

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

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## Research Article

**Keywords:** rice, gravitropism, gravitropic response, growth angle of radicle, genome-wide association study (GWAS)

**Posted Date:** March 18th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-64873/v2>

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**Version of Record:** A version of this preprint was published at Plant and Soil on August 28th, 2021. See the published version at <https://doi.org/10.1007/s11104-021-05087-5>.

# Abstract

**Aims:** Gravitropism is one of the primary determinants of root development, facilitating root penetration into soil and subsequent absorption of water and nutrients. To study the gravitropism of the radicle roots, we conducted this research.

**Methods:** The gravitropism of 226 Chinese rice micro-core accessions and drought-resistant core accessions were assessed through the modified gravity-bending experiment and genome-wide association analysis (GWAS) was used to map the associated QTLs.

**Results:** The average value of gravitropic response speed of radicle roots was 41.05°/h from 16.77°/h to 62.83°/h. Significant difference ( $p < 0.002$ ) in gravity response speed between *Indica* (42.49°/h) and *Japonica* (39.71°/h) subspecies was found. The gravitational response speed of radicle roots was significantly positively correlated with the number of deep roots ( $r = 0.16$ ), the growth speed of radicle roots ( $r = 0.21$ ) and the drought resistance coefficient ( $r = 0.14$ ).

**Conclusions:** In total, 3 QTLs (quantitative traits) associated with gravitropic response speed were identified on chromosome 4, 11 and 12. There are some known QTLs relating to roots traits and drought resistance located nearby the QTLs identified here, which confirms the close relationship between radicle gravitropism and the drought resistance. From within these intervals, 5 candidate genes were screened for qPCR in 6 extreme rice varieties, demonstrating that gene LOC\_Os12g29350 may regulate gravitropism negatively and confirming its 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 attempts 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, gravity-bending is the result of differential accumulation of auxin on opposite sides of the elongation zone, causing differential growth and tip curvature. In addition, the mechanosensitive ion channel hypothesis also could explain some parts of the gravitropism (Ding and Pickard 1993).

Multiple 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; Zhang et al. 2019). 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). The *AGR1* gene involved in root gravitropism could increase root-growth sensitivity to auxin and decrease sensitivity to ethylene in *Arabidopsis* (Chen et al. 1998). The *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, an automated camera that could help researchers to dissect the gravity-response(Mullen et al. 2000).

Despite progress made in recent decades, processes involved in positive root gravitropic response in the root tip remain largely unclear in rice. Since 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 et al (Uga et al. 2013) and Norton and Price(Norton and Price 2009) demonstrate. But there are usually large variances among the results of gravitropic response evaluation. To reduce the variance, we had carried out multiple tests to optimise this methodology. Here, using the optimized method we measured gravitropic response speed of an association mapping population consisting 226 core rice accessions and have been in depth studied on drought resistance (Lou et al. 2015; Ma et al. 2016), and identified several QTLs related to gravitropic response that can be deployed into marker assisted selection programmes.

## Results

### Gravitropic response speed of the natural population

In this study, we detected the bending angle of radicle roots of 226 core rice accessions representing their gravitropic response speed (Table1). 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 of gravitropic response speed of radicle roots.

**Table 1. Gravitropic response speeds of the association mapping population.** The ‘t-test’ is value of student’s test of the gravitropic response speed between *Indica* and *Japonica*.

|                 | Number of accessions | Means°/h | Range°/h    | Standard deviationSD, °/h | Coefficient variation (CV, %) | t-test |
|-----------------|----------------------|----------|-------------|---------------------------|-------------------------------|--------|
| <i>Indica</i>   | 133                  | 42.49    | 16.77-62.83 | 6.41                      | 15.32                         | 0.002  |
| <i>Japonica</i> | 93                   | 39.71    | 21.73-61.64 | 6.30                      | 15.77                         |        |
| Total           | 226                  | 41.05    | 62.83-16.77 | 6.42                      | 15.63                         |        |

Notable differences of gravitropic response speed were found between *Indica* and *Japonica* rice (Table1). 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 mean of gravitropic response speed of *Indica* (42.49°/h) was faster than that of *Japonica* (39.71°/h). Likewise, the range of variation in *Indica* (46.06°/h) was larger than that in *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 between *Indica* and *Japonica* subspecies, and the gravity response speed of *Indica* radicle roots was significantly faster than that of *Japonica* rice.

### 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 an our 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 of DRI was obtained from a previous study in our laboratory(Ma et al. 2016). 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 significance level  $\alpha=0.05$ , 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  $P<0.01$ .

Then, 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/SRR123\\_39601](ftp://ftp-trace.ncbi.nlm.nih.gov/sra/sra-instant/reads/ByRun/sra/SRR/SRR123/SRR123_39601) (Lou et al. 2015).

GWAS was carried out to associate gravitropic response speed to a responsible genomic location using the EMMA model. 3 significantly associated QTLs were detected at the threshold of  $p=10^{-5}$  (**Fig. 2, Table 3**). A QTL named *qGRS4* was mapped on the chromosome 4 (4774373bp), and other 2 QTLs named *qGRS11* and *qGRS12* located on each of chromosomes 11 and 12 (23451244bp and 17439378bp, respectively).

**Table 3. SNP loci related to gravitational response identified by GWAS**

| NO. | QTL           | Lead SNP (bp) | Chr | Maf  | LD Range | P value  |
|-----|---------------|---------------|-----|------|----------|----------|
| 1   | <i>qGRS4</i>  | 404774373     | 4   | 0.08 | 180Kb    | 4.83E-06 |
| 2   | <i>qGRS11</i> | 1123451244    | 11  | 0.07 | 580Kb    | 7.77E-06 |
| 3   | <i>qGRS12</i> | 1217439378    | 12  | 0.20 | 1Mb      | 4.87E-06 |

Chr, chromosome; Maf, minor allele frequency; LD Range, linkage disequilibrium decay distance at  $r^2>0.65$ .

#### qPCR of candidate genes of gravitropic response

According to the GWAS results and based on the annotation information from the Rice Genome Annotation Project (<http://rice.plantbiology.msu.edu/>) and our previous transcriptome data of rice root (Lou et al. 2017), 5 candidate genes in close proximity to the most associated SNPs that may relate with roots gravitropic response were chosen for further expression analysis (**Table 4**). From Chromosome 4 to 12, they are an ethylene receptor, an A49-like RNA polymerase I associated factor family protein, a GRF-interacting factor 2, a wall-associated receptor kinase-like 2 precursors and an ATP binding protein.

**Table 4. Candidate genes of gravitropic response speed and their annotations**

| QTL           | Candidate Gene ID                 | Chromosome | Annotation   |
|---------------|-----------------------------------|------------|--|
| <i>qGRS4</i>  | LOC_Os04g08740<br>( <i>ETR2</i> ) | 4          | Ethylene receptor  |
| <i>qGRS11</i> | LOC_Os11g40090 ( <i>RPA49</i> )   | 11         | A49-like RNA polymerase I associated factor family protein |
|               | LOC_Os11g40100 ( <i>OsGIF1</i> )  | 11         | GRF-interacting factor 2                                   |
|               | LOC_Os11g40430 ( <i>RLCK341</i> ) | 11         | Wall-associated receptor kinase-like 2 precursors          |
| <i>qGRS12</i> | LOC_Os12g29350                    | 12         | 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 lead SNP (1217439378) of *qGRS12* indicated different allele types between 2 groups of accessions, that all three slowest accessions with the G allele, while all three fastest accessions with the alleles of both A and G. The expression patterns of the five genes in these 6 accessions were shown in **Fig. 3**. Demonstrating 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* was 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; Wu 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 out such experiments. However, it remains challenging to maintain an even soil environment to observe the hidden half from the soil. Although there will be obvious differences in the root phenotype of seeds growing in the agar and soil, this agar-based screening system represents more unbiased approach to assess 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 studies (Norton and Price 2009; Uga et al. 2013), we modified the measurement protocol. Before the sowing, all the seeds were screened carefully and cold soaked to normalize their germination vigour. The bending angle was then 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. Since the first 1 hour is the most efficient time in gravity-bending, after this time point the gravitropic response reduces rapidly. 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, 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 gravity-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).

Near the lead associated SNP loci, five candidate genes were selected for further study. A serine/threonine kinase, *ETR2* (LOC\_Os04g08740), was found at a distance of about 40kb from the lead SNP of *qGRS4* (4774373, 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). A further 2 genes were found at an interval within 52 kb from *qGRS11* (23451244,

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 189kb from *qGRS11* (23451244, Chr11) (Vij et al. 2008). Gene LOC\_Os12g29350, an ATP-binding protein, was located approximately 20kb away from the *qGRS12*(17439378, Chr12) QTL. In future studies, we will detect their expression on the different positions of radicle to verify their function in gravity response definitively.

There are 11 known QTLs, that function in roots morphology and drought resistance, close to the 3 associated SNPs' physical position in the genome (<http://qtaro.abr.affrc.go.jp/>) (Table 5). One of them, QTL *11-1* controlling root thickness and number of roots past 100cm near the associated SNP on chromosome 11, was found to be co-segregated with marker C189 (Price 2002). And C189 also co-segregated with root-penetration QTLs (Price et al. 2000) and a radicle root morphology QTL - *SRM11* both 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 seven QTLs were on the chromosome 12(Moncada et al. 2001; Bernier et al. 2007). This work provides further evidence to the hypothesis that gravitropic response speed is correlated with drought resistance are intrinsically related traits.

**Table 5. Co-localisation of QTLs for gravitropic response speed with previously identified QTLs of root and drought resistance.** The information of the reported QTLs is searched from <http://qtaro.abr.affrc.go.jp/>

| QTL/Gene       | Major category      | Character                                  | Chr | Genome Start | Genome End | Reference   | Co-segregated marker    | Gravitropic response speed QTLs | Associated SNP position |
|----------------|---------------------|--|-----|--------------|------------|---|-------------------------|---------------------------------|-------------------------|
| <i>11-1</i>    | Morphological trait | Root thickness, number of roots past 100cm | 11  |              |            | <a href="https://doi.org/10.1016/S0378-4290(02)00010-2">https://doi.org/10.1016/S0378-4290(02)00010-2</a> | C189(23732960-23734930) | <i>qGRS11</i>                   | Chr11-23451244          |
| <i>gpl11.1</i> | Drought Tolerance   | Grains per plant                           | 11  | 17246592     | 23651853   | <a href="http://dx.doi.org/10.1007/s001220051616">http://dx.doi.org/10.1007/s001220051616</a>             |                         |                                 |                         |
| <i>gw11.1</i>  | Drought Tolerance   | 1000-grain weight                          | 11  | 17246592     | 23651853   | <a href="http://dx.doi.org/10.1007/s001220051616">http://dx.doi.org/10.1007/s001220051616</a>             |                         |                                 |                         |
| <i>gw11.1</i>  | Drought Tolerance   | 1000-grain weight                          | 11  | 17246592     | 23651853   | <a href="http://dx.doi.org/10.1007/s001220051616">http://dx.doi.org/10.1007/s001220051616</a>             |                         |                                 |                         |
| <i>qtl12.1</i> | Drought tolerance   | Harvest index                              | 12  | 9895474      | 17758636   | <a href="https://doi.org/10.2135/cropsci2006.07.0495">https://doi.org/10.2135/cropsci2006.07.0495</a>     | RM7195-RM28166          | <i>qGRS12</i>                   | Chr12-17439378          |
| <i>qtl12.1</i> | Drought tolerance   | Panicle number m-2                         | 12  | 9895474      | 17758636   | <a href="https://doi.org/10.2135/cropsci2006.07.0495">https://doi.org/10.2135/cropsci2006.07.0495</a>     | RM7195-RM28166          |                                 |                         |
| <i>qtl12.1</i> | Drought tolerance   | Flowering delay                            | 12  | 9895474      | 17758636   | <a href="https://doi.org/10.2135/cropsci2006.07.0495">https://doi.org/10.2135/cropsci2006.07.0495</a>     | RM7195-RM28166          |                                 |                         |
| <i>qtl12.1</i> | Drought tolerance   | Grain yield                                | 12  | 14257182     | 17546401   | <a href="https://doi.org/10.2135/cropsci2006.07.0495">https://doi.org/10.2135/cropsci2006.07.0495</a>     | RM28048-RM511           |                                 |                         |
| <i>qtl12.1</i> | Drought tolerance   | Biomass yield                              | 12  | 14257182     | 17758636   | <a href="https://doi.org/10.2135/cropsci2006.07.0495">https://doi.org/10.2135/cropsci2006.07.0495</a>     | RM28048-RM28166         |                                 |                         |
| <i>qtl12.1</i> | Drought tolerance   | Plant height at maturity                   | 12  | 14257182     | 17758636   | <a href="https://doi.org/10.2135/cropsci2006.07.0495">https://doi.org/10.2135/cropsci2006.07.0495</a>     | RM28048-RM28166         |                                 |                         |
| <i>qtl12.1</i> | Drought tolerance   | Drought response                           | 12  | 14257182     | 17546401   | <a href="https://doi.org/10.2135/cropsci2006.07.0495">https://doi.org/10.2135/cropsci2006.07.0495</a>     | 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, 3 significant associated QTLs were identified by GWAS. The trait of radicle gravitropic response speed found to be positively correlated 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 this population, 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 et al (Uga et al. 2013) and Norton and Price et al. (Norton and Price 2009), 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 viable 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 taken 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 2cm, 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 was 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). This mapping population has been used in our previous studies for a lot of other important agronomic traits, so its molecular data and analysis method have been ready-made already (Lou et al. 2015; Wu et al. 2015; Ma et al. 2016). Totally, 3038555 SNPs with the minor allele frequency (MAF) of  $\geq 5\%$  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 family relationships within the population and population structure. A kinship matrix was created following the Van Raden protocol within GAPIT, and the first 2 components were used in principal components (PC) adjustment. The 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 for further expression test. The linkage disequilibrium (LD) decay distance in *Indica* and *Japonica* extends to between  $\sim 75$  and 200 kb, so the annotated genes in the range of 200 kb were considered here (Mather et al. 2007). 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, using three extreme accessions with the fastest and three with the slowest gravitropic response speed that had been 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  $-80^{\circ}\text{C}$  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 150bp - 250bp. 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). Actin gene was used as reference gene here. All assays were carried out in triplicate or greater and the expression levels were calculated using the relative quantitation method ( $\Delta\Delta\text{CT}$ ).

## 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; LD: linkage disequilibrium.

## Declarations

**Funding** This work was supported by Shanghai Agriculture Applied Technology Development Program, China (G2015060101), and the Shanghai Natural Science Foundation (19ZR1446900, 18ZR1433300).

**Conflicts of interest/Competing interests** The authors have no conflicts of interest to declare that are relevant to the content of this article

**Ethics approval** Not applicable

**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->

**Code availability** Not applicable

**Authors' contributions** QiaoJun Lou and Liang Chen conceptualized the study; QingSong Li carried out phenotyping studies and curated the data; FangJun Feng performed the GWAS analysis; QiaoJun Lou and YuNan Yang drafted the manuscript under the supervision of LiJun Luo; Liang Chen and Ryan Joynson reviewed and edited the final manuscript. The authors all read and approved the final manuscript.

**Acknowledgements** We thank the Huazhong Agriculture University that provided the SNP information of Chinese core rice accessions.

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## Figures

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### Figure 1

Frequency distribution of gravitropic response speed in the 226 natural rice population. The X axis indicates the first hour's bending angle of radicle after 90° rotation.

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### Figure 2

Manhattan plots of GWAS of gravitropic response speed. The threshold to declare significance is  $p=10^{-5}$ .

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### Figure 3

Relative expression of 5 candidate genes in 6 extreme rice accessions. The left 3 blue columns indicates the fast gravitropic response varieties named F1, F2 and F3, the right 3 orange columns indicates the slow gravitropic response varieties named S1, S2 and S3.

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#### Figure 4

The detailed process of the experiment measuring gravitropic response speed. There are 7 steps to finish the evaluation of gravitropic response speed, and each panel in the figure indicates one step of the method.

## Supplementary Files

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