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Research Article

EASY AND EFFICIENT SPLIT-ROOT METHOD TO STUDY MORPHOLOGY AND ANATOMY OF RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Objective: This study was intended to investigate the efficacy of polyvinyl chloride (PVC) pipe method for split-root experiment in rice (*Oryza sativa* L.) using soil medium at all the growth stages of rice.

Methods: Rice seeds were sown in small PVC pipes and allowed to grow for 1 month, which were then transferred to split-root setup by equally splitting the roots of these seedlings into two halves and were allowed to grow for different time periods of their growth stages to analyze their health and stability.

Results: We report an easy split-root study for rice grown in soil. Unlike the field grown plants, the efficient PVC tube method enables simple and systematic growth and harvesting for proper analysis of the plant samples without damaging the tissue. In our experiments, although the rice plants were transferred to the split-root setup by splitting their roots, they were healthy and stable after 7, 15, 70, and even at 120 days (maturity) of growth in split-root condition.

Conclusion: Morphology and anatomy of plants can be easily and efficiently studied at any growth stage using PVC tube method as opposed to field method where sample harvesting requires an inconvenient process of uprooting the plant while losing and damaging the tissue.

Keywords: Anatomy, Polyvinyl chloride pipes, Rice, Root, Soil, Split-root.

INTRODUCTION

Roots sense their environment, interact with it and respond to any changes in an adaptive manner. These capabilities of roots coupled with the architecture and anatomical structures enable them to carry out the vital function of acquiring water and nutrients from the soil and are highly instrumental in transporting these to the other parts of the plant. Water moves along a path of decreasing potential energy from the soil, through the roots. Before entering the xylem vessels, the water and nutrients from the soil move radially through several concentric layers of the epidermis, cortex, and endodermis, which are, then, longitudinally transported via the xylem vessels to the aerial parts of the plant [1].

Rice (*Oryza sativa* L.) is a dietary staple of more than half of the world and 65% of the Indian population [2,3]. Rice is the only crop which is cultivated and adapted to a wide range of ecosystems. Rice is generally cultivated in semi-aquatic conditions in many parts of the world, but in India, the 44 Mha of total rice area, 33% is grown in rainfed lowlands and 15% in uplands. Since rice is generally grown in fields with submersion, decreasing water availability would threaten the sustainability of this crop. Consequently, scientists have bred ARB6 "aerobic rice," seeds of which are directly sown in soil with no water logging required [4-6]. Irrigation is used extensively to cope with soil water deficit, but salts which come along with irrigation water eventually get accumulated and leads to high salt build up in the soil. All plants tolerate salinity up to a certain threshold level without significant yield reduction, after which an increase in salinity level significantly reduces yield. Although in rainfed lands drought is considered as the major limitation for high crop yield, growing rice is also a challenge in uplands, where salinity is the major production constraint. Rice is very sensitive to salt stress as well [7]. Short-term salinity leads to physiological drought and its long-term persistence causes ionic stress [8]. Na^+ in saline soils causes both osmotic stresses as well as ionic imbalance [9] leading to altered K^+/Na^+ ratios, which affects all aspects of plant growth. The environmental conditions and agricultural practices are changing globally, resulting in an increase in drought and salinity effected lands. Since rice is highly sensitive, it gets adversely affected resulting in a dramatic decrease in yield. Various techniques need to be developed to investigate and understand the responses of crop plants to environmental stresses.

Various techniques, such as the polyvinyl chloride (PVC) pipes and split root tubes, have been used in soybean and vetch [10-12]. Split-root and grafting technique in agar plates had been used in *Medicago truncatula* [13]. Split-root plate assays using plastic dividers supported with 0.6% water agar were used to study the roots of *Lotus japonicus* and *Trifolium subterraneum* [14,15]. The limitations of these techniques are that (i) the studies were conducted in non-soil medium and (ii) they can only be used till vegetative or seedling stage of a plant. Therefore, more techniques need to be developed which allow creating field-like conditions to study the responses of crop plants to environmental stresses.

Since roots are the underground growing part of the plant, therefore, it is difficult to investigate and analyze their responses when growing in field condition. The PVC method has been used by our lab and other researchers for morphological and whole root studies in maize and rice in soil medium under well watered and drought condition [16,17]. Changing environmental conditions such as increasing water shortage, uneven distribution of rainfall, and salinization of soil have become the major constraints for crop productivity of rice varieties. Therefore, it is very important to understand the morphological, anatomical, and physiological responses of rice plants to these changing conditions. Grafting and split-root experiments are emerging trends owing to the importance of studying the effects of environmental and endogenous factors in plants, as they enable for the dissection of function of roots to understand the effect of treatment of one-half of the root on growth, water uptake and long distance signaling. The work reported here is an attempt to use PVC pipe method for growing rice plants with split roots in soil medium, and to evaluate the efficiency of this method in the ease of sampling process of healthy plants. Also to check whether or not this technique would be useful for a study where rice varieties would be tested for drought and salinity responses in split-root condition.

METHODS

Method for seedling growth

For this purpose, the PVC pipes of 40 cm length and diameter of 8 cm were used and filled with a mixture of field soil and organic manure in 2:1 ratio. The soil mixture was watered and compacted using an iron rod till the PVC pipes were tightly packed with soil to mimic the field condition [17]. About 3 seeds of different rice varieties such as ARB6 (drought tolerant), Pokkali (salt tolerant), IR20 (salt sensitive), and Jaya (moderately salt sensitive) were sown in the soil, watered regularly and allowed to grow for a month.

Method for split-root setup

Two adjacent chambers were created by joining two PVC pipes (80 cm length and 16 cm diameter) using a tape, which were then filled with a mixture of soil and manure in the ratio 2:1 as mentioned above. 1-month-old seedlings of four rice varieties were transferred to split-root experimental setup by carefully dividing their roots into two equal halves and then planting half in one chamber (Fig. 1). Three seedlings were transferred per split-root setup where only one healthy seedling was retained after a week of stable growth. To obtain healthy samples, these plants were watered regularly (water/water condition) on both sides of the chambers and analyzed at different intervals. 10 replications per variety were used at each growth stage to evaluate the health and stability of plants in this study.

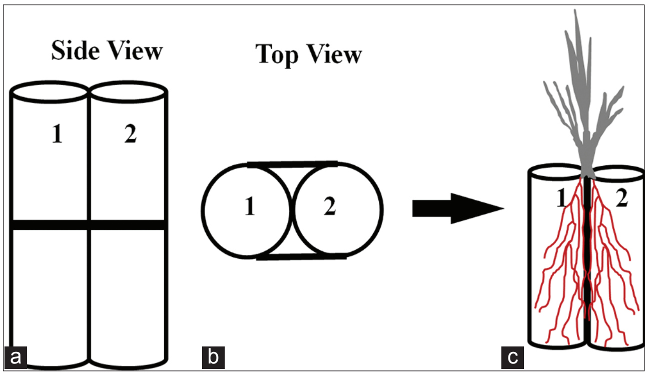

Fig. 1: Schematic representation of split-root setup. (a) Side view and, (b) Top view of the polyvinyl chloride pipes joined together. (c) Seedling transferred in split-root setup

Sample harvesting method

Before plant analysis after 7, 15, 70, and 120 days, the split-root PVC pipes were carefully immersed in a shallow washing tank, plants were carefully taken out of pipes, and the soil was gently washed away from roots. Immediately after washing, the root appearance was analyzed. Free hand sections of these roots were observed under bright field microscope and images were recorded.

RESULTS

The method described in this study is easy and efficient for the growth of rice plants in soil medium using PVC pipes. PVC pipes are easy to handle and transport from one place to another; in addition, the PVC pipes have an added advantage as they help conducting experiments at different locations such as field, greenhouse, and rainout shelter. Since this study was conducted in PVC pipes in field condition (Fig. 2), rice plants were allowed to grow and analyzed after 7, 15, 70, and 120 days in split-root condition (Fig. 3). In our experiments, although the rice plants were transferred to the split-root setup by splitting their roots, they were healthy and stable after 7, 15, 70 days, and even at 120 days of growth in split-root condition (Fig. 3).

Health of a plant is closely related to its root system, therefore, for a plant to survive in unfavorable condition, healthy root morphology and anatomy is important. We observed the health of the roots of 70 days old rice plants which were watered on both sides of the split-root setup; we found the roots to be healthy in appearance (Fig. 4a-c). Anatomy of the roots showed all the cellular layers such as exodermis (ex), cortex (c), aerenchyma (ae), endodermis (en), and xylem vessels (x) to be present and intact (Fig. 4d) in all rice varieties used.

DISCUSSION

The study presents a simple and efficient method of producing soil grown healthy split-rooted rice plants. The advantage is that this study can be conducted in a greenhouse or in an open field or in a rainout

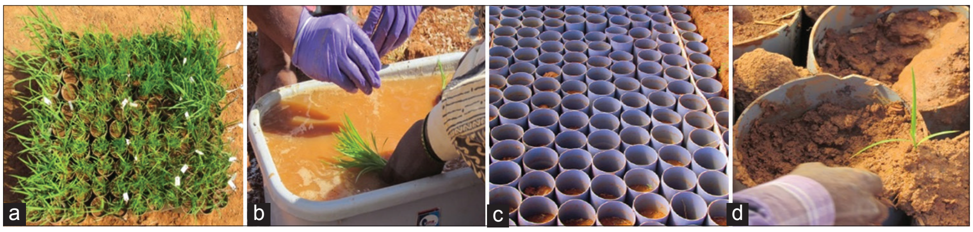

Fig. 2: Pre-split-root experiment process. (a) Raising rice seedlings. (b) Washing seedlings before transfer. (c) Setup of split-root polyvinyl chloride pipes and soil compaction. (d) Transferring of seedlings into split-root setup

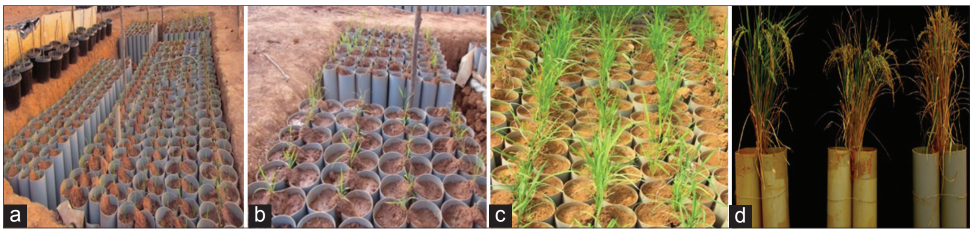

Fig. 3: Rice plants growing in split-root setup in field. (a) 7 days old rice plants in split-root setup. (b) 15 days old rice plants. (c) 70 days old rice plants. (d) 120 days old rice plants

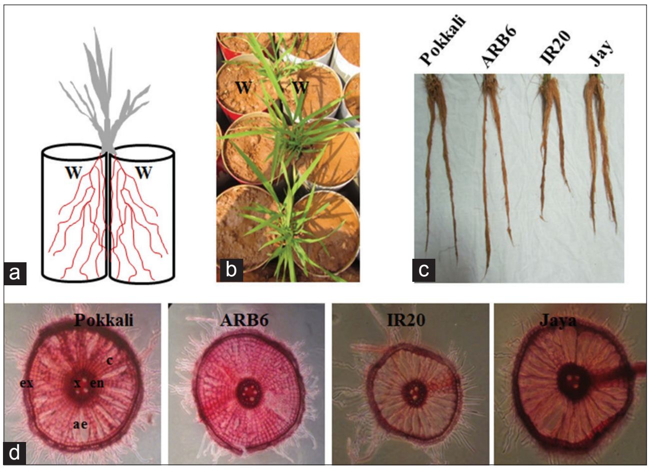

Fig. 4: Split-root setup in field under water/water (W/W) condition. (a and b) Rice plants in W/W condition. (c) Healthy 70 days old rice plant roots. (d) Healthy root anatomy of rice varieties. ex: Exodermis, c: Cortex, ae: Aerenchyma, en: Endodermis, x: Xylem vessels

shelter. Multiple experiments can be set up with same genotypes of rice at these locations for comparative studies. Moisture levels of soil can be maintained as desired. Any number of genotypes can be studied in this method, depending on the pipes available. In addition with split-root setup, the responses of a single plant for two different moisture regimes can be analyzed simultaneously. Given the healthy and stable growth of rice plants in our study, morphology and anatomy of plants can be easily and efficiently studied at any growth stage using PVC tube method as opposed to field method, where sample harvesting requires inconvenient process of uprooting the plant while losing and damaging the tissue.

CONCLUSION

The PVC pipe method can quite efficiently be used for growing rice plants with split-roots in soil medium. It produces healthy plants to work with and helps in easy harvest and evaluation of samples. The dual function of the split-root system makes it more useful technique to test a single genotype for two stresses simultaneously. This split-root protocol can be further used to study other crops also in soil medium.

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