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Review Article
A Review of Phenotypic Evaluation of Rice Root Screening

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Abstract
Rice is considered one of the most important cereal crops for human nutrition. Meeting future demands of food security will require enhanced rice production that is more environmentally sustainable. To achieve this it is important to know the genetic and molecular mechanism controlling the root traits. High throughput phenotyping which can keep pace with genotyping is needed, but for many researchers this needs to be cheap as well as meaningful. The main focuses of this review is comparative evaluation of screening rice root system based on the phenotyping approaches. Moreover, we will review the shortcomings and benefits of current phenotyping methods, as well as the future study to overcome these shortcomings.

Key words: Rice, root, screening, traits, phenotyping, evaluation, QTLs, high throughput

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Based on current predictions of increasing global population, rice production will need to increase by more than 50% to meet the future demand of the rice eating countries[1]. It has already been demonstrated that cereal crop yield could be increased by modifying root architectural traits for production under limited resources like water and nutrients[2-4]. The success of breeding programmes depends to a large extent on the quality of screening technique. The importance of developing a reliable screening technique for drought resistance has been realized very early[5]. Since then some criteria such as experimental design, potentially all interacting factors such as biotic and abiotic stresses and variation of crop morphology have been taken into account for screening systems. There are many different techniques that have been developed for rice root screening and many have been applied to the Bala x Azucena mapping population. Hydroponic screenings of 205 rice genotypes were conducted by Price and Tomos[6]. Different root traits such as root volume, root length were measured with that technique. Glass-sided soil-filled chambers called rhizotrons were developed by Price *et al.*[7] where several root traits were evaluated at different depths after 7 weeks growth. To identify Quantitative Trait Loci (QTLs) for both Seminal Root Morphology (SRM) and gravitropic response by using agar-filled perspex chambers[8]. Hydroponic and rhizotron techniques have been recently compared for rice[9]. Other techniques include x-ray computed tomography or CT-scanner which has been used to visualize root grown in the soil[10]. Basket method was developed by Uga[11] to calculate the root angle in rice. A high throughput phenotyping method Shovelomics (uses the partial digging up of the root system under field condition) was introduced by Trachsel *et al.*[12] to characterize the maize root system such as root biomass in top soil and total number of crown roots, it has yet to be applied to rice.

ROOT PHENOTYPING

High throughput root phenotyping methods are needed that allow meaningful data to be gathered with minimal effort[13,14]. For development of advanced genetics, high throughput field based phenotyping is needed[15]. High throughput techniques should be rapid, accurate and robust[12]. Root traits are particularly difficult to evaluate[16]. A number of techniques have been developed to screen for roots, these include hydroponics, agar plates, aeroponics, paper roll methods, thin soil filled chambers (rhizotrons), soil filled tubes, large soil boxes and field screens (including shovelomics)[6,12,17-20]. Hydroponic has been widely used to evaluate root systems development and for the identification of QTLs[21]. Price and Tomos[6] used a hydroponic system and mapped QTLs for eight root growth characteristics (e.g., maximum root length, root volume and root thickness) in rice[18]. Developed a high throughput gel based two dimensional root systems phenotyping platform by which genetic analysis of root growth and development were evaluated which is suitable for up to 12 days old plants. The shovelomics technique has been used to rapidly evaluate root traits such as root biomass in the top soil and total number of crown roots of field grown plants[12]. A new phenotyping system called rhizoscope developed by Courtois *et al.*[22] which is a two dimensional hydroponic based systems allow the root to growth up to 30 days after sowing. Root growth over time can be examined through this system. This system has been used to phenotype a Japonica rice core collection. All these different techniques have advantages and disadvantages and an evaluation of these methods and the herbicide method used in this review.

USING BURIED HERBICIDE TO ASSESS ROOT DEPTH

A field based technique was developed by Robertson *et al.*[23] by which a large number of genotypes could be tested for deep, extensive roots by banding the herbicide metribuzin at 46, 61 and 76 cm depth. Deep rooted genotypes died more quickly. As well as differences in herbicide symptoms between genotypes they also found there was a correlation with water extraction. To screen deep and shallow peanut cultivars metribuzin was again used by Khalfaoui and Havard[24]. The researcher used the metribuzin in the field at 70 cm depth and found reasonable correlation between herbicide reaction and drought scores. Simazin at 15 cm depth has been used as a field screen for 73 cucumber cultivars[25]. These researchers found no correlation between this field experiment and a pot experiment where the herbicide was placed at 23 cm depth, which might be due to the relatively shallow placement of the herbicide in the field. Seguy *et al.*[26] used atrazine and diuron to test the deep and shallow rooted rice cultivars in the field. In addition Trebuil *et al.*[27] tested three herbicide in the pots and metribuzin in the field experiments on rice. They concluded that injection of the herbicide deep into soil does discriminate cultivars based on rooting depth but that the method was time consuming.

COMPARING THE SCREENING METHODS

The herbicide screening method for rooting depth has been shown to be a reliable, robust and a representative method of assessing root length. This method is a positive test

Table 1: Techniques developed for root screening

Name of technique	Evaluation	References
Hydrophonic	Mapped QTLs for 8 root growth characteristics	Price and Tomos[6]
Paper roll methods	In Paper roll system, the medium is supplied by capillarity in the support paper and that give access to a bi-dimensional view of the root in the early developmental stage	Woll *et al.*[19]
Hydrophonic	Root system development and QTLs identification	Obara *et al.*[21]
X-ray computed tomography	*In situ* capture of the whole root system in soil pot or column	Tracy *et al.*[29]
Shovelomics technique	Evaluate root traits and total number of crown roots in field grown plants	Trachsel *et al.*[12]
Gel-based 2-D root phenptyping system	Genetic analysis of root growth and development, suitable for 12 days old plants	Clark *et al.*[18]
2-D hydrophonic based system-Rhizoscope	Evaluate root growth up to 30 days after sowing	Courtois *et al.*[22]
Buried herbicide method	Characterize the root system architecture specially rooting depth in early vegetative stage	Al-Shugeairy *et al.*[15]

for deep rooting but not a test for shallow roots[15]. The herbicide screening methods proved highly discriminatory between the cultivars when used in 135 Srilankan cultivars[28]. Evidence from the screening methods[15,28] and genetic studies (co-localisation of QTLs) indicate the robustness of the herbicide screening method[15]. At present there are a large number of methods which can be used to screen roots. The herbicide screening methods is compared to a number of the currently used methods. Hydroponic screens have the advantage that the roots can be continually monitored for root growth, however after a period of time the roots become intertwine each other and the pH reduces rapidly[9]. Also, hydroponics is a non-soil based system, so if the roots respond in a solution system to how they would in a soil system could be an issue. Another screening method is rhizotrons, the main advantages are that the plants are grown in soil and that root angle information can be assessed. In addition stresses related to nutrients or water can be measured. Like hydroponics this system can be continually measured, however due to their 2-D construction they do limit root growth in all natural directions as well as being a labour intensive screen, therefore reducing there use in high throughput screens. In Trachsel *et al.*[12] root biomass and number of crown roots can be measured rapidly (8 min plant^{-1}) in the field but this method not estimate root depth and is relient on the plants being screening having well developed roots systems. It does have the major advantage of screening for roots in a natural system. In the paper roll systems[19] bi-dimensional view of the early root systems could be measured but this system is restricted to very early developmental stages and again is not is a soil based system. A brief summary of techniques developed for root screening is shown in the Table 1.

Therefore, when looking at the other methods the herbicide screen fills an important niche, between the high throughput none soil methods (e.g., hydroponics and paper roll) and the lower throughput soil methods (e.g., rhizotrons). The buried herbicide method is a simple screening systems, some shortcomings should be noted. Variation in cultivars sensitivity to herbicide, effectiveness of the method depends on the soil water and plant growth interaction and it is a destructive method (death of tested plants) and the only trait being measured is rooting depth. But it is a low cost method well suited to high throughput root phenotyping. This herbicide screening method has been shown to be a way of rapidly screening large populations of rice accessions to identify deep rooting cultivars. These accessions can then be further studies with the hope of harnessing the natural genetic diversity within rice to breed improved rice cultivars for drought prone regions.

CONCLUSION

In this study, we evaluate a number of root phenotyping methods which demonstrated the high throughput root screening methods. Using these methods phenotyping root screening can be done with many advantages. Though, these methods have some limitations they are very useful for phenotyping root screening. In future, if these limitations can be overcome to achieve the sustainable breeding program in rice by developing a high throughput phenotyping method that can measure relevant trait of the large accessions of rice in a way that is quick, robust and cost-effective.

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