropic response speeds of two rice subspecies

	Number of accessions	Means(°/h)	Range(°/h)	Standard deviation(SD, °/h)	Coefficient variation (CV, %)
Indica	133	42.49	16.77−62.83	6.41	15.32
Japonica	93	39.71	21.73−61.64	6.30	15.77

Correlation among gravitropic response speed, deep rooting phenotype and drought resistant index

Multiple measurements for root phenotypes of these 226 accessions have been measured in a previous study (Lou et al. 2015). The ratio of the yield in dry fields to the yield in paddy fields was calculated as the yield-based drought resistant index (DRI). The original data was obtained from previous studies in our laboratory(Ma et al. 2016). And correlations were calculated between these measurements and the gravitropic response measurements to determine if these traits could be inherently linked. By comparing the correlation coefficient between gravitropic response speed and some agronomic traits (Table 2), we observed that the gravitropic response speed was significantly positively correlated with tiller number (TN), deep roots (DR), growth speed of radicle roots (GSR) and drought resistant index (DRI) with correlation coefficients of 0.13, 0.16, 0.22 and 0.14, respectively. The results indicated that the speed of the gravitropic response of radicle roots was highly significantly positively correlated with the speed of radicle roots growth. The faster the gravitropic response, the faster the growth speed. At α = 0.05, the speed of the gravitropic response of radicle roots was also positively correlated with TN, DR and DRI. The faster the response speed, the larger the deep roots number as well as the drought resistant index. There was no significant correlation between the speed of gravitropic response and plant height (PH), shallow roots (SR), ratio of deep roots (RDR) or roots per tiller (R/T).

Table 2
Correlation coefficient between gravitropic response speed and some
agronomic traits

	Bending angle of radicle
Plant height (PH)	0.03
Tiller number (TN)	0.13*
Deep roots number (DR)	0.16*
Shallow roots number (SR)	0.09
Total roots number (TR)	0.12
Ratio of deep roots (RDR)	0.05
Roots per tiller (R/T)	0.01
Growth speed of radicle (GSR)	0.22**
Drought resistant index (DRI)	0.14*
Note: "*"means significance at P < 0.05,"**"means significance at P < 0.01.	

12 accessions with the fastest speed of gravitropic response were selected for further study, and the growth speed of radicle roots and DRI of this subset were measured (**Supplementary Table 1**). Among them, the DRI of Xiaohonggu, IAC1246 and Zaohandao was 1.06, 1.18 and 1.89, respectively. These 3 accessions could be chosen as donor parental lines for drought-resistant breeding in the future.

GWAS of the gravitropic response speed of radicle roots

The raw sequence data of this population have been uploaded to public databases: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA260762 and ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByRun/sra/SRR/SRR123/SRR12 39601(Lou et al. 2015). GWAS was carried out to associate gravitropic response speed to a responsible genomic location using the EMMA model. 4 significantly associated QTLs were detected at the threshold of $p = 10^{-5}$ (Fig. 2, Table 3). 2 QTLs named *qGRS4.1* and *qGRS4.2* were mapped on the chromosome 4 (404424750 bp and 404774373 bp), and other 2 QTLs named *qGRS11* and *qGRS12* located on each of chromosomes 11 and 12 (1123451244 bp and 1217439378 bp).

Table 3
SNP loci related to gravitational response identified by GWAS

NO.	QTL	Lead SNP	Chromosome	P value
1	*qGRS4.1*	404424750	4	3.52E-06
2	*qGRS4.2*	404774373	4	4.83E-06
3	*qGRS11*	1123451244	11	7.77E-06
4	*qGRS12*	1217439378	12	4.87E-06

qPCR of candidate genes of gravitropic response

According to the GWAS results, and based on the annotation information and our previous transcriptome data of rice root(Lou et al. 2017), 5 candidate genes that may relate with gravitropic response were chosen for expression analysis (Table 4).

QTL	Candidate Gene ID	Chromosome	Annotation
qGRS4.2	LOC_Os04g08740 (*ETR2*)	4	Ethylene receptor
qGRS11	LOC_Os11g40090 (*RPA49*)	11	A49-like RNA polymerase I associated factor family protein
	LOC_Os11g40100 (*OsGIF1*)	11	GRF-interacting factor 2
	LOC_Os11g40430 (*RLCK341*)	11	Wall-associated receptor kinase-like 2 precursors
qGRS12	LOC_Os12g29350	12	ATP binding protein

A serine/threonine kinase, *ETR2* (LOC_Os04g08740), was found at a distance of about 40 kb from the head SNP of *qGRS4*.2 (404774373, Chr4), which is an ethylene receptor and acts as a negative regulator of ethylene signaling. Plants over-expressing *ETR2* display reduced ethylene sensitivity, delayed floral transition and reduced seed set (Hada et al. 2009). 2 genes were found at an interval within 52 kb from *qGRS11* (1123451244, Chr11). One was related with the activity of RNA polymerase called *RPA49* (LOC_Os11g40090), which interacts with *SAD1*. A decline in the function of *SAD1* leads to severe suppression of axillary bud outgrowth, delay in progression of developmental phases and poor root growth (Li et al. 2015). The other was *OsGIF1* (LOC_Os11g40100), which function in floral organogenesis in rice (Liu et al. 2014). LOC_Os11g40430 (*RLCK341*) is a cell wall-associated receptor kinase at a distance of 189 kb from *qGRS11* (1123451244, Chr11) (Vij et al. 2008). Gene LOC_Os12g29350 located about 20 kb away from the *qGRS12*(1217439378, Chr12) QTL, and it is an ATP-binding protein.

Based on gravitropic response speed, the fastest and slowest 3 extreme accessions were selected respectively from the 226 accessions. The fastest 3 extreme accessions were Zaohandao(F1), C22(F2) and Xianggu(F3), and the slowest were BLCO.BRANCO(S1), IPEACO162(S2) and Gaoyangdiandao(S3). The head SNP (1217439378) of *qGRS12* indicated different allele types between 2 groups of accessions, that all three slowest accessions with the SNP type of G, while all three fastest accessions with the SNP type of both A and G. The expression patterns of the five genes in these 6 accessions were shown in Fig. 3. This demonstrate that the expression level of LOC_Os12g29350 in the three accessions with the fastest gravitropic response speed was much lower than that in the three accessions with the slowest gravitropic response speed, indicating that this gene may be involved in the negative regulation of gravitropic response. The expression level of *ETR2*, *RPA49*, *OsGIF1* and *RLCK341*were low in the 6 accessions, and no significant difference was found between the two types of rice with opposite gravitropic response speed.

Discussion

This natural mapping population has been re-sequenced and soundly assessed on root morphological characteristics and drought related traits in our previous research(Lou et al. 2015; Ma et al. 2016). Therefore, it is a good resource to study the relations between gravitropic response and other important agronomic traits, and to explore the genes controlling roots gravitropism.

The gravitropic response of radicle is primarily controlled by genetic factors but is also significantly influenced by environmental conditions(Staves et al. 1997; Norton and Price 2009). Therefore, producing uniform and homogeneous growth environment is the precondition to carry on such experiments. However, it remains challenging to maintain an even soil environment to observe the hidden half from the soil. Despite there will be obvious difference of the root phenotype of seeds growing in the agar and soil, this agar-based screening system still may reflect a biological process, and it has been extensively used to study gravitropic responses (Müller et al. 1998; Debi et al. 2005b). Compared with the method used in other labs(Norton and Price 2009; Uga et al. 2013), we modified the measurement. Before the sowing, all the seeds have to be screened and cold soaked to stratify the seeds resulting in normalized germination vigour. Then, the bending angle was recorded after a shorter period of 1 hour after 90° rotation, that is not only to save time but also to detect early variation in the trait more profoundly. Because the first 1 hour is the most efficient time in gravi-bending, after then the gravitropic response reduces down rapidly. In order to gain an accurate representation of this trait, 50 seeds per accession were used in this study. After removal of non-germinating/infected seeds/ odd roots distribution a minimum of 20 valid samples per accession were assessed.

Root growth angle is an important trait that influences the ability of rice to avoid drought stress (Uga et al. 2015a, b), because the deep roots help plants to absorb water from deep soil. The gravitropic response determines the shape of the root system, especially in the vertical dimension. As we found in this study, the gravitropic response speed significantly positively correlated with the number of deep roots and the drought resistant index. The varieties that have better gravitropic response would therefore infer better drought tolerance. This means that the gravi-bending angle could become an early indicator to predict plants' drought resistance in a cost effective high-throughput manner. Here we highlight three varieties that express the desirable gravitropism trait along with drought resistance that could be promising resources for drought resistant breeding and research (supplementary table1, bold).

There are 11 known QTLs, that function in roots morphology and drought resistance, close to the 4 associated SNPs' physical position in the genome (http://qtaro.abr.affrc.go.jp/, ±1 Mb) (Table 5). One of them, QTL *11 − 1* controlling root thickness and number of roots past 100 cm near the associated SNP on chromosome 11, was found to be co-segregated with marker C189(Price 2002). And C189 also co-segregated with a radicle root morphology QTL - *SRM11* identified by Price et al in the same mapping population (Norton and Price 2009). This interval is therefore very important in the development of the root morphology. The other 10 QTLs are all related to drought resistance, three of which are located on chromosome11, and 7 were on the chromosome 12(Moncada et al. 2001; Bernier et al. 2007). We can conclude that the gravitropic response speed is corelated with drought resistance indeed.

Table 5
Co-localisation of QTLs for gravitropic response speed with previously identified QTLs of root and drought resistance.

QTL/Gene	Major category	Character	Chr	Genome Start	Genome End	Reference	Co-segregated marker
11 − 1	Morphological trait	Root thickness, number of roots past 100 cm	11	23732960	23734930	https://doi.org/10.1016/S0378-4290(02)00010-2	C189
gpl11.1	Drought Tolerance	Grains per plant	11	17246592	23651853	http://dx.doi.org/10.1007/s001220051616	
gw11.1	Drought Tolerance	1000-grain weight	11	17246592	23651853	http://dx.doi.org/10.1007/s001220051616	
gw11.1	Drought Tolerance	1000-grain weight	11	17246592	23651853	http://dx.doi.org/10.1007/s001220051616	
qtl12.1	Drought tolerance	Harvest index	12	9895474	17758636	https://doi.org/10.2135/cropsci2006.07.0495	RM7195-RM28166
qtl12.1	Drought tolerance	Panicle number m-2	12	9895474	17758636	https://doi.org/10.2135/cropsci2006.07.0495	RM7195-RM28166
qtl12.1	Drought tolerance	Flowering delay	12	9895474	17758636	https://doi.org/10.2135/cropsci2006.07.0495	RM7195-RM28166
qtl12.1	Drought tolerance	Grain yield	12	14257182	17546401	https://doi.org/10.2135/cropsci2006.07.0495	RM28048-RM511
qtl12.1	Drought tolerance	Biomass yield	12	14257182	17758636	https://doi.org/10.2135/cropsci2006.07.0495	RM28048-RM28166
qtl12.1	Drought tolerance	Plant heigh at maturity	12	14257182	17758636	https://doi.org/10.2135/cropsci2006.07.0495	RM28048-RM28166
qtl12.1	Drought tolerance	Drought response index	12	14257182	17546401	https://doi.org/10.2135/cropsci2006.07.0495	RM28048-RM511

Conclusion

This study modified the assessment method of radicle gravitropic response to be more efficient and precise. Using a natural population that already has plenty of root and drought resistance data, 4 significant associated QTLs were identified by GWAS. The trait of radicle gravitropic response speed found to be positively corelated with the deep roots and drought resistance. Five candidate genes have been chosen for further verification by qPCR in 6 extreme varieties, and LOC_Os12g29350 was higher expressed in the slow gravitropic response varieties. Some known QTLs of roots traits and drought resistance located nearby the associated QTLs identified in this study, which confirmed the close relationship between radicle gravitropism and the drought resistance.

Materials And Methods

Plant material

The association population used in this study is composed of 131 rice accessions from the mini-core collection of Chinese rice germplasm along with 95 rice accessions from core drought-resistance core rice germplasm collection. Of these 133 accessions are *Indica* rice and 93 accessions are *Japonica* rice. All rice seeds were provided by Shanghai Agrobiological Gene Center and harvested in the same season.

Evaluation of root gravitropism

Based on the root gravitropic curvature experiments described by Uga and Price et al.(Norton and Price 2009; Uga et al. 2013), the gravitropic response speed of radicle roots was measured with some modification. The growth direction of the root tip was first marked when the radicle root grew to 1–2 cm. The root tip was then rotated from the normal vertical axis to the horizontal axis by rotating the agarose plate by 90 degrees. Now under the effect of gravity, the growth direction of radicle root tip was observed, and its position marked again after a growth period of 1 hour. The angle between the two marked root tip growth directions was recorded as the gravitropic response speed. The ratio of radicle root length to the growth period, starting at the date of sowing, was recorded as the radicle root growth speed. The gravitropic response speed of each panel member was calculated after removing the outliers. At least 20 valid seeds for each accession were used to calculate for its average gravitropic response speed.

There were 7 steps to evaluation of root gravitropism, and the details were shown as Fig. 4. (1) Screening seeds. Using salt solution with the specific gravity at 1.1, the sterile and mouldy grains were removed, and about 50 uniform and full seeds were left for further experiment. (2) Sterilization. The seeds were sterilized with a 2.5% sodium hypochlorite solution for 15 minutes. Then, the seeds were rinsed with running water to clear the disinfectant away. (3) Cold soaking. Put the seeds in culture dishes lined with filter paper. Added some tap-water into the dishes to just submerge the seeds. Then covered the dishes with plastic wrap and stored in a 4°C refrigerator for 7 days to make seeds fully soaked and ready for germination. (4) Making agarose gel. The 0.8% agarose was boiled and cooled, then poured into cuboid plastic transparent germination board with a length of 12 cm, a width of 1.3 cm and a height of 10 cm, that have been placed in a container with depth more than 12 cm. The final height of the solid gel was about 9 cm. (5) Sowing. Before sowing, the seeds were placed in a 28 °C growth chamber for about 19 h. After the agar gel was completely cooled and solidified, germination plates were token out from the container and a lid with a length of 12.3 cm, a width of 1.8 cm, and a height of 1.3 cm was added at the bottom. The seeds with the same germination status were selected and sown evenly on the agar plate with embryos downward. 7 seeds per board, and more than 5 boards per accession. The accession name and sowing time were marked on left margin of the board. (6) Rotation after first lineation. After sowing, the plates were placed in an incubator at 28 °C without light. About 1 day later, when most of the radicle roots of the same accession grow to length at 1 to 2 cm, a line tangent to the growth direction of the root tip was marked on the board, and the current time was recorded. Then, the plates were rotated 90°and put into the growth chamber at 28 °C immediately. (7) Second lineation. After 1 hour of growth, another line tangent to the growth direction of the new root tip was marked. The root length and bending angle of the root tip was measured according the two tangent lines, and gravitropic response speed and growth speed of radicle roots were then calculated as described above.

Genome-wide association study

To perform basic statistical analysis on the phenotypic traits, we calculated the average value, standard deviation, coefficient of variation and correlation coefficient as well as to make frequency distribution graph.

The GWAS analysis conducted via the efficient mixed-model association (EMMA) method which is available within the Genome Association and Prediction Integrated Tool (GAPIT) R package(Lipka et al. 2012; Ma et al. 2016). In total, 1019,883 SNPs with the minor allele frequency (MAF) of > = 5% across the panel were used for GWAS. The model was adjusted using a kinship matrix and principal component eigenvectors to remove the confounding effects of hidden familial relationships within the population. A kinship matrix was created following the Van Raden protocol within GAPIT. For PC (principal component) adjustment the first 2 components were taken into account. A threshold of $-\log_{10}^{(P)}$ = 5.0 was used to declare the presence of associated QTLs (quantitative traits loci) for GWAS mapping.

According to the GWAS results, the annotation information of all genes within the range of 200 kb on the two flanks of the lead associated SNP loci was analyzed and the genes whose function are known to may be relate to roots development were selected. Additionally, a further selection step was carried out using transcriptome data of rice root(Lou et al. 2017), where genes that were highly expressed in roots were preferentially selected for further analysis.

RNA extraction and expression verification

To determine if selected candidate genes were differentially expressed between lines in the population, qPCR was conducted. A total of 6 accessions were used for expression verification, with three extreme accessions with the fastest and three with the slowest gravitropic response speed selected from the association population of 226 rice accessions. The radicle roots were sampled when they grew to 1–2 cm and were flash frozen in liquid nitrogen, then stored at -80 °C for later use. The total RNA of 10 pooled radicle roots was extracted using the TRNzol reagent (TIANGEN), and cDNA was synthesized by EasyScript®One-step gDNA Removal and cDNA Synthesis SuperMix following the manufacturers protocol (TransGen Biotech). Primer Premier v5.0 was used to design primers using the genome sequence of Nipponbare as a

sequence reference (**Supplementary Table 2**), the target fragment lengths were expected to be between 150 bp – 250 bp. Real time quantitative PCR was performed in 96-well plates with an Applied Biosystems CFX96 Real-Time PCR Detection System using TransStart Top Green qPCR SuperMix (TransGen Biotech). All assays were carried out in triplicate or greater and the expression levels were calculated using the relative quantitation method ($\Delta\Delta CT$).

Abbreviations

GWAS: genome-wide association analysis; QTLs: quantitative traits; SD: standard deviation; CV: coefficient variation; PH: Plant height; TN: tiller number; DR: deep roots number; SR: shallow roots number; TR: total roots number; RDR: ratio of deep roots; R/T: roots per tiller; GSR: growth speed of radicle; DRI: drought resistant index.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The genetic data of this population can be downloaded from http://www.ncbi.nlm.nih.gov/bioproject/PRJNA260762 and ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByRun/sra/SRR/SRR123/SRR12 39601.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

QJ L and LC conceptualized the study; QS L carried out phenotyping studies and curated the data; FJ F performed the GWAS analysis; QJ L and YN Y drafted the manuscript under the supervision of LJ L; LC and RJ reviewed and edited the final manuscript. The author(s) all read and approved the final manuscript.

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References

1. Amzallag GN, Vaisman J (2006) Influence of brassinosteroids on initiation of the root gravitropic response in Pisum sativum seedlings. Biol Plant 50:283–286. https://doi.org/10.1007/s10535-006-0021-5
2. Baldwin KL, Strohm AK, Masson PH (2013) Gravity sensing and signal transduction in vascular plant primary roots. Am J Bot 100:126–142. https://doi.org/10.3732/ajb.1200318
3. Band LR, Wells DM, Larrieu A et al (2012) Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. Proc Natl Acad Sci U S A 109:4668–4673. https://doi.org/10.1073/pnas.1201498109

4. Bennett MJ, Marchant A, Green HG et al (1996) Arabidopsis AUX1 gene: A permease-like regulator of root gravitropism. Science 273:948–950. https://doi.org/10.1126/science.273.5277.948

5. Bernier J, Kumar A, Ramaiah V et al (2007) A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. Crop Sci 47:507–518. https://doi.org/10.2135/cropsci2006.07.0495

6. Blancaflor EB, Hasenstein KH (1997) The organization of the actin cytoskeleton in vertical and graviresponding primary roots of maize. Plant Physiol 113:1447–1455. https://doi.org/10.1104/pp.113.4.1447

7. Blancaflor EB, Masson PH (2003) Plant Gravitropism. Unraveling the Ups and Downs of a Complex Process. Plant Physiol 133:1677–1690

8. Chang SC, Kim Y-S, Lee JY et al (2004) Brassinolide interacts with auxin and ethylene in the root gravitropic response of maize (Zea mays). Physiol Plant 121:666–673. https://doi.org/10.1111/j.0031-9317.2004.00356.x

9. Chen R, Hilson P, Sedbrook J et al (1998) The Arabidopsis thaliana AGRAVITROPIC 1 gene encodes a component of the polar-auxin-transport efflux carrier. Proc Natl Acad Sci U S A 95:15112–15117. https://doi.org/10.1073/pnas.95.25.15112

10. Cholodny N (1927) Wuchshormone and Tropismen bei den Pflanzen. Biol Zentralbl 47:604–629

11. Debi BR, Chhun T, Taketa S et al (2005a) Defects in root development and gravity response in the aem1 mutant of rice are associated with reduced auxin efflux. J Plant Physiol 162:678–685. https://doi.org/10.1016/j.jplph.2004.09.012

12. Debi BR, Taketa S, Ichii M (2005b) Cytokinin inhibits lateral root initiation but stimulates lateral root elongation in rice (Oryza sativa). J Plant Physiol 162:507–515. https://doi.org/10.1016/j.jplph.2004.08.007

13. Ding JP, Pickard BG (1993) Mechanosensory calcium-selective cation channels in epidermal cells. Plant J 3:83–110. https://doi.org/10.1046/j.1365-313x.1993.t01-4-00999.x

14. Dong Z, Jiang C, Chen X et al (2013) Maize LAZY1 mediates shoot gravitropism and inflorescence development through regulating auxin transport, auxin signaling, and light response. Plant Physiol 163:1306–1322. https://doi.org/10.1104/pp.113.227314

15. Ge L, Chen H, Jiang JF et al (2004) Overexpression of OsRAA1 causes pleiotropic phenotypes in transgenic rice plants, including altered leaf, flower, and root development and root response to gravity. Plant Physiol 135:1502–1513. https://doi.org/10.1104/pp.104.041996

16. Ge L, Chen R (2016) Negative gravitropism in plant roots. Nat Plants 2:1–4. https://doi.org/10.1038/nplants.2016.155

17. Haberlandt G (1900) Über die Perzeption des geotropischen Reizes. Ber Dtsch Bot Ges 18:261–272. https://doi.org/10.1111/J.1438-8677.1900.TB04908.X

18. Hada W, Bo Z, Cao WH et al (2009) The Ethylene Receptor ETR2 delays floral transition and affects starch accumulation in rice. Plant Cell 21:1473–1494. https://doi.org/10.1105/tpc.108.065391

19. Kim SK, Chang SC, Lee EJ et al (2000) Involvement of brassinosteroids in the gravitropic response of primary root of maize. Plant Physiol 123:997–1004. https://doi.org/10.1104/pp.123.3.997

20. Li P, Wang Y, Qian Q et al (2007) LAZY1 controls rice shoot gravitropism through regulating polar auxin transport. Cell Res 17:402–410. https://doi.org/10.1038/cr.2007.38

21. Li W, Yoshida A, Takahashi M et al (2015) SAD1, an RNA polymerase I subunit A34.5 of rice, interacts with Mediator and controls various aspects of plant development. Plant J 81:282–291. https://doi.org/10.1111/tpj.12725

22. Li Y, Dai X, Cheng Y, Zhao Y (2011) NPY genes play an essential role in root gravitropic responses in Arabidopsis. Mol Plant 4:171–179. https://doi.org/10.1093/mp/ssq052

23. Liao H, Yan X, Rubio G et al (2004) Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. Funct Plant Biol 31:959. https://doi.org/10.1071/FP03255

24. Lipka AE, Tian F, Wang Q et al (2012) GAPIT: Genome association and prediction integrated tool. Bioinformatics 28:2397–2399. https://doi.org/10.1093/bioinformatics/bts444

25. Liu H, Guo S, Xu Y et al (2014) OsmiR396d-regulated OsGRFs function in floral organogenesis in rice through binding to their targets OsJMJ706 and OsCR4. Plant Physiol 165:160–174. https://doi.org/10.1104/pp.114.235564

26. Lou Q, Chen L, Mei H et al (2017) Root transcriptomic analysis revealing the importance of energy metabolism to the development of deep roots in rice (Oryza sativa L.). Front Plant Sci 8:. https://doi.org/10.3389/fpls.2017.01314

27. Lou Q, Chen L, Mei H et al (2015) Quantitative trait locus mapping of deep rooting by linkage and association analysis in rice. J Exp Bot 66:4749–4757. https://doi.org/10.1093/jxb/erv246

28. Ma X, Feng F, Wei H et al (2016) Genome-wide association study for plant height and grain yield in rice under contrasting moisture regimes. Front Plant Sci 7:1–13. https://doi.org/10.3389/fpls.2016.01801

29. Mai CD, Phung NTP, To HTM et al (2014) Genes controlling root development in rice. Rice 7:1–11. https://doi.org/10.1186/s12284-014-0030-5

30. Moncada P, Martínez CP, Borrero J et al (2001) Quantitative trait loci for yield and yield components in an Oryza sativa × Oryza rufipogon BC2F2 population evaluated in an upland environment. Theor Appl Genet 102:41–52. https://doi.org/10.1007/s001220051616

31. Mullen JL, Wolverton C, Ishikawa H, Evans ML (2000) Kinetics of constant gravitropic stimulus responses in Arabidopsis roots using a feedback system. Plant Physiol 123:665–670. https://doi.org/10.1104/pp.123.2.665

32. Müller A, Guan C, Gälweiler L et al (1998) AtPIN2 defines a locus of Arabidopsis for root gravitropism control. EMBO J 17:6903–6911. https://doi.org/10.1093/emboj/17.23.6903

33. Němec B (1900) 28. Bohumil Němec: Ueber die Art der Wahrnehmung des Schwerkraftreizes bei den Pflanzen. Ber Dtsch Bot Ges 18:241–245. https://doi.org/10.1111/J.1438-8677.1900.TB04905.X

34. Norton GJ, Price AH (2009) Mapping of quantitative trait loci for seminal root morphology and gravitropic response in rice. Euphytica 166:229–237. https://doi.org/10.1007/s10681-008-9833-z

35. Price A (2002) QTLs for Root Growth and Drought Resistance in Rice. In: Molecular Techniques in Crop Improvement. Springer Netherlands, pp 563–584

36. Rigó G, Ayaydin F, Tietz O et al (2013) Inactivation of plasma membrane-localized CDPK-RELATED KINASE5 decelerates PIN2 exocytosis and root gravitropic response in Arabidopsis. Plant Cell 25:1592–1608. https://doi.org/10.1105/tpc.113.110452

37. Staves MP, Wayne R, Leopold AC (1997) The effect of the external medium on the gravitropic curvature of rice (*Oryza sativa*, Poaceae) roots. Am J Bot 84:1522–1529. https://doi.org/10.2307/2446613

38. Uga Y, Kitomi Y, Ishikawa S, Yano M (2015a) Genetic improvement for root growth angle to enhance crop production. Breed Sci 65:111–119. https://doi.org/10.1270/jsbbs.65.111

39. Uga Y, Kitomi Y, Yamamoto E et al (2015b) A QTL for root growth angle on rice chromosome 7 is involved in the genetic pathway of DEEPER ROOTING 1. Rice 8:8. https://doi.org/10.1186/s12284-015-0044-7

40. Uga Y, Sugimoto K, Ogawa S et al (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet 45:1097–1102. https://doi.org/10.1038/ng.2725

41. Vij S, Giri J, Dansana PK et al (2008) The receptor-like cytoplasmic kinase (OsRLCK) gene family in rice: Organization, phylogenetic relationship, and expression during development and stress. Mol Plant 1:732–750. https://doi.org/10.1093/mp/ssn047

42. Waidmann S, Ruiz Rosquete M, Schöller M et al (2019) Cytokinin functions as an asymmetric and anti-gravitropic signal in lateral roots. Nat Commun 10:1–14. https://doi.org/10.1038/s41467-019-11483-4

43. Went F (1926) On growth-accelerating substances in the coleoptile of Avena sativa. Proc K Ned Akad van Wet 30:10–19

44. Yoshihara T, Spalding EP, Iino M (2013) AtLAZY1 is a signaling component required for gravitropism of the Arabidopsis thaliana inflorescence. Plant J 74:267–279. https://doi.org/10.1111/tpj.12118

45. Amzallag GN, Vaisman J (2006) Influence of brassinosteroids on initiation of the root gravitropic response in Pisum sativum seedlings. Biol Plant 50:283–286. https://doi.org/10.1007/s10535-006-0021-5

46. Baldwin KL, Strohm AK, Masson PH (2013) Gravity sensing and signal transduction in vascular plant primary roots. Am J Bot 100:126–142. https://doi.org/10.3732/ajb.1200318

47. Band LR, Wells DM, Larrieu A et al (2012) Root gravitropism is regulated by a transient lateral auxin gradient controlled by a tipping-point mechanism. Proc Natl Acad Sci U S A 109:4668–4673. https://doi.org/10.1073/pnas.1201498109

48. Bennett MJ, Marchant A, Green HG et al (1996) Arabidopsis AUX1 gene: A permease-like regulator of root gravitropism. Science 273:948–950. https://doi.org/10.1126/science.273.5277.948

49. Bernier J, Kumar A, Ramaiah V et al (2007) A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. Crop Sci 47:507–518. https://doi.org/10.2135/cropsci2006.07.0495

50. Blancaflor EB, Hasenstein KH (1997) The organization of the actin cytoskeleton in vertical and graviresponding primary roots of maize. Plant Physiol 113:1447–1455. https://doi.org/10.1104/pp.113.4.1447

51. Blancaflor EB, Masson PH (2003) Plant Gravitropism. Unraveling the Ups and Downs of a Complex Process. Plant Physiol 133:1677–1690

52. Chang SC, Kim Y-S, Lee JY et al (2004) Brassinolide interacts with auxin and ethylene in the root gravitropic response of maize (Zea mays). Physiol Plant 121:666–673. https://doi.org/10.1111/j.0031-9317.2004.00356.x

53. Chen R, Hilson P, Sedbrook J et al (1998) The Arabidopsis thaliana AGRAVITROPIC 1 gene encodes a component of the polar-auxin-transport efflux carrier. Proc Natl Acad Sci U S A 95:15112–15117. https://doi.org/10.1073/pnas.95.25.15112

54. Cholodny N (1927) Wuchshormone und Tropismen bei den Pflanzen. Biol Zentralbl 47:604–629

55. Debi BR, Chhun T, Taketa S et al (2005a) Defects in root development and gravity response in the aem1 mutant of rice are associated with reduced auxin efflux. J Plant Physiol 162:678–685. https://doi.org/10.1016/j.jplph.2004.09.012

56. Debi BR, Taketa S, Ichii M (2005b) Cytokinin inhibits lateral root initiation but stimulates lateral root elongation in rice (Oryza sativa). J Plant Physiol 162:507–515. https://doi.org/10.1016/j.jplph.2004.08.007

57. Ding JP, Pickard BG (1993) Mechanosensory calcium-selective cation channels in epidermal cells. Plant J 3:83–110. https://doi.org/10.1046/j.1365-313x.1993.t01-4-00999.x

58. Dong Z, Jiang C, Chen X et al (2013) Maize LAZY1 mediates shoot gravitropism and inflorescence development through regulating auxin transport, auxin signaling, and light response. Plant Physiol 163:1306–1322. https://doi.org/10.1104/pp.113.227314

59. Ge L, Chen H, Jiang JF et al (2004) Overexpression of OsRAA1 causes pleiotropic phenotypes in transgenic rice plants, including altered leaf, flower, and root development and root response to gravity. Plant Physiol 135:1502–1513. https://doi.org/10.1104/pp.104.041996

60. Ge L, Chen R (2016) Negative gravitropism in plant roots. Nat Plants 2:1–4. https://doi.org/10.1038/nplants.2016.155

61. Haberlandt G (1900) Über die Perzeption des geotropischen Reizes. Ber Dtsch Bot Ges 18:261–272. https://doi.org/10.1111/J.1438-8677.1900.TB04908.X

62. Hada W, Bo Z, Cao WH et al (2009) The Ethylene Receptor ETR2 delays floral transition and affects starch accumulation in rice. Plant Cell 21:1473–1494. https://doi.org/10.1105/tpc.108.065391

63. Kim SK, Chang SC, Lee EJ et al (2000) Involvement of brassinosteroids in the gravitropic response of primary root of maize. Plant Physiol 123:997–1004. https://doi.org/10.1104/pp.123.3.997

64. Li P, Wang Y, Qian Q et al (2007) LAZY1 controls rice shoot gravitropism through regulating polar auxin transport. Cell Res 17:402–410. https://doi.org/10.1038/cr.2007.38

65. Li W, Yoshida A, Takahashi M et al (2015) SAD1, an RNA polymerase I subunit A34.5 of rice, interacts with Mediator and controls various aspects of plant development. Plant J 81:282–291. https://doi.org/10.1111/tpj.12725

66. Li Y, Dai X, Cheng Y, Zhao Y (2011) NPY genes play an essential role in root gravitropic responses in Arabidopsis. Mol Plant 4:171–179. https://doi.org/10.1093/mp/ssq052

67. Liao H, Yan X, Rubio G et al (2004) Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. Funct Plant Biol 31:959. https://doi.org/10.1071/FP03255

68. Lipka AE, Tian F, Wang Q et al (2012) GAPIT: Genome association and prediction integrated tool. Bioinformatics 28:2397–2399. https://doi.org/10.1093/bioinformatics/bts444

69. Liu H, Guo S, Xu Y et al (2014) OsmiR396d-regulated OsGRFs function in floral organogenesis in rice through binding to their targets OsJMJ706 and OsCR4. Plant Physiol 165:160–174. https://doi.org/10.1104/pp.114.235564

70. Lou Q, Chen L, Mei H et al (2017) Root transcriptomic analysis revealing the importance of energy metabolism to the development of deep roots in rice (Oryza sativa L.). Front Plant Sci 8:. https://doi.org/10.3389/fpls.2017.01314

71. Lou Q, Chen L, Mei H et al (2015) Quantitative trait locus mapping of deep rooting by linkage and association analysis in rice. J Exp Bot 66:4749–4757. https://doi.org/10.1093/jxb/erv246

72. Ma X, Feng F, Wei H et al (2016) Genome-wide association study for plant height and grain yield in rice under contrasting moisture regimes. Front Plant Sci 7:1–13. https://doi.org/10.3389/fpls.2016.01801

73. Mai CD, Phung NTP, To HTM et al (2014) Genes controlling root development in rice. Rice 7:1–11. https://doi.org/10.1186/s12284-014-0030-5

74. Moncada P, Martínez CP, Borrero J et al (2001) Quantitative trait loci for yield and yield components in an Oryza sativa × Oryza rufipogon BC2F2 population evaluated in an upland environment. Theor Appl Genet 102:41–52. https://doi.org/10.1007/s001220051616

75. Mullen JL, Wolverton C, Ishikawa H, Evans ML (2000) Kinetics of constant gravitropic stimulus responses in Arabidopsis roots using a feedback system. Plant Physiol 123:665–670. https://doi.org/10.1104/pp.123.2.665

76. Müller A, Guan C, Gälweiler L et al (1998) AtPIN2 defines a locus of Arabidopsis for root gravitropism control. EMBO J 17:6903–6911. https://doi.org/10.1093/emboj/17.23.6903

77. Němec B (1900) 28. Bohumil Němec: Ueber die Art der Wahrnehmung des Schwerkraftreizes bei den Pflanzen. Ber Dtsch Bot Ges 18:241–245. https://doi.org/10.1111/J.1438-8677.1900.TB04905.X

78. Norton GJ, Price AH (2009) Mapping of quantitative trait loci for seminal root morphology and gravitropic response in rice. Euphytica 166:229–237. https://doi.org/10.1007/s10681-008-9833-z

79. Price A (2002) QTLs for Root Growth and Drought Resistance in Rice. In: Molecular Techniques in Crop Improvement. Springer Netherlands, pp 563–584

80. Rigó G, Ayaydin F, Tietz O et al (2013) Inactivation of plasma membrane-localized CDPK-RELATED KINASE5 decelerates PIN2 exocytosis and root gravitropic response in Arabidopsis. Plant Cell 25:1592–1608. https://doi.org/10.1105/tpc.113.110452

81. Staves MP, Wayne R, Leopold AC (1997) The effect of the external medium on the gravitropic curvature of rice (*Oryza sativa*, Poaceae) roots. Am J Bot 84:1522–1529. https://doi.org/10.2307/2446613

82. Uga Y, Kitomi Y, Ishikawa S, Yano M (2015a) Genetic improvement for root growth angle to enhance crop production. Breed Sci 65:111–119. https://doi.org/10.1270/jsbbs.65.111

83. Uga Y, Kitomi Y, Yamamoto E et al (2015b) A QTL for root growth angle on rice chromosome 7 is involved in the genetic pathway of DEEPER ROOTING 1. Rice 8:8. https://doi.org/10.1186/s12284-015-0044-7

84. Uga Y, Sugimoto K, Ogawa S et al (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet 45:1097–1102. https://doi.org/10.1038/ng.2725

85. Vij S, Giri J, Dansana PK et al (2008) The receptor-like cytoplasmic kinase (OsRLCK) gene family in rice: Organization, phylogenetic relationship, and expression during development and stress. Mol Plant 1:732–750. https://doi.org/10.1093/mp/ssn047

86. Waidmann S, Ruiz Rosquete M, Schöller M et al (2019) Cytokinin functions as an asymmetric and anti-gravitropic signal in lateral roots. Nat Commun 10:1–14. https://doi.org/10.1038/s41467-019-11483-4

87. Went F (1926) On growth-accelerating substances in the coleoptile of Avena sativa. Proc K Ned Akad van Wet 30:10–19

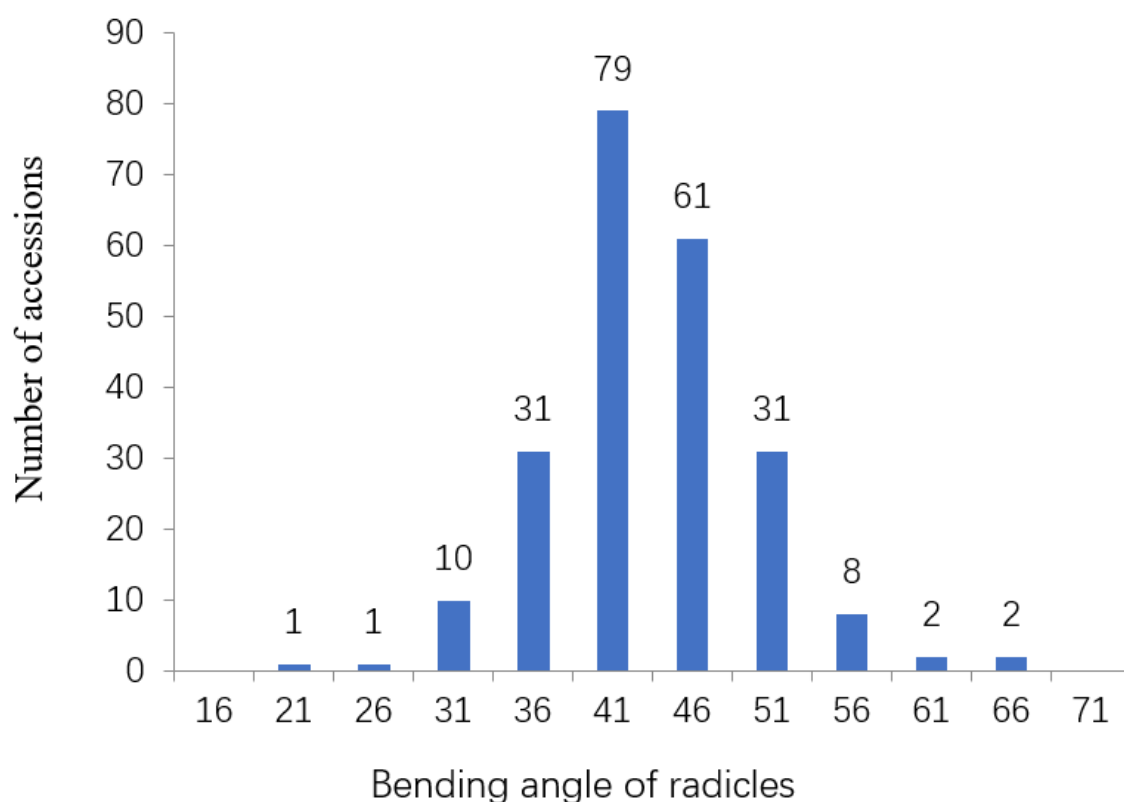
88. Yoshihara T, Spalding EP, Iino M (2013) AtLAZY1 is a signaling component required for gravitropism of the Arabidopsis thaliana inflorescence. Plant J 74:267–279. https://doi.org/10.1111/tpj.12118

Figures

Figure 1

Frequency distribution of gravitropic response in the natural rice population. The X axis is the bending angle of radicle for the first hour after rotation 90.

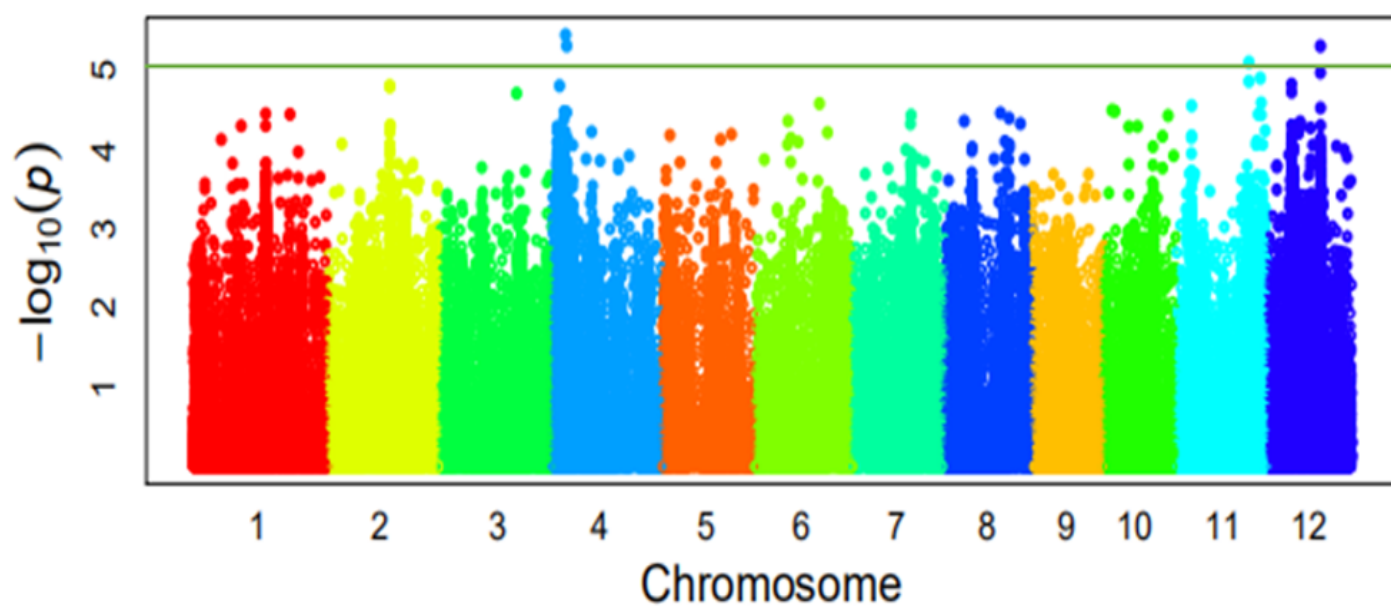



Figure 2

Manhattan plots of GWAS of gravitropic response speed. The threshold is p=10-5.

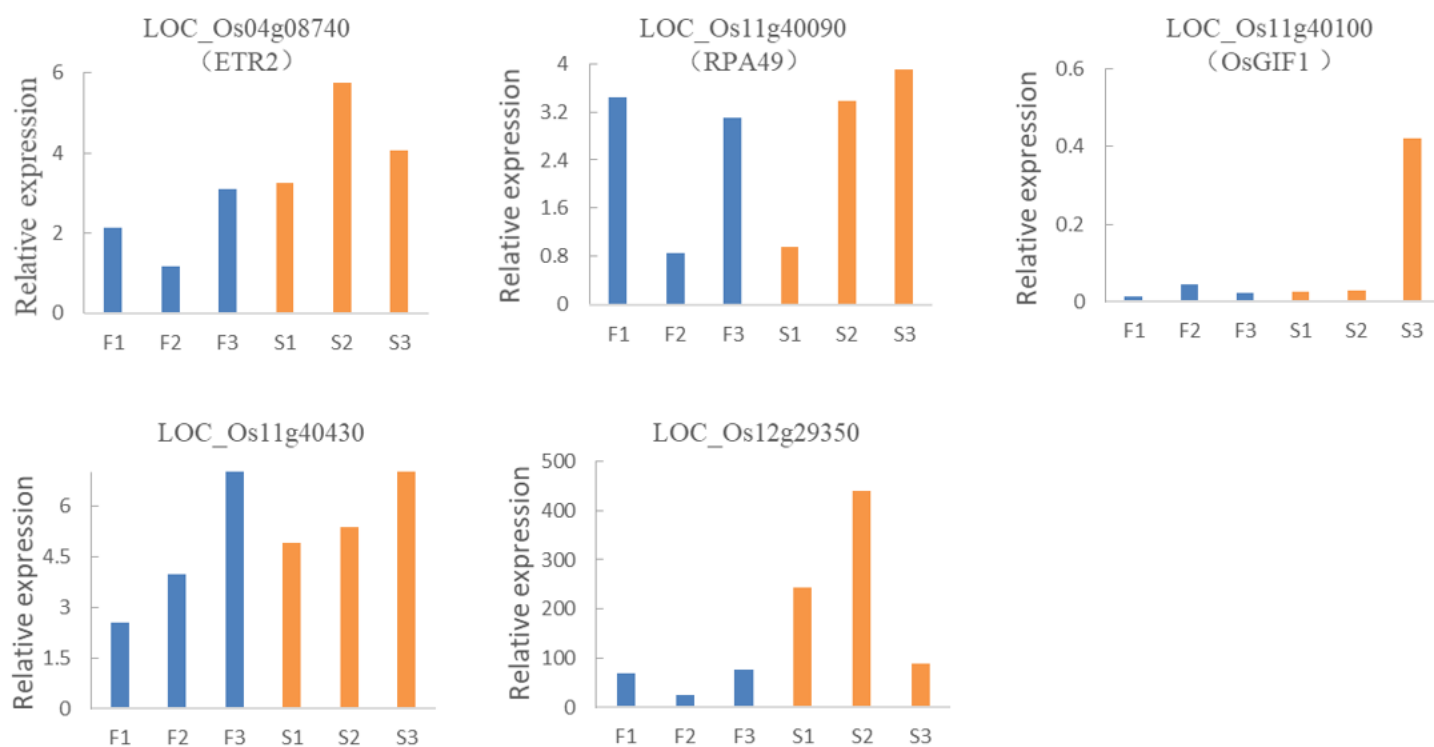



Figure 3

Relative expression of 5 candidate genes in 6 extreme rice accessions. 3 fast gravitropic response varieties indicated with blue, the 3 slow gravitropic response varieties indicated with orange.

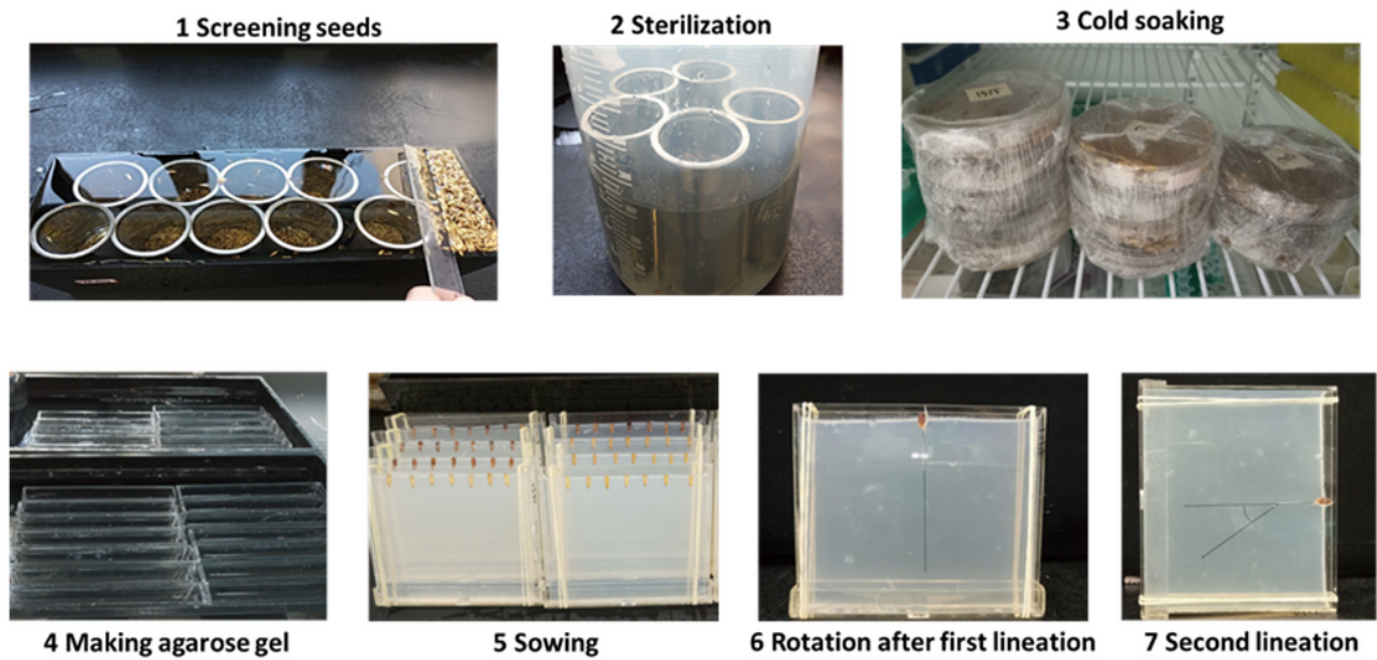



Figure 4

The detailed process of the experiment measuring gravitropic response speed.