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Research Paper

Rolled Towel Assay for seedling root growth evaluation: An effective protocol of delineating juvenile root differences in rice (*Oryza sativa* L.)

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ABSTRACT

Roots define plant productivity under stress and can be effectively harnessed to improve moisture stress resilience in crop plants. However, root traits are being largely ignored in crop improvement programmes, mainly because of the difficulties associated with root recovery and evaluating these traits *in situ*. Among various in vitro methods used, the Rolled Towel Assay (RTA) has been used in several crops like wheat, maize, beans and soybean to assay the juvenile root traits. In the present study, we standardized the rice RTA to allow for maximum germination rates, synchronize seedling growth and minimize fungal contamination. The root towel method provides ample opportunity to screen large germplasm sets for response to hormones, nutrient treatments and stress conditions. We used a diverse panel of 264 temperate rice germplasm and found significant variability in tap root length among the genotypes. The seedlings' tap root length had a mean value of 8.03 cm with a range of 2.80 to 14.57 cm and a CV of 12.44 percent. Genotype GS-33 had the largest seedling root length of 14.57 cm followed by GS-214 (14.2 cm) and GS-62 (13.70 cm). The lowest value (2.80 cm) of root length was recorded in the genotype GS-610. The experimental material used did not suffer from any fungal infection issues and is highly reproducible as has been reported in other cereal crops like wheat and maize. The method is simple, cheap and has high throughput, and can be used in stress breeding programs for the initial screening of genotypes in the diverse germplasm panels.

Key words: Drought stress, rice, rolled towel method, root architecture.

Roots are called as "***hidden half***" of the plant and play an important adaptive role in drought adaptation and resource acquisition. They define plant productivity under stress and can be effectively harnessed to improve moisture stress resilience in crop plants. Drought resistance in rice is correlated with root architecture, which includes its structural and functional elements over time and space, such as root depth, root diameter, total root length, density, hydraulic efficiency and root penetration ability (Nguyen *et al.,* 1997). However, despite the growing evidence for the importance of root traits in drought tolerance, little work has been done to include drought-adaptive root traits in breeding for drought-tolerant rice varieties. Most of the breeding programs have concentrated on above-ground components, particularly for decreasing plant height and increasing harvest index, and have largely ignored root traits, mainly because of the difficulties associated with root recovery and evaluating these traits *in situ*. Besides, the large phenotypic plasticity of root traits in response to changes in soil conditions, and the lack of high-throughput and cost-effective screening techniques make their study highly challenging. As a result, limited information is available on the genetic variability of root traits in rice.

Despite field-based phenotyping of crop root systems providing critical insight into root growth and development under water stress (Pauli *et al.,* 2016; Ruiz-Munoz *et al.,* 2020; Seethepalli, *et al.,* 2020), such studies are obscured by soil and notoriously difficult to access, especially when deficit moisture causes soil compaction. Thus, there has been growing interest in phenotyping root traits in the laboratory using various in-

vitro systems to overcome the hurdles of field-based root phenotyping systems. Such systems are high throughput, easy to conduct as well as provide a uniform and accurate screening environment. Among various in vitro methods used, the rolled towel assay (RTA) has been used in several crops like wheat, maize, beans and soybean to assay juvenile root traits (Bernardi *et al.,* 2018; Stagnati *et al.,* 2019). The RTA for rice seedling growth is an efficient and space-saving protocol for growing and phenotyping seedlings. In the present study, we standardized the rice RTA to allow for maximum germination rates, synchronize seedling growth and minimize fungal contamination. Moreover, the images so taken by normal cameras as well as smartphones can be conveniently processed using freely available software such as ImageJ or Rhizovision.

The major challenge in field-based root phenotyping is the imposition of drought stress by creating managed stress conditions. Polyethylene Glycol (MW=6000) and mannitol have been used to create in vitro stress conditions and have shown a fair amount of correspondence with field-based studies. They are high throughput, cheap, and help to create a highly uniform screening system. The rolled towel method provides ample opportunity to screen large germplasm sets for response to hormones, nutrient treatments and stress conditions. In the present study, the major objective is to standardize the root towel method for use in screening temperate rice germplasm for *in vitro* root growth.

MATERIALS AND METHODS

Plant material: The plant material comprised a rice diversity panel of 266 genotypes. The panel comprised landraces, released varieties and germplasm accessions including check varieties Jehlum, Shalimar Rice-1, Shalimar Rice-2, Shalimar Rice-3 and Shalimar Rice-4 from temperate agro-ecologies.

Experimental setup: The present study was undertaken in 2022 in the Stress Physiology Lab, Faculty of Agriculture SKUAST-K, Wadura. The following steps were used to standardize the RTA protocol.

1. For each genotype, the brown germination paper in a single or stack of three with dimensions of 30 x 30 cm for each towel was used. The papers were labelled with a permanent marker or a preprinted tag label.
2. The towels were dipped into the Captan (0.25% viz., 2.5 g/litre distilled water) solution until the paper was soaked thoroughly to avoid infection during germination and seedling growth in the germinator on account of high humidity.
3. Five healthy and compact seeds were used per towel. The sterilized seeds were arranged linearly along the line 2.5 cm from the top and the portion of the towel was rolled over it followed by rolling from left to right taking care that seed positions are not disturbed. The towels so rolled were loosely tied using a rubber band to ensure that seeds don't get dislocated as well as the roots grow easily through the rolls (Fig. 1).

4.

Fig. 1: Basic experimental set up of rolled towel assay

5. The rolls were arranged vertically in large glass beakers containing distilled water without any nutrient solution. The beakers containing the towels were kept in the growth chamber set at 25ºC and 75% relative humidity for 16/8 hr light/darkness.
6. Two days after planting, we checked the rolls for germination. To do this, we unrolled each roll and inspected the seeds.

Root phenotyping: The pouches were opened carefully on the 7th day. Primary root length can be measured even at two days after germination but laterals can be scored optimally at 5-6 days after germination. The root lengths are measured as the average of five seedlings from each pouch by carefully stretching the plants on a flat surface.

Requirements: Following chemicals/equipment/accessories are required for conducting rolled towel assay

Chemicals: Sodium hypochlorite (Himedia CAS *7681-52-9);* Captaf (Captan 50% Rallis India limited); Distilled water

Equipment: Growth chamber (Temperature and RH set facility as well as light duration darkness control)

Glassware/accessories: Brown germination paper (300 GSM Rough textured); Rubber bands; Permanent markers; Preprinted tag label; Surgical blade; Beakers (1 liter); Scale (30 cm)

RESULTS AND DISCUSSION

The seedlings' main root length had a mean value of 8.03 cm with a range of 2.80 to 14.57 cm (Table 1; Fig. 2 and 3) and a coefficient of variation of 12.44 percent. The mean sum of squares due to genotypes were highly significant ($p < 0.005$). Genotype GS-33 had the largest seedling root length of 14.57 cm followed by GS-214 (14.2 cm) and GS-62 (13.70 cm). The lowest value of seedling root length was recorded in GS-610 (2.80 cm).

Table 1: Descriptive parameters of seedling root length in rolled towel assay

Parameter	Value
Mean	8.03
Range	2.80 – 14.57
C.D.	1.39
C.V.	12.44
σ^2G	5.19
σ^2E	0.99
p-value	< 0.0005

Fig. 2: Representative variability of seedling root length of rice diversity panel

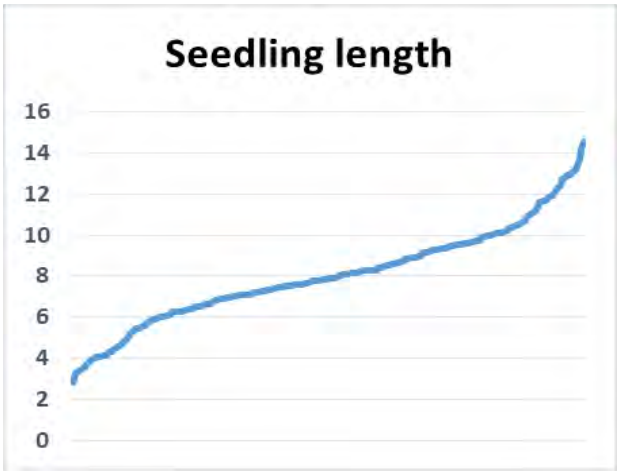

Fig. 3: Range of variation of seedling root length in rice diversity panel

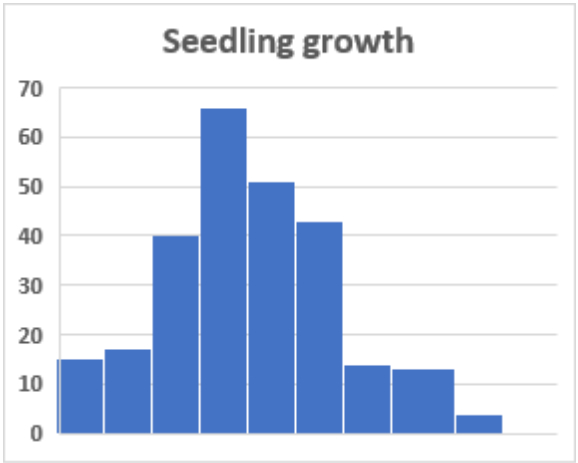

Fig. 4: Distribution of seedling root length in rice diversity panel

The distribution of the trait values of 264 accessions followed a normal distribution (Fig. 4). The microscopic examination of roots indicated that the average diameter of the main root ranged from 0.75 – 0.954 mm and the average diameter of root hairs ranged from 0.016 – 0.025 mm (Fig. 5). There are no major reports of the use of rolled towel experiments in rice for abiotic stresses; however, Sunohara et al (2020) have standardized the root pouch method to study root-knot nematode infection patterns. Zhao et al (2018) used a plastic foam frame method and reported a range of 8.13 – 18.32 cm for root length, with a mean value of 13.71 cm and CV of 12.4 percent in a rice mini core collection of 273 accessions. They reported that the results had significant correspondence with field-based phenotyping for the root-knot nematode. However, the methodology used in this study is much easy and cost-effective and can be used for large germplasm sets. The method has been used effectively in the case of wheat (Djanaguiraman *et al.*, 2019) and maize (Draves *et al.*, 2022).

The rolled towel assay used in the present study has several advantages that make it an ideal choice for screening large germplasm sets to narrow down the set to be taken to further in-depth studies under greenhouse and field conditions where creating managed conditions become progressively difficult (Phamalingam, 1988). As reported in other studies with crops like maize, wheat and soybean (Bernardi *et al.,* 2018; Stagnati *et al.,* 2019), this study also validated the advantages of RTA such as uniform seedling growth, downward roots growth, prevention of water logging due to capillary movement of water, avoiding frequent water additions to experimental set up unless warranted by dry up of beaker containing rolled towels and less time consuming and space-saving. The method is equally good for small, medium, and large seeds. It can be set up in resource-poor research labs as it does not require any costly components. With transparent towel papers becoming available, the root growth can be fairly monitored from outside and experimental set up modified any time, if need arises.

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