RESEARCH ARTICLE

Leaf photosynthesis and antioxidant response in selected traditional rice landraces of Jeypore tract of Odisha, India to submergence

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Abstract Submergence tolerance in rice is important for improving yield under rain-fed lowland rice ecosystem. In this study, five traditional rice landraces having submergence tolerance phenotype were selected. These five rice landraces were chosen based on the submergence-tolerance screening of 88 rice landraces from various lowland areas of Jeypore tract of Odisha in our previous study. These five rice landraces were further used for detailed physiological assessment under control, submergence and subsequent re-aeration to judge their performance under different duration of submergence. Seedling survival was significantly decreased with the increase of plant height and significant varietal difference was observed after 14 days of complete submergence. Results showed that submergence progressively declined the leaf photosynthetic rate, stomatal conductance, instantaneous water use efficiency, carboxylation efficiency, photosystem II (PSII) activity and chlorophyll, with greater effect observed in susceptible check variety (IR 42). Notably, higher activities of antioxidative enzymes and ascorbate level were observed in traditional rice landraces and were found comparable with the tolerant check

variety (FR 13A). Taken together, three landraces such as *Samudrabali*, *Basnamundi* and *Gadaba* showed better photosynthetic activity than that of tolerant check variety (FR 13A) and showed superior antioxidant response to submergence and subsequent re-aeration. These landraces can be considered as potential donors for the future submergence tolerance breeding program.

Keywords Antioxidant · Gas exchange · Traditional rice · Photosynthesis · PSII activity · Submergence

Introduction

Complete submergence due to flash flooding is one of the foremost constraints for rice production, mainly in rainfed lowland areas of South and South-East Asia (Dar et al. 2017; Afrin et al. 2018). Out of ~ 20 million ha of rainfed lowland rice growing areas, 12–14 million ha in India are prone to flash flooding with average productivity of only 0.5–0.8 t ha⁻¹, which is far lower than that of national average productivity (Ismail et al. 2013; Bhowmick et al. 2014). The yield gap is substantially high because of the high yielding rice varieties grown in these areas are susceptible to submergence and perish within 7–14 days (d) of waterlogging (Sarkar et al. 2006; Ismail et al. 2013; Singh et al. 2017). Therefore breeding for submergence tolerance trait will be crucial for maintaining stable yields in these rainfed lowland rice ecosystems (Dar et al. 2017; Goswami et al. 2017). In the last decade, molecular mechanisms underlying flash flood tolerance have been revealed by the identification of major QTL, *Sub1* from FR 13A, a submergence tolerant rice cultivar of Odisha, India (Xu et al. 2006). This QTL has been successfully introgressed into several high yielding varieties and flash flood-tolerant

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varieties has been released for cultivation in India and other parts of Asia (Sarkar et al. 2009; Ismail et al. 2013; Singh et al. 2016). However, these improved varieties are unsuitable for most of the lowland areas where the depth of waterlogging is high and longer duration of flooding occurs. This is mainly attributed to the short plant height and quiescence to submergence combating physiology (Iftekharrudaula et al. 2016; Goswami et al. 2017). Therefore, farmers are hesitating to adopt these genotypes because of poor performance under varying water depths (Goswami et al. 2017; Afrin et al. 2018). Due to the adverse environment in a rainfed ecosystem, many different types of traditional rice cultivars are being cultivated by farmers. The local landraces adapted to rainfed land could be the sources of genetic variation, which can be used to improve the adaptability of rice to submergence (Sarkar et al. 2013; Singh et al. 2016).

Jeypore tract of Odisha, India (20°37' and 17°50' North latitude and 81°27' and 84°01' East longitude) is the home to large number of traditional rice landraces and is considered as the secondary centre of origin of Asian cultivated rice (Mishra et al. 2012; Roy et al. 2016). The tract has its climatic and physiographic features favourable for rice cultivation, in an ample range of agro-ecological situations including hill slopes to very deep-water areas (Arunachalam et al. 2006). The landraces cultivated by traditional farmers might contain considerable genetic diversity and could serve as potential genetic resources for a number of useful traits (Arunachalam et al. 2006; Roy et al. 2016; Panda et al. 2018). In this background, there is limited phenotypic knowledge and profiling reports of traditional rice landraces in relation to submergence tolerance. Understanding the physiological and biochemical mechanisms for cultivar differences in relation to submergence tolerance may facilitate future breeding programmes for the development of adaptable high yielding submergence-tolerant varieties.

The adverse effect of submergence on rice varies with cultivars, such as the developmental stage at which flooding occurs, duration and depth, and other hostile environmental parameters (Colmer and Pedersen 2008; Panda et al. 2008; Das et al. 2009; Afrin et al. 2018). Various interrelated factors are also attributed to varying submergence susceptibility such as limited gas diffusion, reduced irradiance, decrease in adenylate energy charge, cytoplasmic acidification and decrease in membrane barrier function, which cause several visible injuries, damage photosynthetic apparatus and impair plant photosynthesis (Drew 1997; Ram et al. 2002; Panda et al. 2008; Panda and Sarkar 2013; Singh et al. 2017). Maintaining normal photosynthetic characteristics during and after submergence is regarded as one of the adaptive strategy of submergence tolerant cultivar (Panda et al. 2008; Panda and Sarkar

2013). Although, significant progress has been made for studying the impact of submergence on photosynthetic mechanism; there is no unified phenomenon available to explain the exact cause of the reduction of photosynthesis in rice. Moreover, the response of traditional rice plants and mechanism of PSII activity to different durations of submergence is less understood so far.

Oxidative damage caused by submergence generally does not appear immediately but develops soon after water level recedes during recovery from complete submergence (Drew 1997; Sarkar et al. 2006). The post-hypoxic and anoxic injury probably contributes to the generation of reactive oxygen species (ROS) and loss of redox homeostasis of the cell (Ella et al. 2003; Fukao et al. 2011; Upadhyay 2016). Additionally, both photosynthetic and respiratory redox cascades also contribute for production of ROS such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) in a plant cell under submergence (Fukao et al. 2011; Upadhyay 2016). The ROS act as a cellular indicator of submergence stress and they not only have the harmful effect on photosynthesis but also contribute to the down-regulation of many photosynthetic genes (Pfannschmidt 2003; Fukao and Bailey-Serres 2004). To evade the cell level damage during stress, plants possess a number of antioxidative enzymes such as superoxide dismutase, catalase and peroxidase and enzymes of the ascorbate–glutathione (ASC–GSH) cycle or by the effect of a number of low molecular mass antioxidant such as ascorbate, glutathione and phenolic compounds etc. (Damanik et al. 2010). The tolerance level of rice genotypes to submergence is proportional to the potential to neutralize ROS (Gill and Tuteja 2010; Fukao et al. 2011; Panda and Sarkar 2013). Data explaining this relationship between the complete submergence and oxidative damage are not sufficient enough in rice, particularly in traditional rice landraces. The relationship between the photosynthetic gas exchanges, PSII activity and antioxidant defense in submerged rice leaf is unclear. Notably, sparse interest is given to establish the importance of the ascorbate–glutathione cycle and the chief constituents engaged in redox regulation mechanism in rice under submergence.

Therefore, the present study was aimed to access the impact of different duration of submergence and subsequent re-aeration on photosynthetic gas exchange in rice. The physiological results were obtained by chlorophyll fluorescence analysis in order to get an overview of the primary effect of submergence on PSII activity. In addition, changes of some anti-oxidative enzymes were also studied to understand the association of redox regulation of these enzymes with photosynthesis as affected by complete submergence and subsequent re-aeration in rice.

Materials and methods

Plant materials

The experiments were conducted by choosing five suitable traditional rice landraces (*Samudrabali*, *Basnamundi*, *Gadaba*, *Surudaka* and *Dokarakuji*) of Jeypore tract of Odisha, India along with FR 13A (submergence tolerant) and IR 42 (submergence susceptible) as check varieties. The specific characters of studied rice landraces are presented in Table 1. The traditional rice landraces were collected from M.S. Swaminathan Research Foundation, Jeypore and the submergence tolerant and susceptible check varieties were collected from National Rice Research Institute, Cuttack, India. These rice landraces were selected for comprehensive physiological and biochemical characterization after initial submergence tolerance screening of 88 rice landraces collected from different regions of Jeypore tract of Odisha, India (Barik and Panda 2016).

Growth condition and stress treatment

The experiments were conducted during the wet season (June to September) of 2018 at the experimental garden of Central University of Orissa, Koraput, India (82°44'54"E and 18°46'47"N, 880 m above the mean sea level and average rainfall 1500 mm). Uniform sized seeds were selected and sown directly in earthen pots (30 cm in diameter) containing two kg of farm soil and farmyard manure (3:1). The rice seeds were oven dried at 45 ± 2 °C for 5 days prior to sowing. After 5 days, seedlings were thinned and ten plants per pot were maintained. Each pot was supplied with 190 mg single super phosphate (P_2O_5) and 50 mg murate of potash (K_2O). N-fertilizer in the form of urea at 1 g per pot was applied after 10 days of sowing. Plants were regularly irrigated with tap water and subjected to natural solar radiation, with daily maximum photosynthetic photon flux density, air temperature and relative humidity being about $1260 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$, 30.6 ± 2 °C and 65–75%, respectively. Twenty-one days old healthy seedlings were then completely submerged in a concrete tank (3 m $\times$ 3 m $\times$ 1.3 m) under 120 cm depth of water. One more set kept outside under normal conditions served as control. The experiments were carried out by a randomized complete block design in three replications. Each pot was considered as one replication. All the physiological parameters were measured at 7-d and 14-d after submergence and 1-day (24 h) after re-aeration along with the control plants. For re-aeration, the height of water column in the experimental tank was brought down to 10 cm from the level of 120 cm. Experimental setup for

plants grown in control and submergence treatments are presented in Fig. S1.

The floodwater conditions in the submergence tank were monitored every alternate day in morning and evening. The floodwater quality in terms of pH, water temperature and oxygen concentration was determined at 06:00 and 17:00 h by a water analyzer kit (Syland, Heppenheim, Germany). The pH of the flood water varied from 6.6 to 6.8 and oxygen concentration was $2.1\text{--}3.0 \text{ mg L}^{-1}$ at 06:00 h and $3.8\text{--}4.1 \text{ mg L}^{-1}$ at 17:00 h. The temperature was $27.4\text{--}30.3$ °C throughout the period of the experiment.

Measurement of plant height and survival

Plant height was measured in control and submerged plant with the help of measuring scale from the base of the plant to the tip of the uppermost leaf and the value is an average for the surviving seedlings per pot. Seedling survival was counted after 7 days and 14 days of complete submergence treatment followed by 8 days of re-aeration each based on the ability of seedling to produce new growing leaves. The survival was measured from the ratio of number of surviving plants after 8th days of re-aeration to the total number of plants just before submergence and expressed as percentage of survival.

Measurement of leaf gas exchange and chlorophyll content

The leaf gas exchange parameters such as photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s) and internal CO_2 concentration (C_i) were measured on 2nd leaf of five different plants in each replication within 20 min post-submergence treatment by using an open system photosynthetic gas analyzer (CI-340, CID, USA). The measurements were made between 10 and 12 h under normal ambient environmental condition at 27 ± 2 °C air temperature, 70% relative humidity, $1224 \pm 23 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic active radiation, $370 \mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ and 21% O_2 . The instantaneous water use efficiency ($WUE = P_N/E$) and carboxylation efficiency ($CE = P_N/C_i$) were calculated as recently described by Panda et al. (2018). The chlorophyll (Chl) content was determined spectrophotometrically following the method of Arnon (1949). The extraction and measurements of chlorophyll were described by Panda et al. (2018).

Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured on the same leaves used for gas exchange measurements in dark and light-adapted leaves at a mid-day (12:00 h) by using a

Table 1 Details of rice genotypes with their characteristic features as perceived by tribal farmers

Genotype	Specific note	Grains feature
<i>Samudrabali</i>	Lowland aromatic rice variety, maturity duration 180 days, not susceptible to disease and pest, tolerant to lowland flooding ^a
<i>Basnamundi</i>	Lowland rice landrace (grown on flood plain), flood tolerant, maturity duration 160 days, no diseases, 3 ft long straw, non scented ^{a, b}
<i>Gadaba</i>	Lowland rice landraces without awn, long duration of maturity (135–140 days), no diseases, grain shape slender, flood tolerant ^{a, b}
<i>Surudaka</i>	Lowland rice landrace, long duration of maturity (140–145 days), no awning, yellow husk colour, grain shape medium, flood tolerant ^a
<i>Dokarakuji</i>	Lowland rice landraces, maturity duration 125–130 days, not susceptible to any disease, puffed rice, good taste, grain shape slender, flood tolerant ^b
FR 13A	Submergence tolerant rice landrace of Odisha and donor of Sub1 loci, grain deep reddish, bold with distinct awn ^c
IR 42	Submergence susceptible, high yielding semi dwarf rice genotype developed during green revolution, a sister selection of IR 36 (IR 1561-228-1-2/IR 1733//CR 94-13) ^c

^aMishra et al. (2012)^bArunachalam et al. (2006)^cGoswami et al. (2018)

portable chlorophyll fluorometer (JUNIOR-PAM, WALZ, Germany). Different fluorescence parameters like minimal fluorescence (F_o), maximal fluorescence (F_m), variable fluorescence ($F_v = F_m - F_o$) and maximum photochemical efficiency of PSII (F_v/F_m) was measured in 20 min

dark-adapted leaves. In light-adapted leaves at a PPFD of $420 \mu\text{M m}^{-2} \text{s}^{-1}$ (for 15 min), steady-state fluorescence yield (F_s), maximal fluorescence (F_m') after 0.8 s saturating white light pulse and minimal fluorescence (F_o') were measured when actinic light was turned off. Quenching

value due to non-photochemical dissipation of absorbed light energy (NPQ) and the coefficient for photochemical quenching (qP) was also calculated (Maxwell and Johnson 2000).

Measurement of reactive oxygen species content

After measurement of photosynthesis and chlorophyll fluorescence of fully expanded mature leaves, which experienced full submergence stress were collected and used for the measurement of reactive oxygen species content. Superoxide anion ($O_2^{\bullet-}$) production rate was measured by monitoring the nitrite formation from hydroxylamine in the presence of $O_2^{\bullet-}$ as described by Wang and Luo (1990). The $O_2^{\bullet-}$ content was determined and expressed as $mM\ g^{-1}$ fresh weight by using the standard curve of hydroxylamine ($E = 12.8\ mM\ cm^{-1}$). The production of hydroxyl radicals ($OH^{\bullet}$) in the samples was measured by a deoxyribose assay following Halliwell and Gutteridge (1981). The production rate of $OH^{\bullet}$ was expressed as specific absorbency g^{-1} fresh weight. Hydrogen peroxide (H_2O_2) content was measured by the method of Alexieva et al. (2001) and the concentration of H_2O_2 was estimated using the standard curve of H_2O_2 ($E = 0.28\ mM\ cm^{-1}$).

Measurement of antioxidant enzyme activity and lipid peroxidation

Different antioxidant enzyme activities were measured in fully expanded mature leaves of control, submerged and re-aerated plants. The leaves were collected per pot basis, were chopped and mixed, which constituted one replication. A representative sample of fresh leaves in each treatment weighing 500 mg were homogenized in 10 ml of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM EDTA, 1 mM ascorbate, 10% (w/v) sorbitol and 0.1% triton X-100. The homogenate was centrifuged at 0–4 °C at 12,000 g for 15 min and the supernatant was used for enzyme analysis (Mishra and Panda 2017). Superoxide dismutase (SOD) activity was measured by the photochemical method according to Giannopolitis and Ries (1977) with modification suggested by Choudhury and Choudhury (1985). Ascorbate peroxidase (APX) activity was measured according to Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm ($E = 2.8\ mM\ cm^{-1}$). The activity of guaiacol peroxidase (GPX) was assayed according to Rao et al. (1995) by guaiacol oxidation ($E = 26.6\ mM\ cm^{-1}$). Catalase (CAT) activity was measured by monitoring rate of decomposition of H_2O_2 at 240 nm according to Cakmak and Marschner (1992). Glutathione reductase (GR) activity was measured

according to the method of Foyer and Halliwell (1976) by following the decrease in absorbance at 340 nm by NADPH oxidation using an extinction coefficient of $6.2\ mM\ cm^{-1}$. Dehydroascorbate reductase (DHAR) activity was estimated following Nakano and Asada (1981) by measuring the increase in absorbance at 265 nm. Monodehydroascorbate reductase (MDAR) was measured spectrophotometrically by following the decrease in absorbance at 340 nm due to NADH oxidation using an absorbance coefficient of $6.2\ mM\ cm^{-1}$ (Hossain et al. 1984). Lipid peroxidation product in terms of malondialdehyde (MDA) content was measured by thiobarbituric acid (TBA) reactions followed by Heath and Packer (1968).

Measurement of ascorbic acid content

Ascorbic acid content was measured following Shigeoka et al. (1979). Fresh leaf sample weighing 500 mg was homogenized in 3 ml of 5% (w/v) meta-phosphoric acid. The extract was clarified by centrifugation at 2,000 g for 20 min at 4 °C. An aliquot measuring 0.5 ml of extract was mixed with 0.25 ml of 3 mM DCPIP to measure the total ascorbic acid content, whereas an equal volume of water was added when the oxidized ascorbic acid content was to be measured. After keeping the mixture at room temperature for 20 min, 0.5 ml of 1% (w/v) thiourea in 5% (w/v) meta-phosphoric acid and 0.5 ml of DNPH was added. The mixture was then incubated at 50 °C for 1 h, cooled in an ice bath for 15 min while adding 1.2 ml of ice-cold 85% H_2SO_4 . Then absorbance at 520 nm was recorded using a spectrophotometer. The amount of reduced ascorbate was calculated by subtracting oxidized ascorbate content from total ascorbate content.

Statistical analysis

All the physiological and biochemical parameters were subjected to two-way analysis of variance with the variety and treatment level as main factors. Means were compared by ANOVA using CROPSTAT (International Rice Research Institute, Philippines) software's least significant difference (LSD). Duncan's multiple range tests (DMRT) were done by the CROPSTAT software. The standard deviations (SD) and correlation analysis were conducted by *Microsoft Excel 2007*. The Bray–Curtis similarity index of different rice landraces was constructed by dendrogram using different physiological and biochemical parameters under submerged conditions through the unpaired group (UPGMA) by using PAST-3 (Palaeontological Statistics) software.

Results

Test of significance

The analysis of variance of different physiological parameters in studied rice genotypes grown under control and submergence treatments indicated that the variety (V) and treatment (T) effects were highly significant ($P < 0.01$) (Table 2). A highly significant ($P < 0.01$) difference of combined $V \times T$ interaction was observed for all the studied traits. Based on the results, treatment was the major cause of variance for all the studied traits as compared to the variety and interactions of variety $\times$ treatment except Fv/Fm and NPQ, where varietal differences were maximum.

Plant height and survival under submergence

The phenotypic variation of plant growth showed that plant height increased due to submergence in all the genotypes and was significantly ($P < 0.05$) greater in traditional landraces such as *Samudrabali*, *Basnamundi*, *Gadaba*, *Surudaka* and *Dokarakuji* compared to FR 13A and IR 42 (Fig. S2A). The increase of plant height after 14 days submergence was 73–84% in traditional landraces followed by IR 42 (64%) and FR 13A (28%). The survival of genotypes demonstrated that the traditional landraces (*Samudrabali*, *Basnamundi* and *Gadaba*) along with FR 13A were tolerant in 7 days of submerged conditions in the vegetative stage with a survival of 100%, whereas the tolerance level decreased in response to submergence at 14 days. Tolerant cv. FR 13A showed 90% survival after 14 days of submergence and traditional landraces *Samudrabali*, *Basnamundi* and *Gadaba* showed 95–97%, whereas it was less than 10% in IR 42 (Fig. S2B).

Table 2 Analysis of variance (ANOVA) of different photosynthetic and antioxidant parameters in rice seedlings grown under control and submergence

Parameters	Source of variation		
	Variety (df = 6)	Treatment (df = 3)	Variety $\times$ treatment (df = 18)
P _N	320** (7.94)	3533** (87.66)	176.2** (4.37)
E	15.4** (10.87)	118.3** (83.39)	8.1** (5.73)
gs	99,176** (32.77)	166,634** (55.06)	36,764** (12.15)
Chl	2.3** (13.5)	14.0** (81.99)	0.8** (4.41)
Fo	11,138** (4.85)	209,034** (91.05)	9391** (4.09)
Fm	1,087,510** (15.01)	5,673,370** (78.29)	485,827** (6.70)
Fv/Fm	0.3** (53.62)	0.07** (10.91)	0.2** (35.46)
Y(II)	0.03** (27.39)	0.06** (52.69)	0.02** (19.89)
qP	0.15** (20.87)	0.49** (69.71)	0.07** (9.41)
NPQ	4.05** (54.21)	0.695** (9.30)	2.73** (36.49)
SOD	6287** (20.5)	22,678** (73.8)	1728** (5.6)
GPX	6.1** (19.9)	21.5** (70.0)	2.9** (9.45)
CAT	78.9** (36.3)	114.1** (52.4)	24.4** (11.2)
APX	10.1** (38.4)	13.2** (50.2)	3.0** (11.4)
DHAR	9.7** (10.3)	79.4** (84.2)	5.1** (5.4)
MDAR	0.09** (3.8)	2.3** (96.6)	0.03** (1.26)
GR	0.06** (59.2)	0.02** (23.9)	0.02** (16.9)
ASC-T	795,070** (22.7)	2,388,840** (68.1)	326,617** (9.30)
ASC-O	510,023** (22.5)	1,550,910** (68.5)	204,470** (9.03)
ASC-R	41,643** (26.9)	93,524** (60.4)	19,644** (12.7)

Sum square of each trait is the absolute value and percentage of total (in bracket) of main effect resulting from ANOVA. *df* degrees of freedom; total *df* = 83; the *P* of overall ANOVA for variety, treatment and variety $\times$ treatment interaction for each parameters * $P < 0.05$; ** $P < 0.01$

P_N, photosynthetic rate; E, transpiration rate; gs, stomatal conductance; Chl, chlorophyll; Fo, minimal fluorescence at dark-adapted leaf; Fm, maximal fluorescence at dark-adapted leaf; Fv/Fm, maximum quantum efficiency of PSII photochemistry; Y(II), effective quantum yield of PSII photochemistry; qP, photochemical quenching; NPQ, non-photochemical quenching; APX, ascorbate peroxidase; GPX, guaiacol peroxidase; CAT, catalase; SOD, superoxide dismutase; GR, glutathione reductase; MDAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; ASC-T, total ascorbate; ASC-O, oxidized ascorbate; ASC-R, reduced ascorbate

Photosynthetic performance and chlorophyll loss during submergence

The CO_2 photosynthetic rate (P_N) in all the rice seedlings were significantly decreased ($P < 0.05$) under submergence compared to control plants. The differences in P_N among studied genotypes were not significant under the control condition (Fig. 1a). However, significant ($P < 0.05$) variations were observed between the genotypes under 7 and 14 days of submergence and subsequent re-aeration. In particular, susceptible (IR 42) cv. exhibited a sharp reduction of P_N under 7 and 14 days of submergence as compared to tolerant check (FR 13A) cv. and traditional landraces. However, the traditional landraces such as *Samudrabali*, *Basnamundi* and *Gadaba* showed higher P_N than that of FR 13A under submergence and subsequent re-aeration. The percentage of reduction in P_N was 61–79% in traditional rice landraces and tolerant check (FR 13A) variety under 14 days of submergence compared to the control plant whereas, the reduction was 95% in susceptible variety (IR 42). The main cause of variance for

P_N was the treatment which accounted for 87% of total variance compared to the variety (7%) and variety $\times$ treatment (4%) (Table 2). Similarly, a gradual reduction in stomatal conductance (g_s) and transpiration rate (E) was observed in all the genotypes under submergence and significant ($P < 0.05$) varietal difference was observed during 7 and 14 days of submergence and subsequent re-aeration (Fig. 1b, c). The selected traditional landraces and tolerant variety (FR 13A) exhibited a higher value of g_s and E under submergence compared to the susceptible variety IR 42. These parameters were greatly affected by treatment followed by the variety and variety $\times$ treatment (Table 2). Further, significant ($P < 0.01$) decrease of instantaneous WUE and CE were observed within the studied rice genotypes after submergence and subsequent re-aeration compared to control plants (Fig. 1d, e). In particular, the susceptible variety IR 42 exhibited sharp reductions of instantaneous WUE and CE under submergence compared to traditional landraces and tolerant genotype (FR 13A). However, the traditional landraces such as *Samudrabali*, *Basnamundi* and *Gadaba* exhibited

Fig. 1 CO_2 photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), water use efficiency (WUE), carboxylation efficiency (CE) and chlorophyll (Chl) content of studied rice genotypes during control (C), submergence (S) and re-aeration (RA). Data are

the mean of three replications ($n = 3$) with vertical bar representing standard deviation. Means followed by the same letter in a particular treatment are not significantly different at $P < 0.05$ by Duncan's multiple range tests. *LSD* least significance difference

more instantaneous WUE and CE value than that of FR 13A under submergence and subsequent re-aeration. In our study, submergence resulted in significant reduction of Chl in all the studied genotypes, more significantly at 14 days of submergence (Fig. 1f). However, the tolerant cv. FR 13A and traditional landraces maintained a significantly higher amount of Chl during submergence and subsequent re-aeration compared to IR 42. The percentage of reduction in chlorophyll was 52–71% in traditional rice landraces and tolerant check (FR 13A) variety under 14 days of submergence compared to the control plants, whereas reduction was more than 90% in susceptible variety (IR 42). The main cause of variance for Chl was found to be the treatment (81%), followed by variety (13%) and interactions of the variety $\times$ treatment (4%) (Table 2).

Leaf PSII activity under submergence

Different Chl fluorescence parameters were measured to study the variations of PSII activity in the studied rice

seedlings under control, submergence and subsequent re-aeration (Fig. 2). The variations of F_o , F_m and F_v/F_m were not significant ($P < 0.05$) among the variety under the control condition but a significant varietal difference was observed after 7 days and 14 days of submergence and subsequent re-aeration. A significant reduction ($P < 0.01$) of F_o , F_m and F_v/F_m was observed in all the studied landraces under 7 and 14 days of submergence as compared to the control (Fig. 2a, b, c). In particular, susceptible (IR 42) cultivar exhibited a greater reduction of F_o , F_m and F_v/F_m under submergence compared to tolerant check cv. FR 13A and traditional landraces. The main cause of variance for F_o and F_m was the treatment with 91% and 78% of the total variance respectively, whereas for F_v/F_m , the main cause of variance was the variety (53%) followed by variety $\times$ treatment (35%) and treatment (10%) (Table 2). The photochemical quenching (qP) gradually decreased ($P < 0.05$) with increasing duration of submergence compared to control plants (Fig. 2d). In contrast, the submergence increased non-photochemical quenching

Fig. 2 Minimal fluorescence from dark-adapted leaf (F_o), maximal fluorescence from dark-adapted leaf (F_m), maximum quantum efficiency of PSII photochemistry (F_v/F_m), yield of PSII photochemistry ($Y(II)$), photochemical quenching (qP) and non-photochemical quenching (NPQ) of studied rice genotypes during control (C),

submergence (S) and re-aeration (RA). Data are the mean of three replications ($n = 3$) with vertical bar representing standard deviation. Means followed by the same letter in a particular treatment are not significantly different at $P < 0.05$ by Duncan's multiple range tests. LSD least significance difference

(NPQ) in all the genotypes. The major cause of variance for qP was treatment with 69% variation followed by variety (20%) and interactions of variety $\times$ treatment (9%), whereas for NPQ, the varietal difference was more (54%) of total variation (Table 2). The effective quantum yield of PSII photochemistry [$Y(II)$] was also significantly ($P < 0.01$) decreased after 7 days and 14 days of submergence and subsequent re-aeration compared to control plants (Fig. 2d). The selected traditional landraces and tolerant cv. (FR 13A) exhibited a higher value of $Y(II)$ under submergence and re-aeration compared to the susceptible variety IR 42. For $Y(II)$, the main cause of variance was the treatment followed by interactions of variety $\times$ treatment and variety (Table 2).

Reactive oxygen species production and lipid peroxidation under submergence

Variations of ROS such as H_2O_2 , $O_2^{\bullet-}$ and $OH^{\bullet}$ content in different rice genotypes under control, submergence and re-aeration was presented in Fig. 3. There were no significant differences in H_2O_2 production among studied genotypes under the control condition except *Dokarakuji* (Fig. 3c). However, H_2O_2 content was gradually decreased in all the genotypes during the progression of submergence as compared to the control plants and significantly increased during the re-aeration. In particular, the higher H_2O_2 content was observed in susceptible cv. (IR 42) compared to the FR 13A and traditional landraces after 14 days of submergence and re-aeration. Similarly, a marked decrease in $O_2^{\bullet-}$ and $OH^{\bullet}$ content was observed in the studied rice genotypes in response to different days of submergence and also significant ($P < 0.01$) varietal difference was observed (Fig. 3a, b). The $O_2^{\bullet-}$ and $OH^{\bullet}$ content was significantly higher in IR 42 as compared to other genotypes under submergence and subsequent re-aeration. The lipid peroxidation status in rice seedlings under submergence and subsequent re-aeration was evaluated by the TBARS content. Compared with the control, submergence led to a rapid increase in TBARS levels in the leaves of rice seedlings ($P < 0.05$) (Fig. 3d). The susceptible cv. IR 42 showed higher values of TBARS content compared to the other genotypes under 7 and 14 days of submergence treatments. The increase of TBARS content was 2.71–3.3-fold in traditional rice landraces and tolerant check (FR 13A) cv. under 14-days of submergence compared to the control plant whereas, the increase was 6.6-fold in susceptible cv. (IR 42).

Antioxidant enzyme activity under submergence

A gradual decline in SOD activity was observed in all the genotypes under 7 days and 14 days of submergence and

Fig. 3 Superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances (TBARS) of studied rice genotypes during control (C), submergence (S) and re-aeration (RA). Data are the mean of three replications ($n = 3$) with vertical bar representing standard deviation. *LSD* least significance difference

subsequent re-aeration compared to the control plants. The differences in SOD activity among genotypes were not significant under control but significant ($P < 0.05$) varietal differences was observed under 7-days and 14-days of

submergence and subsequent re-aeration. The decrease of SOD activity was 28–41% in traditional rice landraces and tolerant check (FR 13A) cv. under 14 days of submergence compared to the control plant whereas, the reduction was 63% in susceptible variety (IR 42). The studied traditional rice landraces and tolerant check variety (FR 13A) exhibited higher values of SOD under submergence and subsequent re-aeration compared to the susceptible cv. IR 42. More significantly, the landraces *Samudrabali*, *Basnamundi* and *Gadaba* exhibited higher SOD activity than that of FR 13A (Fig. 4a). A similar pattern was also observed for CAT and GPX activity and the studied traditional rice landraces and tolerant check cv. (FR 13A) showed a higher activity of CAT and GPX under submergence and

subsequent re-aeration compared to the susceptible cv. IR 42 (Fig. 4b, c). The major source of variation for SOD, GPX and CAT was the treatment with 73%, 70% and 52% respectively of the total variance followed by interaction of variety $\times$ treatment and variety (Table 2).

The activities of ascorbate–glutathione cycle enzymes such as APX, MDHAR, DHAR and GR of rice genotypes remarkably declined under different durations of (7 days and 14 days) of submergence and subsequent re-aeration compared to the control and also significant ($P < 0.01$) varietal difference was observed (Fig. 5). The traditional rice landraces and tolerant check cv. FR 13A showed a higher activity of APX, MDHAR, DHAR and GR under submergence and subsequent re-aeration compared to the susceptible cv. IR 42. The major source of variation was the treatment followed by interaction of variety $\times$ treatment and variety except MDHAR for activity where variety is the major determinant for variation (Table 2). Among the traditional rice landraces, *Samudrabali*, *Basnamundi* and *Gadaba* showed higher APX and MDAR activity than that of FR 13A under submergence and subsequent re-aeration.

Ascorbate levels under submergence

Changes of oxidized, reduced and total ascorbate content were quantified in the studied rice genotypes under control, submergence and subsequent re-aeration (Fig. 6). The differences of ascorbate level among studied genotypes were not significant under the control condition but significant ($P < 0.05$) varietal differences was observed under 7 days and 14 days of submergence and subsequent re-aeration. The level of oxidized ascorbate was more than that of reduced ascorbate in different rice genotypes. A gradual decline in oxidized, reduced and total ascorbate content was observed in all the genotypes under submergence and subsequent re-aeration compared to the control plants. However, significant greater reduction of ascorbate was observed in susceptible cultivar (IR 42) compared to the other genotype. Among the traditional rice landraces, *Samudrabali*, *Basnamundi* and *Gadaba* showed a higher value of total and oxidized ascorbate than that of FR 13A under submergence and subsequent re-aeration. The main source of variation for oxidized, reduced and total ascorbate was the treatment with 69%, 60% and 68% respectively, of the total variation (Table 2).

Relationship between photosynthetic parameters with antioxidants

The relationship between leaf photosynthetic parameters and antioxidants in rice genotypes were studied by multiple correlation analysis under control, 7 days and 14 days of

Fig. 4 Superoxide dismutase (SOD), guaiacol peroxidase (GPX) and catalase (CAT) of studied rice genotypes during control (C), submergence (S) and re-aeration (RA). Data are the mean of three replications ($n = 3$) with vertical bar representing standard deviation. Means followed by the same letter in a particular treatment are not significantly different at $P < 0.05$ by Duncan's multiple range tests. *LSD* least significance difference

Fig. 5 Ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) of studied rice genotypes during control (C), submergence (S) and re-aeration (RA). Data are the mean of three replications ($n = 3$) with vertical bar representing standard deviation. Means followed by the same letter in a particular treatment are not significantly different at $P < 0.05$ by Duncan's multiple range tests. *LSD* least significance difference

Fig. 6 Total ascorbate, oxidized ascorbate and reduced ascorbate of studied rice genotypes during control (C), submergence (S) and re-aeration (RA). Data are the mean of three replications ($n = 3$) with vertical bar representing standard deviation. Means followed by the same letter in a particular treatment are not significantly different at $P < 0.05$ by Duncan's multiple range tests. *LSD* least significance difference

submergence and subsequent re-aeration (Table 3). The results showed that under control condition, the rate of photosynthesis (P_N), maximum photochemical efficiency of PSII (F_v/F_m) and Chl content were not significantly influenced by different antioxidant enzymes and ascorbate level except APX. However, a significant positive correlation ($P < 0.01$) of different photosynthetic parameters and antioxidants was observed under 7 days and 14 days of submergence and subsequent re-aeration. More significantly, a strong positive correlation was observed between the activities of ascorbate–glutathione pathway enzymes and ascorbate level with P_N , F_v/F_m and Chl under submergence and subsequent re-aeration.

Table 3 Correlation co-efficient (r) value between different photosynthetic and chlorophyll fluorescence parameters with antioxidants in different rice genotypes under control (C), submergence (7dS and 14dS) and re-aeration (RA)

	P _N				Fv/Fm				Chl			
	C	7dS	14dS	RA	C	7dS	14dS	RA	C	7dS	14dS	RA
SOD	– 0.215 ^{ns}	0.670**	0.774**	0.693**	0.195 ^{ns}	0.419**	0.509**	0.661**	0.222 ^{ns}	0.469**	0.572**	0.790**
CAT	– 0.235 ^{ns}	0.566**	0.646**	0.774**	– 0.120 ^{ns}	0.575**	0.648**	0.475**	– 0.246 ^{ns}	0.566**	0.696**	0.793**
GPX	– 0.036 ^{ns}	0.657**	0.765**	0.775**	– 0.133 ^{ns}	0.421**	0.609**	0.661**	– 0.135 ^{ns}	0.559**	0.613**	0.588**
APX	0.494*	0.937**	0.986**	0.976**	0.296*	0.862**	0.848**	0.857**	0.386*	0.967**	0.921**	0.934**
MDAR	0.219 ^{ns}	0.852**	0.930**	0.963**	– 0.130 ^{ns}	0.697**	0.779**	0.843**	0.199 ^{ns}	0.838**	0.902**	0.942**
DHAR	0.227 ^{ns}	0.878**	0.971**	0.988**	– 0.153 ^{ns}	0.785**	0.870**	0.927**	0.141 ^{ns}	0.896**	0.952**	0.985**
GR	0.255 ^{ns}	0.716**	0.797**	0.916**	0.248 ^{ns}	0.632**	0.979**	0.996**	0.083 ^{ns}	0.773**	0.907**	0.937**
ASC-T	– 0.120 ^{ns}	0.973**	0.954**	0.975**	– 0.103 ^{ns}	0.950**	0.978**	0.983**	0.130 ^{ns}	0.945**	0.978**	0.969**
ASC-R	– 0.151 ^{ns}	0.948**	0.939**	0.919**	– 0.208 ^{ns}	0.870**	0.969**	0.924**	0.118 ^{ns}	0.930**	0.976**	0.940**
ASC-O	0.030 ^{ns}	0.971**	0.947**	0.971**	– 0.015 ^{ns}	0.959**	0.968**	0.980**	0.038 ^{ns}	0.942**	0.966**	0.958**

Total degrees of freedom = 83, *significance at $P < 0.05$; **significance at $P < 0.01$

P_N, photosynthetic rate; Chl, chlorophyll; Fv/Fm, maximum quantum efficiency of PSII photochemistry; APX, ascorbate peroxidase; GPX, guaiacol peroxidase; CAT, catalase; SOD, superoxide dismutase; GR, glutathione reductase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; ASC-T, total ascorbate; ASC-O, oxidized ascorbate; ASC-R, reduced ascorbate

Cluster analysis

The cluster analysis based on the Bray–Curtis similarity index on the basis of different physiological parameters classified the landraces into two major clusters (Fig. 7). The rice landraces, such as *Samudrabali*, *Basnamundi* and *Gadaba* along with submergence tolerant check FR 13A were in one cluster having more than 95% similarity. The other two landraces such as *Surudaka* and *Dokarakuji* were present in one sub-cluster with more than 90% similarity with FR 13A. Further, submergence-susceptible cv. IR 42 was present in a separate cluster.

Discussion

Genetic resources are the raw material of any crop improvement program, which enables plant breeders to develop crop varieties that are more suitable for the requirements of different agricultural practices. Traditional rice landraces of Jeypore tract of Odisha have immense genetic potential for adaptation in long-term submergence environments (Patra and Dhua 2003; Roy et al. 2016). The studied landraces of Jeypore tract of Odisha possessing submergence tolerance property with varying potentiality were earlier identified after a rapid submergence tolerance screening (Barik and Panda 2016). In this study, we evaluated the detailed physiological performance of these landraces under submergence for proper characterization of rice lines in relation to submergence tolerance.

Fig. 7 Dendrogram showing the Bray-Curtis's similarity index between the rice genotypes based on physiological parameters under submergence

Submergence tolerance in rice is a complex trait influenced by the interaction between many traits and environmental conditions (Jackson and Ram 2003; Das et al. 2009). In this experiment, different rice landraces showed a distinctly different response to submergence in terms of survival and shoot elongation (Fig. S1, Fig. S2). Among the traditional rice landraces, *Samudrabali*, *Basnamundi* and *Gadaba* showed higher survival under submergence than that of the tolerant check FR 13A. Plant height increased due to submergence in all the genotypes but the height was significantly ($P < 0.05$) greater in traditional rice landraces compared to FR 13A and IR 42. Several reports have indicated that better growth and survival under stress conditions is a useful trait when selecting germplasm to improve grain yield (Sarkar et al. 2006; Sarkar and Bhattacharjee 2011). The rice genotype which shows limited elongation is better than the greater elongation under short-term flash flooding (Setter and Laureles 1996; Xu et al. 2006; Sarkar and Panda 2009). This result confirms that semi-dwarf *Sub1* cultivar (FR 13A) checked elongation growth under submergence may not be suitable for long-term flooding (Goswami et al. 2017; Kuanar et al. 2017; Afrin et al. 2018). In contrast, the present study showed that traditional landraces had more elongation growth along with survival under submergence. Here the ideal response of submergence tolerance (survival underwater) is together with the elongating ability an escape mechanism in the traditional landraces (Sarkar and Bhattacharjee 2011; Goswami et al. 2017). That is probably linked to adaptive changes in its energy metabolism (Yang et al. 2017). As the impact of climate change predicts the intensity of flash flooding is increasing in rainfed lowland and it often has a longer duration of 3–4 weeks. It is pivotal to develop varieties that can withstand more severe and prolonged submergence stress (Singh et al. 2017). Therefore, these traditional landraces with escape strategy may be beneficial for lowland rice growing area where the duration of submergence was more.

Leaf gas exchange is one of the earliest plant responses and highly sensitive to submergence (Panda et al. 2006). A significant decline in the photosynthetic rate (P_N) was recorded for all the studied genotypes under submergence and subsequent re-aeration compared to their respective control plants (Fig. 1a). This was as a result of damage in the photosynthetic apparatus due to the degradation of Chl and/or due to functional variations of the stomata and flood water parameters as has been reported earlier in rice (Panda et al. 2008; Yang et al. 2017). Further, the decrease of transpiration rate and stomatal conductance was observed in all genotypes under submergence. This was probably due to the structural and functional alteration of stomata under low light and limited gas diffusion conditions (Ella et al. 2003; Panda et al. 2008). In this study, a strong

linkage between the P_N and g_s was observed under submergence and it could be inferred that the reduction in the P_N was highly dependent on the stomatal functions and when these parameters are not correlated, the reduction in P_N could be due to non-stomatal traits (Siddique et al. 1999). The result interpreted that non-stomatal limitations such as alteration of pigments and PSII photochemistry are the major cause of inhibition of photosynthesis under submergence (Panda et al. 2006). In the present study, Chl content was remarkably declined under submergence and excess in susceptible variety IR 42. These results were consistent with earlier findings of Chl degradation in rice seedlings under submergence and may be due to oxidative damage (Ella et al. 2003; Sarkar et al. 2013; Yang et al. 2017). The ability to maintain Chl and photosynthetic activity under submergence is of major importance for stress tolerance (Panda et al. 2008). Based on the result, the traditional rice landraces such as *Samudrabali*, *Basnamundi* and *Gadaba* showed better photosynthetic activity than that of tolerant check variety (FR 13A) and showed a more adaptive response to submergence.

The leaf PSII activity is vital to understand the plant response under stress as it is highly sensitive to flooding and water-logging (Panda et al. 2006). To understand the PSII activity under submergence, various chlorophyll fluorescence parameters like F_o , F_m , F_v/F_m , $Y(II)$, qP and NPQ were studied in rice seedling (Murchie and Lawson 2013). In the present study, submergence alters the PSII activity in rice seedling, as reflected in the decrease in the values of F_o , F_m , F_v/F_m , and qP (Fig. 3a–c, e). It indicates the decreasing ability of PSII to reduce the primary acceptor Q_A under different abiotic stress (Hazrati et al. 2016). The decrease of photochemical activity under submergence and subsequent re-aeration may be due to more damage to the photosynthetic apparatus as has been reported earlier in rice (Panda et al. 2006, 2008). The photosynthetic quenching (qP) approximates the proportion of PSII reaction centers that represents the energy consumed in photosynthesis. On the other hand, NPQ is the amount of dissipated excessive irradiation into heat and represents, how effectively the photosynthetic organisms can dissipate excessive irradiation into heat (Pinnola et al. 2013). A gradual decline in qP and increase of NPQ in studied rice genotypes under different duration of submergence observed in the study, suggests that there was decreasing the quantum efficiency of PSII photochemistry either by causing a decrease in the rate of primary charge separation or by increase of heat dissipation under submergence (Fu et al. 2012; Hazrati et al. 2016). The increase of NPQ was more pronounced in susceptible cv. IR 42 under submergence and subsequent re-aeration because of more damage of photosynthetic apparatus leading to loss of photochemistry of PSII (Hazrati et al. 2016). Based on

these results, the studied traditional rice landraces and FR 13A maintained better PSII activity, continued underwater photosynthesis and were resilient to rainfed lowland conditions (Afrin et al. 2018).

Many stress situations including submergence induce generation of reactive oxygen species (ROS) and cause oxidative damage (Ella et al. 2003; Upadhyay 2016). Particularly, during re-aeration, the transition from anoxic to oxygenic environment results in production of ROS (Fukao et al. 2011). In the present study, when the rice seedlings are subjected to submergence, different ROS such as $O_2^{\bullet-}$, H_2O_2 , and OH^- level decreased and further increased during the period of re-aeration in all the genotypes. Particularly, the generation of ROS more pronounced in susceptible IR 42 cv. compared to the traditional landraces and FR 13A. This suggested that submergence prompted loss of redox homeostasis due to overproduction of ROS and is connected to a great extent with the plant susceptibility to submergence (Blokina et al. 2003; Yang et al. 2017). Lower accumulation of ROS in the traditional landraces indicates the less oxidative harm and preserves the redox status efficiently under submergence (Foyer and Noctor 2005). High concentration of ROS might have led to oxidative damage to lipids, forming TBARS as a consequence of cell membrane damage (Vergara et al. 2014). The levels of lipid peroxidation product TBARS gradually increased in all the rice genotypes with the progression of submergence and subsequent re-aeration (Fig. 3d). The increased TBARS content suggests that submergence and re-oxygenation damaged the cell membrane which disturbs metabolic processes and finally inhibits the growth as reported earlier in rice seedlings (Ella et al. 2003; Vergara et al. 2014). This effect was also evidenced in the present findings by the decrease in photosynthetic pigments and survival in rice seedlings under submergence and re-aeration.

To understand the antioxidant protective mechanism in traditional rice landraces under submergence and re-aeration, we measured the levels of the activities of some antioxidant enzymes (Fig. 4, 5). In general, it was observed that all antioxidant enzymes activities were decreased under submergence, however, after exposure to air, the activities of these enzymes were increased only in traditional landraces and tolerant cv. (FR 13A). This suggests that there are mechanisms that control the level of antioxidative enzymes in response to the decrease in the oxygen tension in rice and other crops (Ahmed et al. 2002; Panda and Sarkar 2012). The possible cause of the reduction of these enzymes may be due to the decrease of ROS level under submergence. The susceptible check variety IR 42 was affected severely under submergence and re-aeration period because of less activity of the studied antioxidant enzymes.

The role of the ascorbate–glutathione cycle in the ROS scavenging in plant cells has been well established under different environmental stress including submergence (Foyer and Noctor 2005). The activities of the ascorbate–glutathione cycle enzymes such as APX, MDHAR, DHAR and GR were significantly declined under submergence in all genotypes, however greater maintenance of activity was observed in traditional landraces and tolerant check (FR 13A) compared to susceptible cultivar (IR 42) (Fig. 5). These results highlight the submergence tolerant properties of traditional landraces and were consistent with the earlier observation in different rice landraces (Ella et al. 2003; Damanik et al. 2010; Panda and Sarkar 2012).

Ascorbate is a powerful antioxidant and it gives security to the cell organelles and biomolecules from oxidative damage by specifically scavenging $O_2^{\bullet-}$ and OH^- (Gill and Tuteja 2010). In our investigations, higher content of total ascorbate along with their reduced and oxidized form was noticed under submergence in the traditional landraces, which was however contrasted with the susceptible variety. Ascorbic acid interacts with scavenging ROS in both PSII and PSI sides through xanthophyll cycle and ascorbate–glutathione cycle (Panda and Sarkar 2012). Therefore, decrease in antioxidant enzymes and ascorbate content during submergence and their slow recovery after submergence in the susceptible cultivar cause more oxidative damage to the susceptible cultivars. Based on the results, the three landraces *Samudrabali*, *Basnamundi* and *Gadaba* demonstrated an effective enzymatic reaction than that of tolerant check variety (FR 13A) and have better protection against oxidative damage under submergence. The study also showed that the relationship of antioxidant enzyme activities with photosynthetic parameters was not significant under control condition. However, antioxidant enzymes showed positive significant correlation with P_N , chlorophyll and Fv/Fm under different duration of submergence and subsequent re-aeration (Table 3). Among the antioxidants, enzymes of the ascorbate–glutathione cycle (APX, MDAR, DHAR and GR) and ascorbate level showed higher correlation value with photosynthetic parameters which indicate that the level of ascorbate and enzymes of the ascorbate–glutathione cycle effectively protect the photosynthetic system from oxidative damage and might help for maintenance of underwater photosynthesis in rice.

Characterizing genotypes for submergence tolerance among traditional rice landraces were carried out by cluster analysis using different physiological parameters under submergence and further, these parameters were compared with tolerant and susceptible check varieties. Cluster analysis showed that three landraces such as *Samudrabali*, *Basnamundi* and *Gadaba* are present in one cluster and showed physiologically more nearness with tolerant check

variety (FR 13A) and clearly separated from the susceptible genotypes (IR 42). Submergence tolerance properties in these genotypes are also attributed to the presence of *Sub1A* and *Sub1C* loci along with some unique alleles and potential sources of new submergence tolerance QTLs (Barik and panda 2016). Based on the present findings, it is revealed that these traditional rice lines are expected to possess an adequate level of tolerance to submergence and showed better adaptive fitness than that of FR 13A.

Conclusion

Traditional landraces such as *Samudrabali*, *Basnamundi* and *Gadaba* showed better survival and plant growth under submergence compared to the tolerant check (FR 13A) cultivar. The submergence tolerance adaptation of these landraces seems to be associated with maintenance of better photosynthesis, PSII activity and antioxidant defense under submergence and the subsequent period of re-aeration. In addition, these landraces maintained greater ascorbate level and enzymes of the ascorbate–glutathione cycle under submergence and it might help them for better protection from oxidative damage. Further research is aimed to elucidate the genetic diversity in relation to these physiological traits and the use of these landraces for future submergence tolerance breeding program.

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Compliance with ethical standards

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

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