



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

AKSHAY S SAKHARE¹, SUDHIR KUMAR², RANJEET RANJAN KUMAR³, NARESH KUMAR BAINSLA⁴,
KIRAN GAIKWAD⁵, R K SHARMA⁶ and VISWANATHAN CHINNUSAMY⁷

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

Received: 25 December 2018; Accepted: 16 January 2019

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 ($<40^\circ$), while in wheat, about 67% of the crown roots were in deep rooting angle ($>60^\circ$). 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 $<40^\circ$. Among the rice genotypes, BAM 2574, produced $>60\%$ of crown roots with $>60^\circ$ and identified as deep-rooted genotype. In wheat CL 3791, CL 3817 and CL 3823 were identified as deep-rooted genotypes. This method issuitable 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; Tomita *et al.* 2017). To avoid moisture deficit stress, deeper 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/genes 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), rhizoslides (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

Present address: ^{1,2,7}(sakhare. akshaya@gmail. com, sudhirnpf@gmail. com, viswa. chinnusamy@gmail. com), ³(ranjeetranjaniari@gmail. com), ^{4,5,6}(bainlahau@gmail. com, gaikwadkb@gmail. com, sharma. rk. rk@gmail. com).

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 sandyloam 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/IR 12979-24-1 (BROWN)	Released as IR 55423-01 (NSIC RC 9). Upland, Drought tolerant, Rainfed
BAM-1098	IC346818	
BAM-1264	IC305692	
BAM-2574	IC453681	
BAM-3181	IC545322	
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, WBPH & 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 Cottondora 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
Shabhadghan	IR 55419-4*2/WAY RAREM	IR 55419-04*2. Way Rarem (IR 55419-04 (IR 12979-24-1 (Brown)/UPRLRi5) 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 83383-B-B-129-4 was released as "Sukhadhan 6" in Nepal. Drought tolerant line
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. It has ADAY SEL, IR 64 in the genetic background The sister cultures viz., IR 87707-446-B-B-B released as drought tolerant variety for rainfed shallow low land in Nepal. IR 87707-446-B-B-B released as Tripura Khara Dhan 1 as drought tolerant variety in Tripura, India in 2014. IR 87707-445-B-B-B released as DRR Dhan 42 in India for TN, M.P., CG, JH states in 2014
Nerica-L-44	WAB 1291/5*IR 64	Africa Rice, Africa Rice center, Bouake Derived from BC5 F4, S3
Pusa Basmati 1121	Pusa 3A/Haryana Basmati Introgression from Basmati 370 and Type 3	Semi dwarf, yield 5.5 tonnes/ha
Pusa Basmati –SN 12	Derived from Pusa Basmati 1121	
Nerica L-42	WAB 1291/4*IR 64	Africa Rice, Africa Rice centre, Bouake Derived from BC4 F8, S7

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° from 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°,

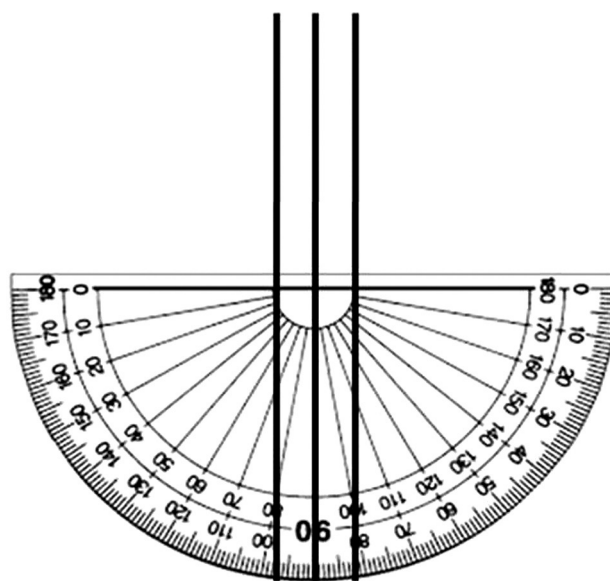


Fig 1. Modified protractor

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 EGPSN 113
CL3782	HD 2967/DPW 621-50
CL3776	UP 2338/WR 1441//10TH EGIPS 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/HD 2733//HUW 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/DW 1425//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/HD 2278
CL3822	HD 2967/PBW 550/ CL 3156
HD2733	ATTILA/3/TUI/CARC//CHEN/CHT O/4/ATTILA
CL3783	DW 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/HD 2009//HD 3003
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 the in 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.

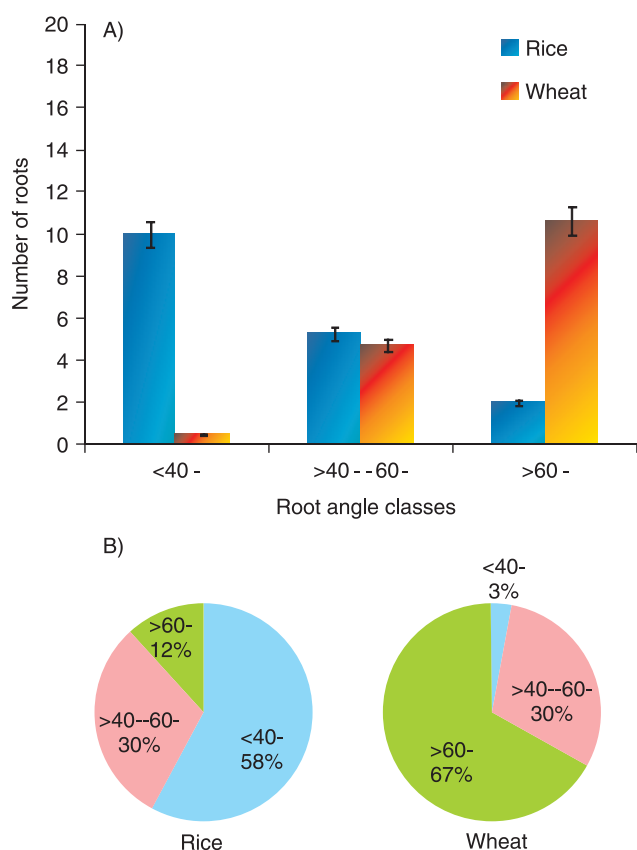


Fig 2. Variation in root angles in rice and wheat. A. Number of roots in different root angle classes, B. Percentage of roots in different root angle classes.

Our method classified rice 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 its 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

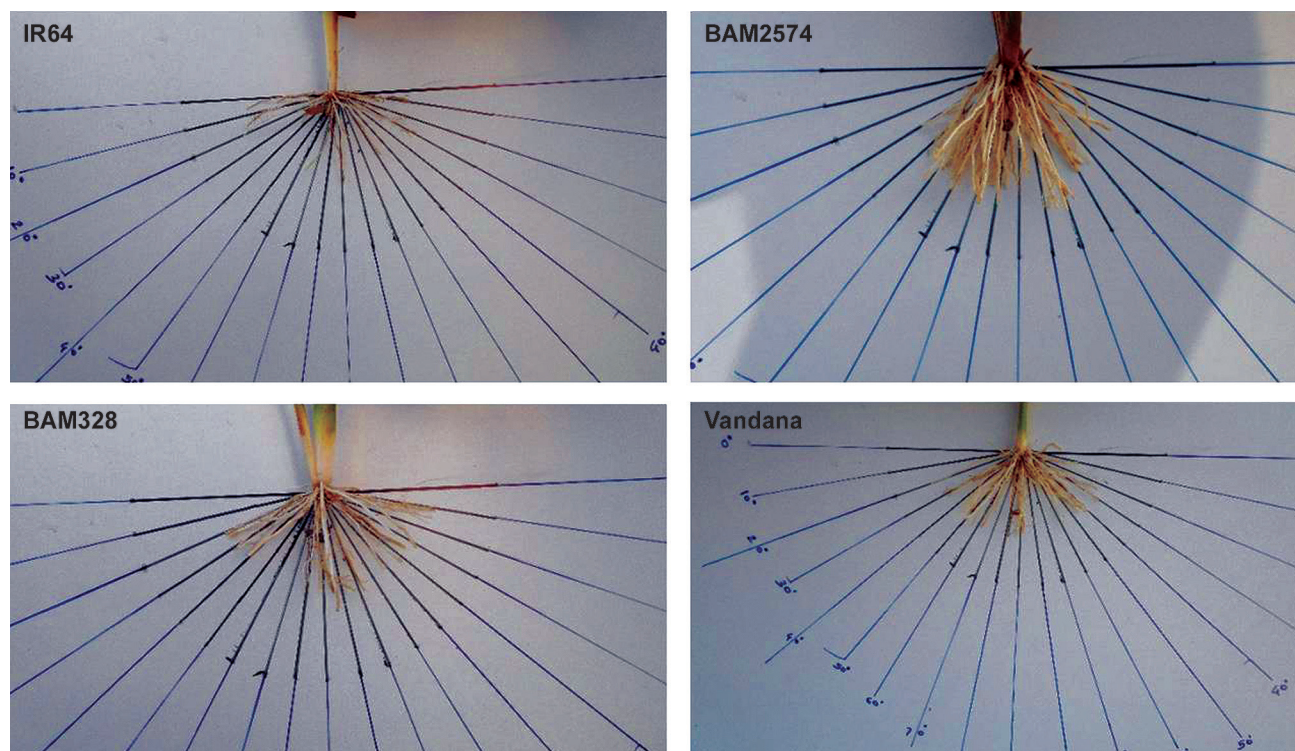


Fig 3. Root distribution in different root angle classes in selected rice genotypes.

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.

ACKNOWLEDGEMENTS

The work was funded by Indian Council of Agricultural Research -National Agricultural Science Fund Grant No. Phen 2015/2011-12. First author is thankful to PG School, IARI, New Delhi for providing scholarship. Dr A K Jha, AKMU, IARI, is acknowledged for statistical analysis.

REFERENCES

- Atkinson J A, Wingen L U, Griffiths M, Michael P, Pound M P, Gaju O, Foulkes M J, Gouis J L, Griffiths S, Bennett M J, King J and Wells D M. 2015. Phenotyping pipeline reveals major seedling root growth QTL in hexaploid wheat. *Journal of Experimental Botany* **66**: 2283–92.
- Bengough A G, Gordon D C, Al-Menaie H, Ellis R P, Allan D, Keith R, Thomas W T B, Forster B P. 2004. Gel observation chamber for rapid screening of root traits in cereal seedlings. *Plant and Soil* **262**: 63–70.
- Hanzawa E, Sasaki K, Nagai S, Obara M, Fukuta Y, Miyao A, Hirochika H, Higashitani A, Maekawa M and Sato T. 2013. Isolation of a novel mutant gene for soil-surface rooting in rice (*Oryza sativa* L.). *Rice* **6**: 30.
- Kolde R. 2018. CRAN - Package & quote; pheatmap: Pretty Heatmaps & quote; Available from: <https://cran.r-project.org/web/packages/pheatmap/index.html>.
- Krishna G K, Vishwakarma C, Thomas P, Aravind J, Kushwaha S, Chinnusamy V. 2018. Association between ABA- and drought-mediated regulation of root traits and identification of potential SNPs in genes for root development in rice. *Indian Journal of Genetics* **78**: 48–58.
- Le Marié C, Kirchgessner N, Marschall D, Walter A, Hund A. 2014. Rhizoslides: paper-based growth system for non-destructive, high throughput phenotyping of root development by means of image analysis. *Plant Methods* **10**: 13.
- Maechler M, Rousseeuw P, Struyf A, Hubert M, Hornik K. 2018. Finding Groups in Data: Cluster Analysis Extended Rousseeuw et al. [R package cluster version 2.0.7-1]. Available from: <https://cran.r-project.org/web/packages/cluster/index.html>.
- Mathieu L, Lobet G, Tocquin P, Périlleux C. 2015. “Rhizoponics”: a novel hydroponic rhizotron for root system analyses on mature *Arabidopsis thaliana* plants. *Plant Methods* **11**: 3.
- Mooney S J, Pridmore T P, Helliwell J, Bennett M J. 2012. Developing X-ray computed tomography to non-invasively image 3-D root systems architecture in soil. *Plant and Soil* **352**: 1–22.
- Nagel K A, Putz A, Gilmer F, Heinz K, Fischbach A, Pfeifer J, Faget M, Blossfeld S, Ernst M, Dimaki C et al. 2012. GROWSCREEN-Rhizo is a novel phenotyping robot enabling simultaneous measurements of root and shoot growth for plants grown in soil-filled rhizotrons. *Functional Plant Biology* **39**: 891–904.
- Oyanagi A, Nakamoto T, Wada M. 1993. Relationship between root growth angle of seedlings and vertical distribution of roots in the field in wheat cultivars. *Japanese Journal of Crop Science* **62**: 565–570.
- Richard C, Hickey L, Fletcher S, Jennings R, Chenu K, Christopher J. 2015. High-throughput phenotyping of seminal root traits in wheat. *Plant Methods* **11**: 13.
- RStudio Team 2016. RStudio: Integrated Development for R. RStudio. Available from: <https://www.rstudio.com/>
- Tomita A, Sato T, Uga Y, Obara M, Fukuta Y. 2017. Genetic variation of root angle distribution in rice (*Oryza sativa* L.) seedlings. *Breeding Science* **67**: 181–190.
- Trachsel S, Kaeppler S, Brown K, Lynch J P. 2011. Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant and Soil* **341**: 75–87.
- Uga Y, Ebana K, Abe J, Morita S, Okuno K, Yano M. 2009. Variation in root morphology and anatomy among accessions of cultivated rice (*Oryza sativa* L.) with different genetic backgrounds. *Breeding Science* **59**: 87–93.
- Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, Motoyama R, Nagamura Y, Wu J, Matsumoto T, Takai T, Okuno K, and Yano M. 2013. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics* **45**: 1097–1102.