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Morphological and Physiological Studies in Rice Cultivars Reveal Critical Role of Root Length and Photosynthetic Rate in Adaptation to Aerobic Conditions

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Abstract

In recent years the aerobic system of rice cultivation is gaining ground principally for its water saving nature. The crop establishment method is direct seeded and the irrigation is need-based with proper management practices. Identification of morphological and physiological traits responsible for such aerobic adaptation will be useful for development of aerobic rice varieties. We investigated the morpho-physiological traits in anaerobic (BPT 5204) and aerobic (CR Dhan 202) adapted rice cultivars grown under anaerobic and aerobic conditions. The root length of CR Dhan 202 significantly increased under aerobic condition which may be attributed to its aerobic adaptation in terms of water acquisition. The photosynthetic rate was significantly higher in CR Dhan 202 as compared to that of BPT 5204 under the aerobic condition. Non-significant differences were observed in the leaf chlorophyll contents under both the conditions implying that aerobic condition did not cause any impact on such parameters. The results showed that the root length, total dry weight and photosynthetic rate are the key parameters for imparting aerobic adaptation. Such traits associated with the adaptation are of paramount importance while designing strategies with regards to water availability and uptake for targeting various rice eco-systems in India.

Key words: Aerobic, anaerobic, photosynthetic rate, rice, root length, total dry weight

Introduction

Rice is traditionally puddled and transplanted and requires standing water which poses a great challenge with reference to water availability in near future. It is estimated that by 2025, fifteen out of 75 million ha of Asia's irrigated rice crop will experience water shortage (Sandhu *et al.,* 2012). Unlike anaerobic transplanted rice, the aerobic system of cultivation of rice involves direct seeded, well-drained, non-puddled and non-flooded fertile soils. This kind of shift in rice cultivation is becoming popular to cope up with the water scarcity as well as for maintaining the ground water table (Tuong *et al.,* 2005; Matsuo and Mochizuki, 2009). In aerobic rice cultivation, 10-50% saving of water has been observed as compared with transplanted rice in India along with reduced labor and lower methane emission (Shashidhar, 2008; Pathak *et al.,* 2011; Kumar and Ladha, 2011).

The morphological and physiological traits are important for adaptation to aerobic conditions. There have been several reports on yield, morphological and adaptability differences in anaerobic and aerobic systems of cultivation but sparse information is available on the physiological traits conferring aerobic adaptation(Kato *et al.,* 2010; Kato and Okami, 2010; Patel *et al.,* 2010; Sandhu *et al.,* 2012; Sandhu *et al.,* 2013). Aerobic rice cultivation largely depends on the initial plant establishment and an important aspect for understanding the adaptation is the response of root growth and development (Bengough*et al*., 2011). This would facilitate the efforts towards integrating the traits responsible for adaptation in popular rice varieties. In the present investigation, we studied the morphological and physiological parameters in root and shoot at panicle initiation stage.

Materials and Methods

Plant Material

Rice genotypes *viz.* BPT 5204 (adapted to anaerobic conditions), CR Dhan 202 (adapted to aerobic conditions) were employed for physiological and morphological studies. Need-based irrigation *i.e.* water in measured volume as and when required to maintain the above-said conditions was provided for aerobic condition. While, anaerobic condition was maintained by a layer of two-five cm water above the soil for a period of 100-120 days in the polyhouse (Barbadikar *et al*., 2016). The morphological and physiological parameters from root and shoot were recorded at panicle initiation (PI) in three replications for three seasons, viz. *Kharif 2015*, *Rabi 2015-16* and *Kharif 2016* (Figure 1).

Figure 1. Rice genotypes (BPT5204 and CR Dhan 202) grown in polythene bags (a, b) under aerobic and anaerobic conditions

Morphological observations

Root and shoot morphological observations (*viz.* root length, shoot length, total plant height, root fresh weight, shoot fresh weight, total plant weight, root dry weight, shoot dry weight, number of productive tillers, root to shoot length ratio and root to shoot fresh weight ratio) were recorded. The entire plants were carefully removed by cutting the polythene bags without damaging the roots (Figure 1). The roots were washed by dislodging all aggregated soil using a high-pressure water pump. Three plants (per replication) were selected as sample size for recording observations. Shoots and roots were cut apart at the node interface.

Physiological observations

At fifty percent flowering stage, LI-6400 Portable Photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA) was used for the measurement of photosynthetic parameters *viz*. net photosynthetic rate (µmol CO_2 m-^{2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), stomatal conductance (mol H_2O m-^{2} s-^{1}), intercellular CO_2 concentration (µmol CO_2 mol-1), water use efficiency and intrinsic water use efficiency (µmol mol-1). Leaf chlorophyll content was estimated using fresh flag leaf samples collected from both under aerobic and anaerobic conditions using solvent extraction method and by SPAD 502 (Soil-Plant Analysis Development) chlorophyll meter (SCMR) (Minolta Co., Ltd, Osaka, Japan). The flag leaf (100 mg) was cut into small pieces and incubated in a 10 ml mixture of 80% acetone for 48 h in dark condition at room temperature to extract chlorophyll. The absorbance of the extracts after 48 hours of incubation was taken at wavelengths of OD_{645nm}, OD_{663nm} using UV-VIS Spectrophotometer. The chlorophyll content was calculated by using the following equations *viz*. Chl A (mg/g FW) = $12.72A_{663} - 2.59A_{645,}$ Chl B (mg/g FW) = $22.9A_{645} - 4.67A_{663,}$ Chl Total (mg/g FW) = $20.31\ A_{645} + 8.05\ A_{663}$ (Hiscox and Israelstam, 1979). The SPAD-502 chlorophyll meter is a portable and convenient meter used for estimation of leaf chlorophyll content and leaf N status. The SPAD-502 chlorophyll meter readings were measured for selected flag leaf from top, middle and lower portion leaf sample of the plant (Yamamoto *et al.*, 2002).

Statistical Analysis

All statistical analyses were conducted with Statistix 8.1 (version 2.0.1) statistical tool. The effects of aerobic and anaerobic conditions on each trait were assessed by two-way ANOVA and least significant difference (LSD). The significant differences between genotype and treatment means were compared with the least significant difference (LSD) at a 5% level of probability ($P \leq 0.05$).

Results and Discussion

Root and shoot morphological traits

The root system architecture, aptly described as the hidden half, critically influences the yield of the crop. The identification of morpho-physiological traits are important for contributing to the development of high yielding rice varieties suitable for aerobic condition (Patel *et al.,* 2010; Atlin*et al.,* 2006; Haryanto *et al.,* 2008; Matsunami *et al.,* 2009). The roots are primarily responsible for the adaptation and responses to various stress situations through complex interactions among the genes. A long and thick root system, the ratio of root weight to shoot weight and root penetration ability of upland rice have been reported to contribute to yield under water-deficit conditions (Price and Tomos, 1997). The root dry weight, root length density, and lateral roots have been found to relate with yield stability and thus their role in root adaptation to different systems of cultivation needs to be emphasized (Wissuwa *et al.,* 2016). In the present study, the root morphological parameters were recorded under aerobic conditions. The interactive effects of the genotypes and the treatments (least significant difference (LSD) for various root and shoot traits *viz.* root length, shoot length, total plant height, shoot fresh weight, root dry weight, shoot dry weight, total dry weight, number of productive tillers, root to shoot length ratio, root to shoot fresh weight ratio and root to shoot dry weight ratio were recorded for the BPT 5204 and CR Dhan 202 rice genotypes at panicle initiation (PI) stage grown under aerobic and anaerobic conditions (Table 1, Figure 2).

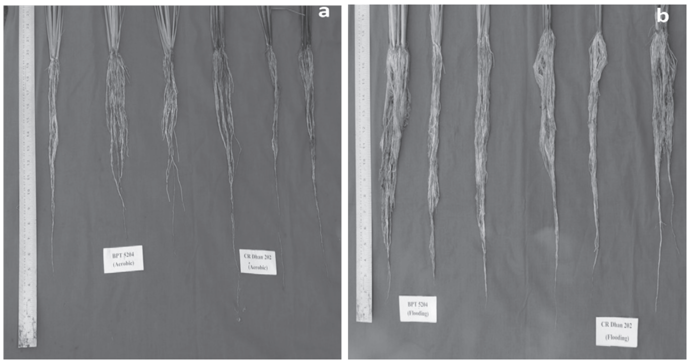

Figure 2. Comparison of root length of BPT 5204 and CR Dhan 202 under aerobic (a) and anaerobic (b) conditions

The root length, root to shoot length ratio and root to shoot fresh weight ratio were significantly ($P \leq 0.05$) higher under aerobic condition as compared to that under anaerobic condition in both the genotypes. Differences in morphological parameters were observed with the cultivars and among the treatments. The shoot length, total plant height and total dry weight were significantly higher in CR Dhan 202 than BPT 5204 under aerobic condition (Table 1). The root length significantly increased in both the cultivars under aerobic condition as compared to the anaerobic condition which may be attributed to the role of deeper roots in water acquisition from soil. Based on the water requirements for growth and development the root length must have increased for efficient uptake and utilization. The higher root to shoot length ratio under aerobic condition in both the cultivars indicated that the root growth was proportionately higher and competed more effectively for nutrients. Root fresh weight, shoot fresh weight, total plant weight, root dry weight and total dry weight were significantly higher under anaerobic condition as compared to aerobic condition in both the cultivars.

This can be explained by the fact that rice has been traditionally adapted to anaerobic conditions with standing water and CR Dhan 202 must have responded likewise even better than BPT 5204. Moreover, increased root length of CR Dhan 202 under aerobic condition may be contributing to its adaptation to aerobic condition. The root related traits *viz.* higher root penetration, root length, and the ratio of root weight to shoot weight contribute to adaption to water deficient conditions in aerobic rice cultivation (Price and Tomos, 1997; Yadav *et al*., 1997; Ali *et al*., 2000; Clark *et al*., 2000). The present results corroborated with the results of Price and Tomos (1997), Martin *et al.* (2007), Patel *et al.* (2010) and Sandhu *et al.* (2012). In upland rice, the root related traits such as deeper root system and root penetration ability have been known to contribute to tolerance to water-deficit conditions (Price and Tomos, 1997). Martin *et al.* (2007) reported that the mega rice varieties (ADT 39 and PMK 3) that were suitable for cultivation under aerobic conditions based on growth performance and yield stability had longer and deeper root system compared to the other rice varieties. Sandhu *et al.* (2012) also reported that the aerobic rice genotypes performed better under water-limited conditions due to its better root length and root biomass. Our results for total plant height, root biomass, tillers number and total dry weight were consistent with the observations of Patel *et al.* (2010) wherein it was reported that plant height, root biomass per hill and total dry weight were significantly higher under anaerobic condition than under the aerobic condition. Such reduction in plant height was ascribed to the limitation in cell elongation that results in a reduction of inter nodal length.

From the root morphological observations, it can be noted that the root system architecture plays a crucial role in sustaining water scarcity and thus it can be inferred that the root length and root dry weight are key parameters for imparting aerobic adaptation. Thus, improvement in aerobic rice can be achieved by identifying varieties having deeper root system which helps in maximizing water uptake and ultimately increase grain yield under water-stress conditions.

Photosynthetic responses

Photosynthesis is a fundamental physiological process and is severely affected by abiotic stresses that alter the ultra-structure of organelles, concentration of pigments and stomatal regulation in all plants (Chaves *et al.,* 2009; Ashraf and Harris, 2013; Osakabe *et al.,* 2014). The early vegetative and reproductive growth depends on assimilating source like light capture, photosynthetic rate and sink constituted by structural growth (*viz.* tiller outgrowth, leaf appearance rate and potential size) (Sandhu *et al.,* 2015).

Similarly our results indicate that, photosynthetic rate was significantly ($P \leq 0.05$) higher under aerobic condition as compared to that under anaerobic condition. Under the aerobic condition, photosynthetic rate was significantly ($P \leq 0.05$) higher in CR Dhan 202 (14.42 µmol CO_2 m^{-2} s^{-1}) compared to BPT 5204 (11.51 µmol CO_2 m^{-2} s^{-1}) (Table 2). The present observations corroborated with the earlier reported results of Patel *et al.* (2010), Mahmod *et al.* (2014) and Nguyen *et al.* (2015). Patel *et al.* (2010)reported that the photosynthesis rate was significantly higher in

Table 1. Comparison of morphological traits studies under aerobic and anaerobic conditions

Genotype	Treatment	Root length (cm)	Shoot length (cm)	Total plant height (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Total plant weight (g)	Root dry weight (g)	Shoot dry weight (g)	Total dry weight (g)	Number of productive tillers	Root/ shoot length ratio	Root/ shoot fresh weight ratio	Root/ shoot dry weight ratio
BPT 5204	Aerobic	44.61^{b}	75.60^{b}	120.21^{b}	12.33^{bc}	19.57^{b}	30.50^{b}	0.61^{b}	5.44^{b}	6.04^{c}	5.44^{ab}	0.59^{a}	0.81^{a}	0.12^{b}
CR Dhan 202		48.74^{a}	93.78^{a}	142.51^{a}	10.93^{c}	23.32^{b}	35.65^{b}	0.76^{b}	6.17^{ab}	6.93^{b}	4.78^{b}	0.51^{b}	0.62^{ab}	0.13^{b}
BPT 5204	Anaerobic	37.36^{c}	83.51^{b}	120.87^{b}	17.33^{a}	35.57^{a}	52.89^{a}	1.43_{a}	7.37^{a}	8.80^{ab}	6.22^{a}	0.47^{bc}	0.55^{b}	0.21^{a}
CR Dhan 202		41.81^{bc}	101.87^{a}	143.68^{a}	15.30^{ab}	35.99^{a}	51.29^{a}	1.56^{a}	7.57^{a}	9.12^{a}	5.78^{ab}	0.41^{c}	0.44^{b}	0.22^{a}
LSD (P≤0.05)														
Genotype		4.05	6.87	8.93	2.10	6.72	7.10	0.24	1.35	1.43	0.71	0.06	0.18	0.05
Treatment		4.05	6.87	8.93	2.10	6.72	7.10	0.24	1.35	1.43	0.71	0.06	0.18	0.05
Genotype X Treatment		5.72	9.71	12.62	2.97	9.51	10.04	0.33	1.91	2.03	1.00	0.08	0.26	0.06
SED		2.80	4.76	6.18	1.45	4.66	4.92	0.16	0.93	0.99	0.49	0.04	0.13	0.03
CV (%)		13.78	11.37	9.95	22.07	34.51	24.49	31.95	29.84	27.26	18.78	16.44	44.17	39.56

The mean values followed by similar lower case letters are not significantly different ($P \leq 0.05$)

Table 2. Comparison of photosynthetic parameters under aerobic and anaerobic conditions

Genotype	Treatment	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mol H_2O m^{-2} s^{-1})	Intercellular CO_2 (μmol CO_2 mol^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Water use efficiency (Pn /TR)	Intrinsic water use efficiency (μmol mol^{-1})
BPT 5204	Aerobic	11.51^{c}	0.10^{b}	186.00^{bc}	6.76^{b}	1.72^{ab}	112.71^{ab}
CR Dhan 202		14.42^{b}	0.16^{ab}	220.31^{ab}	10.31^{ab}	1.42^{bc}	89.40^{bc}
BPT 5204	Anaerobic	17.54^{a}	0.14^{ab}	163.38^{c}	9.27^{ab}	1.92^{a}	125.18^{a}
CR Dhan 202		16.06^{ab}	0.21^{a}	233.79^{a}	12.9^{a}	1.24^{c}	77.01^{c}
LSD (P≤0.05)							
Genotype		2.05	0.05	29.56	2.74	0.29	21.06
Treatment		2.05	0.05	29.56	2.74	0.29	21.06
Genotype X Treatment		2.89	0.07	41.81	3.87	0.40	29.79
SED		1.18	0.03	17.09	1.58	0.17	12.17
CV (%)		9.73	22.36	10.42	19.70	12.85	14.75

Pn: Photosynthetic rate; TR: Transpiration rate

The mean values followed by similar lower case letters are not significantly different ($P \leq 0.05$)

rice varieties under aerobic condition than the anaerobic condition due to maximum light capture in mid-hills ecosystem. The higher photosynthetic rate in CR Dhan 202 under aerobic adaption may be attributed to the maximum light capture and sink constituted structural growth. This needs to be further confirmed at different stages of rice growth as the photosynthetic parameters are single point measurements. Thus, more number of readings needs to be recorded for further understanding of photosynthetic rate under aerobic condition.

Other than the photosynthetic rate, all other parameters did not show any significant difference between the two cultivars under aerobic condition. Under anaerobic condition, the intracellular CO_2 showed significant difference between CR Dhan 202 (233.79 µmol CO_2 mol^{-1}) compared to BPT 5204 (163.38 µmol CO_2 mol^{-1}). Water use efficiency and intrinsic water use efficiency were significantly higher in BPT 5204 than CR Dhan 202 under anaerobic condition (Table 2).

The water use efficiency of BPT 5204 was higher in both aerobic and anaerobic conditions as compared to the cultivar CR Dhan 202. Condition wise, CR Dhan 202 had higher WUE under aerobic condition and BPT 5204 had higher WUE under anaerobic condition. The water use efficiency was found to be similar as reported by Mahmod *et al.* (2014). Mahmod *et al.* (2014) indicated that aerobic rice has high water use efficiency (WUE) compared to the flooded rice under aerobic condition. Nguyen *et al.* (2015) reported that the stomatal conductance was high under anaerobic condition as compared to the aerobic condition. The present observation suggests that the significant increase in photosynthetic rate of CR Dhan 202 under aerobic condition may be attributed to its adaptation in terms of water acquisition.

Leaf chlorophyll content

The leaf chlorophyll content directly determines the photosynthetic potential which is influenced by leaf nitrogen levels under normal or stress conditions in plants (Sanchez *et al.,* 1983). The leaf chlorophyll content has implication in case of certain stress situations like drought, salt, *etc.* A tolerant genotype is expected to retain or maintain the levels of chlorophyll through quenching mechanism (Fillela *et al.,* 1996). The leaf chlorophyll content (*viz.* chlorophyll A, chlorophyll B and total chlorophyll) displayed non-significant differences under both aerobic as well as under anaerobic conditions in both the genotypes (Table 3).Similarly, non-significant differences were recorded in SPAD-502 reading in both the genotypes (BPT 5204 and CR Dhan 202) and between the genotypes under aerobic condition and anaerobic conditions (Table 4).Such non-significant differences in the leaf chlorophyll contents or spectral properties under both the conditions imply that the aerobic condition does not cause any impact on such parameters and physiologically does not burden or increase the metabolic load of the plant.

Table 3. Comparison of leaf chlorophyll content under aerobic and anaerobic conditions

Genotype	**Treatment**	**Chlorophyll A** ($mg\ g^{-1}$)	**Chlorophyll B** ($mg\ g^{-1}$)	**Total chlorophyll** ($mg\ g^{-1}$)
BPT 5204	Aerobic	3.07^a	0.84^a	3.91^a
CR Dhan 202		3.36^a	0.80^a	4.17^a
BPT 5204	Anaerobic	3.25^a	0.77^a	4.03^a
CR Dhan 202		3.06^a	0.81^a	3.87^a
LSD (P≤0.05)				
Genotype		0.48	0.14	0.58
Treatment		0.48	0.14	0.58
Genotype X Treatment		0.68	0.2	0.82
SED		0.33	0.1	0.4
CV (%)		22	25.6	21.39

The mean values followed by similar lower case letters are not significantly different (P ≤ 0.05)

Table 4. Comparison of leaf chlorophyll content using SPAD-502 chlorophyll meter under aerobic and anaerobic Conditions

Genotype	**Treatment**	**SPAD reading**
BPT 5204	Aerobic	37.72^a
CR Dhan 202		37.95^a
BPT 5204	Anaerobic	36.92^a
CR Dhan 202		36.35^a
LSD (P≤0.05)		
Genotype		2.25
Treatment		2.25
Genotype X Treatment		3.18
SED		1.55
CV (%)		8.86

The mean values followed by similar lower case letters are not significantly different (P ≤ 0.05)

Conclusion

The present study revealed that the morpho-physiological parameters *viz.,* root length, total dry weight and photosynthetic rate are critical for aerobic adaption. It was observed that the adaptation in terms of deeper root length in order to acquire and take up the water from the soil operates under aerobic condition. Non-significant difference in the chlorophyll content showed that no major trade-off occurs under aerobic condition as compared to the anaerobic condition. Thus, root length, root dry weight and photosynthetic rate are essential parameters for initial phenotyping of the rice lines/genotypes/landraces in the breeding programmes for aerobic rice varietal development. These observations need to be further tested in more number of genotypes adapted for aerobic and anaerobic cultivation.

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