

# Modulation of Antioxidant Defense System and Polyamine Catabolism in Rice Leaves under Two Planting Conditions

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(Received 6 May 2016; Accepted 7 September 2016;  
Communicated by A. Pécsváradí)

Aerobic rice offers an attractive alternative approach over transplanting system as it consumes less water with low labour expenses. Flag leaf of six rice cultivars, viz. PR 120, PR 115, PR 116, Feng Ai Zan, PAU 201 and Punjab Mehak 1 was analysed for antioxidant defence mechanism and polyamine catabolism under the aerobic and the transplanting conditions. Ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), diamine oxidase (DAO) and polyamine oxidase (PAO) activities increased gradually from tillering to anthesis stage and then declined towards maturity stage under both planting conditions. Apparently, contents of ascorbic acid,  $\alpha$ -tocopherol, proline and polyamines (PAs) also revealed similar trend. The aerobic condition elevated activities of PAO, SOD as well as contents of PAs, lipid peroxide and  $H_2O_2$  whereas the transplanting condition had higher levels of APX, GPX, CAT and total antioxidant activities and contents of ascorbate,  $\alpha$ -tocopherol and proline. Cultivars Feng Ai Zan, PR 115 and PR 120 exhibited superior tolerance over other cultivars by accumulating higher contents of PAs with increasing levels of PAO and SOD activities under the aerobic condition. However, under the transplanting condition PR 116 and PAU 201 showed higher activities of antioxidative enzymes with decreasing contents of lipid peroxide and  $H_2O_2$ . We infer that under the aerobic condition, enhancement of PAs and PAO activity enabled rice cultivars to tolerate oxidative stress, while under the transplanting condition, antioxidative defence system with decreasing of lipid peroxide content was closely associated with the protection of flag leaf by maintaining membrane integrity. In crux, results indicated that  $H_2O_2$  metabolic machinery was strongly up-regulated especially at the anthesis stage.

**Keywords:** antioxidants, aerobic rice, polyamines, rice, transplanted conditions

**Abbreviations:** APX, ascorbate peroxidase; CAT, catalase; DAO, diamine oxidase; GPX, guaiacol peroxidase; PAO, polyamine oxidase; PAs, polyamines; Put, putrescine; ROS, reactive oxygen species; Spd, spermidine; Spm, spermine; SOD, superoxide dismutase

## Introduction

Rice is a semi-aquatic crop that requires eminent amount of water for its proper growth and development. Rice system consumes 66% of irrigation water which resulted in sharp decline in underground water during the last two decades. Alternatively, aerobic rice cul-

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tivation was developed worldwide to reduce water consumption and produce rice with less water (Bernier et al. 2008; Qin et al. 2010). However, this ended up in causing lower plant growth rate, poor grain establishment and hence, less yield. Information regarding effect of antioxidant defence system in relation to polyamine metabolism in rice cultivars raised under transplanted and aerobic conditions is still scanty. Therefore, to have a comparative knowledge about this effect various antioxidative enzymes and osmolyte accumulation were related to polyamine metabolism in the present study. Moreover, the development of high yielding rice genotypes under aerobic conditions have great potential to save water.

Aerobic rice production system refers to the process of establishing a crop from seeds directly without up-holding of water in the field in contrast to transplanting method (Bouman et al. 2007; Parthasarathi et al. 2012). Apparently, rice raised under aerobic condition develops deeper and thickened root system having large xylem vessels to extract water from the soil compared to traditional planting condition (Ahmadi et al. 2007). Under aerobic condition, farmer can skip irrigation if soil moisture status is sufficient for crop even though plant undergoes stress due to less availability of water as compared to traditional methods. Aerobic rice can respond and adapt to water stress by altering their cellular metabolism and evolving various defence mechanisms (Basu et al. 2010). Thus, aerobic genotypes with better antioxidant defence system potentially contribute to water-saving rice cultivation under water-deficit scenarios. Over-production of reactive oxygen species (ROS) due to stress conditions above constitutive level is potentially harmful to all the cellular components as it increases lipid peroxidation, protein and DNA damage (Breusegem et al. 2001; Esfandiari et al. 2007). Though the detoxification of ROS is consequently of prime importance in any defence mechanism but the information regarding the source of ROS is inadequate.

Polyamines (PAs) such as putrescine (Put), spermidine (Spd) and spermine (Spm) act as endogenous plant growth regulators that play an integral role under different environmental stress conditions (Torrighiani et al. 2012). PAs exists as free molecular bases or conjugated with phenolic acids or are often bound to DNA and proteins (Groppa and Benavides 2008). PAs stabilises biological membranes and cellular structures by directly binding to membrane phospholipids, direct scavenging of free radicals, osmotic adjustment, maintaining a cation-anion balance and binding to the antioxidant enzymes and elevating their activities (Roychoudhury et al. 2011). Alteration in PA content and catabolism have been shown in plants under stressful condition (Goyal and Asthir 2010). Diamine oxidase (DAO) and polyamine oxidase (PAO) produces  $H_2O_2$  via catabolism of PAs that is considered as a causative element for apoptotic cell death in plants (Kuehn and Phillips 2005). Likewise,  $H_2O_2$  signalling process in the upstream direction encompasses nitric oxide biosynthesis in response to certain stimuli (Wang et al. 2010). Consequently, elucidation of the occurrence and impact of the interplay among PAs and signalling molecules in stress response is vital for future research.

The detrimental effect of ROS is combated by enzymatic system which include ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD) (Noctor and Foyer 1998). The enzyme SOD converts  $O_2^-$  to  $H_2O_2$  whereas

CAT and peroxidases catabolises  $H_2O_2$  into  $H_2O$  and  $O_2$ . Guaiacol peroxidase requires a phenolic compound guaiacol as electron donor to decompose  $H_2O_2$  while APX uses reduced form of ascorbate to protect cells against damaging effects of  $H_2O_2$ . Role of PAs could be exerted directly or via acting as scavengers of ROS (Zhang et al. 2009). PAs also enhances the expression of genes encoding antioxidant enzymes, and thus increases overall pool size of antioxidants (Wang et al. 2010). However, the key question is whether PAs can regulate expression of antioxidant enzymes to ameliorate the oxidative damage caused by free radicals generated during degradation of PAs by amine oxidases (DAO, PAO). Non-enzymatic antioxidants such as ascorbate, glutathione, tocopherols and carotenoids together have been found to play an important role in the development of tolerance against stress conditions (Jaleel et al. 2009). Ascorbic acid scavenges most dangerous form of ROS, i.e.  $OH^-$ ,  $O_2^-$ ,  $H_2O_2$  through the action of APX and regenerates the  $\alpha$ -tocopherol oxidized by ROS. Accumulation of proline is a widespread plant response to environmental stresses in plants (Chakraborty and Tongden 2005). Proline as an osmolyte, a ROS scavenger and a molecular chaperone, accumulate in rice tissues where it helps in promoting water retention and alleviating the negative effect of drought stress (Mostajeran and Rahimi-Eichi 2009; Goyal and Asthir 2010; Szabados and Savoure 2010). Measurement of antioxidants and osmolyte in response to aerobic (less water availability) condition may provide valuable information on the various strategies of the plant intended to remove ROS.

The pre-requisite for a successful breeding program for aerobic cultivation is the availability of tolerant cultivars. In spite of several studies carried out on aerobic rice system, work regarding the comparative role of antioxidant defense system and polyamine metabolism at different developmental stages is lacking. The present investigation was aimed to study modulation of antioxidative defense mechanism in conjunction with polyamine catabolism in six rice cultivars.

## Materials and Methods

### *Plant material and sampling procedures*

Field experiment was conducted at the Punjab Agricultural University, Ludhiana, Punjab, India in two cropping systems, i.e. aerobic and transplanting conditions for two consecutive years (29<sup>th</sup> May 2010 and 2<sup>nd</sup> June 2011). The soil type of the experimental plot was loamy. Seeds of six rice cultivars PR 120, PR 115, PR 116, Feng Ai Zan, PAU 201 and Punjab Mehak 1 were obtained from Department of Plant Breeding and Genetics, PAU Ludhiana. One set of these seedlings was raised in the field (random block design) under transplanted condition (puddled soil followed by alternate wetting and drying and flood irrigation) and other set under aerobic condition in 1 m × 1 m plot area. Under normal transplanting conditions, field was ponded for the first 15 days and subsequently flood irrigated for two days after the water infiltrates in the soil until two weeks before harvesting. The aerobic condition was maintained by applying irrigation when soil moisture reaches -15 kPa at 15 cm depth. During reproductive stage the irrigation was applied

between  $-10$  kPa soil moisture tension. All enzymatic and non-enzymatic assays were performed on flag leaf at tillering, anthesis and post-anthesis stages, i.e. 7, 15 and 30 days post anthesis (DPA) of plants grown under the aerobic and the transplanting conditions in triplicates.

#### *DAO and PAO enzyme assays*

Leaf tissue (0.5 g) was extracted in 100 mM K-phosphate buffer (pH 6.5) containing 5 mM dithiothreitol and the extract was centrifuged at  $16,000 \times g$  for 20 min at  $4^\circ\text{C}$ . The supernatant was used as source of enzyme. DAO (EC 1.4.3.6) and PAO (EC 1.5.3.11) activities were assayed as described by Asthir et al. (2002) using Put (for DAO) and Spd (for PAO) as substrates. Activities are expressed as nmol (D-pyrroline)  $\text{min}^{-1} \text{g}^{-1}$  FW for DAO and PAO.

#### *Antioxidant enzyme assays*

Leaf samples (0.5 g fresh mass) were homogenized in 50 mM Na-phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone. Ascorbate peroxidase (APX, EC 1.11.1.11) was extracted using same extraction buffer with or without 0.5 mM ascorbate. The homogenate was filtered through four layers of cheese cloth and then centrifuged at  $4^\circ\text{C}$  for 20 min at  $15,000 \times g$ . All operations of enzyme extraction were performed at  $0-4^\circ\text{C}$  and the enzyme assays were carried out at room temperature ( $25 \pm 1^\circ\text{C}$ ) unless otherwise stated.

Superoxide dismutase (SOD, EC1.15.1.1) activity was assayed following the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) as described by Becana et al. (1986). One unit of SOD was defined as the amount of enzyme that produced a 50% inhibition of NBT reduction under assay conditions. Catalase (CAT, EC 1.11.1.6) activity was measured following the decomposition of  $\text{H}_2\text{O}_2$  at 240 nm ( $\epsilon = 39.4 \text{ mM/cm}$ ) and one CAT unit was defined as the enzyme amount that decomposes  $\mu\text{mol H}_2\text{O}_2 \text{ min}^{-1} \text{mg}^{-1}$  protein (Chance and Maehly 1955). Guaiacol peroxidase (GPX, EC 1.11.1.7) activity was assayed by measuring the increase in absorbance at 470 nm,  $\epsilon = 26.6/(\text{mM cm})$  due to guaiacol oxidation and specific activity was expressed as  $\mu\text{mol tetraguaiacol formed min}^{-1} \text{mg}^{-1}$  protein (Chance and Maehly 1955). Ascorbate peroxidase (APX) activity was determined by measuring the decrease in absorbance at 280 nm due to ascorbate oxidation,  $\epsilon = 2.8/(\text{mM cm})$  and the enzyme activity was expressed as  $\mu\text{mol ascorbate oxidized min}^{-1} \text{mg}^{-1}$  protein (Nakano and Asada 1981). Total antioxidant activity (TAA) was estimated according to the method of Prieto et al. (1999) and the activity was expressed as mM ascorbic acid equivalent (AAE)  $\text{g}^{-1}$  FW.

#### *Metabolite analyses*

$\text{H}_2\text{O}_2$  content was estimated according to the method of Sergiev et al. (1997) and was expressed as  $\mu\text{mol H}_2\text{O}_2 \text{ g}^{-1}$  FW. The lipid peroxidation products were determined from

the thiobarbituric acid reactive substances (TBARs) contents resulting from the thiobarbituric acid and the content of TBARs was calculated with  $\epsilon = 155/(\text{mM cm})$  (Larkindale and Knight (2002). Total ascorbate content was measured according to the method of Asthir et al. (2010). Tocopherol extraction involved xylene that reduces ferric ions to ferrous ions to form red colour with  $\alpha, \alpha$ -dipyridyl which can be measured at 520 nm as described by Asthir et al. (2010). Proline was estimated according to the method of Bates et al. (1973). The polyamines (PAs) concentration was measured as described by Dhillon-Grewal et al. (1992).

### *Statistical analysis*

Results were based on at least three replicates. The values were statistically analysed by multifactor ANOVA (CPCSI). Values are presented as a means  $\pm$  SD ( $n = 3$ ). Values were recorded in triplicates for analysing  $P \leq 0.05$  as the level of significance.

## **Results**

Six rice cultivars, viz. PR 120, PR 115, PR 116, Feng Ai Zan, PAU 201 and Punjab Mehak 1 were raised under two different planting conditions, i.e. aerobic and transplanting for studying various biochemical parameters.

### *Determination of the activities of DAO and PAO*

Diamine oxidase (DAO) and polyamine oxidase (PAO) activities were significantly high at initial stages of leaf development and thereafter declined towards maturity in all six cultivars under the transplanting and the aerobic conditions (Fig. S1\*). Interestingly, DAO activity was comparatively low and PAO was significantly high under the aerobic condition in rice leaves. Irrespective of the stage of plant growth, highest activity of DAO and PAO was recorded in Feng Ai Zan followed by PR 115 and PR 120 under the aerobic and in Punjab Mehak 1, PR 116 under the transplanting conditions.

### *Determination of polyamines levels*

Free polyamine especially putrescine (Put) predominated and increased in comparison to spermidine (Spd) and spermine (Spm) in leaves of six cultivars raised under aerobic conditions (Fig. S2). Put, Spd and Spm contents increased significantly under the aerobic condition. Highest content of Put followed by Spd and Spm was found at 7 DPA stage. Interestingly, Spd and Spm contents were found to increase towards leaf maturity. Irrespective of the stage of plant growth highest content of Put was recorded in Feng Ai Zan, PR 115, PR 120 under the aerobic and in Punjab Mehak 1, PR 116, PAU 201 under the transplanting conditions.

\*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

### *Determination of the activities of antioxidative enzymes*

Activities of antioxidative enzymes, viz. ascorbate peroxidase (APX), guaiacol peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) steadily increased from tillering to initial stage of grain filling (7 DPA) and thereafter decreased towards leaf maturity (Fig. S3). However, aerobic condition maintained low levels of all studied enzymes except SOD. Cultivars Punjab Mehak 1, PR 116 and PAU 201 showed higher activities of APX, CAT and GPX at tillering and at 7 DPA stages under transplanting condition and in Feng Ai Zan, PR 115 and PR 120 under aerobic condition. Interestingly, activity of SOD was highest under aerobic condition.

### *Analyses of H<sub>2</sub>O<sub>2</sub>, lipid peroxide levels and total antioxidant activity*

Aerobic conditions led to marked increase in H<sub>2</sub>O<sub>2</sub> and lipid peroxide contents in flag leaf of all six cultivars and the contents of these metabolites increased continuously from initial stage of leaf development up to maturity (Fig. S4). Maximum H<sub>2</sub>O<sub>2</sub> and lipid peroxide contents were found in Punjab Mehak 1, PR 116 and PAU 201 cultivars under the aerobic condition and in Feng Ai Zan, PR 115 and PR 120 under the transplanting condition. Total antioxidant activity increased up to 7 DPA stage in all the cultivars and then declined towards leaf maturity under both planting conditions.

### *Analyses of antioxidants (ascorbate, tocopherol) and proline*

Ascorbic acid,  $\alpha$ -tocopherol and proline contents first increased in flag leaf up to 7 DPA stage and then declined towards leaf maturity in all six cultivars raised under the aerobic and the transplanting conditions (Fig. S4). The concentration of ascorbic acid and  $\alpha$ -tocopherol significantly decreased under aerobic condition in all the cultivars. However, minimum decline in content of these metabolites was recorded in Feng Ai Zan, PR 115 and PR 120.

## **Discussion**

With the predicted climatic changes and global warming, water shortage for the rice crop will increase in a near future (Parthasarathi et al. 2012). Consequently, cultivation of aerobic rice that consumes less water over transplanting ones is an alternate approach to farmers. However, aerobic rice undergoes oxidative stress due to less availability of water which leads to increased production of H<sub>2</sub>O<sub>2</sub> and lipid peroxide contents (Liang et al. 2003). Activities of amine oxidases (DAO, PAO) and antioxidant enzymes (APX, GPX, SOD, CAT) are up-regulated under stress that work co-ordinately (Almeselmani et al. 2006). Apparently, polyamines (PAs) play significant role in stabilization of biological membrane and cellular structures by directly binding to membrane phospholipids, direct scavenging of free radicals, osmotic adjustment, maintaining a cation-anion balance, modulating ion channels and binding to the antioxidant enzymes and elevating their lev-



els (Liu et al. 2004). However, it remains unclear how PAs modulate antioxidant defence system in rice raised under the aerobic and the transplanting conditions. To address this question, six rice cultivars, viz. PR 120, PR 115, PR 116, Feng Ai Zan, PAU 201 and Punjab Mehak 1 were raised in the field to study their inbuilt tolerance response under two planting conditions.

In flag leaf, both DAO and PAO activities got dramatically enhanced at anthesis stage in conjunction with higher content of  $H_2O_2$  in all six cultivars under both planting conditions. However, PAO activity was increased more under the aerobic condition while DAO activity predominated under the transplanting condition. The strong upregulation of PAO under the aerobic condition resulted in higher generation of  $H_2O_2$  that bears some correlation with peroxidases mediated lignification in strengthening of cell walls of leaf as reported earlier in wheat (Asthir et al. 2010). DAO activity was more pronounced in PR 116 and PAU 201 under the transplanting condition while PAO predominated in PR 120 and Feng Ai Zan under the aerobic condition. Both DAO and PAO enzymes are responsible in generation of  $H_2O_2$  that acts as a substrate for APX, GPX and CAT activities. In aerobic rice, PAO and SOD activities were higher in comparison with other studied enzymes leading to higher generation of  $H_2O_2$  and thereby oxidative stress. SOD plays a key role in protecting cells against stress since it dismutate toxic  $O_2^-$  radicals to less toxic  $H_2O_2$  molecule. Upregulation of SOD activity under the aerobic condition was in line with the findings of Qin et al. (2010) in rice indicating involvement of  $H_2O_2$  signalling process in stress tolerance. Though  $H_2O_2$  is considered as a cytotoxic molecule but its involvement as an inducer during aerobic stress is important.

Higher activity of PAO under aerobic condition coincided with the maximum contents of Spd and Spm (Cona et al. 2006) while transplanting condition had predominance of DAO activity along with Put content which clearly indicated that enzyme activity is not limited by substrate supply. The higher concentration of Spd and Spm coincided with the maximum activities of arginine decarboxylase, S-adenosylmethionine decarboxylase and Spd synthase during grain filling stages of rice (Yang et al. 2008). In general, when cellular PA contents are increased, their catabolism also increased which leads to the enhancement of ROS especially  $H_2O_2$ , hence their role in preventing the cellular damage is customary (Gill and Tuteja 2010). In fact, PAs may also be involved in activating APX, GPX and SOD activities thereby elevating antioxidant levels (ascorbate,  $\alpha$ -tocopherol), to diminish free radical generation and thereby prevent membrane collapse (Yang et al. 2007; Zhang et al. 2009; Sudhakar et al. 2015). Although, PAs enhances the expression of antioxidant enzymes at the translational level (Jaleel et al. 2009), higher ability of tolerant plants to upregulate antioxidative genes reflect the involvement of reduced glutathione content mediated by glutathione reductase activity (Noctor and Foyer 1998). The levels of ascorbate was increased more in transplanted rice to regenerate  $\alpha$ -tocopherol which provides synergistic protection of the membranes (Kanwischer et al. 2005). Cultivars Feng Ai Zan, PR 115 and PR 120 are critically efficient in eradicating free radicals due to higher scavenging ability of endogenous PAs. We infer that stress-tolerant cultivars revealed higher PA titers than sensitive ones similar to the observations of Alcazar et al. (2010) and Moschou et al. (2008).

Similar to amine oxidases, activities of APX, GPX, CAT and SOD also revealed increasing trend from tillering to 7 DPA stage. However, these activities declined with leaf maturity in correspondence with PAs contents. Cultivars raised under transplanting condition had better ability of scavenging ROS by producing higher levels of antioxidative enzymes, antioxidants, and thereby maintaining membrane integrity. In comparison, aerobic condition experienced more oxidative stress due to higher accumulation of  $H_2O_2$  generated by PAO and SOD activities. In fact  $H_2O_2$  production is a highly regulated and orchestrated process in which several enzymes APX, GPX, CAT along with DAO, PAO and SOD are involved, which metabolise  $H_2O_2$ . Consequently,  $H_2O_2$  has dual role in stimulating or deactivating the synthesis of antioxidative enzymes (Basu et al. 2010), and thus plays a major role under the stress conditions. Likewise, lower content of  $H_2O_2$  in cultivars Feng Ai Zan and PR 120 corresponded well with increased activities of antioxidant system under aerobic condition clearly support leaf development by overcoming stress. Low levels of  $H_2O_2$  and lipid peroxide contents were also observed in water stressed wheat seedlings (Chakraborty and Pradhan 2012). Total antioxidant activity (TAA) which outlines overall capacity of antioxidant defence response also revealed higher activity in PAU 201, Punjab Mehak 1 and PR 116 under the transplanting condition reflecting amelioration of oxidative stress (Liang et al. 2003). This encompasses several other factors also comprising accumulation of low molecular weight osmolytes particularly proline which contributes to osmotic adjustment at the cellular level (Qin et al. 2010).

Lipid peroxidation as reflected by MDA content, is related to levels of antioxidant activity. The induced antioxidative defence mechanism under the transplanting condition lowers MDA concentration and thus provide protection against ROS. However, under the aerobic condition, antioxidant defence system failed to combat oxidative stress leading to higher accumulation of MDA content. Similar to our findings, Nagesh and Devaraj (2008) also reported increased levels of  $H_2O_2$  and lipid peroxide contents under salinity condition. In parallel to antioxidant enzymes, the contents of ascorbate,  $\alpha$ -tocopherol, proline and total antioxidant activity also increased at anthesis stage and decreased thereafter under both planting conditions. Higher contents of these metabolites in PR 116 and PAU 201 under the transplanting condition indicated their higher stress tolerance mechanism in terms of radical scavenging that maintain homeostasis (Munne-Bosch and Falk 2004).

Overall, the aerobic condition elevated the activities of PAO, SOD as well as contents of PAs, lipid peroxide and  $H_2O_2$  whereas the transplanting condition had elevated levels of APX, GPX, CAT, total antioxidant activity and contents of ascorbate,  $\alpha$ -tocopherol and proline. Cultivars Feng Ai Zan and PR 120 were more efficient due to higher PAs metabolism while PR 116 and PAU 201 had higher antioxidant defence system based on less peroxide and  $H_2O_2$  contents. Therefore, we infer that under the aerobic condition, enhancements of PAs contents and PAO activity enabled rice cultivars to tolerate oxidative stress, while under the transplanting condition, antioxidative defence system with decreasing content of lipid peroxide was responsible for enhancing membrane stability in flag leaf. In crux, results indicated that  $H_2O_2$  metabolic machinery was strongly up-regulated especially at anthesis stage of plant. Future studies will explore new possibility of



finding key regulatory steps underlying the enhanced tolerance during stress via enzymes and metabolites at molecular level.

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### Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at <http://www.akademai.com/content/120427/>

Electronic Supplementary *Figure S1*. Activities of DAO (A) and PAO (B) at tillering (T), anthesis (A), and 7, 15 and 30 days post anthesis stages in flag leaf of six rice cultivars raised under aerobic and transplanted conditions. A, Cultivars; B, Conditions; C, Stages. CD (5%): DAO – A 3.28, B 5.41, C 3.57, AB 1.48, AC 1.65, BC 1.99; PAO – A 4.58, B 6.91, C 4.07, AB 1.48, AC 1.65, BC 1.89.

Electronic Supplementary *Figure S2*. Contents of Put (A), Spd (B) and Spm (C) at tillering (T), anthesis (A), and 7, 15 and 30 days post anthesis stages in flag leaf of six rice cultivars raised under aerobic and transplanted conditions. A, Cultivars; B, Conditions; C, Stages. CD (5%): Put – A 0.50, B 0.92, C 0.63, AB 0.18, AC 0.35, BC 0.49; Spd – A 0.68, B 0.71, C 0.47, AB 0.18, AC 0.45, BC 0.59; Spm – A 0.58, B 0.91, C 0.67, AB 0.14, AC 0.25, BC 0.42.

Electronic Supplementary *Figure S3*. Activities of APX (A), GPX (B), SOD (C) and CAT (D) at tillering (T), anthesis (A), and 7, 15 and 30 days post anthesis stages in flag leaf of six rice cultivars raised under aerobic and transplanted conditions. A, Cultivars; B, Conditions; C, Stages. CD (5%): APX – A 0.51, B 0.61, C 0.79, AB 0.28, AC 0.45, BC 0.24; GPX – A 0.18, B 0.28, C 0.27, AB 0.38, AC 0.26, BC 0.49; SOD – A 0.16, B 0.09, C 0.07, AB 0.08, AC 0.15, BC 0.09; CAT – A 0.18, B 0.41, C 0.17, AB 0.28, AC 0.35, BC 0.29.

Electronic Supplementary *Figure S4*. H<sub>2</sub>O<sub>2</sub> (A), LPX (B), Ascorbate (C),  $\alpha$ -tocopherol (D), TAA (E) and proline (F) at tillering (T), anthesis (A), and 7, 15 and 30 days post anthesis stages in flag leaf of six rice cultivars raised under aerobic and transplanted conditions. A, Cultivars; B, Conditions; C, Stages. CD (5%): H<sub>2</sub>O<sub>2</sub> – A 0.78, B 0.45, C 0.57, AB 1.18, AC 1.35, BC 0.91; LPX – A 2.98, B 2.01, C 2.27, AB 4.28, AC 5.35, BC 3.29; Ascorbate – A 0.68, B 0.71, C 0.27, AB 0.428, AC 0.55, BC 0.69;  $\alpha$ -tocopherol – A 0.18, B 0.91, C 0.47, AB 0.38, AC 0.25, BC 0.19; TAA – A 0.18, B 0.41, C 0.17, AB 0.28, AC 0.35, BC 0.29; Proline – A 2.48, B 1.1, C 1.17, AB 1.28, AC 2.35, BC 1.29.