ORIGINAL RESEARCH

Variant biochemical responses: intrinsic and adaptive system for ecologically different rice varieties

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Abstract

India has a diverse range of agro-ecological conditions which support the cultivation of different rice varieties differing in the adaptation which is so important for sustainable development of rice crop. Specific ecotypes of rice adapted to diverse conditions have divergence in their morphology, physiology, biochemistry, molecular function, agronomy, and stress response. In the present study, 12 different rice varieties viz., PB-1, PB-1509, Pusa-RH-10, CSR-30, HKR-47, PR-126, Govind, Sharbati, ADT-37, ADT-39, ADT-45, White Ponni, were selected for the study of intrinsic biochemical behaviour and these varieties belong to different Agro-ecological zones and basmati or non-basmati rice varieties. Amongst intrinsic biochemicals activity, the differential response of radical scavenging, superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POX) activities, were observed in the selected rice varieties at 14 days old seedling stage, developed under controlled growth conditions. Comparatively, North India region rice varieties displayed an enhanced intrinsic biochemical response than south India region rice varieties. Similarly, basmati rice varieties showed increased biochemical response compared to non-basmati rice varieties. Thus, the differential biochemical responses (radical scavenging, SOD, CAT, and POX activities) observed creates a significant difference between rice varieties and provides valuable information about rice ecotype-biochemical interaction for sustainable adaptive value under different ecological conditions.

Keywords Rice · Agro-ecological zone · Basmati or non-basmati · Antioxidant enzyme

Introduction

India is known to have a diverse range of agro ecological zones for widespread rice cultivation and different types of ecosystems are available for rice farming within the country. Amongst different rice ecosystems, rainfed uplands occupy a major area (37%) followed by rainfed lowlands (34%), flood-prone (26%) and irrigated areas (24%) (Prasad et al. 2001). Rice cultivation in India also extends from 8° to 35° N latitude and from sea level to as high as 3000 m with different altitude and climate conditions (https://farmer.gov.in). Accordingly, five major regions are broadly classified within India that are North-Eastern, Eastern, Northern, Western, and Southern Regions which are categorized based on agro-ecological zone and climatic conditions (Tripathi et al. 2011). Due to diverse ecological conditions, rice farming not only varies greatly on location to location within India but also varies from country to country. Generally, rice crop prefers hot and humid environment while, excessive humidity, extended sunshine and proper water supply are best for rice cultivations. Also, seasons variations are very important factor in India and thus, temperature, soil types, rainfall condition, water availability, intensity of light and moisture content in the atmosphere are most influencing parameters for rice farming.

Rice belongs to Gramineae (Poaceae) family of the *Oryza* genus which comprises 25 recognized species. Out of 25 species, 23 species belong to wild species while *O. sativa* and *O. glaberrima* are most cultivated or domesticated species. According to nine distinct genome classification (A, B, C, D, E, F, G, H and J) for various rice species, the genus *Oryza* is grouped into distinct species complexes (Vaughan et al. 2003). The *O. sativa* complex belongs to "A" genome and comprises two domesticated species (*O. sativa* and *O. glaberrima*) and six wild species (*O. rufpogon*, *O. nivara*,

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O. barthii, O. *longistaminata*, O. *meridionalis* and *O. glumaepatula*) (Tripathi et al. 2011; Nadir et al. 2018). *Oryza sativa* is known as Asian rice and due to its widespread cultivation, two different ecological groups have developed namely; subspecies *Indica* and subspecies *japonica* (Oka 1988). *Indica* rice is preferably cultivated in tropical and subtropical environments at lower latitudes or altitudes, whereas more temperate environments at higher latitudes or altitudes are specified for *Japonica* rice farming (Yang et al. 2014; Nadir et al. 2018). Under different ecological conditions, both the subspecies of *Oryza sativa* exhibit great diversification in their morphological, physiological, biochemical, genome sequence, genetic relationship and agronomic traits and also display variation in their yield, quality, and stress-resistant responses (Kawakami et al. 2007; Lu et al. 2009; Hu et al. 2014; Kusano et al. 2015).

Rice crop growth and development under various agro-ecological conditions shows considerable variation at their morphological, physiological, and molecular levels and these variations are associated with adaptability to a different environmenst and climatic zones (Glaszmann 1987; Oka 1988; Wang et al. 1992). Therefore, ecological, and evolutionary pressures are continuously customizing adaptability in natural populations (Romero 2004; Korte et al. 2005). Moreover, the effect of genotype, environment and mixed performance of genotype and environment interaction influence the phenotypic performance of the species that represent their general and specific adaptability to different environmental conditions (Balakrishnan et al. 2016). Conversely, any influential conditions that transform plant growth performance depends upon the altering of physiological and metabolic processes through changes in the various enzymatic, biochemical, and molecular phenomenon. Importantly, the generation of reactive oxygen species and their counter action by antioxidant enzymes regulate the metabolic and oxidative homeostasis of plant cells (Hussain et al. 2018). Because antioxidant enzyme plays a vital role in regulating physiological and metabolic changes and their differentiating response with their different levels of amount reveals a significant difference between plant species. Moreover, the study of complex environmental pressures on plants provides a specified level of information for analyzing the impact of any constraint during the growth period and improves our understanding of their effect and sustainable development.

In the present study, the 12 distinct rice varieties belonging to a different agro-ecological zones were used to study the variation in their biochemical response during their growth and development at 14th day old seedlings. The generation of free radical species and their counter action by antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX)] were examined under controlled conditions amongst 12 different rice varieties. The

present study provides information about differences in their intrinsic biochemical response in the selected rice varieties beside of their distinct morphology, physiology, and ecology under normal culture conditions.

Materials and methods

Plant material collection and culturing

The different agro-ecological zones based twelve rice varieties namely; PB 1, PB 1509, Pusa RH 10, CSR 30, HKR 47, PR 126, Govind, Sharbati, ADT-37, ADT-39, ADT-45 and White Ponni, were selected for the present study. These varieties belong to different climatic conditions and their farming is very commonly done in north and south regions of India (Table 1). For the experiment, seeds were dehusked manually followed by rinsing several times with distilled water. Surface sterilization was performed with 4% sodium hypochlorite (NaClO) solution for 20 min followed by 3 times washing with distilled water to remove traces of disinfectant (Haq et al. 2019). Further, the seeds were aseptically surface sterilized with mercuric chloride (0.1% w/v) solution (Merck, India) for 3 min then washed with distilled water for 3–4 times. Sterilized seeds from 12 different varieties were cultured on the water-agar environment for 14 days in triplicates under controlled conditions in the culture chamber.

Free radical scavenging activity

The methanolic extracts of 14 days old seedlings of 12 rice varieties were used for free radical scavenging activity through diphenyl-picrylhydrazyl (DPPH) assay. The test was performed in a triplicate manner with 0.002% DPPH in methanol and 0.250 ml plant extract was used for the analysis in 3 ml reaction volume. After 30 min incubation at room temperature, the absorbance was recorded at 517 nm and the blank was set with 0.002% DPPH methanolic solution (Bemani et al. 2012). The Free radical scavenging potential was measured by the formula:

$$\% \text{ inhibition of DPPH radical} = \left(1 - A_{\text{sample}}/A_{\text{blank}}\right) \times 100.$$

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the sample/reference.

Enzyme extraction and antioxidant enzyme assay:

The enzyme extraction was carried out from 14 days old seedling of 12 different rice varieties. The homogenization was executed in ice chilled 50 mM Sodium phosphate buffer (pH 7.0) containing 2 mM EDTA, 5 mM β-mercaptoethanol

Table 1 Comparative details of 12 different rice varieties belonging to the distinct agro-ecological zones of India

Serial no.	Variety name	Crop maturation duration	Eco-system	Special characteristics	Cultivation zones
1	PB-1	130–135 days	Irrigated	Semi-dwarf, high yielding and basmati rice or scented variety with soft texture and pleasant aroma	North India Province: Punjab, Haryana, Uttar Pradesh, Uttarakhand
2	PB 1509	115–120 days	Irrigated	Semi-dwarf stature, high yielding and basmati rice variety	North India Province: Haryana, Punjab, Delhi, Uttar Pradesh
3	PB-RH-10	100–115 days	Irrigated	Early maturing, high yielding, fine grain and basmati rice variety	North India Province: Punjab, Haryana, Delhi, Uttar Pradesh, Uttarakhand
4	CSR-30	140 days	Irrigated	Long slender, highly scented grains and basmati rice variety with salt tolerant nature	North India Province: Haryana, Punjab, Uttarakhand, Uttar Pradesh
5	HKR-47	135 days	Irrigated	Semi dwarf, long slender grain and non-basmati rice variety	North India Province: Haryana, Uttar Pradesh
6	PR-126	93–125 days	Irrigated mid-early rice ecosystem	Early maturing, non-basmati rice variety and resistant to several pathogens	North India Province: Punjab
7	Govind	95–110 days	Rainfed Early	Dwarf, long slender grain and non-basmati rice variety	North India Province: Uttar Pradesh
8	Sharbati	125–135 days	Irrigated	High yielding and semi dwarf and non-basmati rice variety	North India Province: Punjab, Haryana, Uttar Pradesh
9	ADT-37	105 days	Irrigated Early	Short duration, semi dwarf, non-basmati rice variety and resistant to various pathogens	South India Province: Tamil Nadu, Pondicherry and Andhra Pradesh
10	ADT-39	120–125 days	Irrigated Medium	Early maturing, dwarf, non-basmati rice variety and resistant to various pathogens	South India Province: Tamil Nadu, Pondicherry
11	CO-45	135 days	Irrigated low land	Dwarf, long slender grain, non-basmati rice variety and resistant to various pathogens	South India Province: Tamil Nadu, Pondicherry
12	White Ponni	135–140 days	Irrigated	Tall, non-basmati rice variety and resistant various pathogens	South India Province: Tamil Nadu, Andhra Pradesh

and 4% PVP-40. Then, the homogenate was centrifuged at 15,000 rpm for 30 min at 4 °C and supernatant was collected in separate vials and stored at − 20 °C till further analysis. The supernatant was used for antioxidants enzyme assay which are as follows:

Superoxide dismutase (SOD, EC 1.15.1.1)

SOD activity was calculated through the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) by enzyme extract. The reaction mixture of 1 ml comprised 50 mM potassium phosphate buffer (pH 7.8), 2 mM riboflavin, 75 mM NBT, 13 mM DL methionine, 100 mM EDTA and enzyme extract (50 µl) (Beauchamp and Fridovich 1971). The reaction was initiated by illuminating for 30 min at 25 °C and absorbance was recorded at 560 nm. One unit of activity was determined as the amount of enzyme required to inhibit the photo-reduction of NBT to blue formazan by 50% and was expressed as SOD units per gm fresh weight (Fw).

Catalase (CAT, 1.11.1.6)

Catalase activity was measured at 25 °C through the decrease in absorbance of H_2O_2 at 240 nm for 3 min by the method described by Aebi 1984. The reaction mixture of 1 ml contained 10 mM H_2O_2 in 50 mM phosphate buffer (pH 7) and 50 µl enzyme extract (Aebi 1984). CAT activity was expressed as U (µmol of H_2O_2 decomposed min^{-1}) per gm Fw using the extinction coefficient 39.4 mM^{-1} cm^{-1}.

Peroxidase (POX; EC 1.11.1.7)

Peroxidase or Guaiacol peroxidase activity was determined by the oxidation of guaiacol to a colored tetra-guaiacol at 25 °C which led to an increase in absorbance and measured through spectrometric analysis. The reaction mixture of 1 ml contained 50 mM phosphate buffer (pH 7), 9 mM guaiacol, 10 mM H_2O_2 and 50 µl of enzyme extract (Fernández-García et al. 2004). The increase in absorbance is recorded at 470 nm for 3 min at 30 s min interval and blank was

without enzyme extract in the reaction mixture. The enzyme activity was expressed as U (μmol of guaiacol oxidized or guaiacol dehydrogenation product (GDHP) min^{-1}) per gm Fw and extinction coefficient of its oxidation product is 26.6 mM^{-1} cm^{-1} (ε).

Statistical analysis

All the experiments were carried out in triplicates and data are expressed in figures with mean ± standard deviation followed by one-way ANOVA fulfillment was performed for statistical differences among the means of two or more groups using Microsoft Excel program 2007. Further, the post hoc pairwise comparisons test was performed through the Tukey–Kramer test and P value < 0.05 was considered as statistically significant. Moreover, the principal component analysis (PCA) was used to identify the covariance structure and relationships of the samples based on the information from the variables. The result from biochemical responses such as DPPH, SOD, CAT and POX of different rice seedlings were utilized for the PCA requirement. Moreover, cluster analysis was achieved using results from biochemical responses (DPPH, SOD, CAT and POX) for better understanding of hierarchical associations, on the basis of unweighted pair group method with arithmetic mean (UPGMA) and Euclidean distance methods. The Tukey–Kramer test, principal component analysis (PCA) and cluster analysis (CA) were performed using PAST statistical software (Hammer et al. 2001).

Result and discussion

The present study was conducted on twelve different rice varieties belonging to distinct agro-ecological zones and ecotypes which are cultivated specifically in north and south province of India, respectively (Fig. 1). To get an understanding, the 14 day old rice seedlings were developed on
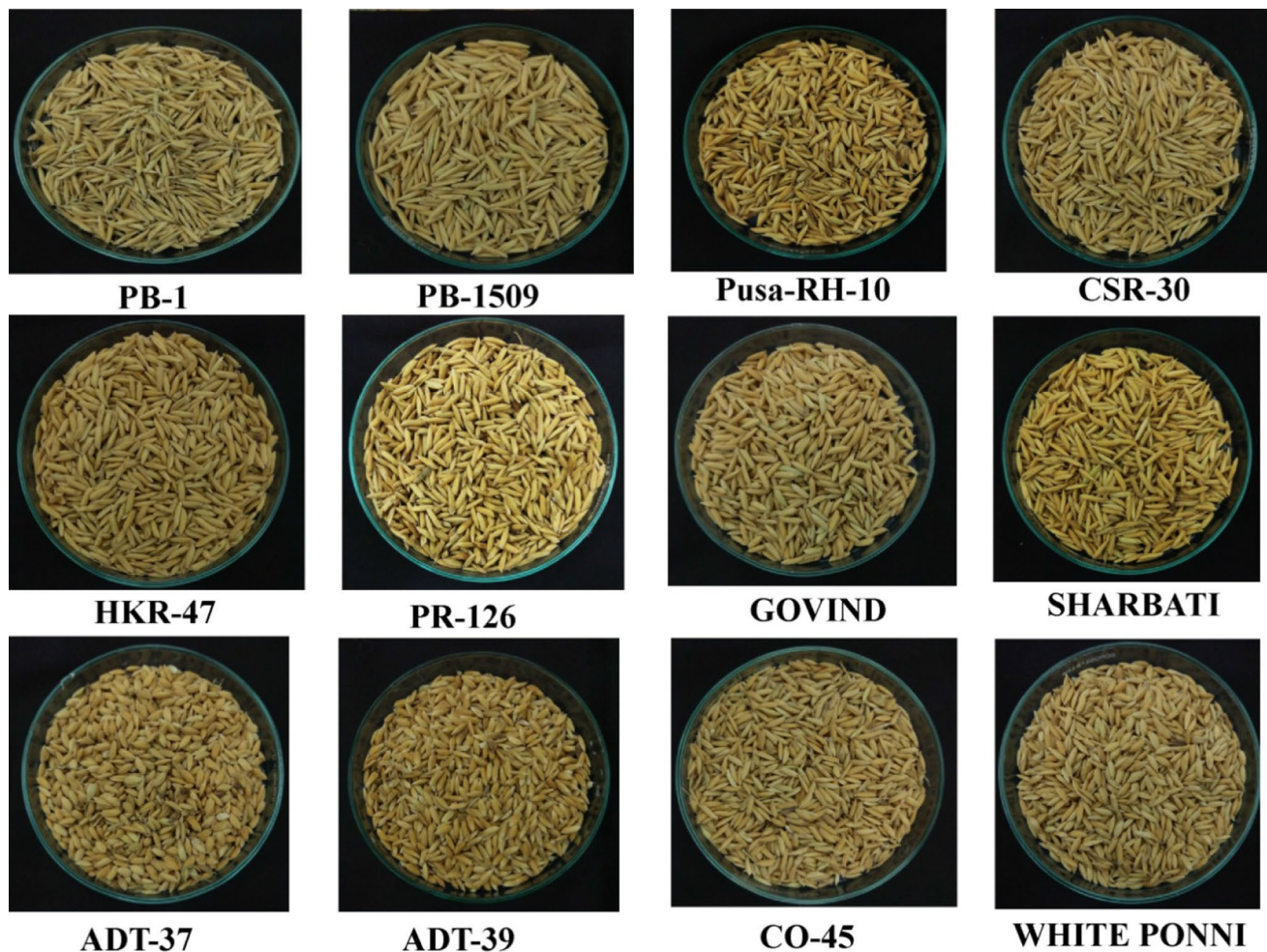
Fig. 1 Twelve different rice varieties used for the studies which represent distinct morphological and physiological characteristics and belong to different ecological zones of India

water-agar environment under controlled system and were employed for the identification of free radical scavenging capacity and antioxidant enzymes comprising superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (POX). In the present study, the comparative overview is represented not only rice varieties belonging to different agro-ecological conditions but also between basmati and non-basmati rice varieties. The several biochemicals studied here are very important for vital functioning in plant and have an impact directly and indirectly on normal growth and development.

Radical scavenging activity

The free radical scavenging activity was performed on 14th days old rice seedlings using DPPH methods. Presently, the methanolic extracts of 12 different rice seedling comprising PB 1, PB 1509, Pusa RH 10, CSR 30, HKR 47, PR126, Sharbati, Govind, ADT-37, ADT-39, ADT-45, and White Ponni, were evaluated for their radical scavenging activity. The average of DPPH radical scavenging activity of rice seedlings was 74.20% which were ranged from 66.65% (CSR-30) to 85.39% (ABT-45) (Fig. 2). This percentage-based radical scavenging analysis is in accordance with earlier studies reported in different plant species and radical scavenging potential were ranged between from 0.19 to 94.51% (Dudonne et al. 2009; Ghosh et al. 2013; Haq et al. 2019). Additionally, non-basmati varieties displayed increased radical scavenging activity than basmati varieties and India south region varieties showed enhanced radical scavenging potential compared to India north region varieties. For instance, PB 1509 variety showed increased DPPH percent inhibition and lowest was seen in CSR-30 variety amongst basmati varieties and amongst

non-basmati varieties, ABT-45 represented enhanced radical scavenging potential while lower was seen in Govind variety. For north region varieties, Sharbati variety showed an enhanced DPPH activity and reduction was observed in Govind variety comparatively. Amongst south region rice varieties, the improved DPPH potential was identified in ABT-45 and reduced DPPH value was found to be noted in ABT-39. Earlier, a similar study has been reported that DPPH were ranged from 13.0 to 76.4% amongst nine red and three black rice varieties belonging to different geographical condition comprising Thailand, China and Sri Lanka (Sompong et al. 2011).

Presently, we used electron transfer (ET) principle-based DPPH methodology for radical scavenging activity which was determined by calculating the percentage of antioxidant activity through reduction of DPPH into yellow colored diphenyl picryl hydrazine compound using a spectrophotometer (El Bedawey et al. 2010). This change in colour of DPPH can be correlated with the presence of antioxidant molecules in the biological extract that quench DPPH free radicals by providing electron donation and convert them into bleached product resulting of decrease in absorbance at 515–520 nm (Ribeiro et al. 2006). Commonly, hydrogen atom transfer (HAT) and electron transfer (ET) principle are two common methodologies which are used for identification of in vitro radical scavenging activities in the biological extracts and both methods are responsible for the neutralization of free radicals. Most of HAT-based assays are kinetics based and involve in competitive reaction system in which antioxidant and substrate compete for radicals while ET based methods evaluate the ability of an antioxidant in the reduction method and measure the colour change during reduction (Apak et al. 2007).
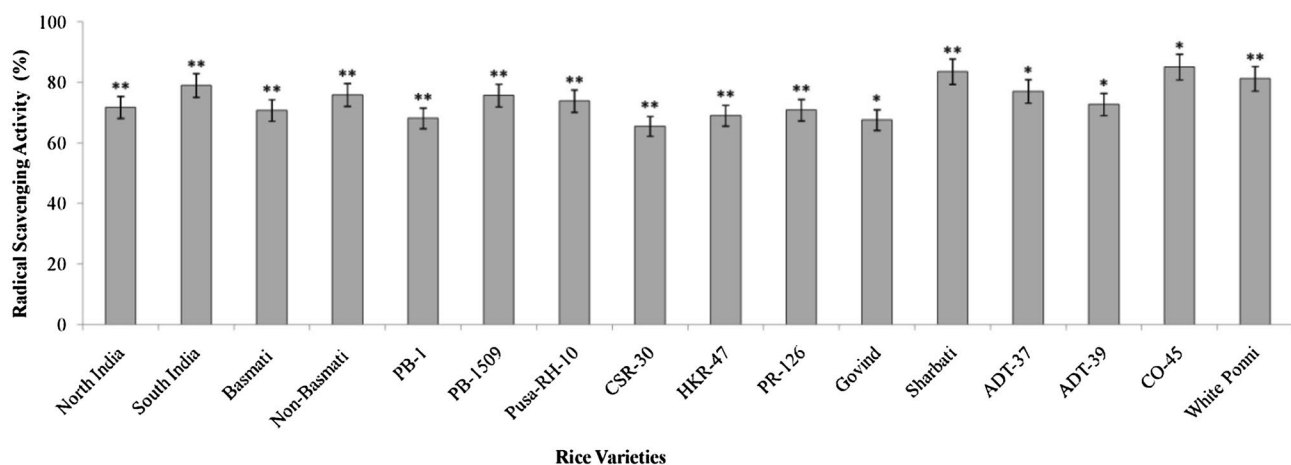
Fig. 2 Intrinsic DPPH radical scavenging activity amongst 12 ecologically divergent rice varieties. The data represent as mean ± SD ($n = 3$) and asterisks * and ** above the mean bar indicate significant and significantly different, respectively, at $P < 0.05$ based on the post hoc multiple comparison Tukey–Kramer test

In the present study, 12 different rice varieties represented a distinct level of their antioxidant capacities through DPPH assay which might be correlated to their different geographical localizations, ecosystem, developmental processes, physiological response, and genetic background. Moreover, this differential nature of antioxidant capacity can also be linked to a diverse range of phenolic compounds in their methanolic extracts because phenolic compounds are known as natural antioxidants in plants (Vichapong et al. 2010; Gonçalves et al. 2017; Huyut et al. 2017). Phenolic compounds are the class of secondary metabolites having a diverse range of chemical structure and function with either in bound or free form in plants. The free forms of phenolic compounds comprise flavonoids or proanthocyanidins while bound forms of phenolic compounds are known as ester linked to cell wall polymers (Bonoli et al. 2004). During germination, it has been observed that rice seeds contain an increased level of α-tocopherols (Moongngarm and Saetung 2010), total phenolic (Tian et al. 2004) and anthocyanin contents (Sutharut and Sudarat 2012) which are believed to be potent antioxidants and have free radical quenching capacities (Mittler 2002; Tian et al. 2004; Umnajkitikorn et al. 2013; Huyut et al. 2017). Flavonoid compounds comprising catechin, kaempferol, myricetin and quercetin are seen more commonly in rice (Du et al. 2012). Further, phenolic compounds were found to be as major contributors of variations amongst black rice, red rice and white rice grain extract (Fidrianny et al. 2016). Apart from this, biochemical interactions often generate a diverse range of free radicals which act as necessary intermediates for a variety of cell signaling and biological process under controlled way but an excess of their productions wreak havoc or oxidative damage to the biological cell (Mittler 2017; Haq et al. 2019). Amongst different types of free radicals, oxygen-derived radicals are most concerned widely and collectively known as reactive oxygen species (ROS) which is formed by sequential reduction of molecular oxygen to the formation of a group of ROSs. Recently, several studies have been documented the involvement of ROSs in signaling reactions, cellular proliferation and differentiation, biotic and abiotic stresses, seed germination, root/shoot development and flowering process etc. (Tsukagoshi 2016; Du and Scheres 2018; Mhamdi and Breusegem 2018).

Antioxidant enzyme activity

In the present study, the baseline for antioxidant enzymes was measured amongst 12 different rice varieties at 14 day old rice seedlings which were cultured on water-agar environment under controlled system. The antioxidant enzymes confined to superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POX) were examined because these enzymes play a fundamental role in the normal progression and defense machinery in the plants. During normal growth and metabolism, the limited amounts of reactive oxygen species (ROSs) are generated in different plant cellular organelles like chloroplast, mitochondria, and peroxisomes. The ROS level in the cells not only act as a secondary messenger but also get regulated by antioxidant defense machinery. Therefore, both ROSs and antioxidant system are required as an essential component for normal growth and cellular homeostasis in the organism (Das and Roychoudhury 2014).

Superoxide dismutase (SOD)

The distribution of SOD activity was identified amongst 12 different rice varieties belonging to different geographical locations and climatic conditions. Presently, the SOD activity was varied from 180.68 to 228.20 U/g FW with an average of 207.63 U/g FW. The deviation in SOD activity was observed which create separation in the action of SOD enzyme into 12 rice seedlings during their normal growth and development. SOD results of present study are in the accordance with earlier studies (Cavalcanti et al. 2004; Wang et al. 2018). SOD is most important primary enzymatic agent that transforms the superoxide into peroxide through dismutation of 2 molecule of superoxide ($O_2^{.-}$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Further, the level of peroxide (H_2O_2) is regulated by other antioxidant enzymes (CAT, APX, GPX, POX) accordingly while a certain level of H_2O_2 concentration is maintained within the cells for signaling purpose which is responsible for regular physiological and biochemical processes in plants (Niu and Liao 2016). SOD is identified as first-line defense machinery in all the aerobic organisms and play an important role in protecting from the harmful effect of ROS which is overwhelmingly generated under various situation including environmental adversity, diseases, pathogenic infection, biotic and abiotic stress and oxidative damage etc. (Hasanuzzaman et al. 2013; Caverzan et al. 2016; Xie et al. 2019).

Moreover, Basmati rice varieties showed comparatively increased SOD activity than non-basmati rice varieties but within Basmati, PB RH 10 and PB-1 varieties represented the highest and lowest SOD activity, respectively. Amongst non-basmati rice varieties, HKR-47 displayed increased SOD level while lowest SOD activity was seen in ABT-45. According to geographical distribution, it seems that India north region rice varieties displayed more SOD activity compared to India south region rice varieties (Fig. 3). The deviation in the distribution of SOD activity was observed within and across selected rice varieties and distinct ecological groups, respectively. The variation in the minimum threshold value amongst rice varieties might be due to different demographic, geographic, climatic condition, and genetic adaptation. Further, the deviation in SOD values can
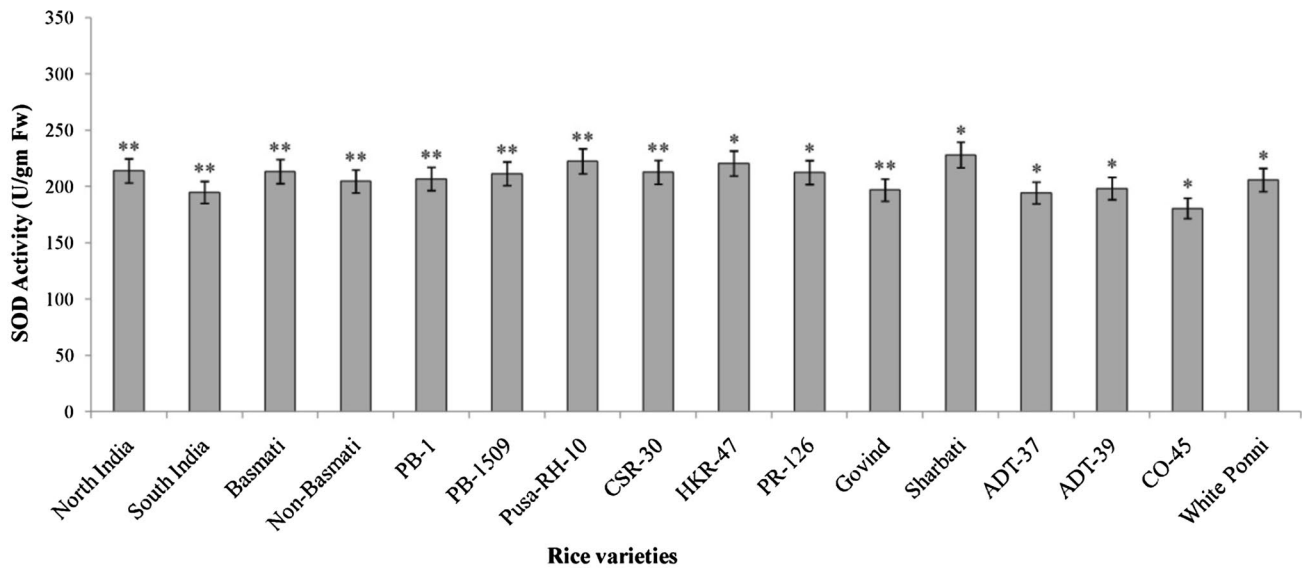
Fig. 3 Intrinsic superoxide dismutase (SOD) activity amongst 12 ecologically divergent rice varieties. The data represent as mean ± SD ($n = 3$) and asterisks * and ** above the mean bar indicate significant and significantly different, respectively, at $P < 0.05$ based on the post hoc multiple comparison Tukey–Kramer test

also be implicated by different morphological, physiological, biochemical, and molecular makeup which are differentially required for a constant level of growth and development amongst different rice cultivars (Hasanuzzaman et al. [2013]; Xie et al. [2019]). Additionally, metal cofactor categorized SOD into three different isoforms like, Cu/Zn-SOD, Fe-SOD and Mn-SOD which are separately localized in different subcellular compartments (Del Río et al. [1998]) and their activities occur distinctively for combating different conditions for survival and maintenance of plant growth. Therefore, all these SOD isoforms have been also identified differentially in various rice seedlings and deviation in SOD activity is also correlated with differential distribution of SOD isoforms (Sharma and Dubey [2005]; Sharma et al. [2013]; Kaur et al. [2016]). In the present study, the deviation in the SOD activity might also be extrapolated with variation in the ecology involved amongst different rice varieties such as soil salt conditions, pH, waterlogging, lightening, temperature, and atmospheric pressure (Gill and Tuteja [2010]; Das et al. [2013]; Zhang et al. [2015]; Kaur et al. [2016]; Rossatto et al. [2017]; Abdelaziz et al. [2018]; Xie et al. [2019]).

Catalase (CAT) Activity

Catalase (CAT) activity was estimated for the selected rice varieties belonging to diverse ecological conditions. A tetrameric heme-containing CAT enzyme plays a key role in dismutation of H_2O_2 into H_2O and O_2 after SOD action within all living tissues. It is a unique antioxidant enzyme having very high turnover rate (6×10^6 molecules of H_2O_2 to H_2O and O_2 min^{-1}) and reveal more affinity for H_2O_2 but lesser specificity for organic peroxides (Mittler [2002]). An average CAT activity was found to be 18.04 U/g FW which were ranged from 11.33 (Sharbati) to 29.51 (PB-1) U/g FW. Presently, the action of CAT enzyme amongst the selected rice varieties are in compliance with earlier reports in different rice cultivars (Huang and Guo [2005]; Jha and Subramanian [2013]). Peroxisomes, a membrane-bound cytoplasmic organelle present in most eukaryotic cells and are believed as a major source for H_2O_2 productions by metabolic reactions like β-oxidation of fatty acids, photorespiration, and purine catabolism (Mittler [2002]; Schrader and Fahimi [2006]). This generated H_2O_2 is promptly degraded by peroxisomally CAT enzyme which help to oxidative balance within the cell (Walton and Pizzitelli [2012]) nevertheless other subcellular compartment (cytosol, chloroplast and the mitochondria) are also found to have a role in oxidative balance within the cells through significant CAT activities (Mhamdi et al. [2010]). Notably, the low concentration of H_2O_2 have tendency to normalize cellular functions like signal transduction, cell proliferation, cell death, redox-balancing, gene expression modulations and other processes but an extensive increased level of H_2O_2 leads to have deleterious effect on metabolic dysfunction, affecting normal cellular functions and gigantic damage to biomolecules (DNA, lipids and proteins) (Petrov and Breusegem [2012]; Hossain et al. [2015]; Niu and Liao [2016]).

In the current study, basmati rice varieties exhibited increased CAT activity compared to non-basmati rice varieties. Among basmati varieties, PB-1 revealed the highest

CAT value and lowest was seen in CSR-30 variety. Amongst non-basmati varieties, PR-126 rice variety showed exceptionally high CAT activity and lowest CAT value was seen in Sharbati rice variety (Fig. 4). Depending upon the geographical distribution of rice varieties, the enhanced CAT activities found to be seen within North India region rice varieties compared to south India region rice varieties in the present study. Because of these results, it can be assumed that the growth and progression of species belonging to different genetic constitution experience certain kind of pressures which are essentially required for normal growth and development. Therefore, behavioral constraint amongst species represents their uniqueness, adaptively to given environment and also creates a separation amongst species. Furthermore, this deviated CAT activity might be correlated with catalase isoenzymes (CAT 1, CAT 2 and CAT 3) which have been identified in higher plants and their differential distribution was noticed in the distinct compartment of the cell amongst angiosperms (Das and Roychoudhury 2014). It has been also documented that CAT activities can be affected by distinct factors such as environmental signal (ozone, temperature, UV irradiation, and SO_2), exogenous constraint, temporally and spatially regulation of CAT genes, differentially expression of CAT gene and hydrogen peroxide signaling (Scandalios 2005; Kwon et al. 2007; Hossain et al. 2015; Hu et al. 2016).

Peroxidase (POX) activity

Similarly, guaiacol peroxidase activity was examined in the selected rice varieties at the 14 days old seedling stage. The

POX activity ranged from 5.72 (Govind) to 14.54 (Pusa RH 10) U/g FW with an average of 8.97 U/g FW. In the present study, the guaiacol peroxidase activity is in accordance with previous studies reported in different rice varieties which also reported that POX activity continuously increase during day to day growth in normal as well as in stressed condition but comparatively increased POX activity is observed under stressed condition than normal condition (Sharma and Dubey 2007; Lotfi et al. 2009; Singh et al. 2012; Poli et al. 2018). Guaiacol POX belongs to heme-containing enzyme family composed of 40–50 kDa monomers and ubiquitously present in fungi, plants, and vertebrates. This enzyme is considered as an active representative during normal metabolism as well as defense mechanism against biotic and abiotic stresses (Ghamsari et al. 2007) hence, it is known as stress enzyme (Sharma et al. 2012). It preferably oxidizes aromatic electron donors such as guaiacol and pyragallol by utilization of H_2O_2 (Asada 1999). The several kinds of POX enzymes are found in a different compartment of cell [intra cellular (cytosol, vacuole), extracellular (apoplast) and cell wall] and numerous physiological functions are also assigned to plant peroxidases, for example, lignification, suberization, cross linking of cell wall proteins, stress response, defense against pathogens, salt tolerance and senescence (Hiraga et al. 2001; Ghamsari et al. 2007; Gill and Tuteja 2010; Hamann 2012; Pandey et al. 2017; Wu et al. 2019).

Significantly, non-basmati rice seedlings showed reduced POX activity compared to basmati rice seedlings and amongst basmati varieties, the Pusa RH 10 cultivar revealed maximum POX activity but the minimum was found in PB-1 variety. Similarly, the top-notch POX
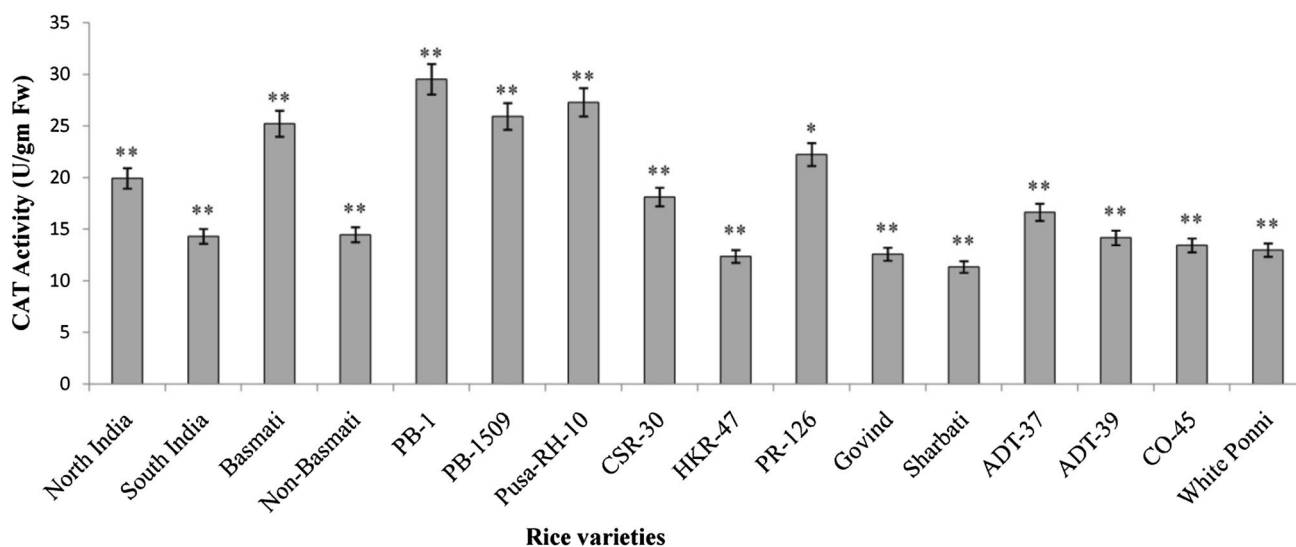
Fig. 4 Intrinsic catalase (CAT) activity amongst ecologically divergent rice varieties. The data represent as mean ± SD (*n* = 3) and asterisks * and ** above the mean bar indicate significant and significantly different, respectively, at *P* < 0.05 based on the post hoc multiple comparison Tukey–Kramer test

activity found to be observed in PR 126 but Govind variety displayed a lowest POX value amongst non-basmati cultivars (Fig. 5). According to geographic condition, south India region rice cultivars displayed enhanced POX activity compared to north India region rice cultivars. In the present study, lower POX enzyme activity was observed than CAT activity amongst 12 different rice seedlings but deviation within POX activities was also identified in different rice seedlings belonging to distinct ecology. The discrepancies within POX values is characterized by species-specific condition of POX enzyme, nature of constraint and transform developmental response amongst cultivars during growth and development. The low level of POX activity might be also correlated to normal growth progression under no stress and minimum level of oxidation of phenolic compounds are also responsible for POX action (Taggar et al. 2012). Accordingly, a large set of POX enzyme family are present in higher plants (Welinder 1992) and their expression pattern are differentially orientated, organ specific and developmentally regulated (Klotz et al. 1998; Hiraga et al. 2000). The diversification in the POX function is explained not only by the presence of several isoforms of POX enzyme which results from gene duplication actions during evolution but also depends on the developmental process for organs buildup in distinct sections of plants which explicates an existence and diversification of POX enzymes (Ghamsari et al. 2007; Passardi et al. 2004). Therefore, variation in the Guaiacol POX activities have been confirmed in the previous studies and this change depends upon plant species, stresses and various environmental conditions (Yamane et al. 2009;

Gill and Tuteja 2010; Panda et al. 2019; Sarkar et al. 2013; Banerjee and Roychoudhury 2019; Sharma et al. 2020).

## Principal component analysis (PCA) and clustering analysis

The principle component analysis (PCA) was carried out to examine relationship amongst different rice varieties using data from DPPH, SOD, CAT and POX activities in shoots, based on covariance matrix at 14 days old seedling stage. Under the multivariate statistical methods, PCA a popular statistical procedure which is used for data exploration with reduced data dimensionality, representation of the information in data of high dimension and finding relevance between objects (Çam et al. 2009; Wang and Hu 2011). Presently, the outcome of PCA was obtained in several principle components (PCs) and loading of PC1 and PC2 accounted for 72.09% of total variance whereas, PC 1 revealed high eigenvalue of 2.4 with 48.25% of total variance and PC 2 showed 1.2 eigenvalue with 23.84% of total variance, respectively (Fig. 6). The results of loading plot of PC1 and PC2 are shown in Fig. 7 and PC1 mostly showed a harmony amongst basmati rice varieties while PC2 showed positive relationship amongst non-basmati rice varieties. A degree of differentiation was found in basmati and non-basmati rice varieties belonging to diverse ecological nature. Therefore, the present study represented relationship amongst ecologically different rice varieties on the basis of their biochemical (DPPH, SOD, CAT, and POX) responses at 14 days seedling stage. Earlier, the several studies have shown high dimensionality data exploration for number of
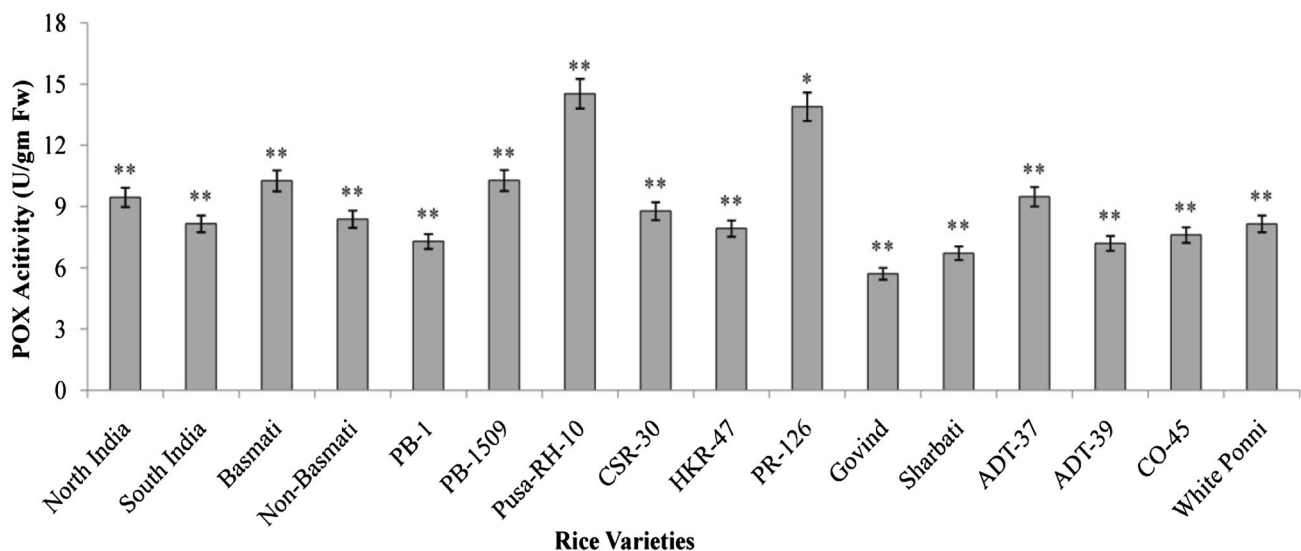
Fig. 5 Intrinsic peroxidase (POX) activity amongst ecologically divergent rice varieties. The data represent as mean ± SD (n = 3) and asterisks * and ** above the mean bar indicate significant and signifi- cantly different, respectively, at P < 0.05 based on the post hoc multiple comparison Tukey–Kramer test

Fig. 6 Detail of eigenvalues of each principal component that stand for the amount of variation
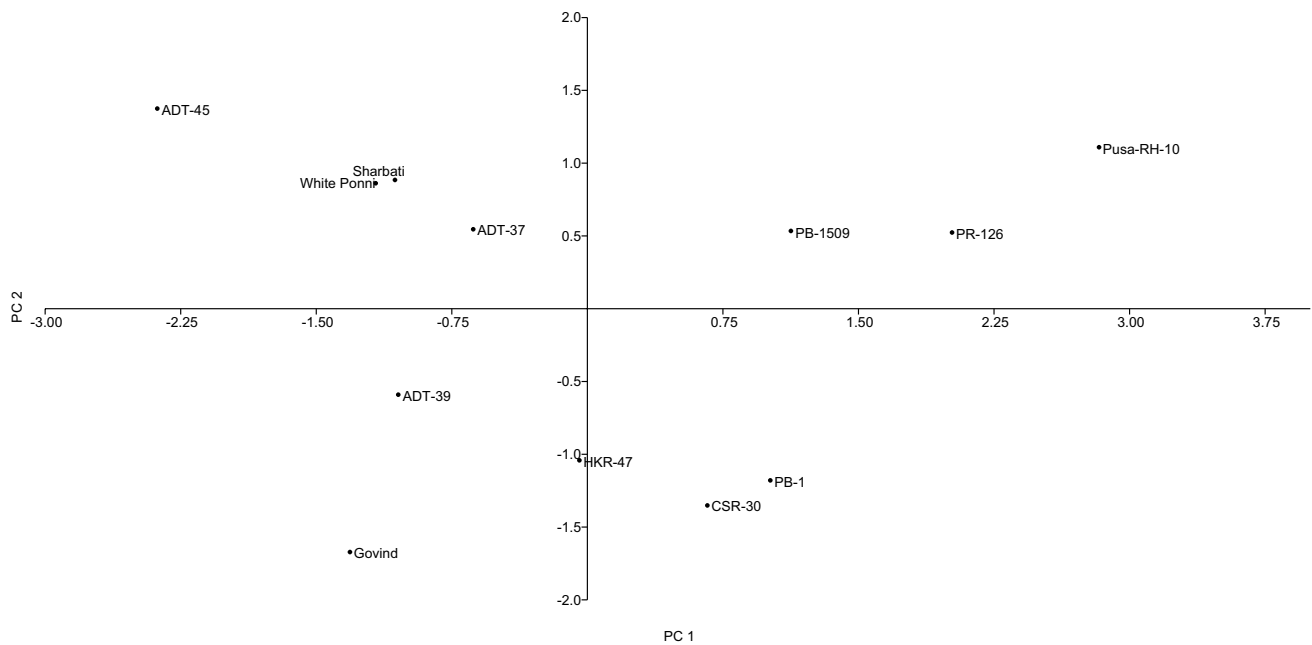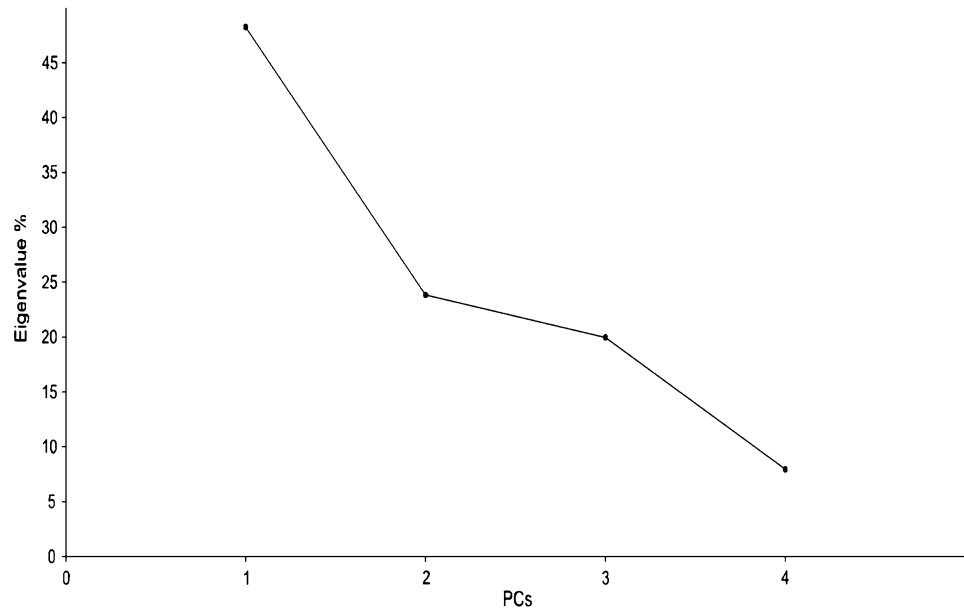

Fig. 7 Biplot obtained from PC 1 and PC 2 showing the relationship amongst 12 ecologically different rice cultivars based on the result from DPPH, SOD, CAT and POX activities in a shoot at 14th days old seedling stage

different variables used and their relationship between the variables using principle component analysis amongst different plant species (Çam et al. 2009; Wong et al. 2006; Boeing et al. 2014). Additionally, cluster analysis was performed amongst 12 different rice varieties on the basis of association amongst DPPH, SOD, CAT, and POX activities in the shoot at 14 days old seedling stage through unweighted pair group method with arithmetic mean (UPGMA) and Euclidean distance methods. Broadly speaking, two major groups

were observed from the cluster analysis and somewhat, these groups revealed partition between basmati and non-basmati rice varieties. While group I was mostly concentrated with non-basmati rice varieties including India's North and South region rice varieties except CSR-30 and group II showed a major association of basmati rice varieties except PR-126 (Fig. 8). Thus, cluster analysis represented a resemblance and divergence amongst ecologically different rice varieties and grouped these varieties according to their basmati and

Fig. 8 UPGMA and Euclidean distance-based cluster analysis showing hierarchical association amongst 12 ecologically different rice varieties using data from biochemical response (DPPH, SOD, CAT and POX) in shoot at 14th days old seedling stage
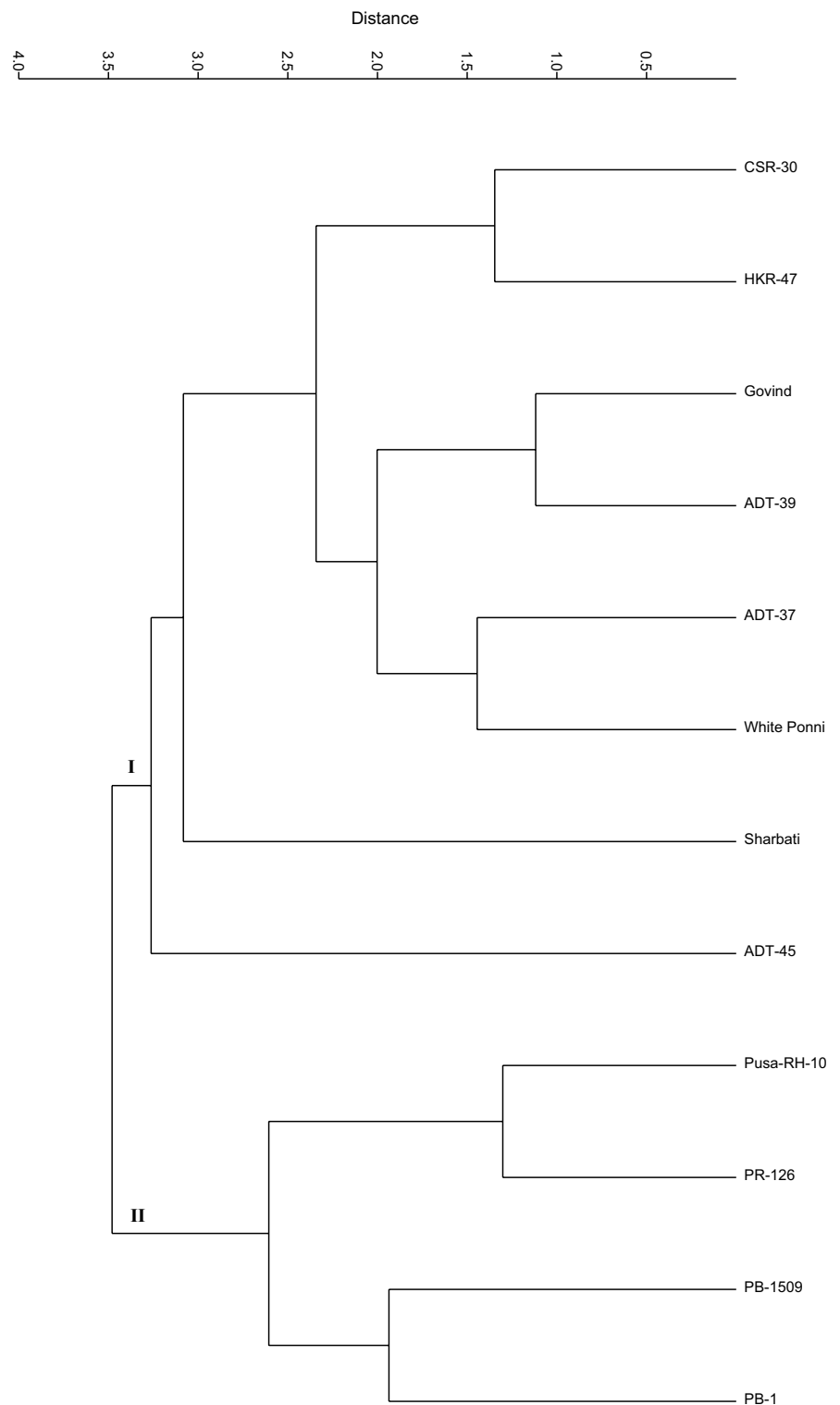
non-basmati nature within distinct edges, respectively, using biochemical responses.

In conclusion, aerobic organisms are exhibited into distinct groups on the basis of their morphological characteristics, physiological processes, molecular functions, evolution pattern and their ecology. These differences create an

imposition on the living organism to adapt with the environment and to produce the varied level of reactive oxygen species (ROS) which are essentially required for normal metabolism, signaling and physiological functions but high concentration exacerbate the toxicity of ROS which result in an oxidative burst. Because, ROS are highly reactive

molecules of oxygen and can have a vital impact on cell damaging not only by altering biomolecules entities such as lipids, proteins, carbohydrates, and nucleic acids but also by disturbing their functions. Significantly, aerobic organisms have well organized antioxidant systems including both enzymatic and non-enzymatic antioxidant arrangements that usually participate in controlling or blocking harmful effects of ROS. In the present study, 12 different rice varieties were used for intrinsic radical scavenging activity and antioxidant enzymes (SOD, CAT and POX) distribution at 14 days old seedling stage which were developed on water-agar system under controlled environment. Consequently, varied levels of radical scavenging potential and antioxidant enzyme functions were observed amongst 12 rice varieties belonging to different morphological, geographical distribution and ecotypes. Hence, a distinct level of ROS and their antioxidant enzyme nature exhibits remarkable differentiation amongst various rice varieties and unveil their sustainable development adaptability according to a different environment, geographical localization, ecotypes, and genetic differences.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this paper.

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