



Differential responses of rice genotypes and physiological mechanism under prolonged deepwater flooding



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ABSTRACT

Submerge tolerant genotypes and genotypes representing wide range of ecosystems were compared in normal and prolonged flooding situation during 2011 and 2012. Discriminant function analysis discriminated tolerant from intolerant genotypes by shoot length, grain yield per plant, number of tillers, EBT and number of nodes per stem. Cultivar-by-biplot analysis revealed that grain yield strongly correlated with filled grains per panicle, number of EBT, number of nodes, spikelet fertility percent or basal culm girth under prolonged flooding. Further, based on biplot analysis, two rice genotypes – Puzhuthiikar and IR 72593 were selected for pot experiment to study their physiological mechanisms of escape from prolonged flooding by evaluating photosynthesis, transpiration, inter cellular CO₂ and morphometric traits. Under prolonged flooding, cv Puzhuthiikar exhibited significant increase in leaf blade length, sheath length and area and decrease in leaf blade area. Consequently, photosynthetic rate, transpiration and intercellular CO₂ were increased. Therefore, breeders who involved in breeding rice cultivars for prolonged flooding situation should emphasize on parameters leaf blade length, sheath length and area to breed genotype suitable for prolonged flooding condition.

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1. Introduction

Semiaquatic plants endure in flood plains and along river beds by keeping their part of foliage above the rising waters to avoid submergence (Kende et al., 1998). This group includes rice (*Oryza sativa* L.), which is cultivated in four different ecosystems viz., irrigated, rainfed low land, upland and flood-prone (Halwart and Gupta, 2004). Deepwater rice is characterized as rice grown in flooded condition with more than 50 cm deep for one month or longer during the growing season (Catling et al., 1988). This rice is widely grown in the river basins of Ganges and Brahmaputra in India, Irrawaddy Delta region of Burma, Chao Phraya in Thailand, Mekong in Vietnam, Niger deltas of West Africa and Amazon deltas of South America. Deepwater rice is a subsistence crop of millions of people and grown on about 13 million hectares globally. Eastern part of India alone has 3.9 million hectares under deepwater rice. The modern high yield rice cultivars yields an average of 6 tons/ha, whereas, deep water rice yields 2 tons/ha (Vergara et al., 1976; Catling, 1992).

In recent years, the deep water rice crop area has declining due to non-availability of suitable rice varieties, low profit due to low productivity, changes in rainfall pattern due to climate change, change in flooding patterns with building of roads and embankments, introduction of fish, shrimp farms and farm ponds, and the utilization of land for industrialization and urban expansion leads farmers to abandon these lands. Therefore, developing cultivars with increased yield and growth potential is of major agronomic importance as deepwater rice is the only crop that can be grown in this fragile ecosystem.

Limited knowledge on mechanisms of deep water rice, particularly of their physiological mechanisms, biochemical interactions, inheritance and the lack of effective screening techniques are the major constraints, which have slowed breeding progress. On the other hand, physiological mechanisms of submergence tolerance and elongation ability of rice are known, their functions and expressions under different soil conditions varies and are not studied well (Bhuiyan et al., 2004). Studies on mechanism and genetic basis for submergence-promoted internodal elongation of deepwater rice have received relatively little attention. The elongation of internode, as well as the degree of elongation during submergence is regulated by environmental and hormonal factors viz., ethylene, GA and ABA (Vriezen et al., 2003). Further, Hattori et al. (2009) reported

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that this trait is controlled by two major *SK* genes driven by the rice actin promoter. There are few reports available that specify the pattern of shoot elongation/internode elongation under deep-water. However, very scanty reports are available on response of physiological (leaf area, photosynthesis and non-structural carbohydrates) and morphological (shoot length, tillers, culm thickness, spikelet fertility, shoot biomass and grain yield) traits under deep-water rice (Amante, 1986; Mallik et al., 1995; Sakagami et al., 2009, 2013; Vergara et al., 2014). Therefore, this little information on these traits is not sufficient for deep water rice breeder to develop effective breeding strategies for deep water rice situation. Genotypes with tolerance for these flooding stresses might be available in indigenous landraces as they are adaptable over diverse environment. The experiment was planned with following objectives to study differential responses among diverse 332 rice cultivars from normal to prolonged deepwater flooding conditions and to find responses and expression pattern of leaf blade and sheath relative to their position and stem morphology between tolerant and susceptible genotypes under prolonged flooding.

2. Materials and methods

We conducted field and pot experiments at Annamalai University (11°24' N, 79°43' E, 5.79 mean sea level), India, in 2011 and 2012 representing rice growing area in Cauvery delta zone of Tamil Nadu state. Field experiments were carried out in 2011 and 2012 (experiment 1) and pot experiment was conducted in 2012 under controlled condition (experiment 2). The soil is Typic Haplusterts, belongs to order vertisol, texture is clay with pH 7.35.

2.1. Experiment 1: Field experiment (physio-morphological responses of rice genotypes to prolonged flooding)

Physio-morphological, grain yield and yield component traits were evaluated using 332 rice (*Oryza sativa* L.) cultivars (Table S1 Supplementary) representing different rice ecosystems such as low land, medium deep and deep. Reference genotypes of prolonged flooding tolerant (IR70215-2-CPA2-1-B-2 (IR70215) and IR67495-M-2-1-1-1-1 (IR67495)) and complete submerge tolerant (Cihorang Sub 1 and PSB RC82 Sub 1) genotypes was provided by the International Rice Research Institute, Philippines. These genotypes were grown under two treatments were: (1) seedlings transplanted into the field with ~5 cm water as control (non-flooding) throughout the growth period, and (2) transplanted seedlings were maintained under prolonged flooding condition. The experiment was laid out in a randomized complete block design with two replications. Twenty-five-day-old seedlings were transplanted in 6 m long row 15 cm apart and 20 cm between rows of 3.6 m² plot with 33 hills m². Plots were maintained with recommended fertilizer doses of 150 kg nitrogen ha⁻¹, 50 kg phosphorus ha⁻¹, and 50 kg potash ha⁻¹. Manual weeding was carried out twice. In prolonged flooding treatment, 15 days after transplanting (DAT) (*i.e.* 40th day after seeding), water level was increased in the plot by allowing 20 cm of water and further it was increased to 40 cm on 30 DAT (55th day after seeding) and 60 cm on 55 DAT (80th day after seeding) with an interval of 15 and 25 days respectively. The water level was maintained by means of an overflow system. After 35 days (90 DAT/115th day after seeding) with 60 cm water, water was drained out completely for grain maturity. The water used for flooding was with the pH 7.61 (model HI 9124, Hanna Instruments, Woonsocket, RI) and EC 0.47 dsm⁻¹ (model HI 9033, Hanna Instruments). The same experiment was repeated in the following year (2012).

Supplementary Table S1 related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.fcr.2014.11.007>.

During the experiment in 2011, the average daily solar radiation with photosynthetic photon flux density, relative humidity, maximum and minimum air temperature were approximately 1312 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 75.8%, 31.8 °C and 23.9 °C respectively. On the other hand, during the experiment in 2012, the average daily solar radiation with photosynthetic photon flux density, relative humidity, maximum and minimum air temperature were approximately 1216 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 71.7%, 32.1 °C and 23.9 °C respectively. Fifty five days after transplanting at active vegetative phase, three plants in the center from each genotype of each replication were selected to study the shoot length (cm), blade length (cm), sheath length (cm), number of leaves in primary stem (externally visible leaves), number of nodes and internodes in primary stem were determined in prolonged flooding and non-flooding plots. At maturity phase, observations on days to 50 per cent flowering (DFF), shoot length (cm), number of tillers per plant, number of ear bearing tillers (EBT), panicle length (cm), basal culm girth (mm), number of leaves in primary stem (externally visible leaves), number of nodes in primary stem, number of filled grains per panicle, spikelet fertility per cent, 100 seed weight (g), grain yield (g), and whole plant dry weight (DW) (g) were measured. Culm width was measured approximately 2–3 cm above the soil surface with a caliper. The whole plant was collected and dried in an oven for 72 h at 70 °C for DW measurement. Two season field trials were pooled and subjected to statistical analyses.

An initial descriptive statistics, including mean, standard deviation, minimum and maximum values, and distribution pattern was performed by box plot technique. Paired *t*-test was performed to establish significant differences between the variables of 332 rice genotypes under prolonged flooding and non-flooding conditions. Principal component analysis (PCA) was performed to estimate Euclidean distance between genotypes and traits responsible for prolonged flooding and grouping pattern of rice genotypes in vegetative and maturity phase of crop growth. Biplot figure explains variances of the variable, correlation between the variables, Euclidean distance between two genotypes in the multivariate space and adaptation of genotypes to specific environment (Anandan et al., 2011). These analyses were performed using the Windowstat 7.5 software (Indostat Services, Hyderabad, India).

Further, an attempt was made to find the best variable(s) that can discriminate high and low yielding genotypes under prolonged flooding. For this, the genotypes were divided into two groups (tall plant with >10 g grain yield and tall/intermediate/semi dwarf with <10 g grain yield) based on the broad trend observed in the PCA. Subsequent analyses involved forward stepwise discriminant function analysis (DFA) to understand the combination of variables which can best explain the grouping in STATISTICA ver. 10 (StatSoft, Inc., Tulsa, OK, USA). In DFA, eigenvalue gives information about the effectiveness of the discriminant functions. The eigenvalue is the ratio of the attributable sum of squares to the within groups or error sum of squares. The size of the eigenvalue is helpful for measuring the spread of the group centroids in the corresponding dimension of the multivariate discriminant space. Larger eigenvalue indicate that the discriminant function is more useful in distinguishing between the groups.

2.2. Experiment 2: (Pot experiment)

The objective of the pot experiment was to confirm the results of the field experiment. Therefore, two varieties Puzhuthiikar and IR72593-B-3-2-2-2-B (IR72593) were selected for further study. Puzhuthiikar and IR72593 exhibited increased shoot length, blade length, number of nodes and internode length under prolonged flooding and differ in grain yield. Puzhuthiikar and IR72593 matures in 140 and 135 days respectively. Seedlings were raised in field nursery and transplanted into earthenware pots (30 cm height

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