Studies on root anatomy, morphology and physiology of rice grown under aerobic and anaerobic conditions

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Abstract With the changing climate and rainfall aberrations, there is a gradual shift in the system of rice cultivation from traditional transplanted anaerobic to aerobic system. Studies on the root anatomical and morpho-physiological traits provide insights about the adaptation under aerobic conditions. We investigated the root anatomical and morpho-physiological traits in anaerobic (BPT 5204) and aerobic (CR Dhan 202) adapted rice genotypes grown under anaerobic and aerobic conditions. It was observed that the formation of fewer aerenchyma, thickened root and larger xylem area were critical anatomical traits associated with aerobic adaptation as compared to anaerobic 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. The morpho-physiological results showed that the root length, total dry weight and photosynthetic rate are the key parameters for imparting aerobic adaptation. These root anatomical and morpho-physiological traits associated with the adaptation can be used as screening criteria for phenotyping and selection of genotypes suitable for aerobic system of cultivation. Such study is expected to expedite the development of rice aerobic varieties in aerobic breeding programmes.

Keywords Rice · Aerobic · Anaerobic · Photosynthetic rate · Root length · Total dry weight

Introduction

Rice (*Oryza sativa* L.) is grown in non-puddled, non-flooded fertile soils under aerobic system of cultivation (Bouman and Tuong 2001). Aerobic rice cultivation is gaining popularity in several regions to cope up with water scarcity (Tuong et al. 2005; Matsuo and Mochizuki 2009). 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). The increasing shortage of water resources is accelerating the adoption of aerobic rice system wherein up to 45% water saving has been reported (Atlin et al. 2006; Shashidhar 2007; Haryanto et al. 2008; Matsunami et al. 2009). The response of root growth and development including efficient uptake of water and nutrients is an important aspect for understanding aerobic adaptation (Bengough et al. 2011). The root system architecture, aptly described as the hidden half, critically influences the yield of the crop. 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 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). Thus it is very much essential to study the anatomical, morphological and physiological features of roots under anaerobic and aerobic systems of cultivation (Kato et al. 2010; Kato and Okami 2010; Patel et al. 2010; Sandhu et al. 2013, 2016).

Root anatomical studies reveal the associated traits that are responsible for adaptation and yield under different

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water regimes. The number of aerenchyma (air cavities that comprise gas-filled spaces) and size of aerenchyma in the root are influenced by the diverse water regime viz. flooding (anaerobic) or hypoxia conditions. Aerenchyma contributes to the transport of essential gases from the above ground parts to the roots (Armstrong and Drew 2002; Colmer 2003). The supply of oxygen from the roots to shoots and shoots to roots is facilitated by the formation of aerenchyma, which is essential for the survival of plants under water logging conditions (Evans 2003). Aerenchyma quantification is essential to know the presence of aerenchyma formation in the rice genotype habitat with the diverse water regime conditions. The number and size of aerenchyma have known to be associated with the drought tolerance (Redillas et al. 2012; Jeong et al. 2013). Certain anatomical changes in rice roots viz. cell wall thickening, lignification of the exodermis, endodermis and sclerenchyma has been observed under aerobic conditions (Mostajeran and Rahimi-Eichi 2008).

The morphological and physiological traits are important in relation to aerobic adaptation. 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, 2013, 2016). Aerobic rice cultivation largely depends on the initial plant establishment which is an important aspect for understanding the adaptation which is largely depend on root growth and development (Bengough et al. 2011). With this background, we analyzed root anatomical, morphological and physiological traits which contribute to aerobic adaptation in two rice genotypes which are best adapted for aerobic and anaerobic systems of cultivation.

Materials and methods

Plant material and treatments

Rice (*Oryza sativa* L.) genotypes viz. BPT 5204 (anaerobic adapted mega genotype), CR Dhan 202 (aerobic adapted genotype) were employed for root anatomical, physiological and morphological studies. Need-based irrigation i.e. measured water applied as and when required was provided to maintain 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. The procedures followed were essentially as described earlier (Barbadikar et al. 2016; Phule et al. 2018). The genotypes were grown in the polythene bags containing 15 kg soil under aerobic and anaerobic conditions in the greenhouse (Fig. 1). The root tissue

samples were harvested at panicle initiation (PI) stage (60–70 days after germination) in three independent biological replicates from each genotype per treatment. 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 2015–2016* and *Kharif 2016* (Fig. 1).

Comparative root anatomy

The root tissues of BPT 5204 and CR Dhan 202 at panicle initiation stage under aerobic and anaerobic conditions were harvested and cut into thin sections in water using sharp surgical blade. The sections were stained using acridine orange and observed at 10 × and 40 × magnification under a simple light microscope (Olympus CH-20i Research Binocular Microscope, Olympus Corporation, Japan). A detailed comparative root anatomical study was carried out using scanning electron microscope (SEM; Ruska lab, College of Veterinary Science, PVNRTVU, Hyderabad). The root tips from crown roots were cut using ultra-microtome and 10 µm root sections were studied in three replicates. The root sections were visualized using the scanning electron microscope at 120 × magnification and the vascular bundles (stellar region) were observed at 330 × magnification. Root section images were captured using a digital camera connected to a microscope. The root anatomical parameters viz. aerenchyma cell number, radius of cross section (µm), vascular bundle diameter (µm), phloem number, xylem area (µm) and root thickness (µm) were recorded for comparative analysis.

Morphological observations

The entire plants were carefully removed by cutting the polythene bags without damaging the roots (Fig. 1). The roots were washed by dislodging all aggregated soil using a high-pressure water pump. Shoots and roots were cut apart at the node interface. 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 (three plants per replication).

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 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal

Fig. 1 Rice genotypes (BPT 5204 and CR Dhan 202) grown in polythene bags (**a**, **b**) under aerobic and anaerobic conditions

conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), intercellular CO_2 concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$), water use efficiency and intrinsic water use efficiency ($\mu\text{mol mol}^{-1}$). Leaf chlorophyll content was estimated using fresh flag leaf samples using solvent extraction method and by SPAD 502 (soil–plant analysis development) chlorophyll meter (SCMR; Minolta Co., Ltd, Osaka, Japan), a portable and convenient meter used for estimation of leaf chlorophyll content and leaf N status. 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 h of incubation was taken at wavelengths of OD645 nm, OD663 nm using UV–VIS Spectrophotometer. The chlorophyll content was calculated by using the following equations viz. $\text{Chl A (mg/g FW)} = 12.72\text{A}_{663} - 2.59\text{A}_{645}$, $\text{Chl B (mg/g FW)} = 22.9\text{A}_{645} - 4.67\text{A}_{663}$, $\text{Chl Total (mg/g FW)} = 20.31 \text{A}_{645} + 8.05 \text{A}_{663}$ (Hiscox and Israelstam 1979). 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

Statistical analyses were conducted with Statistix 8.1 (version 2.0.1) statistical tool. The responses of the genotypes (BPT 5204 and CR Dhan 202) under aerobic and anaerobic condition on the level of 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

Comparative root anatomical studies

The comparative root anatomical studies under aerobic and anaerobic conditions aids in associating the adaptations to the root morphological traits. Xylem area and root thickness were significantly ($P \leq 0.05$) higher under aerobic condition as compared to anaerobic condition. Significant differences in aerenchyma cell size and number were observed under simple microscope. The aerenchyma cell number, vascular bundle diameter, xylem area and root thickness were significantly higher in CR Dhan 202 than the BPT 5204 under aerobic condition. In BPT 5204, the aerenchyma cell number, radius of cross section, vascular bundle diameter and xylem area were significantly higher than the CR Dhan 202 under anaerobic condition (Table 1 and Fig. 2).

Aerenchyma in roots provide transport of essential gases from the above ground parts to the roots (Armstrong and Drew 2002; Colmer 2003). Our results for aerenchyma cell number were consistent with Suralta et al. (2008), Henry et al. (2012), Kundur et al. (2015) and Pradhan et al. (2016). Suralta et al. (2008) also observed that the aerenchyma formation was up to a lesser extent under drought condition than under the flooded condition in both aerobic adapted and lowland genotypes. Kundur et al. (2015) revealed highly significant variance among diverse genotypes of 20 days old seedlings for aerenchyma cell number and xylem cross-section area in root under aerobic condition as compared to anaerobic condition. The submergence condition in rice roots showed prominent aerenchyma formation (Pradhan et al. 2016). Our results suggest that

Table 1 Comparison of root anatomical traits studies under aerobic and anaerobic conditions

Genotype	Treatment	Aerenchyma cell number	Radius of cross section (μm)	Vascular bundle diameter (μm)	Phloem number	Xylem area (μm^2)	Root thickness (μm)
BPT 5204	Aerobic	26.66 ^d	236.33 ^b	233.00 ^b	22.00 ^{ab}	46.26 ^b	17.20 ^c
CR Dhan 202		28.66 ^c	260.67 ^b	261.33 ^a	23.66 ^a	59.96 ^a	32.90 ^a
BPT 5204	Anaerobic	40.33 ^a	327.33 ^a	271.33 ^a	20.33 ^b	48.36 ^b	23.66 ^b
CR Dhan 202		35.00 ^b	249.00 ^b	228.00 ^b	20.66 ^b	37.50 ^c	27.10 ^b
LSD ($P \leq 0.05$)							
Genotype		1.39	18.56	18.14	1.94	5.69	4.06
Treatment		1.39	18.56	18.14	1.94	5.69	4.06
Genotype $\times$ treatment		1.97	26.25	25.65	2.75	8.05	5.74
SED		0.81	10.73	10.48	1.12	3.29	2.34
CV (%)		3.02	4.90	5.17	6.34	8.39	11.39

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

Fig. 2 Rice root anatomy at panicle initiation (PI) stage under aerobic and anaerobic conditions

the formation of aerenchyma, being a constitutive process, fewer aerenchyma must have formed under aerobic condition as compared to the anaerobic condition as an essential anatomical adaptive trait for survival under aerobic conditions in rice.

The observations for xylem area and root thickness are consistent with the results of Nguyen et al. (1997) and Uga et al. (2009). It was reported that a deeper and thickened root system having large xylem vessels has been shown to allow upland rice varieties to extract more water from the

soil, resulting in a higher yield potential under water scarce conditions (Nguyen et al. 1997; Uga et al. 2009). The aerobic adapted genotype, CR Dhan 202 was characterized by fewer aerenchyma formation, thickened root and larger xylem area contributing to the aerobic adaptive anatomical traits and would be useful anatomical traits for further improvement of aerobic adaptive rice genotypes.

Root and shoot morphological traits

The identification of morpho-physiological traits are important for contributing to the development of high yielding rice varieties suitable for aerobic condition (Atlin et al. 2006; Haryanto et al. 2008; Matsunami et al. 2009; Patel et al. 2010). 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). 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 2, Fig. 3).

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 within the genotypes 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 2). The root length significantly increased in both the genotypes 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 genotypes indicated that the root growth was proportionately higher and must have 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 genotypes.

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

Table 2 Comparison of root 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	Aerobic	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	Anaerobic	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 ($P \leq 0.05$)														
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 × 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 ($P \leq 0.05$)

Fig. 3 Comparison of root length of BPT 5204 and CR Dhan 202 under aerobic (a) and anaerobic (b) conditions

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 for 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 as compared to the other rice varieties. It was reported that aerobic rice genotypes performed better under water-limited conditions due to its better root length and root biomass (Sandhu et al. 2012). 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 was noted that the root system 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 lines or varieties having deeper root system which helps in maximizing water uptake and ultimately increase grain yield under water limited 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 (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). Our results indicated 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 ($14.42 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) as compared to BPT 5204 ($11.51 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (Table 3). The present observations corroborated with the earlier reported results of Patel et al. (2010), Mahmood et al. (2014) and Nguyen et al. (2015). Patel et al. (2010) reported that the photosynthesis rate was significantly higher in rice varieties under aerobic condition than the anaerobic condition due to the maximum light capture in mid-hills ecosystem.

Table 3 Comparison of photosynthetic parameters under aerobic and anaerobic conditions

Genotype	Treatment	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	Intercellular CO_2 ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)	Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	Water use efficiency (Pn/TR)	Intrinsic water use efficiency ($\mu\text{mol mol}^{-1}$)
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 ($P \leq 0.05$)							
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 $\times$ 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

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

Pn photosynthetic rate, TR transpiration rate

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.

Except the photosynthetic rate, all other parameters did not show any significant difference between the two genotypes under aerobic condition. Under anaerobic condition, the intracellular CO_2 showed significant difference between CR Dhan 202 ($233.79 \mu\text{mol CO}_2 \text{ mol}^{-1}$) as compared to BPT 5204 ($163.38 \mu\text{mol CO}_2 \text{ mol}^{-1}$). Water use efficiency and intrinsic water use efficiency were significantly higher in BPT 5204 than CR Dhan 202 under anaerobic condition (Table 3).

The water use efficiency of BPT 5204 was higher in both aerobic and anaerobic conditions as compared to the genotype CR Dhan 202. Condition wise, CR Dhan 202 had higher WUE under aerobic condition and BPT 5204 had higher WUE under anaerobic condition. The results of water use efficiency was found to be similar as reported by Mahmud et al. (2014). Mahmud et al. (2014) indicated that aerobic rice has high water use efficiency (WUE) as 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 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 (Fil-lela et al. 1996). The leaf chlorophyll content (viz. chlorophyll A, chlorophyll B and total chlorophyll)

Table 4 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 ($P \leq 0.05$)				
Genotype		0.48	0.14	0.58
Treatment		0.48	0.14	0.58
Genotype $\times$ 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 ($P \leq 0.05$)

Table 5 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		36.92 ^a
CR Dhan 202		36.35 ^a
LSD ($P \leq 0.05$)		
Genotype		2.25
Treatment		2.25
Genotype $\times$ treatment		3.18
SED		1.55
CV (%)		8.86

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

displayed non-significant differences under both aerobic as well as under anaerobic conditions in both the genotypes (Table 4). 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 5). 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.

Conclusion

The present investigation demonstrated that the root anatomical traits viz. fewer aerenchyma formation, thickened roots and larger xylem area and the morpho-physiological parameters viz. root length, total dry weight and photosynthetic rate are critically associated with aerobic adaptation. 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. These anatomical, morpho-physiological parameters can form the preliminary basis of root phenotyping for the development of suitable rice genotypes for aerobic adaptation. Also, the lines or genotypes to be identified as donors for aerobic breeding programmes should be phenotyped for these traits. These observations need to be further tested in more number of genotypes adapted for aerobic and anaerobic cultivation.

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Author contribution PAK, MSM and KMB conceived and designed the experiments. ASP, KMB performed the experiments. ASP, KMB, PS and DS analyzed the data. ASP, KMB and MSM wrote the manuscript. PAK, MSM and KMB supervised the study. All authors reviewed and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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