Review article

IRRI's drought stress research in rice with emphasis on roots: accomplishments over the last 50 years

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Abstract: Early recognition of the importance of roots for drought resistance, and the diversity in rice root architecture, provided a strong foundation for drought research at the International Rice Research Institute (IRRI). IRRI was founded in 1960, and large efforts for research on root growth in response to drought were ongoing by the mid-1970s, with an emphasis on deep root growth, formation of coarse nodal roots, and the root pulling force method. In the 1980s, aeroponic studies on root morphology and anatomy and line-source sprinkler field studies were commonly conducted. The use of crosses to better understand the genetics of root traits started in the 1980s. Further characterization of the genetics behind root traits was conducted in the 1990s, specifically the use of molecular markers to select for root trait QTLs. A shift toward rainfed lowland experiments in addition to upland conditions began in the 1990s, with increased recognition of the different types of drought stress environments and characterization of root water uptake. In the 2000s, drought breeding efforts moved from selection of root traits to direct selection for yield under drought. Today (the 2010s), we have identified two major drought-yield QTLs to be related to root traits, and phenotyping for association mapping of genes related to root traits and functions is underway. After direct selection for yield during the past decade that is now approaching impact at the farm level, we are seeing that root traits are indeed involved in improved yield under drought.

Keywords: drought, IRRI, rice (*Oryza sativa* L.), root

Abbreviation: IRRI, International Rice Research Institute

Introduction

The International Rice Research Institute (IRRI) is a non-profit organization with a mission to conduct scientific research to help rice farmers improve yields in a range of agroecosystems. For drought-prone environments, IRRI's research program has included plant breeding efforts to improve yield under drought as well as the physiological investigation of the mechanisms behind drought resistance, such as root traits. Although root/drought research has been ongoing at IRRI for decades, many of the results from the early work are summarized only in institutional reports or publications and, although valuable, these are difficult to access for those outside of the institute. In this paper, specific root/drought research methods and results are presented for each decade of research, as well as the contribution of the root research to the drought breeding program. Note that the physiological root/drought conclusions made at the time are reported here, but in some cases these conclusions may not reflect the current thinking among root/drought researchers. This paper is meant to share the previous root/drought work at IRRI with a wider audience, and to give an overview on the evolution of scientific perspectives and research strategies for a long-term goal: the improvement of rice yield through root/drought research.

The 1960s: A focus on high-input, irrigated agroecosystems at IRRI

During the first decade following the foundation of IRRI in 1960, the major research effort was to support the Green Revolution for fighting imminent famine in South and Southeast Asia, particularly with the release of high-yielding variety IR8 for high-input environments in which irrigation and agrichemical applications were used. No work was conducted specifically on drought as the research emphasis at the

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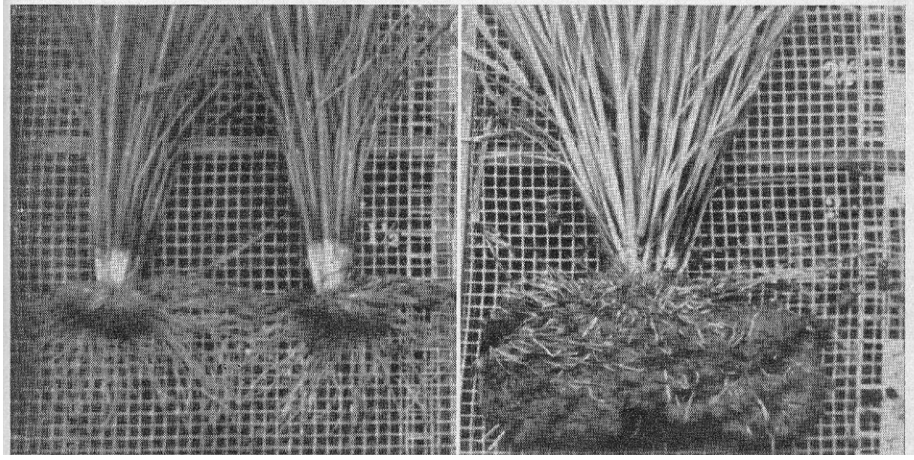


Fig. 1. One of the earliest photos of root studies from the IRRI annual reports: effects of row spacing on root growth (IRRI, 1964).

time was for high productivity, but some root studies were conducted including the use of autoradiographs to examine the effects of shading on the movement of carbon to roots, the effect of row spacing on root growth (IRRI 1964; Fig. 1), and the oxidizing power of rice roots (IRRI 1967).

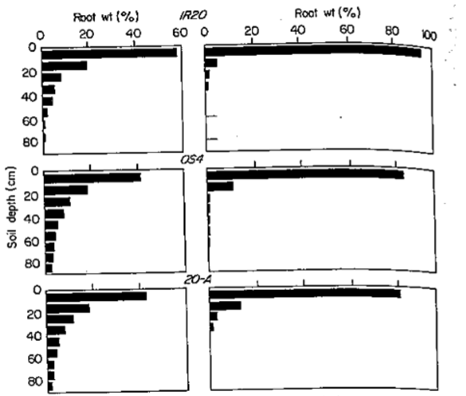
The 1970s: Large-scale screening revealed extensive diversity for rice root traits and drought response

The first mention of drought in IRRI annual reports was in 1970 (IRRI 1971), and the drought program at IRRI grew from then on through the mid-1980s. Root/drought research at IRRI during the 1970s emphasized 1) characterizing rice diversity in terms of root growth and drought response; 2) exploring plant parameters that could be used for drought screening; 3) using root study methods, including tanks, root boxes, and root pulling force; and 4) describing a plant ideotype for improved yield under drought. Starting in 1974, a special section on "Root studies" was included in the IRRI annual reports. The target environments of most drought research at the time were upland agroecosystems, since these were the systems where drought stress most frequently occurred, productivity was lowest, and the poorest farmers resided.

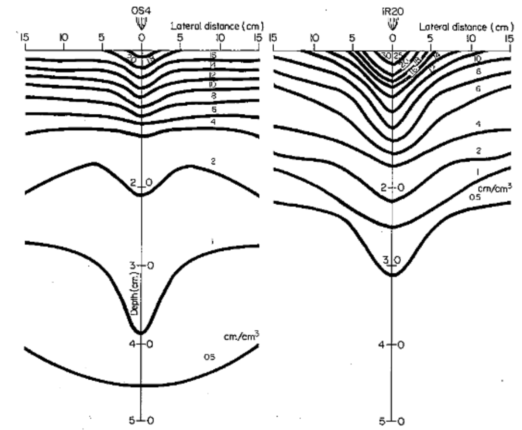
Characterizing rice diversity in terms of root growth and drought response

Early reports indicated that drought-tolerant upland varieties, including OS4 and Palawan, typically showed "long and thick" root systems (Chang and Vergara 1975). This root ideotype of long/deep and thick (large diameter nodal) roots remained the target drought/root phenotype for about the next four decades at IRRI. However, it was also recognized that although upland varieties typically showed deeper roots and could avoid drought, they were not as tolerant of stress as lowland varieties. Notably, Moroberekan was identified as one of the least tolerant at the IRRI farm, despite being rated as deep-rooted (IRRI 1976). Other genotypes commonly cited as deep-rooted were FR13A, Khao Dawk Mali 105 (KDML 105), Salumpikit, Azucena, and Dular. Most of these genotypes are still being used in IRRI drought experiments today, and root traits in addition to deep root growth, such as increased lateral root growth, have since been reported for some (Bañoc et al. 2000).

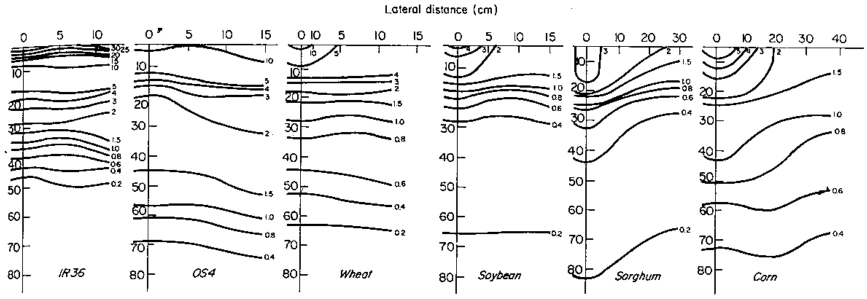
Several genotypes were highlighted during the 1970s for their response to drought or were commonly used as drought-tolerant checks. Salumpikit was a commonly used check line in screening trials. OS4 received much attention for physiological characterization. In a study on the effects of water supply, soil texture, and soil compaction on root growth comparing IR20 and OS4, root:shoot ratio increased due to decreased shoot biomass under drought and was greater in OS4, which showed better vertical distribution of roots under drought stress (Fig. 2). Soil texture was not reported to affect root:shoot ratio (IRRI 1975). Over time, root growth of IR20 stayed stable, but OS4 decreased the growth of shallow roots and increased the growth of deep roots after drought (IRRI 1979). OS4 and Palawan stood out as having denser and thicker roots among genotypes excavated from an upland field (Chang et al. 1972).



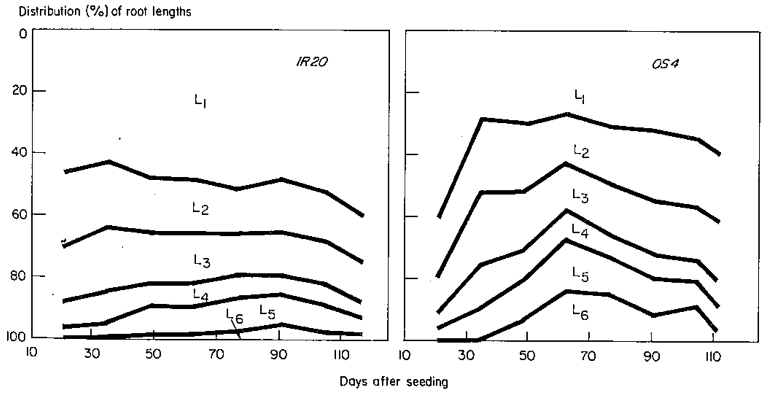
11. Vertical root distribution (by weight) at soil depths of three varieties grown in root boxes and in the field. IRRI, 1976.



9. Iso-root density diagram of rice varieties OS4 and IR20 grown in the field, IRRI, 1977.



. Vertical root distribution of different crop species. Number at end of each curve is root density (cm/cm^3). IRRI, 1978 dry eason.



10. Percentage distributions of root lengths in different soil layers for IR20 and OS4. Depths (cm): L_1:0–5; L_2:5–10; L_3:10–15; L_4:15–20; L_5:20–40; L_6:40–90. IRRI, 1978.

Fig. 2. Figures from IRRI annual reports using genotype OS4 as a drought-resistant (deep-rooted) check. These density diagrams were a popular way of expressing root data during the 1970s and 1980s.

Moroberekan was cited often due to its unique combination of showing deep root growth but low drought tolerance when root depth was restricted, and was even used as a drought-susceptible check in some screenings (IRRI 1979). Kinandang Patong was highlighted in the root/drought sections of IRRI annual reports in the 1970s for showing deep root growth. Because Kinandang Patong showed no severe mid-day internal water deficit and higher dawn leaf water potentials, it was hypothesized to have a root system "superior" to that of IR28 (IRRI 1979), characterized by greater root length density at depth

(Cruz and O'Toole 1985).

In terms of traits that were studied during the 1970s in addition to maximum root depth, large diversity for deep root:shoot ratio (based on roots below 30 cm) was observed in relation to drought response (IRRI 1977). An equation was derived for estimating root mass from shoot mass data (IRRI 1978). Radiolabeling was used to demonstrate that tillers become independent of the main shoot and rely on their own root system (IRRI, 1978). Some work was conducted on the interactions between drought and aluminum toxicity, which limits root growth (IRRI 1980). Genetic differences in the ability to penetrate a compacted layer were noted, and more research on penetration of compacted layers was suggested to be necessary (IRRI 1975). There was some mention of xylem vessel diameter (Chang and Vergara 1975), root branching, root diameter, and root hairs (IRRI 1978) during the 1970s, but no strong conclusions about these traits were made in terms of drought response. A low tiller number was reported to be related to deep rooting habit (Yoshida and Hasegawa 1982).

Exploration of plant parameters that could be used for drought screening

During the 1970s, IRRI's research groups were divided into genetic evaluation and utilization (GEU) teams that typically included an agronomist, pathologist, entomologist, physiologist, and a soil and cereal chemist (IRRI 1980). Though the target ecosystems for drought were upland agroecosystems until 1976, the importance of drought in rainfed lowland systems was recognized, although this recommended shift in experimental conditions to rainfed lowland drought was not implemented until the 1990s. Drought resistance identified by plant breeders in the 1970s was largely dependent on avoidance (IRRI 1976). As such, research efforts on roots were focused on linking root growth in containers with drought scoring in the field.

Comparisons of root growth in containers with scoring/performance in the field included comparisons of root mass distribution with depth between root boxes and field studies (IRRI 1977). The shallower root distribution in the field was thought to be due to genetic predetermination, lack of oxygen, or the presence of a hardpan. We have still not explained exactly why roots grow deeper in cylinders than in the field, even at similar soil bulk densities. Other studies revealed root growth in root boxes to be well correlated with soil water extraction in a field line-source irrigation trial, detected by neutron probe readings (Puckridge and O'Toole 1981). Deep root:shoot ratio (based on roots below 30 cm) was reported to be closely related to the drought resistance ratings made by breeders (IRRI 1976).

During the 1970s, physiologists were screening root growth of large numbers of genotypes, as required by the breeding program. Using the root box technique, 200 varieties were screened for root depth in 1975 (IRRI 1976); 768 were screened for root depth in 1977, of which 256 were classified as deep, mostly upland varieties (IRRI 1978); and 1081 were screened for deep root:shoot ratio in 1979 (IRRI 1980). A number of traits were explored that were hypothesized to be representative of deep root growth, including root pulling force in flooded paddies and time to flowering, for which shorter time to flowering was reported to be correlated with deep root:shoot ratio.

Root study methods at IRRI in the 1970s

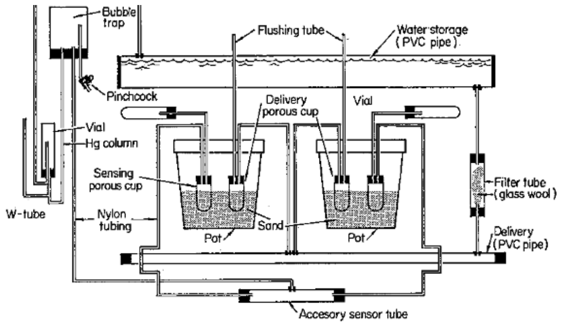
Of all the root techniques used at IRRI during the 1970s, perhaps the most distinct was "root pulling force" (Fig. 3), in which individual hills were vertically pulled out of the soil, and the force required to do so was reported to be correlated with root growth. This measurement was conducted in flooded paddies, using about 10 hills per plot. Other root measurements in the field included core sampling coupled with the use of photographs to estimate total root length (IRRI 1978). Both vertical and lateral root distribution were assessed, and the need for uniformity of field soil was emphasized for root studies.

Greenhouse root studies during the 1970s included the use of tanks (hydroponic systems) and soil-filled boxes, and a unique greenhouse setup for maintaining constant soil water potential (Fig. 4). Containers were also used to conduct large-scale screenings on drought tolerance under limited rooting depth (IRRI 1979).

Description of a plant ideotype for improved yield under drought

Water stress at different growth stages was recognized to affect yield differently (IRRI 1971). A collection of terms was used to describe the different types of drought stress responses that allow a plant to produce grains under stress (O'Toole and Chang 1978): 1) escape (e.g., early flowering, or matching crop duration and development to the rainy season length), 2) avoidance (e.g., deep root growth to allow continued water uptake), 3) drought tolerance (e.g., the ability to withstand very negative soil water potentials), and 4) drought resistance, an overall term for the ability to produce grains through any of the above mechanisms (Fig. 5). Root morphology and rate of development were thought to be the most important characteristics for drought resistance (Krupp et al. 1972).

Fig. 3. Various setups to measure “root pulling force,” a method used at IRRI to assess root growth in the field that was reported to be linked to root architecture. Photos are from the IRRI annual reports (IRRI 1977 and 1984) and John C. O’Toole.



10. An automatic setup for maintaining a predetermined and constant soil moisture tension in a pot. IRRI, October 1974.

Fig. 4. Greenhouse setup for maintaining a constant soil water potential (IRRI 1975).

The 1980s: Initiation of genetic studies on root/drought traits

During the 1980s at IRRI, more emphasis in general was given to research programs for irrigated systems rather than stress-prone environments. In the drought program, the screening strategy was for survival of severe drought stress in the field and in containers limiting root depth. Although the rainfed lowland environment was emphasized in the breeding program, most screening was still done in upland vegetative-stage drought conditions. Common root methodologies included the use of aeroponics in the greenhouse and root pulling force in the field. Canopy temperature was added as a screening parameter in the 1980s (IRRI 1984 and 1986) and was determined to be a good selection tool for reproductive-stage drought stress screening (Garrity and O’Toole, 1995). Genetic studies for roots began in the 1980s. Although the root/drought program seemed to be quite active throughout the 1980s, 1985 marked the end of the special section on “Root studies” in the IRRI annual reports.

Root trait studies in the 1980s – aeroponics, anatomy, and root pulling force

Studies using aeroponic culture started in the 1980s, with a focus on nodal root growth in terms of diameter (thickness) and maximum root depth (Fig. 6; Armenta-Soto et al. 1982). Trends in aeroponics were reported to parallel those from field, tube, or root box studies (IRRI 1982). The “deepest and thickest” roots were observed in drought-resistant traditional dryland varieties, bulu varieties were noted to vary markedly in root length and thickness, and semidwarf varieties stood out for root number only (IRRI 1982; Zuno et al. 1990). Genetic variation was explored in terms of root diameter and the relationship with elongation rates (IRRI 1984). In terms of root growth patterns with time, Dular stood out as having significantly increased growth at 17 days after sowing (DAS), and all genotypes studied showed a slowed elongation rate at 38 DAS (IRRI 1985). Lines with root length equal to or longer than that of Moroberekan were identified, including Azucena (IRRI 1987). Root length was reported to be correlated with shoot length (IRRI 1985), and significant genetic variation was observed for all root traits except root:shoot ratio (IRRI 1990).

Root anatomy was characterized during the 1980s

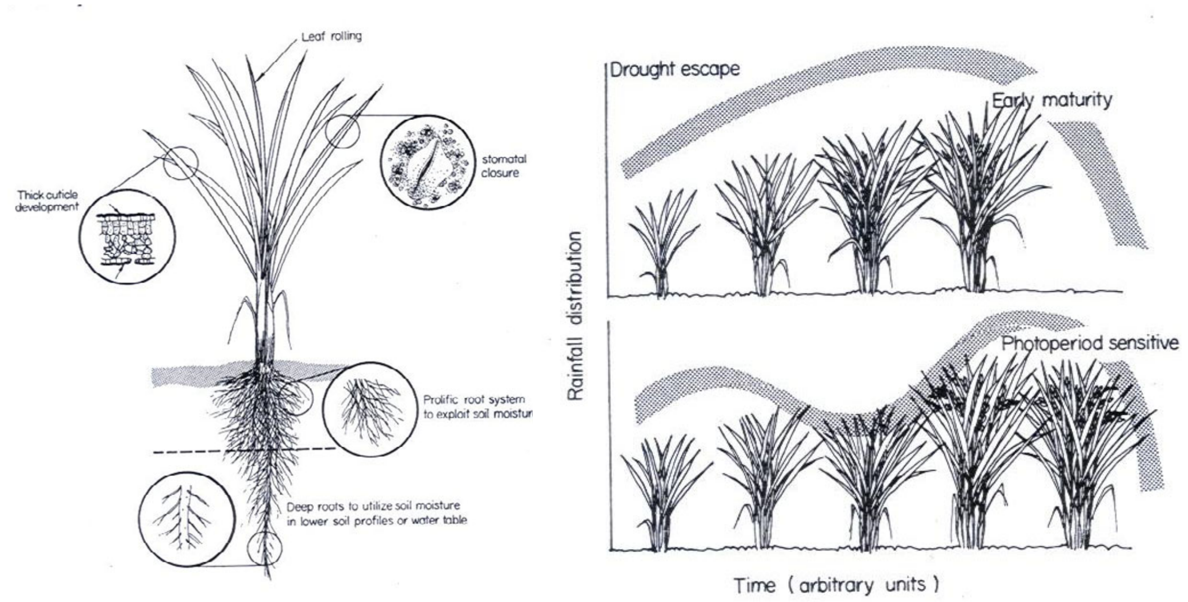
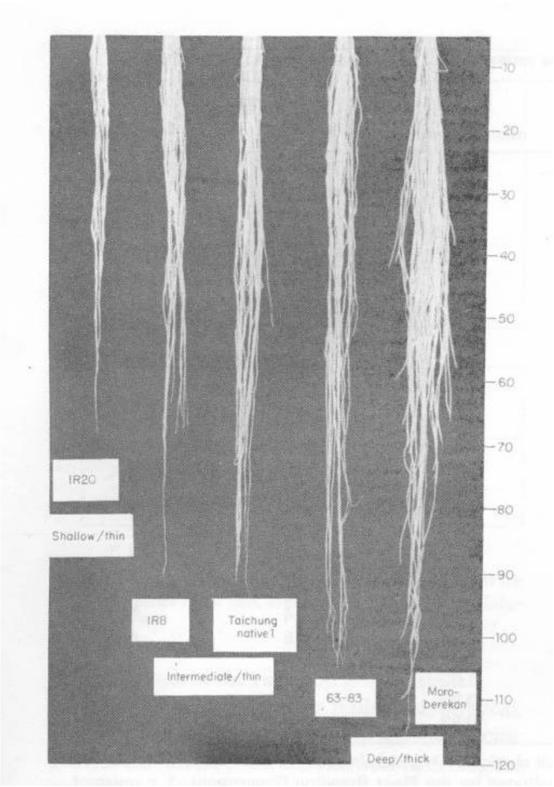


Fig. 5. Figures illustrating key traits/mechanisms for drought response in rice (O'Toole and Chang 1978).

with the hypothesis that large-diameter xylem vessels could be a drought resistance trait. Varietal differences in the number and diameter of xylem were explored, and upland cultivars were reported as having larger xylem vessel diameters (IRRI 1986; Bashar et al. 1990). The relationships between leaf water potential and transpiration as affected by root xylem anatomy were investigated, and, although some positive correlations were reported, experimental error was high for these studies (IRRI 1988). Despite the genetic variation in xylem diameter that was identified, the conclusion was that "increasing root xylem vessel radii probably will not increase drought resistance" (Yambao et al. 1992).

In terms of root diameter, coarse nodal roots ("thick roots") were hypothesized to be important for penetration of hardpans, and the response of roots and shoots to soil mechanical impedance received increasing attention at IRRI in the mid-1980s (IRRI 984; Hasegawa et al. 1985). Using line-source irrigation experiments in the field, soil was characterized as having increased mechanical impedance when moisture levels decreased (IRRI 1984), and root length density was reported to be related to soil mechanical impedance (IRRI 1985). Container studies that limited rooting were thought to be useful for environments where rooting depths are restricted, such as in the case of impenetrable hardpans. Drought tolerance screening under limited rooting depth was conducted on 108 lines in 1984 (IRRI 1985) and 105 lines in 1985 (IRRI 1986), in which drought was imposed until the susceptible check – usually Leb Mue Nahng – died, followed by rewatering. However, it was noted that lines with poor drought tolerance ratings from the field recovered well in these container studies.

In the field, root/drought studies during the 1980s were conducted using the line-source sprinkler system, which included a central irrigation line that provided decreasing amounts of water at distances farther from the center of the field, planted under upland conditions. Root experiments were conducted using the line-source sprinkler system on the assessment of divergent rice ecotypes at the vegetative phase (IRRI 1984), the relationship between root length density and soil moisture content (Puckridge and O'Toole 1981; Fig. 7), and interactions between drought and nutrient uptake (IRRI 1985). It was observed that root morphology changed more than root weight among line-source zones (IRRI 1985). Additionally, some root studies on rainfed lowland rice in puddled soil were conducted that described the use of a root length scanner (IRRI 1984), as well as root pulling force. A monolith sampler was used for taking soil-root samples in flooded, puddled soil (Fig. 8; IRRI 1985). Soil sections that contained roots were put into a sieve and gently washed with a hose until only the roots remained (K. Ingram, personal communication). Reduced root growth under drought conditions was associated with increased mechanical impedance of the soil (Ghildyal and Tomar, 1982; Cruz et al. 1986; Thangaraj et al. 1990).



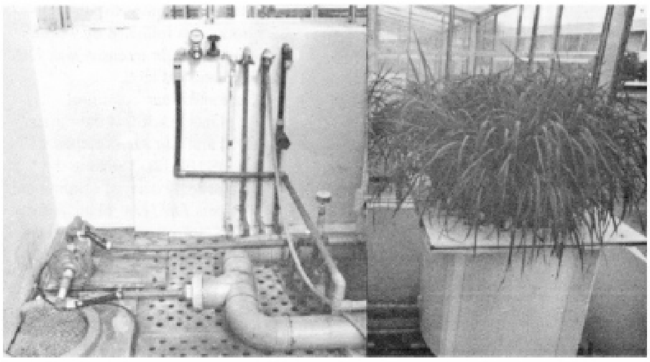

General view of the aeroponic device inside a phytotron glasshouse at IRRI.

Fig. 6. Genotypic variation in nodal root growth in terms of length and diameter (left; IRRI, 1983) was commonly studied using an aeroponic system in the 1980s (right; Armenta-Soto et al. 1982).

Roots and drought breeding in the 1980s: initiation of genetic studies

Genetic studies started in the 1980s to improve selection methods for root characters. In an 8-parent set of crosses, F_1s commonly exceeded the maximum root length of parents (IRRI 1982). Moroberekan was reported as having recessive alleles controlling thick root tips and dominant alleles for root dry weight, whereas OS4 was reported to have dominant alleles for thick root tips. Among F_2 lines, more lines were observed with many long roots, as opposed to very thick roots, leading the scientists to conclude that these results may explain the difficulty in crossing lines with contrasting root phenotypes, since the traits appeared to be controlled by combinations of dominant and recessive alleles. IR20 × Kinandang Patong and IR20 × Moroberekan crosses were made in an effort to select for lines with deep and thick roots (IRRI 1984). Genetic studies of root systems on crosses of five deep-rooted and one shallow-rooted variety concluded that multiple genes are responsible

Fig. 7. Line-source sprinkler studies (left) were popular setups for upland drought studies in the 1970s and 1980s at IRRI. Genetic effects on soil moisture were characterized by a neutron probe (right). Photos are from Ofelia Namuco.

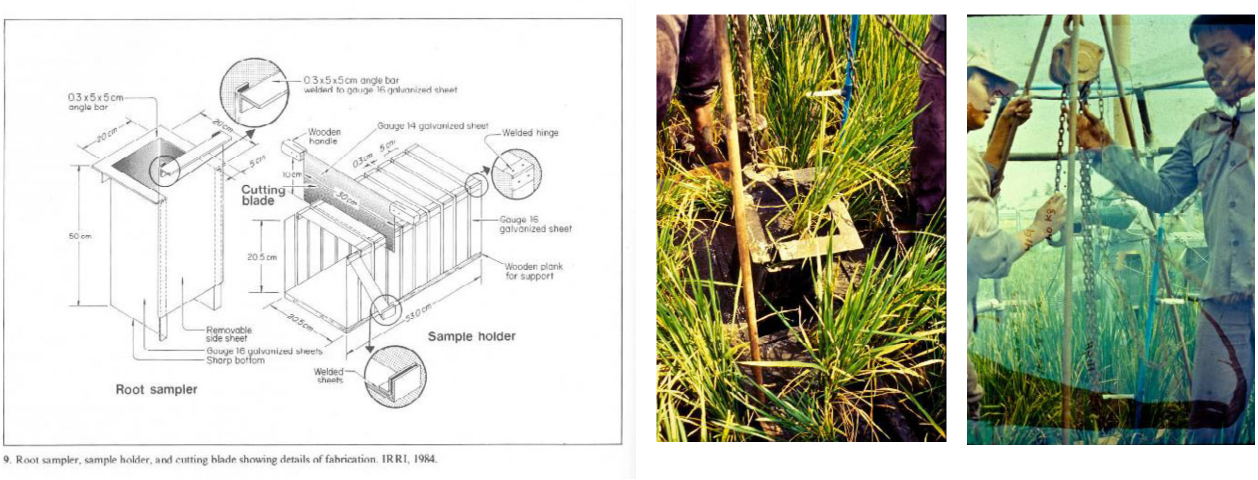


Fig. 8. IRRI monolith sampler setup (left; IRRI 1985). Monolith samplers were used in well-watered treatments and lifted from the soil profile using a suspended chain block (right; photos from Ofelia Namuco).

for root length, thickness, and weight, and that heritability was high (75–90%) (IRRI 1985). Analysis of an IR20 × MGL-2 population indicated all root traits to be polygenic with relatively high heritability (Ekanayake et al. 1985).

Plant ideotype: emphasis on "deep and thick roots"

During the 1980s, the plant ideotype for drought included a deep and thick root system (referring to characteristics of nodal roots), moderate tillering ability, short and narrow leaves, and an intermediate plant height. Root length, thickness, and xylem vessel number and area were found to be positively correlated with drought resistance score in the field, whereas root number was not (Haque et al. 1989). A number of statements about roots were made in the IRRI annual reports regarding drought response. In some cases, root characteristics did not correlate well with drought resistance in the field. There were speculations about thin roots not being able to extract or store enough water to meet atmospheric demand, concluding that thicker roots, which also had larger diameter xylem vessels, would confer drought resistance (IRRI 1983 and 1987). Although deep root growth was emphasized, drought tolerance at shallow rooting depths was mentioned to be important for the ability to recover from drought (IRRI 1983). It was recognized that earliness alone or root thickness alone could not guarantee drought tolerance, since both shallow- and deep-rooted plants varied in their drought response in the field (IRRI 1989), and it was mentioned that drought tolerance was not necessarily correlated with good drought recovery (IRRI 1984).

The 1990s: Characterization of environmental effects (G×E) on rice root growth and mapping root QTLs

During the 1990s, root/drought research at IRRI expanded to consider environmental effects (G×E analysis), including the addition of the rainfed lowland program. Annual reports included separate sections for rainfed lowland and upland research. Increased attention was given to rainfed lowlands for drought research as it was recognized that the total land area affected by drought was greater in rainfed lowland than in upland systems. The drought breeding strategy during the 1990s was selection for traits, and as such considerable effort was put into identifying and introgressing QTLs, particularly for root traits, and generating targeted drought treatments in screening trials that were relevant to the different upland environments (Courtois and Lafitte 1999). Drought researchers at IRRI were also collaborating with/being influenced by scientists outside of IRRI who published important papers that contributed to the definition of the overall IRRI drought strategy, including Boyer (1982), Passioura (1983), Fukai and Cooper (1995), and Nguyen, Chandra Babu, and Blum (1997). A large part of IRRI's drought research at the time was funded by the Rockefeller Foundation.

Physiology studies: differences among isozyme groups and studying root function for water uptake

Root methodologies continued to advance in the 1990s, including the use of a minirhizotron and Comair scanner for root length quantification. Large (about 1 m tall) PVC cylinders were used in green-

Fig. 9. Large (about 1 m tall) PVC cylinders were used in greenhouse root studies starting in the 1990s. Photo from Brigitte Courtois.

house root studies (Fig. 9), and researchers recognized the importance of studying root distribution under drought, which would require observing root growth in different soil layers rather than sampling the entire root system together (IRRI 1996). The use of herbicides at depth in the field and greenhouse revealed similarities with their previously characterized rankings in terms of root growth, but the herbicide techniques used were not concluded to be an efficient screening method at that time (Trebuil et al. 1996). Root types by isozyme group were described as indica types showing thin, shallow roots; japonica types showing thick and deep roots; and aus types showing roots of intermediate thickness and deep root growth (Lafitte et al. 2001).

A characteristic of drought-resistant cultivars was reported to be a quick response in root growth to changing soil water status, since variety N22 showed trends of having its greatest total root length before stress, but showed an early decrease in root length after the onset of stress, which subsequently increased again quickly after rewatering (IRRI 1991). A study on root growth per tiller in upland conditions pointed to a link between tillering and nodal root development in the spatial expansion of the rice rooting zone (IRRI 1996). The concept that different drought stress severities have different effects on root growth was examined in pots with treatments of severe water deficit after panicle initiation or with a slow and progressive drought treatment during tillering (IRRI 1996). Although not consistently apparent with xylem vessel diameter and root length density, yield under drought was reported to be correlated with a rapid response of root growth to changing soil moisture status and root length density below 20 cm (Ingram et al. 1994).

In addition to root architecture, studies on root function for water uptake increased during the 1990s. Genetic variation for water extraction rate was reported to be related to osmotic adjustment ability (Kamoshita et al. 2000). Rice was reported to have lower water uptake ability per length of root than maize (Kondo et al. 2000). Root branching and lateral root growth were investigated in the context of drought response, and genetic variation was reported for these parameters (Bañoc et al. 2000; Azhiri-Sigari et al. 2000).

An emphasis on genotype × environment effects

Environmental effects on root growth were emphasized during the 1990s through increased attention to rainfed lowland environments as well as upland drought studies. In rainfed lowland environments, a set of lines was identified as differing in hardpan penetration capacity, osmotic adjustment, and gross root morphology (IRRI 1996). Reasons behind the generally shallow distribution of roots in rainfed lowlands compared with uplands were hypothesized to be oxygen supply, nutrient distribution, rate of onset of stress, root signals, or soil acidity. Researchers also questioned whether the few roots that were thought to be present below 10 cm would be enough to meet transpiration demand as drought increased in rainfed lowland soils.

Several studies during the 1990s focused on studying root penetration of hard layers (Yu et al. 1995; Clark et al. 2002). Comparison of a set of genotypes in 36 environments across Asia identified a subset of lines with the most stable yields that included deep-rooted genotypes (Wade et al. 1999). Root/drought studies were conducted in rainfed lowland fields at partner sites in addition to those in the Philippines (Fig. 10), including characterizing root penetration of hardpans in Bangladesh, where genetic variation for hardpan penetration was reported to be distinct from that for water uptake below 15 cm (Samson et al. 2002), and root growth response to soil texture in Thailand, where soil texture was reported to affect root:shoot ratio (Pantuwan et al. 1996). Studies on management practices at IRRI concluded that wet-seeded rice was more drought-resistant than transplanted rice due to greater total root length (Tuong et al. 2002). In a study using several upland sites, genotype had the strongest effect on nodal root number, specific root weight, and root:shoot ratio, whereas the environment had the strongest effect on deep root ratio and total root dry weight (Kondo et al. 2003).

Genetic studies: QTL mapping for root traits

In the 1990s, mapping populations were developed in both the upland and rainfed lowland research pro-

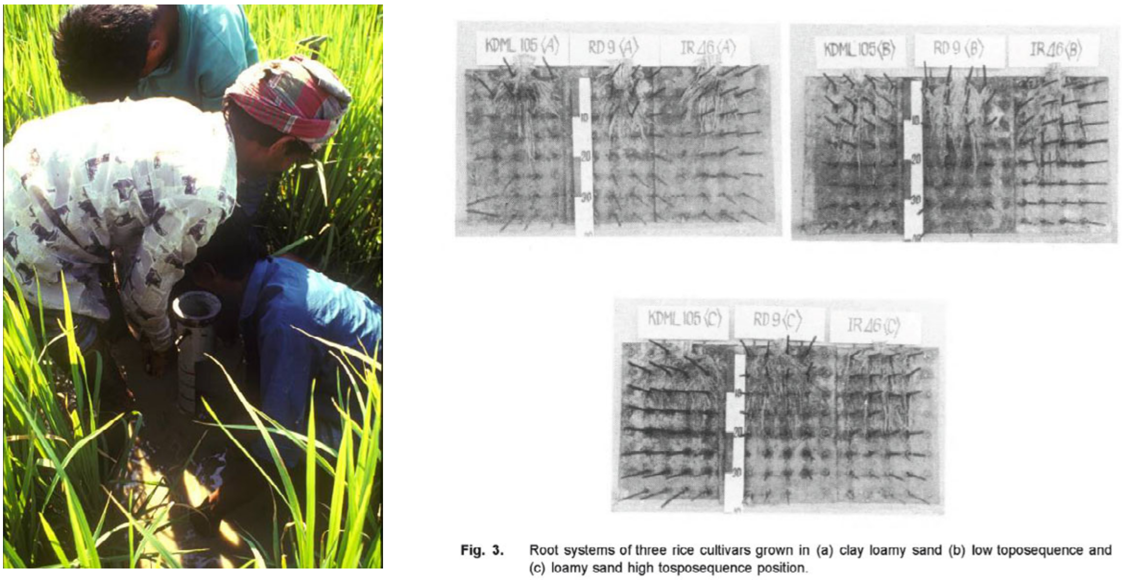


Fig. 3. Root systems of three rice cultivars grown in (a) clay loamy sand (b) low toposequence and (c) loamy sand high tosposequence position.

Fig. 10. Increased attention to environmental effects on root/drought response involved root studies at partner sites, including Rajshahi, Bangladesh (left; photo from Ben Samson), and Ubon Ratchathani, Thailand (right, photo from Pantuwan et al. 1996).

grams. Molecular markers for root traits were identified that could aid in the introgression of root traits into different genetic backgrounds (Champoux et al. 1995). In the upland program, QTLs for five traits (root thickness, maximum root depth, root dry weight (RDW), deep RDW, root:shoot ratio) were identified in an IR64 × Azucena population (Yadav et al. 1997), which were common QTLs with a Co39 × Moroberekan population (IRRI 1996; Courtois et al. 2000). Common QTLs for root penetration ability (as assessed using a wax-layer system) were also found between those two populations (Zheng et al. 2000). A multi-location mapping exercise indicated several root QTLs from a Bala × Azucena population (Price et al. 1999). Subsequently, marker-assisted transfer of QTLs for root depth to IR64 was conducted, although the rate of QTL transfer was reported to be low (IRRI 2000). This work resulted in the development of several near-isogenic lines in the IR64 background showing differences in root mass or length (Shen et al. 2001).

Mapping of root traits was done in upland japonica × lowland indica crosses (CT9993 × IR62266; Kamoshita et al. 2002 and IAC165 × Co39; Courtois et al. 2003) and within lowland indica (IR58821 × IR52561) populations, leading researchers to conclude that constitutive root traits could be improved by crossing lowland indicas with lowland indicas, or by introgression with upland japonicas.

The 2000s: Direct selection for yield under drought, followed by a renewed focus on root/drought studies

In the 2000s, physiology studies on root function for water uptake became more detailed. The basis of many physiological studies was to identify traits that could be used to screen diverse sets of genotypes or that could be mapped to improve selection efforts by drought breeders (Lafitte and Bennett 2003). Screening of deletion mutants was also conducted to identify several lines with improved water uptake and root growth under drought (Cairns et al. 2009). Perhaps the major innovation during the 2000s pertaining to drought research at IRRI was the shift in strategy from breeding by selecting for traits to breeding for direct selection for yield under drought. This new direction resulted in the identification of major-effect drought-yield QTLs, at least one of which was subsequently linked to water uptake. Later in the decade, the Drought Frontiers Project strategized the integration of breeding with physiology, genomics, transformation, modeling, and agronomy (Serraj et al. 2011), with an emphasis on precise phenotyping methodologies in the field, greenhouse, and newly-built IRRI rainout shelters. Through this strategy, a renewed focus on roots began during the late 2000s.

Physiology studies on root function under drought

Physiology studies at IRRI during the 2000s moved toward the topic of root function for water uptake, and

10. In IRRI's new drought screening greenhouse, the drought resistance team hopes to screen from 1,000 to 3,000 rices per year. Left to right: Dr. Te-Tzu Chang, IRRI geneticist; Dr. Shouichi Yoshida, plant physiologist; Dr. John C. O'Toole, associate agronomist; and Dr. Surajit K. De Datta, agronomist.

Fig. 11. IRRI Greenhouse BG02 as a facility for drought screening in soil-filled tanks in the 1970s (left; IRRI, 1975) and as a semi-automated lysimeter facility for simultaneous monitoring of water uptake and leaf area (2011).

many studies were conducted in collaboration with other institutes. Xylem vessel cavitation was observed, and nighttime root pressure was hypothesized to be important for refilling of cavitated xylem vessels (Stiller et al. 2003). Root pressure was assessed by measuring the amount of sap exuded from single cut tillers, and initial analyses indicated lower root pressure to be associated with drought sensitivity (Lafitte and Courtois 2002). Significant variation among locations for soil penetration resistance was observed (Cairns et al. 2011), further emphasizing the importance of environmental characterization in root studies. Rice was reported to have high root hydraulic resistance compared with other crops (Miyamoto et al. 2001), and the water flow in roots was thought to be most affected by the endodermis (Ranathunge et al. 2003) and predominantly through the apoplast (Ranathunge et al. 2004). Studies with limited rooting depth concluded that root attributes limit rice performance more than shoot attributes under drought, based on physiological responses of rice leaves compared with those of other species (Parent et al. 2010). However, root growth kinetics studies in slant-tubes grown over a range of drought severities indicated rice leaf elongation to be much more sensitive to drought than root growth (Mabesa 2010). In the same IRRI greenhouses used for drought screening in the 1970s, a lysimeter facility was set up using 1-m-tall PVC cylinders that allowed for monitoring of water uptake dynamics while estimating shoot growth through digital imaging (Fig. 11).

Drought breeding: identification of major-effect drought-yield QTLs

In the drought breeding program, a major shift was made away from selecting for traits, and breeders instead started conducting direct selection for grain yield under drought. This strategy facilitated the identification of several major-effects QTLs for yield under drought (Kumar et al. In Press). Physiological characterization of $qtl_{12.1}$ in the IRRI lysimeter facility revealed an effect of the QTL on water uptake (Bernier et al. 2009).

Efforts toward association mapping began during the 2000s, in which detailed genetic characterization was linked to phenotypes. This work started with the characterization of the OryzaSNP panel, a set of 20 diverse genotypes that were mapped for approximately 160,000 SNP markers (McNally et al. 2009). In field root architecture studies (Henry et al. 2011) and greenhouse lysimeter studies at IRRI (Gowda et al. 2012), aus lines stood out for having greater root growth at depth and greater water uptake under drought. Further screening of the OryzaSNP panel also began through a multi-institutional root/drought network.

Current status of root/drought research at IRRI

Research networks with partners continue to be important for root studies and for understanding/screening in target environments. Studies on rice root physiology under drought are progressing, including studies linking root hydraulic conductance, anatomy, and aquaporin expression (Henry et al. 2012). To improve recovery of fine roots during root washing, the root washing method has been changed from rinsing over a screen to a flotation method (Fig.

Fig. 12. Although the same facility is still used, root washing methods at IRRI have evolved from 1995 (left; photo from Brigitte Courtois) to 2010 (right) in order to improve the recovery of fine lateral roots.

12). Studies on characterizing the mechanisms behind major-effect drought-yield QTLs identified in the drought breeding program indicate that several QTLs – although not all – are directly linked to root traits (Kumar et al. In Press). The distinct mechanisms conferred by these QTLs that were identified by the IRRI drought breeding group are now being pyramided. However, because these studies have revealed some unexpected traits to be associated with improved yield under drought, our view of a "rice drought ideotype" is probably less defined than in previous decades.

The identification of QTLs and genes for drought resistance is advancing both at IRRI and in other institutes. Kinandang Patong has now been used as a source for a gene controlling deep root angle, and it also shows improved root elongation ability and yield under drought (Uga et al. 2011). N22 is the source of a major-effect drought-yield QTL, $qDTY_{1.1}$ (Vikram et al. 2011). Notably, root/drought screening studies in the first decades of IRRI research had highlighted these genotypes, pointing to the strength and foresight of those early root/drought studies.

Conclusions

Many of the promising deep-rooted or generally drought-resistant genotypes that were identified in the early decades after IRRI's foundation are still in use as important drought donors today. "Deep and thick" nodal roots were focused upon over several decades at IRRI as important drought traits and they are still considered important traits for drought resistance. However, we now also consider lateral (fine) root growth, anatomy, and molecular attributes as key traits linked to yield under drought, based on the traits observed thus far behind major-effect drought-yield QTLs. For a future direction, efforts to improve our understanding of root function for water uptake should continue. There is also a strong need for more detailed characterization of the kind of drought that is occurring in farmers' fields, in order to understand how the lines being released from the breeding program might respond to different drought environments. Finally, pyramiding of drought traits could result in complementary or synergistic responses for improving drought resistance in rice beyond that already accomplished through the introgression of major-effect drought-yield QTLs.

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* All IRRI books have now been re-released with free access on the Google Books Web site: http://books.irri.org/

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Fig. 13. Some of the key people behind IRRI root/drought research during the first 50 years.
Shown from top left:
Row 1: T.T. Chang, O. Namuco, J. O'Toole, R. Cruz, S.K. De Datta, E. Yambao, G. Loresto, M. Thangaraj
Row 2: S. Yoshida, D. Puckeridge, M. Maguling, M. Haque, F. Bueno, J. Malabuyoc, P. Singh, M. Yamauchi
Row 3: J. Padilla, K. Ingram, M. Kondo, B. Courtois, B. Samson, L. Wade, A. Kamoshita, J. Siopongco
Row 4: R. Lafitte, J. Cairns, J. Bernier, R. Serraj, V. Gowda, R. Mabesa, R. Torres