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 CO_2 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 CO_2 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

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 deepwater. 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 deepwater 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 (Ciherang Sub 1 and PSB RC82 Sub1) 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 μmol m⁻² 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 μmol m⁻² 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

and 20 cm diameter) containing well puddled clay soil (pH 7.37, EC 0.44 dsm^{-1}) and farmyard manure in 3:1 ratio. Seven plants of 40 day old seedlings were placed in a concrete water tanks under a water depth of 50 cm (30 cm pot height and 20 cm absolute water depth) as a prolonged flooding plot. Soon after, water level was increased to 70 cm (30 + 40 cm) in 55 day old seedlings and 90 cm (30 + 60 cm) in 80 day old seedlings with an interval of 15 and 25 days respectively. After 35 days (115 days old plant), water was drained and observations were recorded. Tap water was used for each experiment having the pH 7.46 and EC 0.40 dsm^{-1}. Controls were setup without prolonged flooding and maintained with 5 cm water level until the end of experiment (non-flooding). Each experiment was repeated three times.

The average maximum and minimum air temperature above the water surface were 32 °C and 23.1 °C respectively, mean photosynthetic photon flux density at water surface was 1006 μmol m^{-2} s^{-1}, and relative humidity of 72%. Five plants from each genotype per replication were selected from both treatments for measurement of shoot length (cm), blade length (cm), blade area (cm^2), sheath length (cm), sheath area (cm^2), internode length (cm), root length (cm) and dry weight (g). The leaf and sheath area were measured using Weiber leaf area meter (model Acm-52301-j, ACMS, New Delhi, India). Photosynthesis, stomatal conductance, transpiration and inter cellular CO_2 were measured for each leaf that appear above the water surface in intact plants of both the treatments for five plants in three replications using portable photosynthesis system (LI-6400XT Portable Photosynthesis, Lincoln, Nebraska USA) during morning from 9:00 to 11:00 on 115 days old plant. The photosynthetically active radiation (PAR) was 1500 μmol m^{-2} s^{-1}, the CO_2 concentration of the air was 375 μl l^{-1}, relative humidity was 73.5% and air temperature was 33.07 °C.

Statistical analyses were performed for each parameter studied using Windowstat 7.5 software (Indostat Services).

3. Results

3.1. Experiment 1

A paired t-test analysis found highly significant difference for all the traits studied ($p < 0.001$) between genotypes under prolonged flooding and non-flooding conditions at both phases (vegetative and maturity) (Table 1). During vegetative phase, shoot length, number of leaves per stem, blade length, sheath length, number of nodes per stem and number of internodes were greater in plants grown under prolonged flooding condition. On the other hand at maturity phase, days to 50 per cent flowering, number of tillers, EBT, panicle length, filled grains, spikelet fertility percent, DW and grain yield per plant were greatly reduced under prolonged flooding. Shoot length varied from 75 to 210 cm in prolonged flooding condition and from 44 to 185 cm in normally raised plants. Compared with the number of leaves in normally grown genotypes, prolonged flooded plant leaf number has increased to 1.25 times.

3.1.1. Variation in physio-morphological traits under prolonged flooding and non-flooding condition

The distribution pattern of 332 rice genotypes for six traits in vegetative phase and 13 traits in maturity phase were presented in Fig. S1 (a–h) (Supplementary). The genotypes studied in the present experiment have a wide diversity of morphological and physiological traits. Except spikelet fertility percent under normal (non-flooding) condition and number of tillers, ear bearing tiller (EBT), grains per panicle and grain yield per plant under prolonged flooding, other traits were found to be normally distributed. Tiller numbers and grain yield per plant were found to be deviate significantly from the normal distribution with positive skewness ($S > 1$),

Table 1
Paired t-test analysis of 332 rice genotypes under prolonged flooding and control conditions at two different phases.

Traits	Mean		S.E
	Control	Prolonged flooding	
Phase 1: Vegetative phase			
Shoot length (cm)	59.57	109.28	1.03
No leaves	4.29	5.31	2.77
Blade length (cm)	26.81	45.38	0.65
Sheath length (cm)	15.67	22.85	0.25
No of nodes	4.29	5.46	1.66
No of internode	3.29	4.46	1.67
Phase 2: Maturity phase			
DFF	93.65	90.92	10.18
Shoot length (cm)	108.98	131.14	1.01
No tillers	17.71	7.77	0.32
EBT	15.53	6.50	0.31
Panicle length (cm)	21.31	20.78	2.80
No of leaves	3.62	4.54	1.18
Filled grains	126.53	72.51	19.83
Spikelet fertility %	84.17	55.10	21.47
100 seed wt (g)	2.17	2.17	0.28
DW (g)	25.83	17.93	0.23
Grain yield (g)	8.85	5.16	0.25
Culm girth (mm)	4.47	6.25	2.49
No of nodes	4.50	5.63	1.39

All the traits between normal and submergence were significantly different at $P < 0.001$; s.e.: standard error.
DFF: days to 50 per cent flowering, EBT: number of ear bearing tillers, DW: whole plant dry weight (DW).

while spikelet fertility was negatively skewed ($S < 1$). Among the traits studied in vegetative phase, shoot length under prolonged flooding varied from 40.74 cm to 158.50 cm as measured in Gasmal 735 and AC41620 respectively with mean of 109.28 cm and AC41620 exhibited 1.45 times of the mean value. Genotypes in prolonged condition at maturity phase exhibited minimum differences for most of the traits except, spikelet fertility percent (3.00–90.45%), dry weight (1.52–18.48 g) and basal culm girth (1.38–12.00 mm). Coefficients of variation (Cv) indicating variability among the genotypes were >60% for grain yield per plant under prolonged flooding and >30% for EBT, number of tillers, spikelet fertility and 100 seed weight. On the other hand, grain yield per plant, filled grains per panicle, tiller numbers and 100 seed weight exhibited high Cv of >30% under non-flooded condition.

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

3.1.2. Traits and grouping pattern of genotypes

Principal component analysis (PCA) was performed to identify traits responsible for prolonged flooding and grouping pattern of rice genotypes in vegetative and maturity phase of crop growth. PCA in the first and second component explained 85.28% and 11.34% (totaling 96.62%) of the variance, respectively under non-flooding condition (Fig. 1a and b). While, under prolonged flooding first component explained 76.39% and second component explained 19.50% (totaling 95.89%). During vegetative phase, under prolonged flooding condition, cultivar-by-trait biplot indicated that the traits were grouped into two, based on counts (number of nodes, internodes and leaves per stem) and length (shoot length, sheath and blade length) (Fig. 1b). However, the genotypes with elongating character and escape strategy were grouped together in quadrant 4, whereas genotypes with Sub1 (resilience mechanism) were scattered into quadrants 2 and 3.

In PCA at maturity phase under non-flooding condition, first and second component explained 75.73% and 13.3% (totaling 89.03%) of the variance respectively (Fig. 2a). Based on the loading and biplots under non-flooding conditions, it appeared that few traits are the
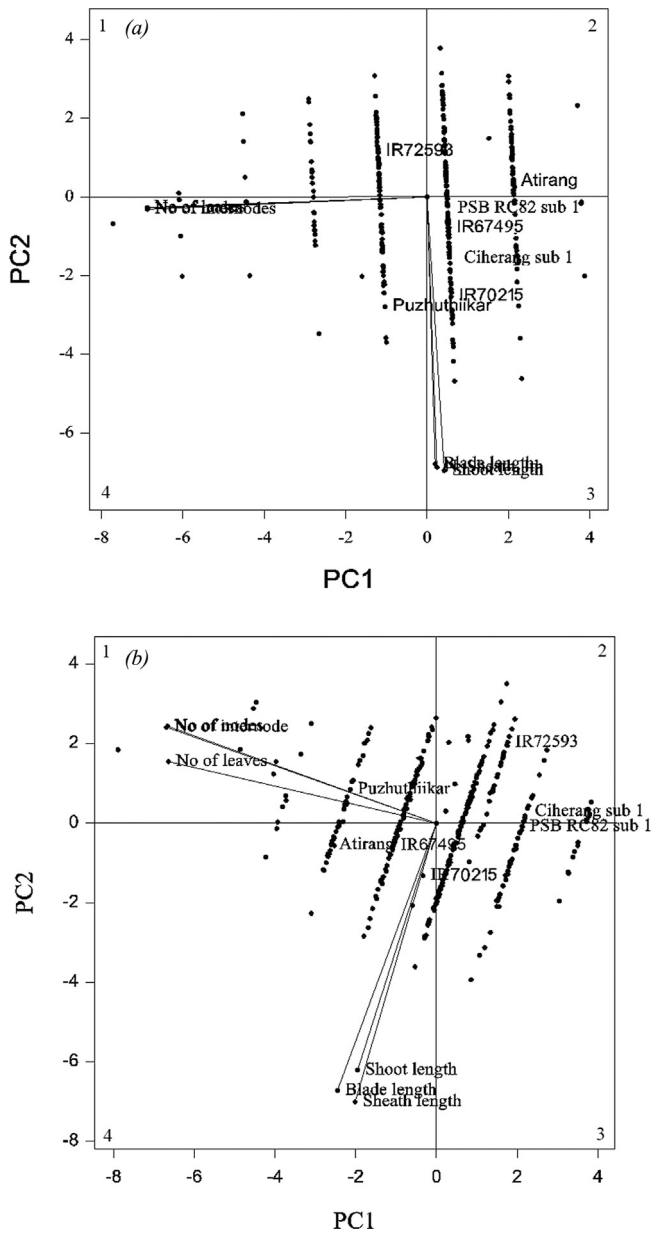
Fig. 1. (a, b) Spatial distribution of 332 rice genotypes for shoot length, blade length, sheath length, number of leaves in primary stem, number of internodes and nodes during vegetative phase for the first two principal components. (a) Nonflooding and (b) prolonged flooding conditions (experiment 1).

Fig. 2. (a, b) Spatial distribution of 332 rice genotypes for days to 50 per cent flowering (DFF), shoot length, number of tillers per plant, number of ear bearing tillers (EBT), panicle length, basal culm girth, number of leaves in primary stem, number of nodes, number of filled grains per panicle, spikelet fertility %, 100 seed weight, grain yield and whole plant dry weight (DW) during maturity phase for the first two principal components. (a) Nonflooding and (b) prolonged flooding conditions (experiment 1).

major determinants for differentiating genotypes, among which number of tillers, number of EBT, shoot length and DW plays an important role. Cultivar-by-trait biplot (Fig. 2a and b) indicated that the pairwise trait of genotype exhibited an acute angle among the yield component traits. However, grain yield per plant significantly correlated with filled grains per panicle ($r = 0.570$) and EBT per plant ($r = 0.512$) or tillers per plant ($r = 0.528$) under non-flooding condition.

The PC_1 accounted for about 81.42% and PC_2 accounted for 9.17% of the total variance (totaling 90.59%) of maturity stage under prolonged flooding condition. From Fig. 2b, it is inferred that the longest vector loading such as shoot length, DW, panicle length, filled grains per panicle and spikelet fertility are the major discriminators for grouping the genotypes under prolonged flooding conditions. Cultivar-by-biplot figure revealed relationship between variables by vector angle. Among them, number of nodes
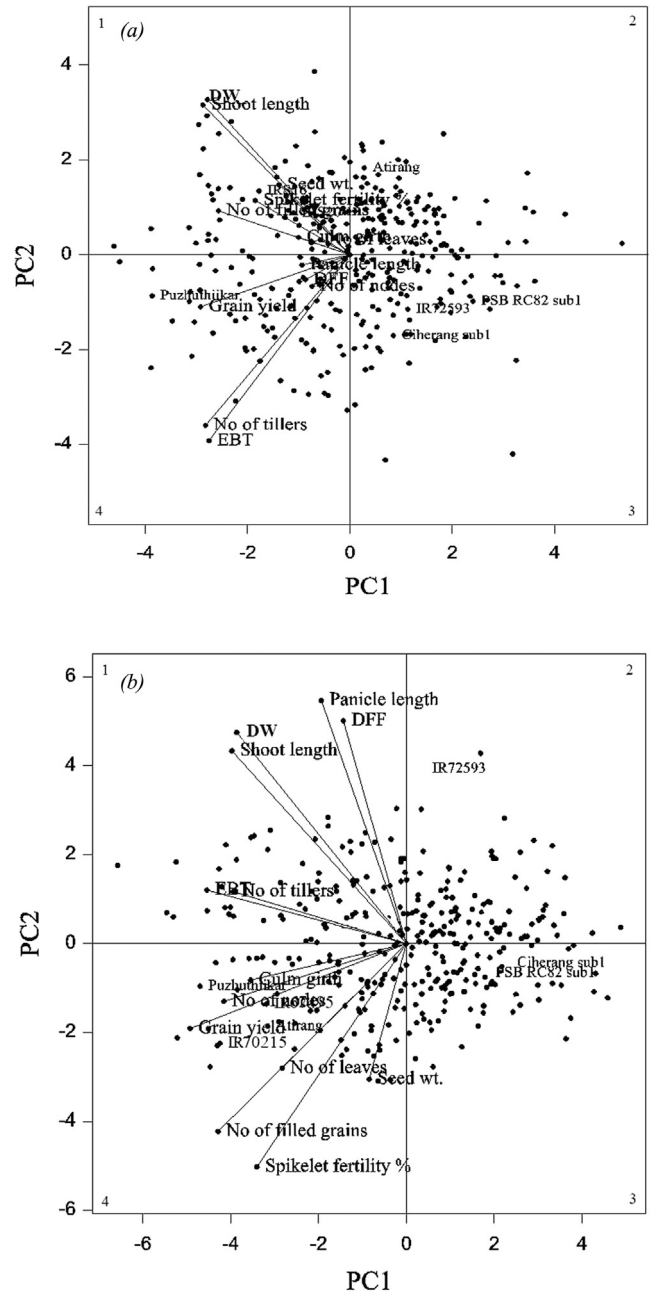
significantly correlated with number of leaves ($r = 0.627$). Similarly, grain yield per plant strongly correlated with filled grains per panicle ($r = 0.683$), number of EBT ($r = 0.645$), number of nodes ($r = 0.769$), spikelet fertility percent ($r = 0.589$) or basal culm girth ($r = 0.721$).

The position and perpendicular projection of genotypic positions onto variable vector can be used to identify genotypes having characters adoptable to specific environment. Genotypes in quadrant 1 (Fig. 2b) showed tallness, increased shoot length in response to prolonged flooding, whereas genotypes in quadrant 3 (opposite to quadrant 1) had failed to contact external atmosphere with no

elongation. In contrast genotypes in quadrant 4 showed increased node numbers, basal culm girth, grain yield per plant, filled grains, spikelet fertility percent and number of leaves with partial elongation, whereas in quadrant 2, genotypes exhibited low grain yield due to reduced spikelet fertility percent and filled grains per panicle.

3.1.3. Comparison between higher and lower yielding genotypes under prolonged flooding condition

In the present study, discriminant function analysis has effectively discriminated prolonged flooding tolerant from intolerant genotypes. Further, it found traits *viz.*, shoot length, grain yield per plant, number of tillers, EBT and number of nodes per stem to be best in explain the grouping. The discriminant function is statistically significant with high canonical correlation ($R = 0.895$), indicates that function has discriminated well. In the present investigation, eigenvalue was found to be larger 4.039 with Lambda of 0.077 has a significant value ($p < 0.001$), thus it indicates that the corresponding function explain the group membership well.

The graphical representation of PCA analysis was applied to identify trends among high yielding rice genotypes and the traits responsible for main source of the variability under prolonged flooding (Fig. 3). The first and second PC accounted for 86.04% and 8.44% of the variance respectively. The vector angles of biplot in Fig. 3 shows grain yield per plant significantly ($p < 0.01$) and positively correlated with DW ($r = 0.523$), filled grains per panicle ($r = 0.602$), number of leaves per stem ($r = 0.676$), shoot length ($r = 0.529$), number of nodes per stem ($r = 0.657$), number of tillers per plant ($r = 0.577$) or spikelet fertility percent ($r = 0.517$). Inferring from Fig. 3, the panicle length has the highest variance among the variables in the biplot, while the number of nodes per stem has the lowest. Further, it was observed that length of the vector differs when high yielding genotypes analysed separately from the whole genotype group (Figs. 2b and 3) with increased vector length of
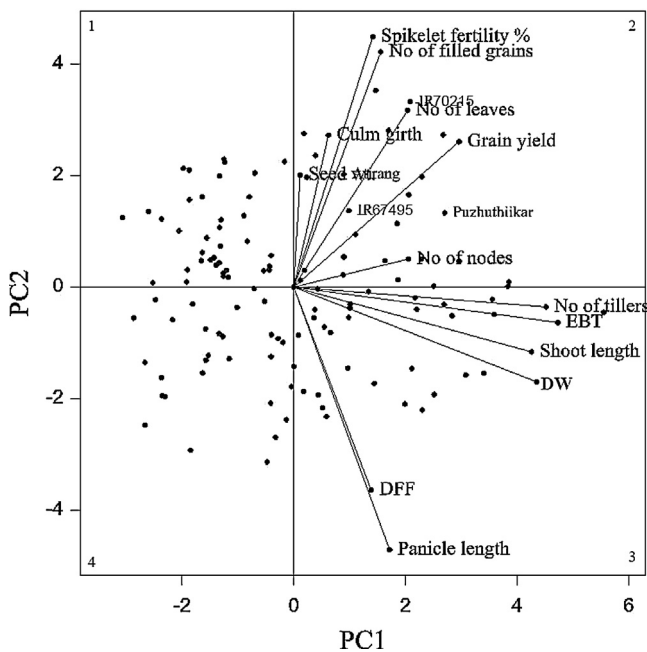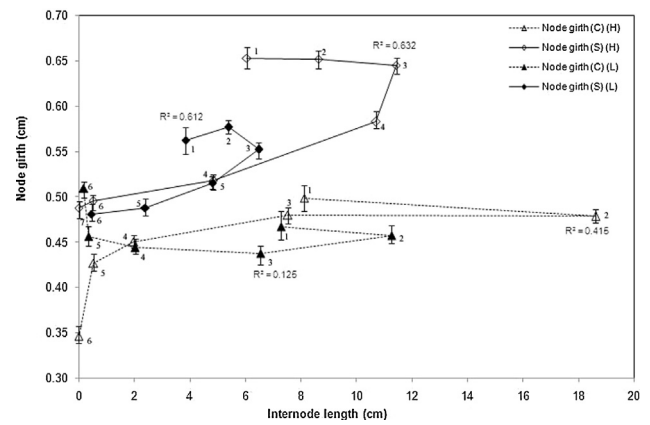

Fig. 4. Relationship between internode length and culm girth at each node under nonflooding and prolonged flooding condition (experiment 1). The vertical bars represent s.e. N-nonflooding, P-prolonged flooding, H-high yielding and L-low yielding. Number 1–7 represents order of nodes from base.

panicle length followed by number of leaves and decrease in number of nodes.

The node number and internode length had a great impact on shoot elongation during prolonged flooding situation and they correlated significantly ($p < 0.01$). Under prolonged flooding situation, linear regression between order of node and internode length revealed to be $R^2 = 0.962$ against non-flooding ($R^2 = 0.432$). First three internodes from the base had increased in length during prolonged flooding, while in non-flooding condition the first two internodes elongates and further reduction in internode length was observed in both conditions. In prolonged flooding situation, culm girth at each node varied from 0.49 to 0.65 cm in better performing (high yielding) genotypes with 0.65, 0.65, 0.64 and 0.58 cm thick node from base (Fig. 4). Whereas, 0.56, 0.58, 0.55 and 0.52 cm wide node was observed from base in poor performing (low yielding) genotypes with culm girth range of 0.48–0.56 cm. Culm girth at each node in non-flooded condition ranged from 0.35 to 0.50 cm and 0.44 cm to 0.47 cm in high and low yielding genotypes respectively.

3.2. Experiment 2

To ascertain the relationships among growth parameters under prolonged flooding and non-flooding of Puzhuthiikar and IR72593, the following parameters *viz.*, shoot length, blade length and area, sheath length and area, internode length, root length and DW were studied and presented in Figs. 5a–c and 6a–e. The maturity period slightly varies in both the cultivars between treatments. The cultivar Puzhuthiikar reached its maturity in 133 days, one week ahead of non-flooding, while IR72593 matured in 138 days by delay in three days.

3.2.1. Differences in plant height, root length, and dry weight between the two contrasting genotypes under prolonged flooding and non-flooding situation

Fifteen days after flooding of 55 day old seedling, the shoot length of Puzhuthiikar and IR72593 increased 1.10- and 1.09-fold higher than its non-flooding counterpart respectively. The shoot length of prolonged flooded and non-flooded plants of IR72593 and Puzhuthiikar increased at an almost identical rate until 15 days of flooding. Thereafter, the shoot length of both the plants continued to increase under prolonged flooding due to increase in water level (Fig. 5a). The shoot elongation rate of both cultivars at 80 and 115 days were significant ($p < 0.01$) and greater in flooded plants than its non-flooded counterparts.

Fig. 3. Spatial distribution of tall high yielding rice genotypes for days to 50 per cent flowering (DFF), shoot length, number of tillers per plant, number of ear bearing tillers (EBT), panicle length, basal culm girth, number of leaves in primary stem, number of nodes, number of filled grains per panicle, spikelet fertility %, 100 seed weight, grain yield and whole plant dry weight (DW) during maturity phase for the first two principal components under prolonged flooding conditions (experiment 1).
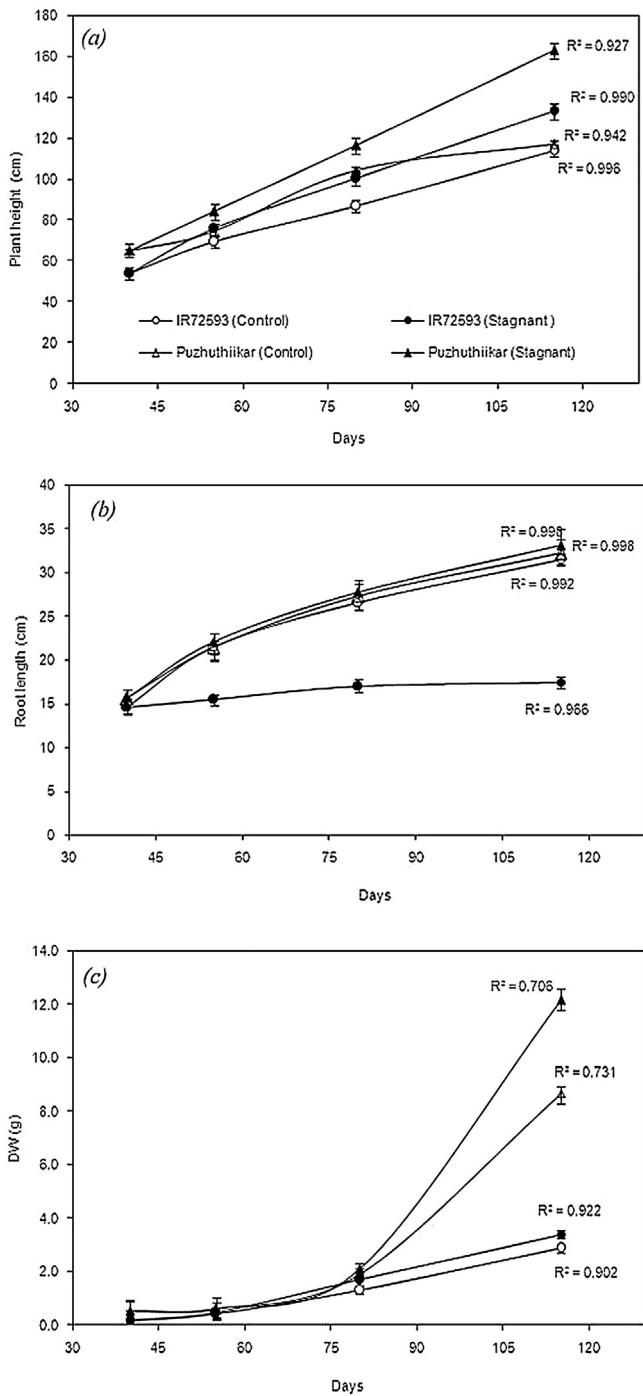
Fig. 5. (a–c) Effect of prolonged flooding on plant height, root length and dry weight (DW) of 40th, 55th, 80th and 115th day after sowing. Bars indicate the standard deviation (experiment 2).

Compared with the length of roots in non-flooded control with prolonged flooding treatment, no statistical significant difference was observed between treatments of Puzhuthiikar, but cv IR72593 exhibited statistically significant ($p < 0.01$) reduction by 0.28, 0.36 and 0.44 times than that of its control after 15 (55 DAS), 40 (80 DAS) and 75 (115 DAS) days respectively after flooding (Fig. 5b). The total DW increased significantly in both the cultivars under prolonged flooding condition than its non-flooded counterparts (Fig. 5c). Puzhuthiikar showed increased in DW by 1.33, 1.24 and 3.58 times of the IR72593 at 55, 80 and 115 days after flooding treatment respectively.

3.2.2. Differences in leaf and stem morphology between the two contrasting genotypes under prolonged flooding and non-flooding situation

Compared with the length of blade in non-flooding control with prolonged flooding, a highly significant difference was observed between them in both cultivars. Blade length of the prolonged flooded plants increased to 1.03, 1.04 and 1.35 fold higher that of the control after 15, 40 and 75 days after flooding respectively in Puzhuthiikar (Fig. 6a–e). However, in cv IR72593, the blade length of prolonged flooded plant decreased after 15 and 40 days of flooding to 0.23 and 0.19 times respectively. Later, there after 75 days of flooding, the blade length of prolong flooded IR72593 plant increased at an almost identical length to its non-flooded counterparts in 115 day old plant. Concern to the elongation of blade length at each position, Puzhuthiikar exhibited its elongation of leaf blade from third leaf, but in contrast, blade length of cv IR72593 decreased at all position except boot leaf. Comparing the area of leaf blade in non-flooded control with prolong flooded treatment, highly significant difference was observed between treatments in both cultivars. Decrease in blade area was observed at 15 and 40 days after flooding in both the plants than its non-flooded counterparts (Fig. 6b) and later it was increased 1.28 and 1.08 fold higher after 75 days of flooding in 115 day old plant of Puzhuthiikar and IR 72593 respectively. Seventy-five days after flooding, 6th and 7th (boot leaf) leaf blade area of Puzhuthiikar was increased to 1.16 and 5.94 fold than its non-flooded counterpart respectively. Similarly, increase in blade area of 6th and 7th leaf of cv IR72593 was observed to be 1.08 and 2.55 fold than control. Significant ($p < 0.05$) difference was observed among the flooded and non-flooded treatments in both cultivars for sheath length (Fig. 6c). Average lengths of sheath was increased 1.10, 1.15 and 1.22 times in prolong flooded plants of 55, 80 and 115 day old respectively, compared to the non-flooded control in Puzhuthiikar at all levels of sheath. In contrast, the reduction in length of sheath was observed in IR72593 with 0.05, 0.04 and 0.11 times of non-flooded control in 15 (55 DAS), 40 (80 DAS) and 75 (115 DAS) days after prolonged flooding respectively. However, it displayed increase in length of sheath only at 6th position after 40 days of prolonged flooding. Comparing the area of sheath in non-flooded control with treatment of prolonged flood, IR72593 and Puzhuthiikar exhibited significant difference at 1% and 5% level respectively. Average area of sheath was increased 1.14, 1.18 and 1.28 times under prolong flooding at 55, 80 and 115 day old plants respectively, compared to the non-flooded control in Puzhuthiikar (Fig. 6d). In contrary, the area of sheath in IR72593 was reduced under prolonged flooding than its non-flooded counterpart at the rate of 0.05, 0.25 and 0.34 times on 15 (55 DAS), 40 (80 DAS) and 75 (115 DAS) days after flooding respectively. Related to the individual sheath area, Puzhuthiikar observed increase in area from 3rd position onwards. On the other hand, cv IR72593 exhibited decrease in area of sheath against control at all levels. The order of internode length at each level of both cultivars were significantly ($p < 0.01$) greater in prolong flooded plants than its non-flooded counterparts. Development of internodes in Puzhuthiikar on 15, 40 and 75 days under prolonged flooding, exhibited an average length of 2.24, 2.26 and 1.48 times greater respectively (Fig. 6e). Further, Cv IR72593 showed an increase in average internode length of 2.30, 1.90 and 1.17 times, than its counterpart of non-flooded control at 55, 80 and 115 day old plant respectively.

3.2.3. Differences in photosynthesis parameters between the two contrasting genotypes under prolonged flooding and non-flooding situation

Leaf photosynthetic rate (Pn), stomatal conductance to CO_2 (Gs), transpiration (Trmmol) and intercellular CO_2 concentration (Ci) showed variation between prolonged flooding and non-flooding condition. The genotypes in prolonged flooding exhibited higher
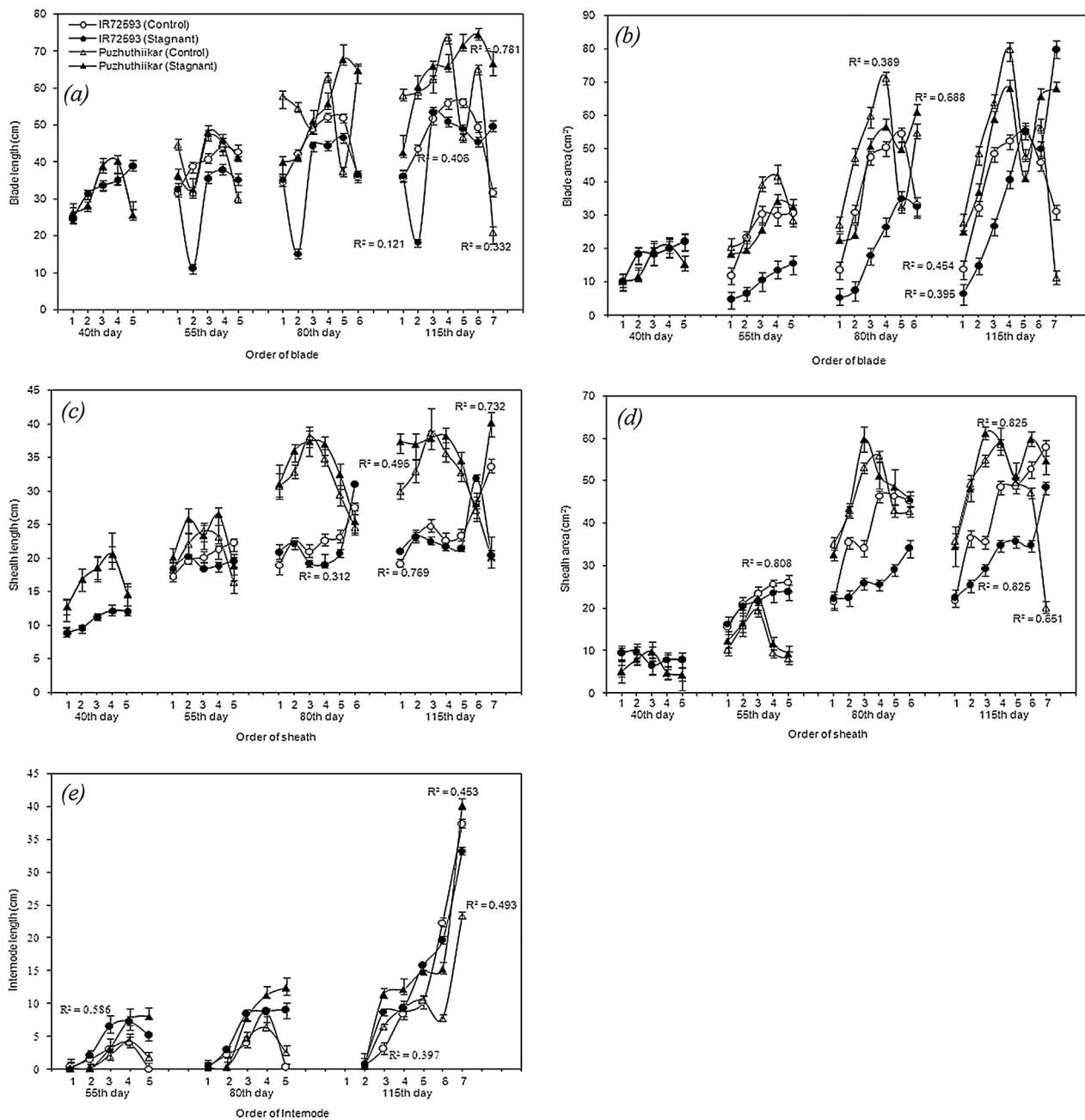
Fig. 6. (a–e) Effect of prolonged flooding on blade length and area, sheath length and area and internode length of 40th, 55th, 80th and 115th day after sowing. Number in x-axis represents order of blade/sheath/internode (1 represents basal blade/sheath/internode, 5 or 6 represents terminal blade/sheath/internode). Bars indicate the standard deviation (experiment 2).

Pn and Gs and reduction in transpiration rate and Ci, than its corresponding non-flooded counterparts. However, Ci in Puzhuthiikar under prolonged flooding (316.5 μmol CO_2 mol air^{-1}) was not significantly differed from control (316.6 μmol CO_2 mol air^{-1}). Comparing the Pn, Gs, Trmmol and Ci in Puzhuthiikar with IR72593, a highly significant difference was noted among them in prolonged flooding condition. The cv IR72593 intolerant under prolonged flooding state, photosynthesis was decreased from terminal to basal leaf (22.2, 14.6, 3.23 μmol m^{-2} s^{-1}) and similar situation was also observed for stomatal conductance (1.3, 0.74, 0.06 mol m^{-2} s^{-1}) (Figs. 8 and 10). In contrary, photosynthesis (13.8, 21.1, 14.1, 13.2 μmol m^{-2} s^{-1}) and stomatal conductance (0.8, 0.9, 0.6, 0.9 mol m^{-2} s^{-1}) were uniform at all levels in Puzhuthiikar (Figs. 8 and 10).

4. Discussion

In rice, growth response in deepwater situation is regulated by genetic and environmental factors. Amongst the wide range of landraces, there are genotypes that can tolerant environmental stress, resulting in highly adaptable to low-input agricultural systems. Our analysis indicates that the traits studied varied considerably among the 332 lines of rice genotypes under prolonged flooding, indicated sufficient genetic variability is present for exploitation through breeding. Shoot length and spikelet fertility percent exhibited maximum variability under prolonged flooding during vegetative and maturity phase respectively. A statistical significant difference was observed between genotypes in prolonged flooding and non-flooding condition for all the traits except 100 seed weight.
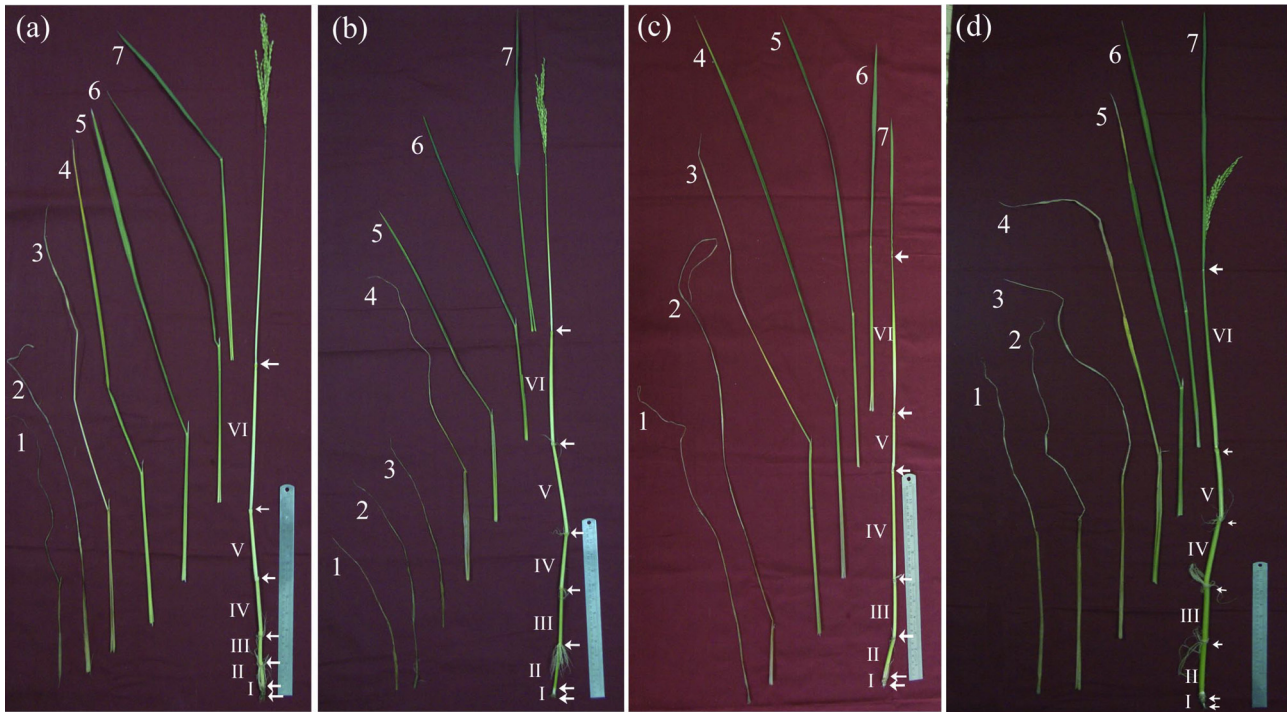
Fig. 7. (a, b) Photograph of 115 day old rice plant of IR72593 (a, b) and Puzhuthiikar (c, d). *a* and *c* are air grown plant (nonflooded), *b* and *d* are prolonged flooded plant. Arabic numerical indicates leaf number with sheath in ascending order (old leaf as 1 and young leaf as 6). Roman letters indicate internode position and arrow indicate the positions of the node (experiment 2).

4.1. Genotypic responses to prolonged flooding at vegetative and maturity phase

In the present work, PCA analysis during vegetative phase, vectors of the variables under non-flooded (normal) and prolonged flooding condition, leaf blade, shoot length and sheath length were formed a separate group (highly correlated). Similarly, number of internodes, nodes and leaves were formed another group (highly correlated) under non-flooded condition. While under prolonged flooding, vector of number of leaves had separated from the group of nodes and internodes. The reason might have been due to, leaf

senescence caused by ethylene under flooding. The position and perpendicular projection of genotypic points onto variable vector under prolonged-flooded condition in vegetative phase, identifies Puzhuthiikar maintains its vigor by increase in node and internode number and leaf number coupled with shoot length, blade and sheath length (Fig. 1b). In contrary, under flooding IR72593, PSB RC 82 Sub1 and Ciherang Sub1 moved to quadrant 2 (opposite to quadrant 4, where exhibits increase in shoot length, blade and sheath length), where the shoot length, blade and sheath length were repressed by prolonged flooding. Genotype Atirang (a known prolonged flooding tolerant) fell in quadrant 2, exhibited less shoot length than cv Puzhuthiikar under normal condition and increased
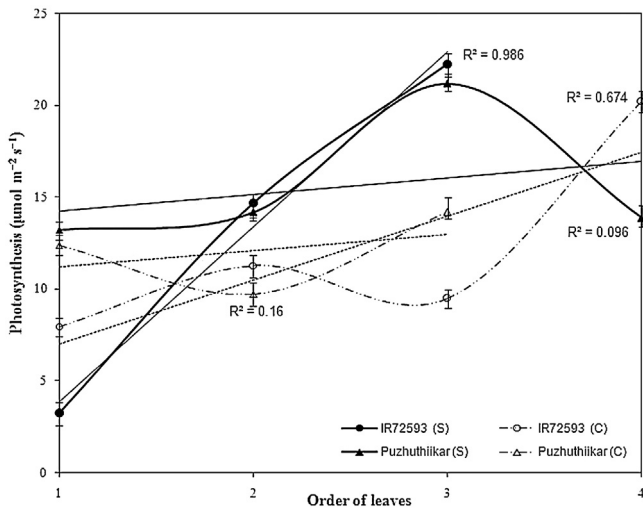

Fig. 8. Photosynthetic efficiency of IR72593 and Puzhuthiikar under nonflooding and prolonged flooding condition. Numbers in *x*-axis represents order of leaves (1 represents basal leaf, 4 represents terminal leaf). N-nonflooding, P-prolonged flooding. The vertical bars represent s.e. (experiment 2).

Fig. 9. Transpiration of IR72593 and Puzhuthiikar under nonflooding and prolonged flooding condition. Numbers in *x*-axis represents order of leaves (1 represents basal leaf, 4 represents terminal leaf). N-nonflooding, P-prolonged flooding. The vertical bars represent s.e. (experiment 2).

Fig. 10. Stomatal conductance of IR72593 and Puzhuthiikar under nonflooding and prolonged flooding condition. Numbers in x-axis represents order of leaves (1 represents basal leaf, 4 represents terminal leaf). N-nonflooding, P-prolonged flooding. The vertical bars represent s.e. (experiment 2).
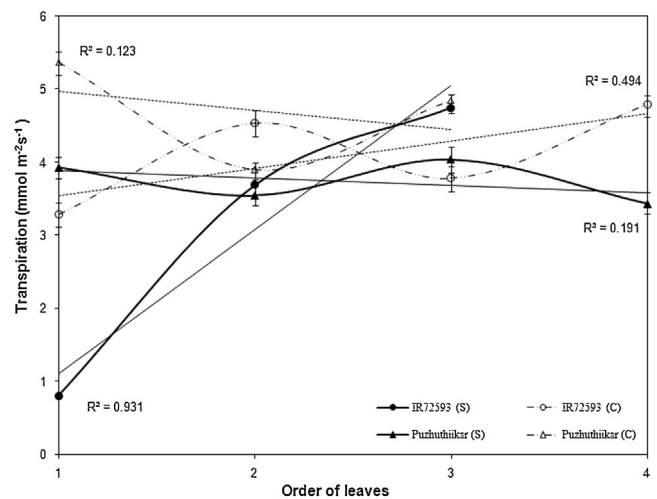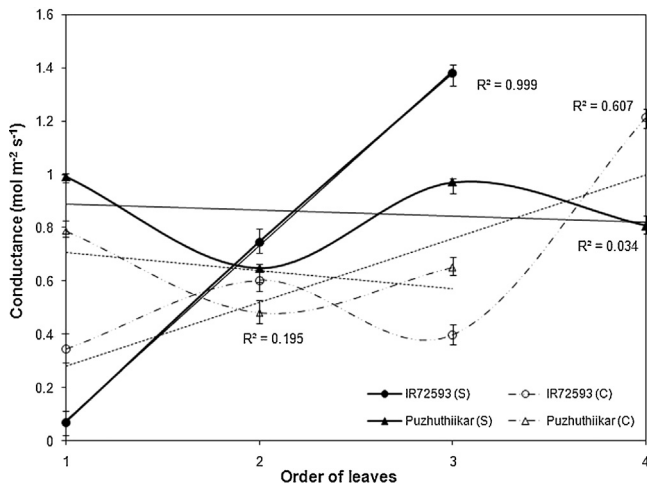
in its shoot length and number of leaves under flooding situation (quadrant 4). This suggests that, prolonged flooding tolerant genotype should probably have early vigor trait (Nguyen et al., 2013). IR70215 and IR67495 improved genotypes exhibited their submergence avoidance by increase in shoot length under flooding but they have less number of leaves. This might have been due to senescence of early formed leaves or less number of nodes. Therefore, increase in node and live leaf number coupled with moderate shoot elongation under prolonged flooding situation might be considered as one of the selection criteria at the vegetative phase.

At maturity phase, under non-flooding condition vectors of the variables, number of nodes, culm girth, number of leaves, panicle length, days to fifty percent flowering, seed weight, number of filled grains, spikelet fertility percent and grain yield had exhibited less variability. In contrast, these traits exhibited higher variance under prolonged flooding situation. This suggests that, expression of these traits varied among cultivars and highly influenced by prolonged flooding. DW and shoot length were exhibited strong correlation with number of tillers, EBT and panicle length and weak correlation with spikelet fertility percent, number of filled grains, number of leaves and grain yield under prolonged flooding. The cause for weak correlation suggests that, much of the photoassimilates and stored carbohydrates might have been consumed for rapid elongation of shoots and tillers by cell division and cell elongation to adapt strategy to avoid submergence from rapid increase in the water level and there by it decreases the spikelet fertility percent. Extensive shoot elongation exhaust energy and result in tall, spindly plants that usually lodge and disintegrate in floodwater. On the other hand, the slow elongating types have low survival and tillering, possibly because most leaves stay under water resulting in their degradation and leading to poor aeration and assimilate supply. However, the tolerant cultivars demonstrated a greater increase in tiller number and EBT under prolonged flooding.

Prolonged flooding tolerant cultivars IR70215 and IR67495 showed high shoot elongation and positioned in quadrant 1 under non-flooded condition. Whereas, Sub1 cultivars and IR72593 plotted in opposite quadrant 3. This suggests that, genotypes breed for prolonged flooding grows to its maximum shoot length even under non-flooded condition. On the other hand, landraces like Atirang and Puzhuthiikar exhibited adequate level of shoot length under normal situation with differences in their grain yield. The biplot graph (Fig. 2b) of genotypes raised under prolonged flooding, exhibited the complete change in position of genotypes compared to

the biplot graph of genotype grown under normal condition. Genotypes showed an increase in shoot elongation depends on water depth and increased spikelet fertility, where plotted in quadrant 4 and IR72593 with reduced spikelet fertility per cent positioned in opposite quadrant 2. From this position, it is inferred that the physiological characteristic of genotypes in quadrant 4 confers tolerance under prolonged flooding by an increase in leaf number, number of nodes, culm girth, spikelet fertility per cent, number of filled grains and moderate elongation rates of 15–30% (Vergara et al., 2014) compared to their height under normal conditions, reaching 125–192 cm under prolonged flooding. Therefore, plants with these traits are expected to possess vigor under prolonged submergence with lodging resistance mechanism of avoiding submergence situation. Sub1 cultivars with quiescent strategic mechanism plotted in between quadrant 2 and 3.

4.2. Variables that responsible to discriminate high and low yielding groups under prolonged flooding condition

Prolonged flooding significantly affected rice plants with 1.4 fold decrease in dry weight. Suppression of tiller emergence, EBT and leaf number during flooding are the possible reasons for reduction in dry weight. Flood water inhibits tiller buds of rice plants due to anaerobic condition under prolonged flooding, leading to dormancy or death of tiller buds. Leaf number is important for the survival of plants under prolonged flooding and for the maintenance of biomass in the photosynthetic CO_2 fixation. Flooding increases shoot length of rice plants to 1.2 fold. Therefore, rice can survive in flooding stress through rapid shoot elongation and extension of leaves (Anandan et al., 2012; Sakagami, 2012).

To get further insight on knowledge of tolerant mechanism of prolonged flooding tolerant genotypes, taller genotypes with good spikelet fertility percent were plotted into a separate biplot graph (Fig. 3). The biplot graph shows the traits such as number of leaves, tillers and EBT has maximum variability. This suggests that, flooding tolerant genotypes had greater variance in partitioning of photoassimilate and carbohydrate for survival vs productivity. Strong correlation between grain yield and number of nodes or grain yield and number of leaves under deep water suggests that leaf number is necessary for the maintenance of shoot biomass in the photosynthetic CO_2 fixation process.

The high yielding genotypes possess thick culm and good elongation of internodes of first three numbers from the base (Fig. 4). While, in low yielding genotypes internode elongation and thick culms were observed for first two nodes. However, plants under flooding exhibited internode elongation at the base in response to water depth and subsequently the number of node had increased. This might have been due to the sequential increase in water level (Anandan et al., 2012). Hattori et al. (2009) demonstrated differences in the capacity for internode elongation in lines carrying the SNORKEL genes. In their study, SK1 elongated one to three internodes, while SK2 elongated one to seven internodes. Therefore, genotypes suitable for prolonged flooding should have capacity to elongate three internodes or it should posses SK1 gene. However, in total genotypes under prolonged flooding increases culm girth than genotypes under control condition. The increase in culm girth in tolerant genotypes is essential to store carbohydrates and partitioning of photoassimilates for initial plant establishment and to increase chance of survival of genotype by avoiding lodging in standing water and degeneration of leaves by submergence. Therefore, we suggest that good internode elongation and thick culm from the base are the important criterion for selection of genotypes suitable for prolonged flooding. Thick culm contributes to the rigidity and higher lodging resistance with higher starch content (Ishimaru et al., 2008). The increase in culm girth also increases stem aerenchyma under prolonged flooding which could further

enhance aeration of roots and flooded shoot parts (Kirk et al., 2014).

4.3. Physiological mechanism of tolerant genotype under prolonged flooding

Prolonged deepwater flooding adapted rice can survive flooding stress through vigorous upward elongation of shoot and leaves (Anandan et al., 2012; Sakagami, 2012). In the present experiment, it was observed that there was greater shoot elongation in Puzhuthiikar and IR72593 during prolonged flooding than under non-flooding. However, shoot elongation was more in Puzhuthiikar than IR72593. Vigorous upward elongation of shoot during prolonged flooding was associated with blade length, sheath length and sheath area, but the same was not observed in IR72593 (Fig. 7). The tolerant genotype Puzhuthiikar exhibited its elongation of leaf blade and increase in sheath area from third leaf onwards, but cv IR72593 lacks that capacity. Further, it exhibited increase in blade area at all levels and sheath length at 6th and 7th position. Sakagami et al. (2009) reported that vigorous leaf elongation during prolonged submergence is critical for ensuring shoot emergence from deepwater flooding. Certain cultivars lacks either of sheath elongation (Anandan et al., 2012) and internodal stem elongation (Singh et al., 1989) under prolonged submergence but they have a capacity for leaf elongation under flooding. The genotype that has the capacity to elongate not having the capacity of expanding sheath length and area could case culm weakness and tend to lodge. Leaf blade width has got thinner in both cultivars under prolonged flooding until 40 days after flooding (80 days after seeding) and later it was increased. This suggests that, rapid shoot elongation clearly uses photosynthesis mechanism to increase blade length by restoring contact between the leaves and air (Mazaredo and Vergara, 1982; Sakagami et al., 2009). The response of Puluzhiikar and IR72593 at all three stages showed significant differences for internodes length compared to control. Elongation of internodes was observed in all internode levels. The increase in node number and internodal length is an important factor to adopt an escape strategy in environments where photosynthesis is limited. The root length under prolonged flooding decreased in IR72593 and maintained in Puzhuthiikar (Fig. 5b). Colmer (2003) demonstrated that roots of rice form extensive aerenchyma and an inducible barrier to radial oxygen loss (ROL) enhanced longitudinal oxygen diffusion to the root tip. Oxygen lost at root tip, forms an aerobic zone around the tip and enables respiration (*i.e.* oxidative phosphorylation) to continue in roots even when in water logged condition (anaerobic soils). The production of ATP enables root growth and functions to continue, resulting in deeper rooting (Armstrong et al., 1994). Aerobic zone creates oxidation rhizosphere which, enhances root penetration into anaerobic substrate, detoxifies iron and manganese and supports nitrification.

4.4. Photosynthetic capacity, transpiration rate, intercellular CO_2 concentration and stomatal conductance under prolonged flooding

Flooding increases greater stress for source activity and photosynthetic rate in upper leaves above the water surface (Setter et al., 1987; Yamaguchi et al., 1989; Hirano et al., 1995). The intolerant genotype IR72593 clearly demonstrate the upper leaf (terminal leaf) has high photosynthetic ($R^2 = 0.986$), stomatal conductance ($R^2 = 0.999$) and transpiration rate ($R^2 = 0.931$) than other leaves (Figs. 8–10). The level of photosynthesis and stomatal conductance varies according to leaf position in intolerant cv IR72593 under prolonged flooding and this has been confirmed by inverse-regression line (rate of photosynthesis *vs* order of leaves). However, tolerant cultivar Puzhuthiikar was unaffected for photosynthesis and

stomatal conductance by flood water (Figs. 8–10) and uniformity was observed at all levels of leaf appears above the water level. Sakagami et al. (2013) reported that dynamic allocation of photosynthetic products through increasing stomatal conductance in upper leaves affects the plant survival under prolonged flooding. This line is very much correlated with the intolerant rice genotype IR72593 by its inverse relationship. The total DW of plant has increased in both the genotypes under prolonged flooding than its non-flooding counterparts. This happens when plant has abundant water, it has consequence for a growth response to elevated CO_2 because of Ci is still larger and hence photosynthesis is enhanced. Higher stomatal conductance increases CO_2 diffusion into the leaf and favors higher photosynthetic rates. Higher photosynthetic rates could favor higher biomass.

5. Conclusions

Screening of large number of genotypes in the present study against prolonged flooding brings out much valuable information and explains that *Oryza sativa* L. genotypes possess tolerance against deepwater flooding. This study demonstrated that, increase in node and live leaf number coupled with moderate shoot elongation at vegetative phase and increased tiller number and EBT at the maturity phase should be used as selection criteria. Increase in node number has effect on leaf number and has direct effect on shoot biomass in the photosynthetic CO_2 fixation process. Tolerant genotypes possess thick culm and good elongation of internodes to avoid lodging and storing more carbohydrate and starch. Further, they had increased blade length, sheath length and sheath area. Therefore, by understanding such escape mechanisms used by rice plants to survive under deepwater flooding condition expected to help breed and select superior lines for genotypes grown in deep water flood-prone areas.

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