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# Progress in the genetic studies on root development in *Oryza sativa*

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## ABSTRACT

Rice (*Oryza sativa*) root system at seedling stages consists of primary (seminal)-, crown- and many lateral roots, and carries out diverse important functions for the maintenance of the plant. In spite of the importance of the root system, almost of the genetic and molecular biological bases of rice root morphogenesis are still unclear. Recently, genetic studies to verify the mechanisms of root development started, and several root type-specific mutants have been reported. In this review, we introduce currently available rice root mutants and discuss about the needs of more mutants with molecular tags. The criteria and system for screening the specific root mutants is also suggested in the frame of the strategies to genetically dissect the root developmental mechanisms. We also discuss the future directions of rice root studies using diverse genomic resources.

**Key words** : root development, mutant, tagging, monocot, rice *(Oryza sativa)*

Plant root systems play two major roles for absorption of water and mineral nutrients from soil, and for mechanical support. Furthermore, roots have functions for storage of nutrients, chemical secretion, supply of air, and even for the movement of plants (1). The formation of proper architecture is the prerequisite to carry out the physiological functions of the root system. Therefore, understanding the root morphogenesis is indispensable to understand and utilize the root functions. For the last 15 years, some important breakthroughs in the research of root development have been achieved in Arabidopsis as a dicot model plant (2). Certainly, the knowledge has contributed to understanding the root developmental mechanisms also in crop plants. However, there is a limitation in directly applying the research data from Arabidopsis to rice root system, because of the differences in the architectures of root systems between dicot and monocot plants (3,4). Therefore, there is a demand for studies on the root development in monocot crop plants themselves. Furthermore, when considering the agricultural importance of monocot crop plants including rice (*Oryza sativa*) and maize (*Zea mays*), the rice root system needs to be studied with great priority especially in Asia, where the rice is the staple food for human beings

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## The architecture of rice root system

Rice root system consists of the primary-, crown-, and lateral roots, branching out from pre-existing roots (**Fig 1**). The primary root is seminal, i.e. it is included in the embryo, and the crown- and lateral roots are post-embryonic. The crown roots start to develop from the nodes after several days from the germination. Lateral roots are also post-embryonic as crown roots. However, they develop from the mature region of the pre-existing other root types. Sometimes, adventitious roots develop from the upper nodes, when rice plants experience low oxygen by submergence (5). The surface of all functional roots is covered with root hairs, outwardly elongated epidermal cells, which increase the contacting and absorbing area. Maize root system is also similar to that of rice, except the presence of two or more additional roots in the embryo, the seminal roots (3; **Fig 1**). The terminology of seminal roots indicates the additionally existing roots in maize besides the primary root. In rice, however, the seminal root indicates very often the primary root without any confusion, because rice embryo contains only one, the primary root that is seminal. In rice and maize, major functional task is carried out by the crown roots developing from several nodes. This is the major difference between Arabidopsis and monocot crop plants; Arabidopsis has a tap root system where the primary root grows continuously and carries out its functions (2,3).

## Root developmental mutants in rice

Physiological studies on the development of plant roots have been hindered by the extreme sensitivity of root system to the given environmental conditions. Even under the highly controlled environment, miscellaneous changes caused by unexpected external factors could severely change the root system, e.g., local drop of temperature in the growth chamber could dramatically affect the length of crown roots in rice plants in darkness (Jung, Hwang and Park, unpublished). Therefore, genetic studies using root-specific mutants have been regarded as more promising than physiological analyses, so several root developmental mutants in rice have been found and made (**Table 1**).

**Table 1.** Examples of some rice mutants showing aberrant root development.

| Mutants | Described phenotype | Mutagen | Literature |
|---|---|---|---|
| RM1, RM2 | Short root | Radiation ($\gamma$-ray) | (5) |
| *crl1, crl2* | Crown root deficient | Chemical (MNU*) | (6) |
| *rrl1, rrl2* | Reduced root length | Radiation ($\gamma$-ray) | (7) |
| *srt5* | Inhibition of root elongation | Chemical ($NaN_3$) | (8) |
| *alf1* | Short lateral roots | Transposable element (*Tos17*) | (9) |
| NB208 | Temperature-sensitive defect in root elongation | T-DNA | (10) |

* MNU: N-methyl-N-nitrosourea

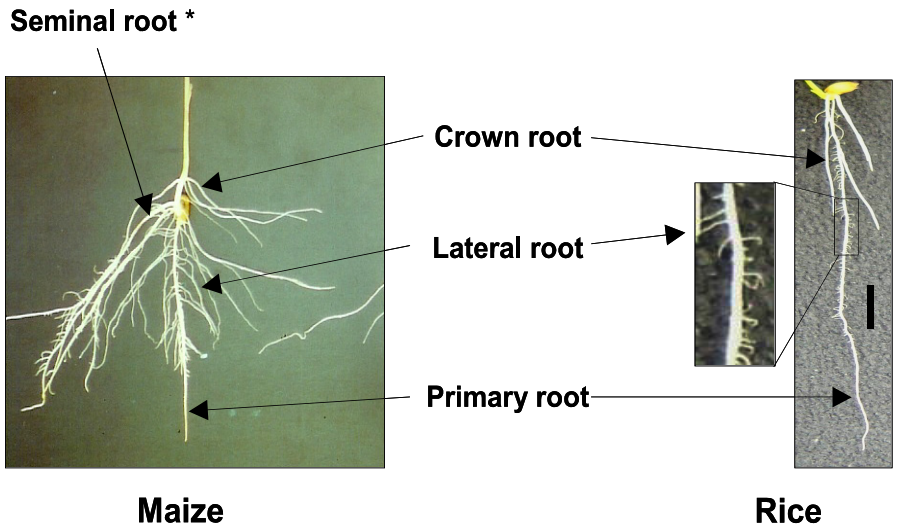


**Fig 1.** Root systems of two major monocot crop plants, maize and rice. Bar indicates 1 cm.
* Seminal root indicates the primary root in rice.

The root mutants displayed in **Table 1** could be classified by the following root-types that are affected by the mutations. Most commonly, mutation affects the length of the primary root (6,8,9,11). There are also mutants affected in crown- (7) or lateral roots (10). Some of the mutants displayed the existence of root type-specific regulation mechanisms. For example, the mutant *crl1* without crown roots seems to have aberration in the crown root-specific auxin-regulated developmental controls. The *crl1* gene was cloned by map-based chromosomal walking (12) and identified as the gene for a lateral organ boundaries protein (13) that is one of the targets of auxin response factor (ARF). This finding indicates that auxin controls crown root-specific development.

## Needs for root mutants to be screened by strict criteria: specificity

Mutants with defects in growth regulation, e.g., phytochrome control (14) could also have root phenotypes as well as the examples listed in **Table 1**. However, mutants with pleiotropic phenotypes including roots are not so helpful for dissecting the regulatory mechanisms of root development. Because diverse physiological networks, e.g., plant hormones, affect root development, mutation in indirectly linked steps can make problems more confusing. Therefore, the most important aspect of the mutant is the specificity to a certain root trait, i.e., specificity to a certain root-type or to a developmental stage in the root. In maize, the mutant phenotype *slr1* and *slr2* are specific to the elongation of lateral roots (15), and the mutation of *lrt1* is specific to the initiation of laterals (16). We need these kinds of mutants for primary-, crown and lateral roots affected in initiation, elongation or maturation steps also in rice root morphogenesis.

## Needs for tools to clone the mutated genes: molecular tags

Although there are several root-specific mutants in rice, the genes affected are still largely unidentified. One of the major difficulties is that molecular tags, which can be utilized for the isolation of their flanking sequences, are lacking, because the reported rice mutants were obtained by radiation or chemical mutagens (see **Table 1**). T-DNA and transposable elements are currently available mutagens attaching molecular tags to the genes to clone the mutated genes in rice. Currently, several tagging populations are available (or will be available for public in the near future). One of the largest populations is prepared by T-DNA insertion (17; G. An, genean@postech.ac.kr, http://postech.ac.kr/life/risd). This population has been successfully utilized to investigate diverse plant development. Another population is prepared by the transposable element *Tos17* (18; H. Hirochika, hirohiko@nias.affrc.go.jp). Total of 50,000 *Tos17*-induced mutant lines carrying 250,000 independent insertions were collected. The flanking sequence database is available on the web site (http://tos.nias.affrc.go.jp). Drawbacks of *Tos17* lines are the low tagging rate and the difficulty of regeneration. However, utilizing already prepared populations with known flanking sequences is free from the difficulties. There is another rice population tagged with the *Ds* element. Preparation and the sequencing of the flanking sequences are currently in the progress. The genomic resources will be open to the public (M. Eun, myeun@rda.go.kr, http://www.niab.go.kr). Although this *Ds* population is still incomplete, the mapping results of more than 1,000 insertion sites revealed the unbiased distribution of *Ds* all over the rice genome, indicating that this population is ideal for mutant selection (19). Other genomic resources are well reviewed in a recent publication (20). All these tagging lines are potentially available for the screening of new developmental mutants in rice roots.

## Needs for screening system for root developmental mutants

Even with a good population, the preparation of plants for the screening of rice root mutant is still challenging, because the root system is extremely sensitive to environmental conditions. Therefore, careful control of temperature, light, air circulation and humidity is necessary. Furthermore, rice root development can be severely affected by kinds of media that surround the roots (**Table 2**), because growth media affect the local conditions of water and air supply. The media should support plants very well even in wet conditions without disturbing the growth to keep the roots in a

**Table 2.** Comparison of various media for the screening system of root developmental mutants in rice (Jung, Hwang and Park, unpublished).

| Media | Water supply | Air supply | Support | Easiness for observation | Recovery of roots |
|---|---|---|---|---|---|
| Agar | G | B | G | G | B |
| Soil | G | M | G | B | B |
| Nutrient solution (hydroponic culture) | G | B | B | G | G |
| Filter paper* | G | M | B | M | M |
| Paper towel | G | M | M | M | M |
| The Korean paper** | G | G | G | G | G |

* Cellulose filter paper is not a suitable growth medium for mutant screening. However, special filter papers (e.g., from Sartorius) are good as the Korean paper

** The Korean paper indicates the paper (thick type) for calligraphy

Abbreviations: G; good, M; middle, B; bad

good form for morphological analyses. For further cultivation of the plants after the first observation and sample preservation, the media should not deter restoring the plants. For mass screening, the price of the media is also a factor that cannot be ignored. Currently, the Korean paper for calligraphy (thick type) meets all the requirements, and is successfully being used in our lab. After soaked rice seeds are lined on the paper, the paper is rolled up and set in a beaker or flask as the paper roll system used for maize root mutant screening (21). To get homogenous plant samples, synchronization of the germination is essential. This can be achieved by soaking for 2 days in water (even better at 4°C) and moving to the Korean paper system (Jung, Hwang and Park, unpublished). Proper surface disinfection before soaking is a basic skill in all rice laboratories (22).

## Conclusion

Although there several rice root mutants are available, we need much more mutants that can be used for the verification of root type-specific and developmental stage-specific molecular mechanisms of rice root development. These mutants should be screened based on a strict criterion to guarantee the specificity. Good populations with molecular tags for cloning and a good screening system to overcome various environmental disturbances are necessary. Specific root mutants obtained by the previously described processes will reveal the regulation mechanisms of rice root development and open new possibilities for rice quality improvement.

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