A facile and cheaper method to measure root angle of rice and wheat

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ABSTRACT

Genetic improvement in root system architecture (RSA) is an important trait to achieve stability of grain yield in water-deficit stress environments. Deep rooting is a major component trait that contributes to dehydration avoidance under drought in most crops. Due to the difficulty in the phenotyping for deep rooting, genetic variability in deep rooting is less exploited in genetic improvement programme. Root angle is a surrogate for deep rooting. Here, we report a novel method to measure root angle which is easy, robust and cheaper. By using this method, 56 wheat and 29 rice genotypes were phenotyped for root angle under field conditions. Wide variability in root angle was observed among rice and wheat genotypes. In rice, about 58% of crown roots were having shallow angle (<40°), while in wheat, about 67% of the crown roots were in deep rooting angle (>60°). This method could categorize the previously known shallow rooted rice cv. IR6 4 in to shallow root category with >90% of its crown root with an angle of <40°. Among the rice genotypes, BAM 2574, produced >60% of crown roots with >60° and identified as deep-rooted genotype. In wheat CL 3791, CL 3817 and CL 3823 were identified as deep-rooted genotypes. This method is suitable for high throughput phenotyping of root angle in natural field conditions.

Key words: Root system architecture, Rice, Wheat, Root angle phenotyping, Drought tolerance

Cereals are most important component of human diet providing 50% of human calorie intake. Although cereal demand is increasing due to increasing population, the relative rates of yield increase in cereals are declining. Abiotic stress particularly moisture deficit stress restricts the productivity of cereals globally. To cope-up with the food demand for projected population increase commensurate increase in crop yields in drought-prone areas is necessary.

Crop productivity is dependent on the availability of moisture and mineral nutrients in rhizosphere and uptake by plants. Spatial and temporal distribution of roots in rhizosphere, i.e. root system architecture (RSA), determines the ability of plant to extract moisture and mineral nutrients from the soil. Root angle and length are important components of RSA that determines rooting depth. Although variability in root length have been extensively studied in cereals, only limited efforts have been made to identify the genetic variability in root angle (Uga et al. 2009). Root angle determines the volume and area of soil from which root can acquire moisture and minerals. To acquire minerals/agricultural inputs such as P shallow rooting can be helpful (Hanzawa et al. 2013). Moreover, quite a lot number of QTLs of root traits overlap with those of moisture and nutrient uptake and yield (Atkinson et al. 2015). Therefore, screening genotypes for identification of variability in root angle is a way forward to identify QTLs/gene for root angle. For screening we need easy, robust and inexpensive method, especially when we have to screen large number of germplasm. Currently soil based methods such as basket method (Oyanagi et al. 1993), soil sampling (Trachsel et al. 2011), X-ray computed tomography (Mooney et al. 2012) and rhizotrons (Nagel et al. 2012) and non-soil techniques such as gel chamber (Bengough et al. 2004), hydroponics (Mathieu et al. 2015), rhizosoles (Le Marié et al. 2014), Clearpot (Richard et al. 2015) and Growth pouch method (Richard et al. 2015) are available to measure root angle.

In this study we have developed a simple, easy, robust and inexpensive method, to measure root angle of plants grown in natural soil in field and laboratory conditions. We have used a modified protractor drawn on the plywood board for measuring root angle. Here we use angle range of 0°-90°. 0° implies the horizontal or parallel to soil surface and 90° is perpendicular to soil surface and parallel to the direction of gravity.

MATERIALS AND METHODS

Rice: Twenty-nine rice genotypes were sown on 7th October 2015 in the research farm of Division of Plant Physiology of IARI, New Delhi. Plastic mesh baskets
were buried in the soil 20 cm apart with RBD design. The dimensions of the circular mesh basket were 19 cm upper diameter, 15 cm base diameter and 5.6 cm height. Three seeds were sown in the center of the basket. Finally, healthiest among three seedlings was allowed to grow. The soil type was sandy loam soil. Fifty percent of the recommended dose of fertilizers (120:60:40 N:P:K respectively) was applied as basal dose. Soil was kept near saturation by regular irrigation. After 37 days of germination, plants were uprooted by pulling up the plastic pots which helped to uproot the plant roots completely without disturbing the root system architecture of the plants. Roots were washed gently and soil was removed. Root angle was measured.

Wheat: Fifty-six wheat genotypes were sown on 17th November 2015 in five replications in the research farm of the Division of Genetics, IARI, New Delhi. The plot size was 1.2 m × 6 m having six rows with row-row spacing of 20 cm. A seed rate of 100 kg/ha was used. The recommended dose of fertilizers (120: 80:60 N: P: K, respectively) and irrigation was given to raise a healthy crop. The soil type was sandy loam. Five plants per genotype per replicate were sampled 37 days after germination. A 15 cm × 15 cm area of soil along with the plant in the center of the square was excavated from the soil. The roots were carefully washed with water and the root angle and number were measured.

Table 1. Details of rice genotypes phenotyped for root angle

| Genotype  | Parentage/Accession | Remarks                                                                 |
|-----------|---------------------|-------------------------------------------------------------------------|
| APO       | UPL RI 5/1R 12979-24-1 (BROWN) | Released as IR 55423-01 (NSIC RC 9). Upland, Drought tolerant, Rainfed |
| BAM-1098  | IC346589            |                                                                          |
| BAM-1264  | IC305692            |                                                                          |
| BAM-2574  | IC4593689           |                                                                          |
| BAM-3181  | IC554322            |                                                                          |
| BAM-328   | ICIC132765          |                                                                          |
| BAM-4138  | ICIC330600          |                                                                          |
| CR143-2-2 | One of the parental line of variety Sneha (IET 11482). Sneha variety derived from cross of Annada/CR 143-2-2 (CR 19-2) | Sneha variety is released in Orissa for rainfed upland conditions. Early variety with 50 days to 50% percent flowering with 3.5 t/ha yield. |
| BAM -5449 | IC21340             |                                                                          |
| BAM-766   | ICRCPL 1-3C         |                                                                          |
| IR 64     | GAM PAI 30-12-15/Taichung Native 1 | IR-5857-33-2-1 x IR-2061-465-Semi dwarf (100 cm), grains: LS, white, resistant to blast, BLB, RTV, BPH, GLH, WPBH & GM; Yield: 58 Q/ha. |
| CR 2624   | UPL RI 5/IR 12979-24-1 | CR2624 -IR5523-01 (IET 21214) released as Pyari (CR Dhan 200) in 2011 as Aerobic rice for Orissa state. 85-90 days to 50% flowering, short bold grains and 4.5 t/ha yield. |
| Moroberekan | Germplasm introduced from Guinea to IRRI Gene bank | Drought tolerant Variety from Guinea, West Africa, also known in the international literature as a genotype of good root development, good root penetration capacity on soil, tolerant to drought and toxic aluminum. |
| MTU1010   | Krishnaveni × IR-64 | Semi-dwarf (108 cm), grains: LS, white, AWA, resistant to blast & tolerant to BPH; Yield: 74 Q/ha. Also known as Cottonsora Sannalu (IET 15644), released in 2000 for AP state for Irrigated early variety possessing Long slender grains, 90 days to 50% flowering and 6.7 t/ha yield. |
| Nagina 22 | A selection from Rajbhog | Tall, grains: short bold, susceptible to blast, BLB and resistant to drought, Yield: 20-25 Q/ha. Released for UP state in 1978 for rainfed upland variety with 70 days to 50% flowering, short bold grains with 2.5 t/ha yield. |
| Rasi      | TAICHUNG NATIVE 1/CO 29 | Upland variety IET 1444 |
| Shabaghidhan | IR 55419-4*2/WAY RAREM | IR 55419-04*2 Way Rarem (IR 55419-04 (IR 12979-24-1 (Brown)/UPLRI5) from BC2F4 S3. Released in India as drought tolerant variety in 2010 for rainfed upland, rainfed shallow low land ecosystem. It is also released in Bangladesh as BRRI Dhan 56 for drought. |

Contd.
Table 1 (Concluded)

| Genotype        | Parentage/Accession | Remarks                                                                 |
|-----------------|---------------------|-------------------------------------------------------------------------|
| Swarna          | VASISTHA/MAHSURI    | Also known as MTU 7029; IET 7041. Released in 1979 in AP state. Late duration variety with 125 days to 50% flowering. Released for rainfed shallow lowland ecosystem. Tolerant to BLB. |
| WAY rarem       | IR 9669/B 981       | Drought related                                                          |
| BAM-3154        | IC IC458319         |                                                                         |
| Vandana         | CO 22 x KALAKERI    | IET 12304 (RR 167-982), Released for Bihar and Orissa                   |
| IR-77298-14-1-2-10 | IR 64 (WH)/ADAY SEL//3*IR 64 | Single plant selection from BC3 (F5, S4)                                |
| IR-83383-B-B-129-4 | IR 72022-46-2-3-3-2/IR 57514-PMI 5-B-1-2 | Single plant selection                                                  |
| IR-83388-B-B-108-3 | IR 72022-46-2-3-3-2/SWARNA |                                                                         |
| IR-87707-445-B-B-B | IR 77298-14-1-2-10/IR 77298-5-6-11 | Drought and aerobic rice.                                                |
| IR-87707-445-B-B-B | IR 77298-14-1-2-10  |                                                                         |
| IR-87707-446-B-B-B | IR 77298-14-1-2-10/IR 77298-5-6-11 |                                                                         |
| Nerica-L-44     | WAB 1291/5*IR 64    | Africa Rice, Africa Rice center, Bouake                                  |
| Pusa Basmati 1121 | Pusa 3A/Haryana Basmati | Derived from BC5 F4, S3                                                  |
| Pusa Basmati –SN 12 | Derived from Pusa Basmati 1121 | Semi dwarf, yield 5.5 tonnes/ha                                           |
| Nerica L-42     | WAB 1291/4*IR 64    | Africa Rice, Africa Rice centre, Bouake                                  |

Root angle measurement: The plant was cut 5-10 cm above the ground level. Pullout the plastic mesh pot (in rice) or dig out the root by using shovel without causing damage to root near the base of the stem. Roots were washed gently without damaging and the soil particles were removed. To reduce the weight of the roots, roots were cut at approximately 2-3 cm distance from the base of the stem. Entangled lateral roots were removed. Water from roots was blotted by using tissue paper. The natural spatial orientation of root is restored after reducing the extra mass and entangling owing to the elastic properties of root. Thereafter root sample was ready to measure the angle. Modified protractor (on protractor three black parallel lines are drawn; these lines are drawn onto it to help to hold the plant perpendicular to the protractor) was used for angle measurement. Modified protractor has angle range from 0°-90° form both the sides of 90° line. This helps to measure angle from both the side (Fig 1).

For ease, a protractor was drawn or printed on the plywood board to measure root angle. The shoot along with the root was held on to the protractor. For measuring angle, one root was considered, hold that root in plane of protractor, measure the angle and note it down. Then, that root was cut from the base of the stem and removed to avoid the double measurement. Likewise, the procedure was repeated for all the roots. A table containing columns of angle classes such as 0°-10°, 10°-20°, 20°-30°, 30°-40°,
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Table 2. Details of wheat genotypes phenotyped for root angle

| Genotype | Genetic background/ parentage |
|----------|-----------------------------|
| CL3794   | ADVANCED BREEDING LINE      |
| CL3812   | HD 2998/HD 2160/DBW 50      |
| CL3803   | HD 2967/HD 2189/DW 1403     |
| CL3788   | MUNAL/WESTONIA              |
| CL3810   | DBW 50/PBW 550              |
| CL3797   | HD 2998/HD 2733/DW 1432     |
| CL3818   | HD 2967/BL 1724/VHW 4668    |
| CL3809   | HD 2998/HD 2733/DW 1432     |
| DBW17    | CMH 79A. 95/3*CNO 79/RAJ 3777|
| CL3775   | CL 2762/ HP 1744/ERA F 2000|
| CL3800   | ADVANCED BREEDING LINE      |
| CL3777   | DW 1411/HP1744/DW1440       |
| CL3807   | HD 2998/CL1682/HD 2964      |
| CL3779   | EC 635534                   |
| CL3821   | HD 2967/10TH EGIPSN 113     |
| CL3782   | HD 2967/DPW 621-50          |
| CL3776   | UP 2338/WR 1441/10TH EGIPSN 80|
| CL3785   | CL 2566/DW 1367/HD 2285     |
| CL3813   | ADVANCED BREEDING LINE      |
| CL3787   | HD 2824/CL1682/HD 2946      |
| CL3825   | HD 2682/CL 3156/WH 542      |
| CL3790   | DBW 14/IC296743             |
| HD3086   | DBW 14/HW 468               |
| CL3793   | KBRL 22/K 8027/LBRL 1       |
| CL3819   | ADVANCED BREEDING LINE      |
| CL3796   | HD 2967/LBE 2003-1/LBRL 11  |
| CL3804   | ADVANCED BREEDING LINE      |
| CL3799   | ADVANCED BREEDING LINE      |
| CL3786   | HEILO/SUNCO/2*PASTOR        |
| CL3802   | ADVANCED BREEDING LINE      |
| CL3778   | HD 2998/DPW 621-50          |
| CL3805   | ADVANCED BREEDING LINE      |
| CL3784   | DW 978/ HD 3024             |
| CL3808   | ADVANCED BREEDING LINE      |
| CL3795   | KBRL 22/HD 2009/DW 1391     |
| CL3811   | ADVANCED BREEDING LINE      |
| CL3798   | RNB 246/IC296743            |
| CL3814   | ADVANCED BREEDING LINE      |
| CL3780   | HD 2967/DW 1395             |
| CL3817   | ADVANCED BREEDING LINE      |
| CL3791   | HD 2687/VAS 415/PBW 607     |
| CL3820   | ADVANCED BREEDING LINE      |
| CL3789   | DW 987/DL 955-1             |
| CL3823   | ADVANCED BREEDING LINE      |
| CL3816   | DBW 50/DBW 17               |

Contd.

Table 2. (Concluded)

| Genotype | Genetic background/ parentage |
|----------|-----------------------------|
| HD2967   | ALD/COC//URES/HD 2160M/HW 2278|
| CL3822   | HD 2967/PBW 550/ CL 3156     |
| HD2733   | ATTILA/3/TUI/CARC/CHET O/4/ATTILA|
| CL3783   | EW 1422/WR 544/HRLSN 7     |
| WH1105   | MILAN/S 87230/BABAX          |
| CL3792   | CL 2762/ HP 1744/ERA F 2000|
| CL3801   | HD 2998/CL 3021/CL 3010     |
| CL3781   | VL 796/HW 2009/HW 2003      |
| CL3806   | HD 2998/DW 1403/DPW 621-50  |
| CL3824   | HI 1479/PBW635              |
| CL3815   | HD 2967/ERA F 2000/DW 1451  |

40°-50°, 50°-60°, 60°-70°, 70°-80° and 80°-90° can be used to record the data (Table 1). At least 3 plants per replicate per genotype per treatment were measured to reduce the experimental error due to soil heterogeneity. On the basis of root angles, genotypes were classified in three classes 0°-40° (shallow), 40°-60° (medium), 60°-90° (deep) classes (Table 2).

Statistical analysis: Analysis of variance was done using SAS 9. 2. The comparative and combined analysis of root traits was done using different command lines of R-software (RStudio Team 2016). K means analysis was done by using R package “cluster” (Maechler et al. 2018). The heatmap of two way cluster diagram was visualized by using R package “pheatmap” using inbuilt commands for visualizing the heat map and with hierarchical clustering method using Euclidean distances (Kolde 2018).

RESULTS AND DISCUSSION

This method was used to phenotype rice and wheat germplasm lines grown under natural field conditions. The root angles measured with this protocol revealed sufficient variation among the genotypes. Genotypes were broadly categorized in the three root angles classes, viz. less than 40°, 40° to 60° and more than 60°.

The mean total root number of 29 rice genotypes was 17.2 (±0.58), while that of wheat was 15.8 (±0.03). Inconsistent with their growth habit, these two cereal crops showed contrasting pattern of distribution of roots in different root angle classes (Fig 2). The number of roots in shallow angle to deep angle increased in wheat, while that of rice decreased. At deep angle, the mean number of roots of rice genotypes was only 2.0 (±0.12), while that of wheat was 10. 6 (±0.67). Thus, the rice crop which is grown in more near saturation soil condition has evolved more roots (58%) in shallow angle as compared to the wheat which is grown in soil moisture often less than that of field capacity has evolved to produce more roots (67%) in deeper layer (Fig 2).

Interestingly, the mean number of roots in medium angle class (>40° to <60°) was about 30% in both crops.
of less than 40°. Uga et al. (2013) also estimated vertical root distribution of IR64 and showed that IR64 is a shallow rooting variety. However, Tomita et al. (2017), have shown that the roots of 14 days old seedlings of IR64 collected from seedling tray were distributed between angle class 20°to 90° with maximum frequency at root angle 52°. Our study and the study of Uga et al. (2013) were conducted in field conditions with relatively older plants, which might have contributed to the consistency. In rice genotypes, BAM2574 produced highest number of roots and about 51% of the roots in deep root angle (>60°), while Vandana produced most of it roots (76%) in shallow angle (Fig 3 and Supplementary Fig 1-2).

BAM328 is another genotype which has shown the root distribution in all the three angle classes. Sahbhagi Dhan, a variety released for drought conditions, was categorized as shallow to medium rooting. Sahbhagi Dhan has high adaptability to its environment (Krishna et al. (2018). In moisture deficit environment it may produce roots with deeper angles. Since in our cultivation condition, moisture-deficit stress was not imposed, Sahbhagi Dhan might have not produced root in deeper angles. Although IR64 with Sahbhagi Dhan had similar number of roots in the root angle category >40° but in the root angle category of >40° to <60°, Sahbhagi Dhan showed more number of roots than that of IR64. The 29 rice genotypes could be classified into different categories based on root angle data. The phenotypic variability of genotypes was visualized by two-way clustering dividing genotypes into three mega clusters (Supplementary Fig 2A). The K means clustering also divided the genotypes into three optimal clusters and the two principal components explained 78.1% variability.

Our method classified rdrive cv. IR 64 as shallow rooting genotype with maximum number of roots in angle class of less than 40°. Uga et al. (2013) also estimated vertical root distribution of IR64 and showed that IR64 is a shallow rooting variety. However, Tomita et al. (2017), have shown that the roots of 14 days old seedlings of IR64 collected from seedling tray were distributed between angle class 20° to 90° with maximum frequency at root angle 52°. Our study and the study of Uga et al. (2013) were conducted in field conditions with relatively older plants, which might have contributed to the consistency. In rice genotypes, BAM2574 produced highest number of roots and about 51% of the roots in deep root angle (>60°), while Vandana produced most of it roots (76%) in shallow angle (Fig 3 and Supplementary Fig 1-2).

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amongst the genotypes (Supplementary Fig 2B). BAM2574, BAM3181, CR-143-2-2 clustered along with the drought tolerant rice cv. Nagina 22.

Among 56 wheat genotypes analyzed, wheat genotypes, viz. CL3791, CL3817 and CL3823 were categorized as profusely rooting genotypes with >80% of roots in deeper angle class. The wheat mega variety HD2967 produced about 91% of roots in deep root angle class. Genotypes CL3782, CL3804 and CL3797 were having root in all three angle classes though maximum of them were in medium to deeper angle classes (Supplementary Fig 3 and 4).

The 56 wheat genotypes could be classified into different categories based on root angle data. The phenotypic variability of genotypes could be visualized by two-way clustering dividing genotypes into three mega clusters (Supplementary Fig 4A). The K means based clustering also divided the genotypes into three optimal clusters and the two principal components explained 80.1% variability amongst the genotypes (Supplementary Fig 4B).

The proposed protocol is easy, robust and less expensive method. Root angle is measured in plants grown in soil under natural field environments and no growth media required. Root angle at any stage of plant can be measured and no orientational/spatial restriction is imposed on root growth. Data on root number can also be recorded. The observations should be recorded before the roots dry. Natural variation among genotypes for root angle was captured by this method accurately. It is easy, robust and cheaper method. As it does not require special training, larger number of genotypes can be phenotyped in short span by increasing the number of the workers and with experience. Thus, our method could display the genotypic variation in number of root and root angle in rice and wheat and identify genotypes which produce more roots with deeper root angle.

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