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Research Article

Physiological characterization of SUB1 trait in rice under subsequent submergence and re-aeration with polyamine and salicylic acid interaction

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Abstract: In the present study, the sensitivity of two chemical elicitors: polyamine (PA) and salicylic (SA) acid were exercised for submergence sensitivity in Swarna Sub1A rice variety. Under 5 days of submergence, the antioxidation responses were more distinguished in plants against control. Along with the anti-oxidation modules, significant changes in biomolecule loss were registered through lipid and protein oxidation by 1.91 and 1.46 -fold respectively. PA and SA treated plants were the reliever to recover the membrane potential. Total carbohydrate and reducing sugars were varied under submergence by down regulation of 36.66 and 44.44% as compared to control. This was also supported by regulation of α-amylase activity under submergence that also recovered significantly with PA and SA treatments against submergence. In association with carbohydrate metabolism, Under submergence Swarna Sub1A recorded to be prone with oxidative stress through $O_2^{\cdot-}$ (1.55 fold) and peroxide (1.70-fold) over-accumulation but recovered as PA and SA applied. In both cases, sustenance of non-enzymatic anti-oxidant like total carotenoid and lycopene content were also contributory through down-regulation. The enzymatic anti-oxidation paths like SOD, GPX, CAT and GR were regulated by 11.11, 19.54, 13.65, 10.03% declined respectively and thoroughly discussed with reference to PA and SA interactions.

Keywords: Rice; antioxidative enzymes; polyamine; salicylic acid, submergence, ROS

Abbreviations: **CAT**, Catalase; **DNPH**, 2,4-Dinitrophenylhydrazine; **DNS**, Dinitrosalicylic acid; **EDTA**, Ethylenediaminetetra acetic acid; **GPX**, Guaiacol peroxidase; **GR**, Glutathione reductase; **H_2O_2**, Hydrogen peroxide; **MDA**, Malondialdehyde; **NBT**, Nitroblue tetrazolium; **$O_2^{\cdot-}$**, Superoxide; **PA**, Polyamine; **PMSF**, Phenyl methane sulphonyl fluoride; **PVP**, Polyvinyl pyrolidine; **ROS**, Reactive oxygen species; **SA**, Salicylic acid; **SOD**, Superoxide dismutase; **TBA**, Thiobarbituric acid; **TCA**, Trichloroacetic acid; **β-ME**, β -Mercapto ethanol.

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Introduction

Rice, the most staple crop in the world has been with the serious concern for its growth and sustainability from kinds of abiotic stresses. Excess of water in the form of submergence in rice fallows sets a bottleneck for both survival and productivity of indigenous land races. On an average 20% curtail of productivity is experienced globally in rice cultivation. In global rice cultivation, it covers 16% of total area under submergence stress with varying depth and durations. Submergence stress may be compounded with two major facets: induced water stress and anoxic/hypoxic stress. Due to abundance of water in soil, roots are suffered with

poor hydraulic conductivity. Thus, osmotic potential of the tissues becomes depleted resulting water deficit stress (1). Alternatively, the dissolve oxygen in stagnant flood water for prolong days with turbidity and microbial invasion causes hypoxic/anoxic condition (2). This is more affected with an oxidative eruption of redox and causes accumulation of various Reactive Oxygen Intermediates/species (ROI/ROS). Typically rice plants with anoxic and hypoxic condition in submergence suddenly are exposed to high oxygen tension on recede of water level. The exposure of high intercellular oxygen concentration, particularly, with high irradiance creates an elevated redox with profuse ROS (3). Degeneration of cell membrane, loss of membrane permeability, disruption of energy yielding metabolism and finally oxidation of macromolecules are the outcome of such oxidative stress under submergence.

Plants are responsive to different chemical elicitors those influence the perception, transduction and cellular reactions to abiotic stress factors. In fact, the degrees of tissue sensitivity become a governing factor to the stress responses are variable to plant developmental stages as well as tissue types (4). Those chemical elicitors often modulate some cellular metabolic reactions and are reflected in changes of plant tolerance stresses. Out of few low molecular weight indigenous moieties, polyamines (PA) are the specific with features like aliphatic in nature, varied with unsaturation, soluble in cell sap, easily diffusible over the membrane (5). PA through its polycationic nature is mostly offered to bind cell and organelle membrane can influence the permeability, gene expression, modulation of different biochemical reactions. Metabolism of PA and with its expression profile is quite variable in sensitive and tolerant cultivars of crops subjected to different abiotic stressors. While osmotic, salinity, metal, temperature and oxidative stress are most discussed with PA interactions in cereal crops (mostly referred with rice), the submergence stress is less discussed. Submergence with its induced water deficit and accompanying oxidative exposure needs to be investigated with interfaces of PA interference. On the other hand, Salicylic acid (SA), a phenolic compound, is well justified with its efficiency mostly to biotic stress, in developing of ROS. In view of submergence related oxidative stress, the role of SA may also be important to the insights of cellular responses. In the present paper, the role of SA and PA in independent manner on influence of submergence stress reaction is highlighted. Swarna Sub1A, a recommended submergence tolerant line of rice is addressed with some contributing cellular responses under the influence of PA and SA in a precise concentration. The information may be useful for their selection rice varieties against submergence stress through some cellular reactions influenced by PA and SA activities.

Materials and Methods

Experimental Plant Material:

The experiment was performed in the laboratory of Plant Physiology and Plant Molecular Biology Unit, Department of Botany, University of Kalyani, W.B, India. Rice seeds of the variety Swarna Sub1A were collected from Rice Research Institute, Chinsurah R S, Hooghly, West Bengal 712103, India. Initially the seed's viability tests were done according to Hong and Ellis (2004) (6) under laboratory condition at temperature 26 ± 2˚C and RH 80% in a seed germinator. After sprouting, 7 days old seedlings were divided into 4 sets: Set 1 (control-without submergence and any treatments), Set 2 (submergence of seedlings without any treatment), Set 3 (submergence of seedlings pre-treated with 1mM SA) and Set 4 (submergence of seedlings pre-treated with 2mM Putrescine as PA). Set 3 and Set 4 were pre-treated with 1mM SA and 2mM PA for 24 hours before submergence (7,8). On completion of treatments excluding control (not under submergence) seedlings were fully submerged under water in a cemented tank for 5 days. After 5 days of submergence Set 2, Set 3, Set 4 (without control) were kept in aeration for 1 day for recovery of seedling and stored at -80° C for further use. This is to monitor the extent possible recovery of submergence (hypoxic/anoxic condition) to oxygenic condition of the seedlings.

Biochemical Analysis:

The biochemical analyses were done from shoot portions of the plant sample under various treatments according to standard methods.

Total carbohydrate content:

Determination of total carbohydrate was done by anthrone method according to Hedge and Hofreiter (1962) (9). 100mg of plant sample was taken in a test tube with 5 ml of 2.5 N HCl in a water bath for hydrolysis for 15 min and cool to room temperature. Then added some sodium carbonate for neutralize it until the effervescence disappear. Extraction makes up to 50 ml by added distilled water and centrifuged 10000 x g for 15 minutes. Then supernatant was collected and 1 ml aliquots taken for reaction in test tubes. Thereafter, 4 ml of anthrone reagent was added in every test tube and kept in a boiling water bath for 10 minutes. Tubes were cooled rapidly by tap water and read the absorbance of green to dark green color in a UV-VIS spectrophotometer (Cecil) at 630nm. Then the amount of total carbohydrate was calculated from standard graph of glucose.

$$\text{Total carbohydrate present in 100mg of sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

Reducing sugar content:

Reducing sugars were determined by DNS method according to Miller (1972) (10) with slight modification. 100 mg of sample was homogenized with 5 ml of 80% of ethanol in a mortar pestle. The supernatant was collected and kept in a water bath for evaporation at 90°C. When extract came to near 1 ml, samples were taken out and make up to 10 ml solution to dissolve sugars with distilled water. 1 ml of extract pipetted out in test tubes and added 2 ml of DNS reagent. Then the samples were heated for 5 minutes in a boiling water bath and 1 ml of 40% Rochelle salt was added when the reaction mixtures were still warm. Thereafter, test tubes were cooled by tap water and absorbance of the dark red color of the reaction mixtures were read at 510nm in a UV-VIS spectrophotometer (Cecil). The amount of reducing sugars was calculated from the standard graph of glucose.

α-Amylase activity:

α-Amylase activity was determined according to Kruger (1972) (11) with slight modification. The 1g of plant sample crushed with 10mM of ice-cold calcium chloride solution and kept for 3 hours at room temperature. The extract was centrifuged at 12000 x g at 4°C for 20 minutes and the supernatant was collected and used as enzyme extract. In test tubes, 1 ml of enzyme extract and 1 ml of 1% starch solution were added and incubated at room temperature for 20 minutes. The reaction was stopped by addition of 2 ml of dinitrosalicylic acid (DNS) reagent and heated for 5 minutes in a boiling water bath. While the tubes were still warm, 1 ml of Rochelle salt (40%) was added. Then solution was cooled by running tap water and volume make up to 10 ml by addition of distilled water. The absorbance was read at 560nm by a spectrophotometer using 1 ml of water instead of enzyme extract in a test tube as blank. Enzyme activity was expressed as mg of maltose (calculated from standard graph of maltose) produced per min.

Total carotenoid and lycopene content:

The experiments were determined by the method of Zakaria et al. (1979) (12) with slight modification. The 0.5g sample was homogenized in a mortar pestle and then saponified with 3 ml of 10% alcoholic potassium hydroxide in a water bath at 70°C for 30 min. The extract was transferred in a separating funnel with 10 ml petroleum ether and then mixed thoroughly. Then the upper layer containing carotenoids and lycopene was collected and lower layer was transferred in another separating funnel for further purification and repeat this step until the aqueous layer become colorless. For removing the extra moisture from petroleum ether extract added small amount of anhydrous sodium sulphate until effervescence disappear. The final volume was noted for further calculation. The absorbance of the assay mixture was measured at 450nm and 503nm for carotenoid and lycopene content respectively in a UV-VIS spectrophotometer (Cecil). Total carotenoid and lycopene content was determined using the following formulae:

$$\text{Amount of total carotenoids} = \frac{A_{450} \text{ x Volume of the sample x } 100 \text{ x } 4}{\text{Weight of the sample}}$$

$$\text{Amount of lycopene} = \frac{A_{450} \text{ x Volume of the sample x } 100 \text{ x } 3.12}{\text{Weight of the sample}}$$

Amount of total carotenoids and lycopene content were expressed as mg/g of the sample.

Lipid Peroxidation:

Determination of malondialdehyde was done from fresh plant sample according to R. L. Heath and L. Packer, 1968 (13) with some modification. 1g of plant sample homogenized in liquid nitrogen and extracted with 10% TCA containing 0.5% TBA followed by centrifugation at 12000x g for 15 min at 4°C temp. The extract was heated at 95°C for 30 min and the reaction was stopped by cooling in an ice bath. The absorbance of the contents was measured at 532 nm and 600 nm in a UV-VIS spectrophotometer (Cecil). MDA concentration was calculated by using following formula:

A_{532}-A_{600}= εCL

ε=Extinction coefficient (155 is extinction coefficient of MDA)

C=Concentration

L=Path length (Distance of the cuvette=1cm)

Protein Oxidation:

Determination of carbonyl content was done by Dinitrophenyl hydragene (DNPH) reagent according to Foyer and Noctor (2005) (14). 1.0 g of fresh plant samples were homogenized in liquid nitrogen by addition of 10 % (w/v) sodium dodecyl sulphate (SDS) incubating at room temperature for 20 min. 10 mM DNPH and 1.5 mM TCA were added followed by gentle agitation. The pellet was suspended with 200 mM sodium phosphate buffer (pH 7.0) and the absorbance was read at 360 nm. The carbonylated derivative of protein was calculated using the molar extinction coefficient (22000 M^{-1} cm^{-1}) of DNPH.

Superoxide ($O_2^{\cdot-}$) content:

Generation of $O_2^{\cdot-}$ content from treated plant samples were determined according to Elstner and Heupel (1976) (15). 1 g of plant tissue was homogenized in liquid nitrogen by adding 65 mM phosphate buffer (pH 7.8). The homogenate was centrifuged at 7000 g for 20 min at 4°C. Thereafter,

the supernatant was collected and added within 3 ml of assay mixture containing 65mM phosphate buffer, 10mM hydroxyl-amine and supernatant. The reaction was incubated for 30 min at 25°C. Thereafter, 10mM sulphanilamide and 7mM α-napthyl amine were added and incubated for 20 min at room temp. The absorbance was read at 530 nm and the concentration of $O_2^{\cdot -}$ was derived by using NO_2^- as standard.

Hydrogen peroxide (H_2O_2) content:

H_2O_2 content of treated plants was done according to Ghosh et al. (2011) (16). 500 mg of plant tissue was homogenized in liquid nitrogen by adding 2 ml of 1 % (w/v) trichloro acetic acid (TCA). The homogenate was centrifuged at 10,000 g for 15 min at 4°C. Thereafter, the supernatant was collected and added within assay mixture containing 10 mM potassium phosphate buffer (pH 7) and 1 mM potassium iodide (KI) followed by incubation in dark for 20 min. The absorbance was read at 390 nm. The H_2O_2 content was derived using a standard solution.

Superoxide dismutase activity:

SOD activity was done Cakmak and Marschner (1992) (17) by measuring the ability to inhibit the photochemical reduction of nitro blue tetrazolium. The fresh plant samples were homogenized within liquid N2 and extracted using the extraction buffer containing 100mM phosphate buffer (pH=7.0), 100mM EDTA, 10mM PMSF, 10mM MgCl2, 10mM β-ME and 4% PVP. The assay mixture containing 100mM phosphate buffer (pH=7.0), 10mM methionine, 75mM NBT, 100mM EDTA and 100µl enzyme extract. The reaction was started after addition of 2 µM riboflavin and thereafter the experimental tubes were illuminated under 20 W fluorescent lights for 15 min. The absorbances of the illuminated tubes were measured at 560 nm. The SOD activity was expressed as Unit/mg of protein.

Guaiacol peroxidase activity:

Peroxidase activity was determined by the oxidation of guaiacol according to Verma and Dubey (2003) (18). The reaction was started with a 3ml reaction mixture containing 100mM phosphate buffer (pH=7.5), 10mM guaiacol, 200µl enzyme extract and 2mM H_2O_2. The activity was measured at 470nm at 30 sec intervals to determine the change in absorbance 0.1 and specific activity of enzyme was expressed as unit/gm f.wt.

Catalase activity:

CAT activity was done according to Verma and Dubey (2003) (18). The enzyme was extracted within liquid N_2 using the above-mentioned extraction buffer in a prechilled mortar and pestle. The reaction mixture containing 200 mM potassium phosphate buffer (pH=7) and 10 mM H_2O_2. The reaction was started by adding 200µl of enzyme extract and the decrease in absorbance was recorded at 240 nm per min and activity was detected using the extinction coefficient of 39.4M-$1cm^{-1}$ for H_2O_2.

Glutathione Reductase activity:

GR activity was done by determining the glutathione dependent oxidation of NAD(P)H according to Cakmak and Marschner (1992) (17). The enzyme was extracted using the above-mentioned extraction buffer. The assay mixture containing 100 mM phosphate buffer (pH= 7.5), 5mM EDTA, 2mM NAD(P)H, 30mM oxidized glutathione. The reaction was started by the addition of 200µl enzyme extract and the decrease in absorbance was recorded at 340nm for 2 min. The activity was calculated using molar extinction coefficient for NAD(P)H of 6.22 $mM^{-1}cm^{-1}$ and expressed as nM NAD(P)H oxidized $min^{-1}mg^{-1}$ of protein.

Statistical analysis:

The data obtained were statistically tested with ANOVA using the general linear model. The variations between the means of treatments with three replications were compared using Duncan's multiple range tests (at $P \leq 0.05$).

Results

The results of the present experiment are focused with the most possible changes of SUB1 trait when there is a transit from submergence to de-submergence. Rice varieties under submerged condition, particularly, for those of tolerant cultivars are displayed with two strategies: either quiescence or elongation ability. Whatever of those, when the varieties are transfer from submergence to aerated condition the plant might resume its normal growth through aerobic respiration (19). In the present experiment, it is of those cellular responses expressed under sudden transit from hypoxic/anoxic to aerobic condition are embedded herein. Moreover, the responses are under regulation of two chemical elicitors, PA and SA with the standard dose to check any influence on cellular responses. PA and SA have quite been deciphered mostly with water, salinity, oxidative stress than that of flooding or submergence stress.

Initially the Swarna Sub1A was marked with a significant variation in total carbohydrate content under 5 days of complete submergence followed by 1-day de-submergence to recover. Swarna Sub1A maintain 36.66 % down-regulation to accumulate to total carbohydrate in its culm and leaf sheath in case of submergence as compared to control which again under gone recovery by 1.31-fold and 1.26-fold by PA and SA respectively (Fig. 1). In support of carbohydrate accumulation

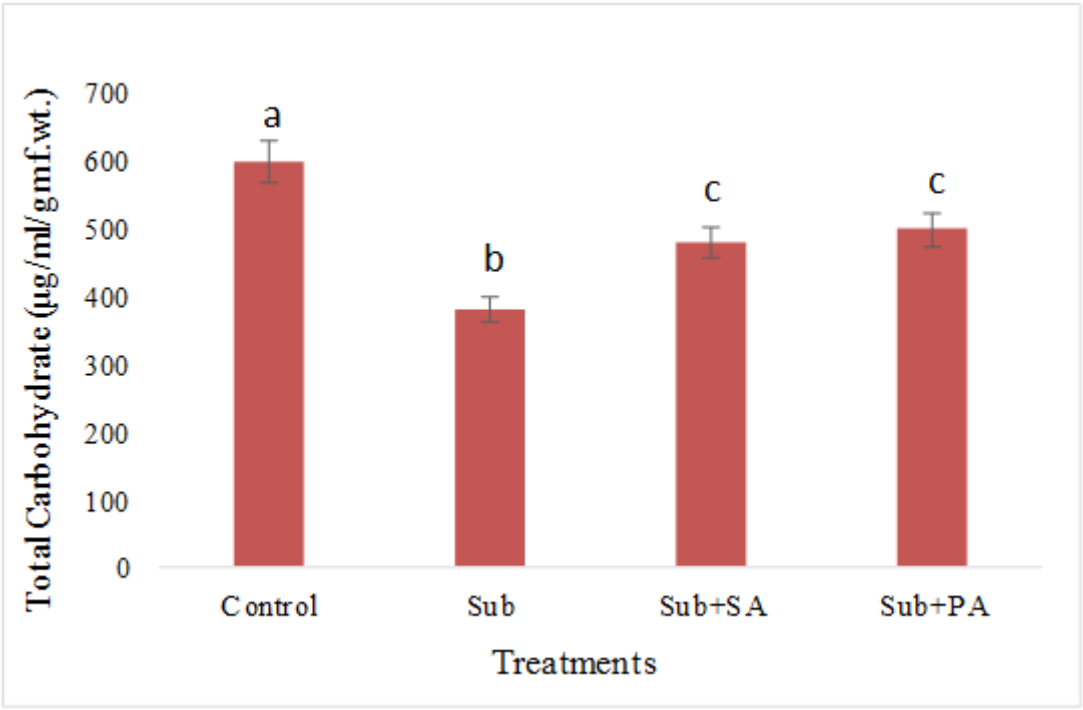

Fig. 1: Assay of total carbohydrate content in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

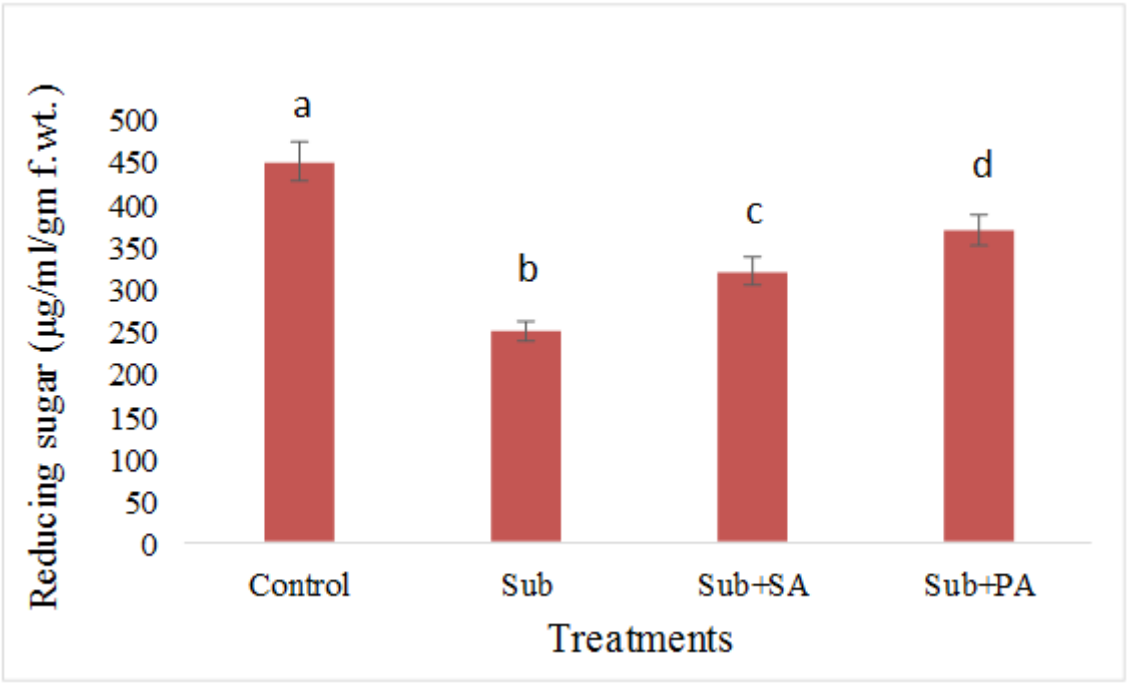

Fig. 2: Assay of reducing sugar content in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

through current photosynthesis the soluble sugar, mostly the reducing sugar as photosynthates were also consistent to justify the plant responses. The decline in reducing sugar was significant ($P\leq0.05$) by 44.44% which denotes solubilization of total carbohydrate including non-structural residues were under controls. The strategy to regulate the expense of soluble sugar is quite expected from SUB1 locus and that also moderated by PA (1.48 fold) and SA (1.28 fold) (Fig. 2). Activity of α-amylase is often contextual in description of SUB1 possessing allele rice land races. Questions still there whether this locus is realized in quiescence strategies either through constitutive or inductive expressions? For the later, it becomes more prudent that stringency is prevent in release of

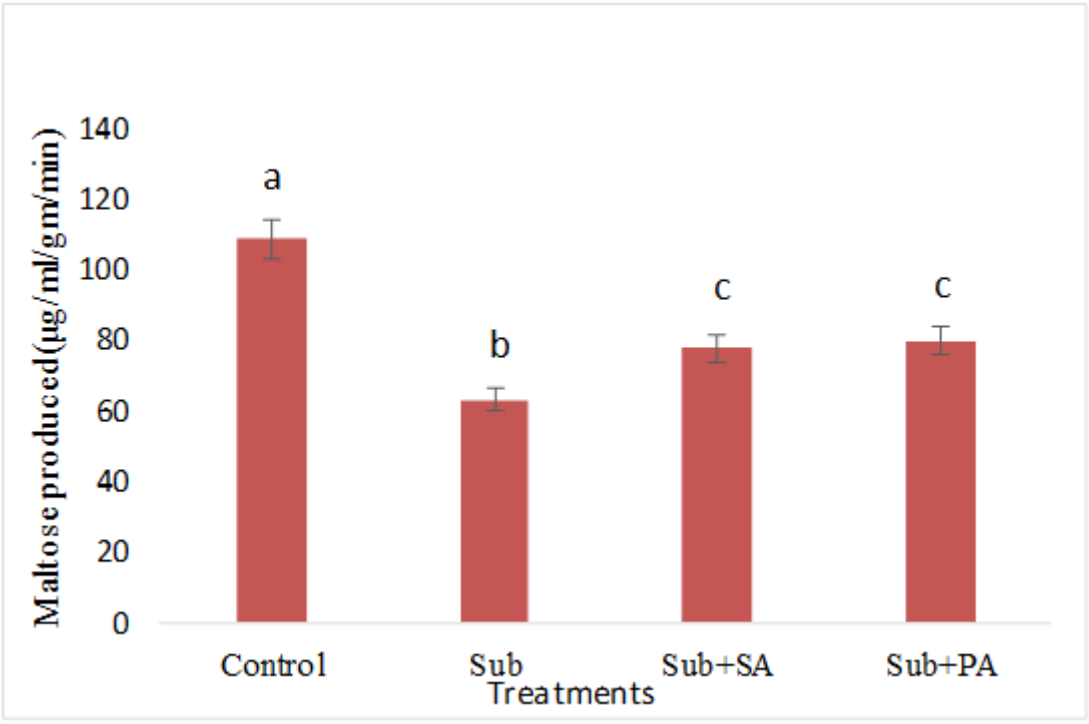

Fig. 3: Assay of α-amylase activity in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

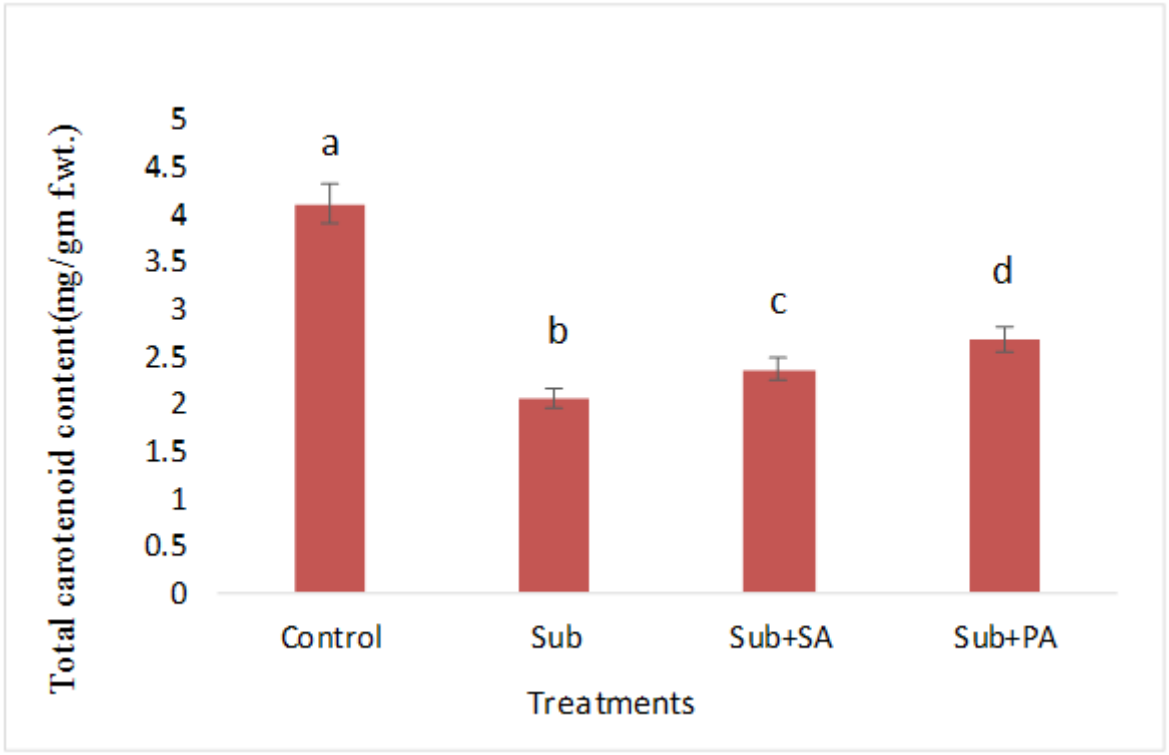

Fig. 4: Assay of total carotenoid content in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

soluble sugars for developing culm and leaf sheath under hypoxic /anoxic condition of submergence. Fig. 3 establish this fact by a significant down-regulation of α-amylase activity by 42.27% over control. However, both PA and SA have recover some effect on submerged tissues by 1.26 & 1.23-fold (Fig. 3) respectively in enzyme activity compared to submerged plants but was not significant between themselves ($P\leq0.05$). Still, the effect of these elicitors may be understood as moderation of SUB1 to resume the activities in support of plant growth and development in a

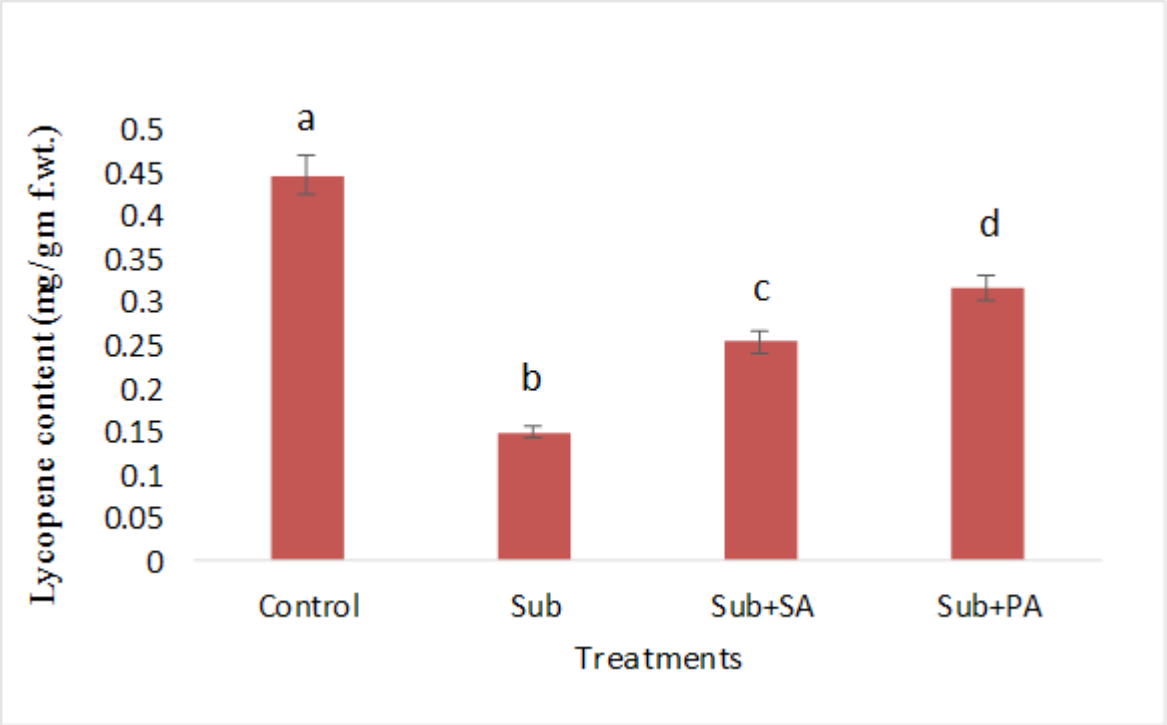

Fig. 5: Assay of lycopene content in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

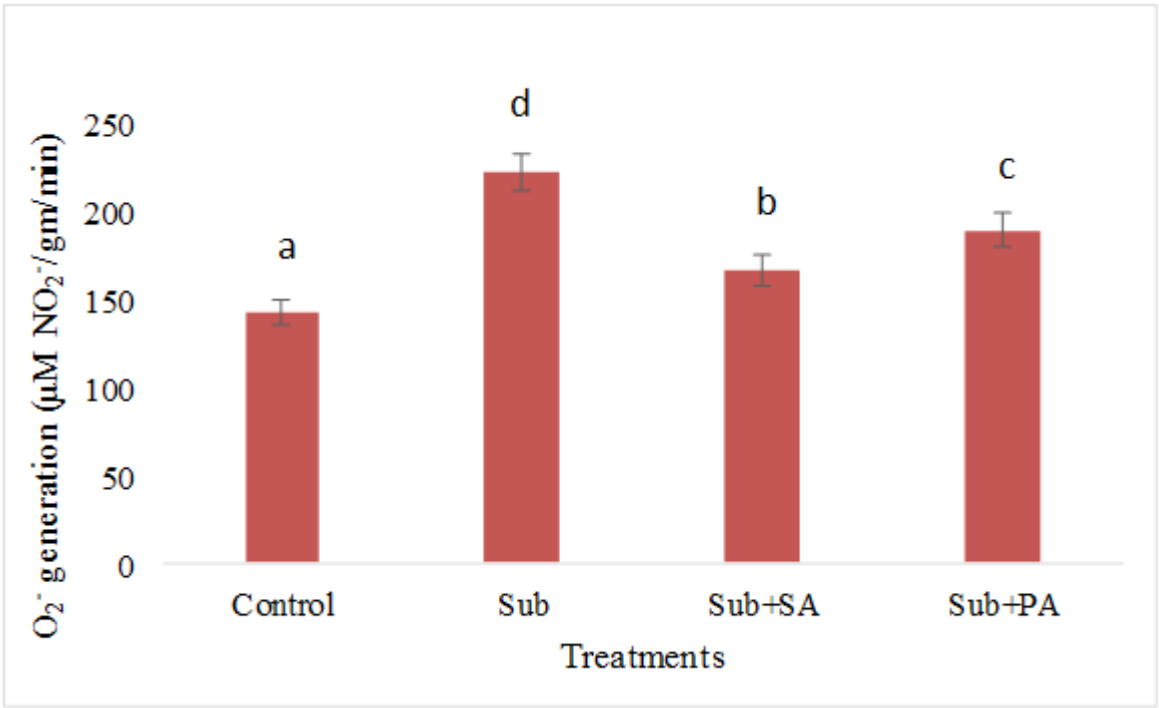

Fig. 6: Assay of $O_2^{\cdot-}$ generation in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

more non-significant manner. The important photosynthetic pigments like carotenoid and lycopene which helps the plant to recover photo-oxidative damages caused by different stressors. In the present experiment, total carotenoid and lycopene contents are being down-regulated by 50.00% and 66.59% respectively over control but when PA and SA are treated, the total carotenoid and lycopene content over up-regulated by 1.30 & 2.12-fold and 1.14 & 1.70-fold respectively as compared to submerged plants (Fig. 4 & 5).

The most critical factor set with sustainability under submergence of plant is a transient oxygenic burst while water level recedes.

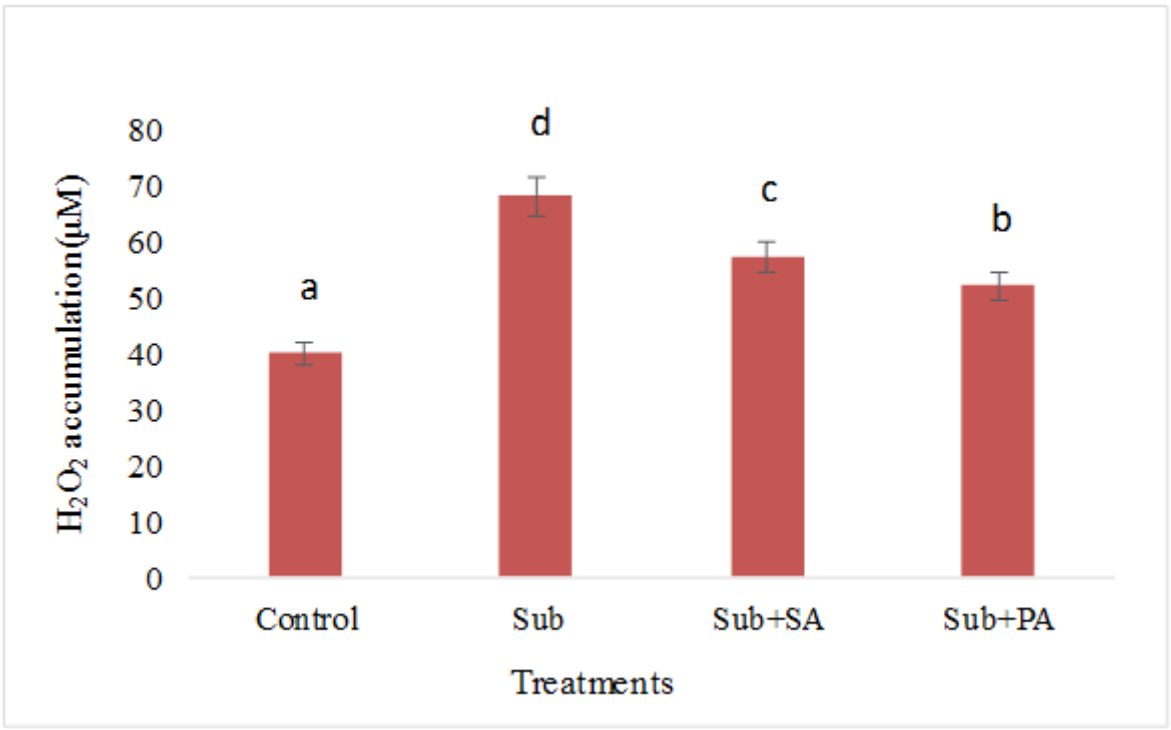

Fig. 7: Assay of H_2O_2 accumulation in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

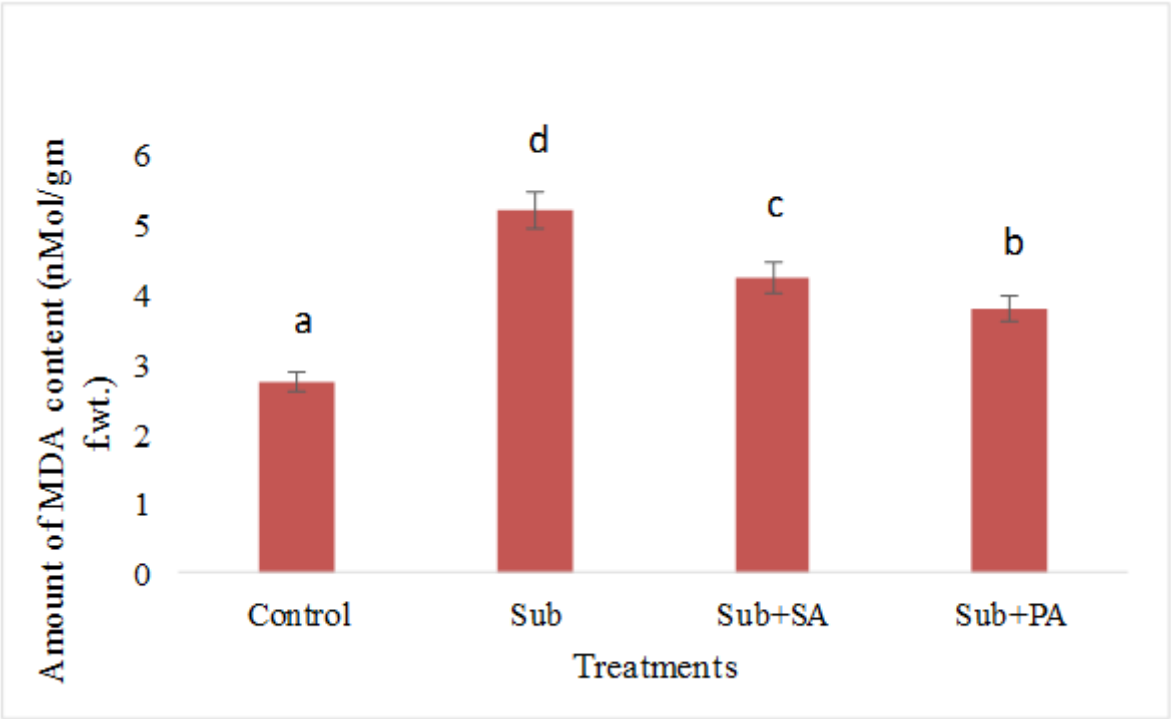

Fig. 8: Assay of MDA content in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

In most of the cases the tolerant land races are quite stable under submergence while that goes in vulnerability when comes up de-submergence. The changes of redox were markedly observed in the present experiment when c.v. Swarna Sub1A accumulated a significant amount of ROS (Fig. 6 & 7). However, it is the merit of SUB1 QTL that it overcomes the consequences of ROS through cellular degeneration of bio-molecules. Thus, when plant accumulated 1.55-fold over expression of apoplastic superoxide ($O_2^{\cdot-}$), a concomitant induction of lipid peroxidation (1.91-fold) was the

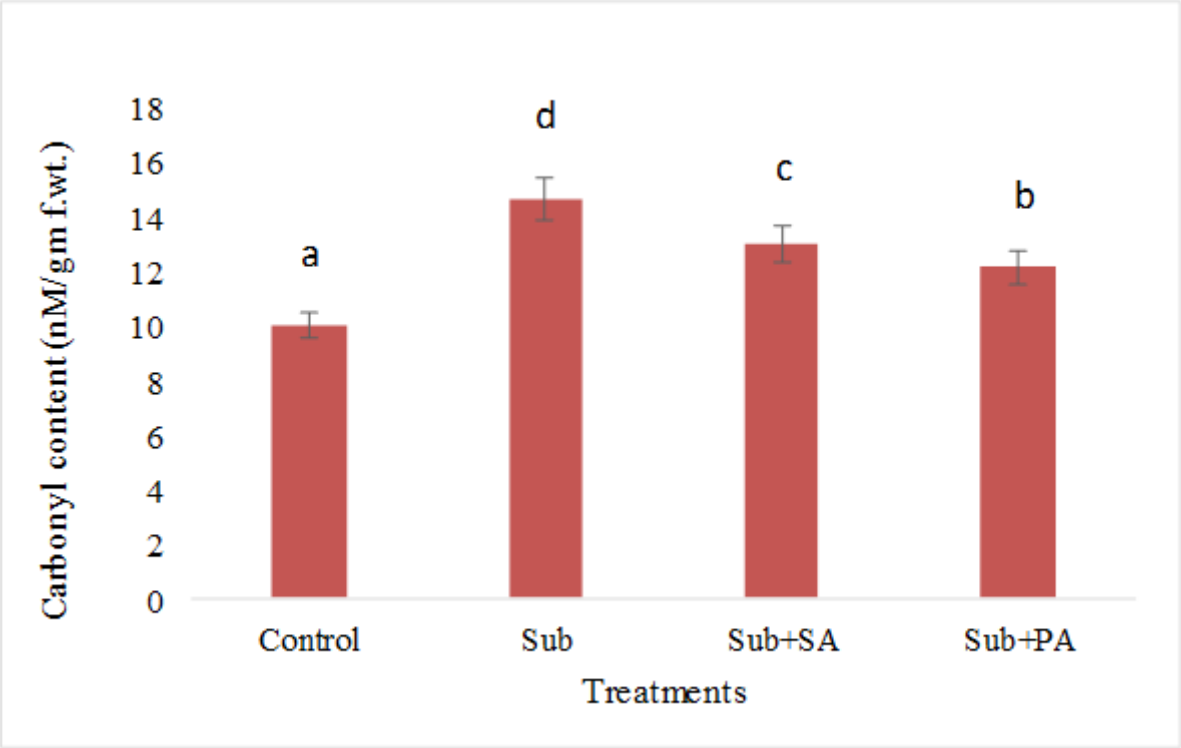

Fig. 9: Assay of carbonyl content in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

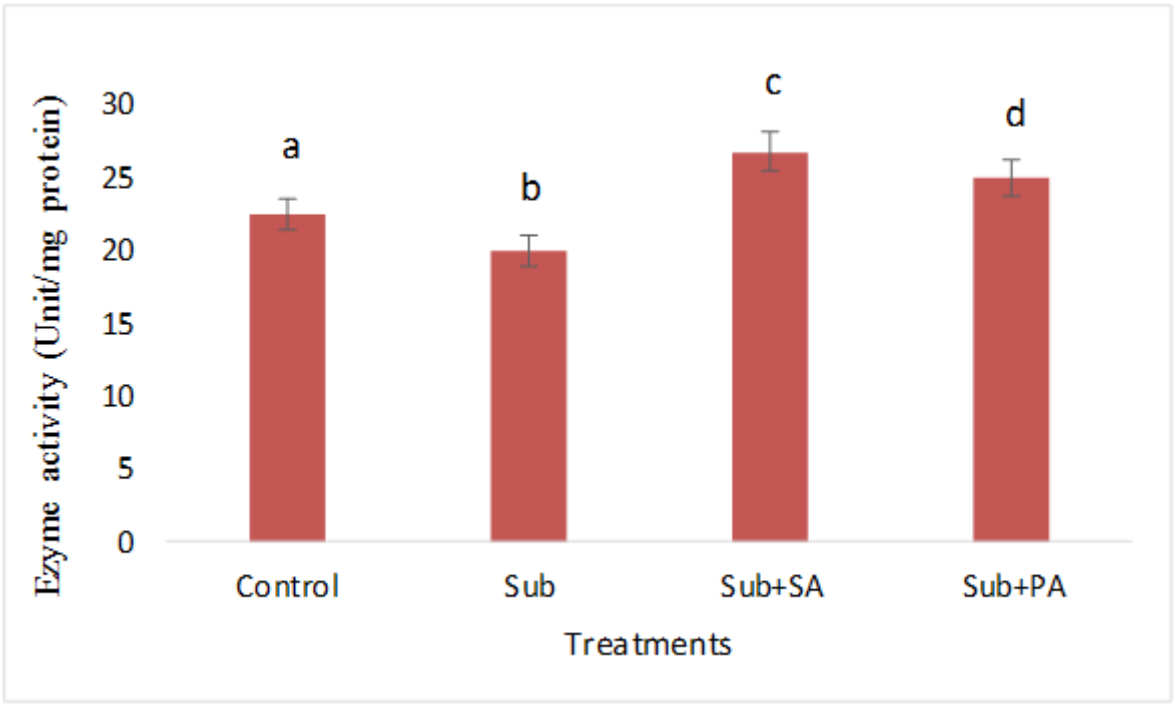

Fig. 10: Assay of SOD activity in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

result (Fig. 6 & 8). At significant level ($P\leq0.05$), the amount of peroxides by ROS induced reactions characterize the oxidative stress. H_2O_2, another ROS however, not a free radical realized the similar trend in their accumulation (1.70-fold than control) in rice varieties (Fig. 7). The product of H_2O_2 mediated protein-carbonylation was also accompanied with lipid peroxide by 1.46-fold and also support the oxidative stress under de-submergence. But both PA and SA at the compatibility to moderate level, down-regulate the $O_2^{\cdot-}$ generation (14.80% & 25.11% respectively) and

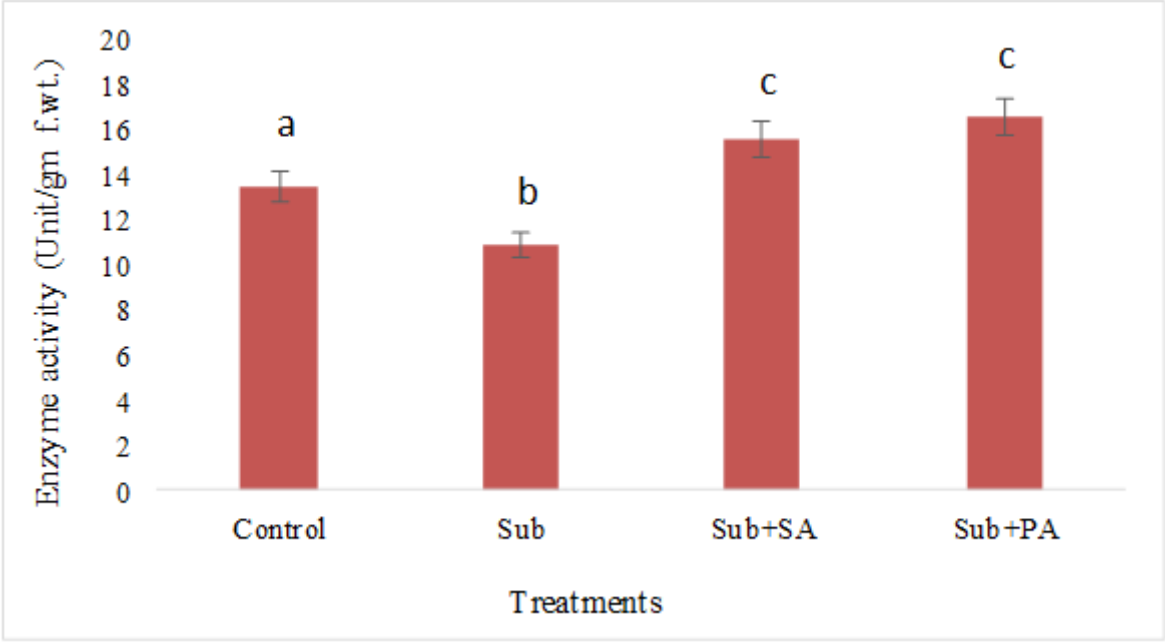

Fig. 11: Assay of GPX activity in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

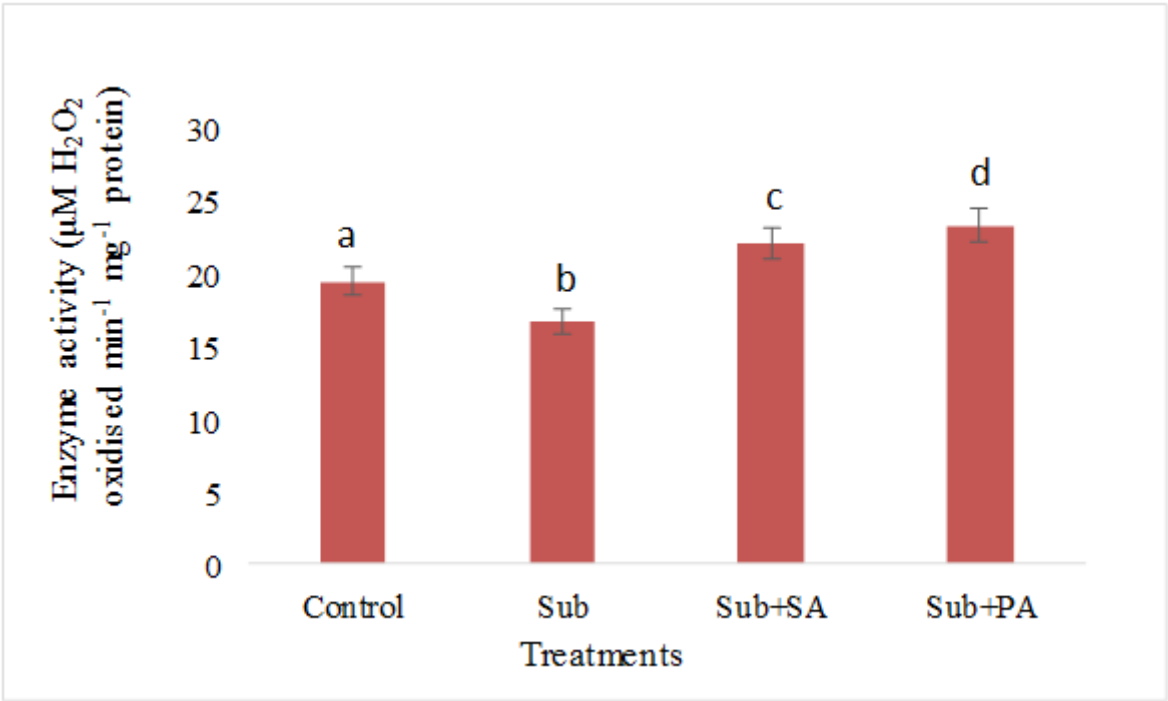

Fig. 12: Assay of CAT activity in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

H_2O_2 generation (23.53% & 16.17% respectively) in a significant manner to sustain oxidative stress (Fig. 6 & 7). Regardless of these two, the relief of macromolecule oxidation was satisfactory for MDA (Malondialdehyde) and carbonylated derivatives by the 27.58%, 18.77% & 17.03%, 11.44% respectively by PA and SA (Fig. 8 & 9).

The chemical messengers as PA and SA are supposed to be good reliever of the oxidative stress of Swarna Sub1A is evident from anti-oxidative enzyme activities. SOD is the first line of defense to ROS lyses the $O_2^{\cdot-}$ into H_2O_2. Thereby, the elevated activity as recorded in present experiment undoubtedly scores the plant potential to tolerance. Thus, PA and SA mediation activities are modulated by upregulation with 1.25-fold and 1.34-fold respectively (Fig. 10). Development of the peroxide and its downstream effect is another criterion for oxidative stress from the tissue

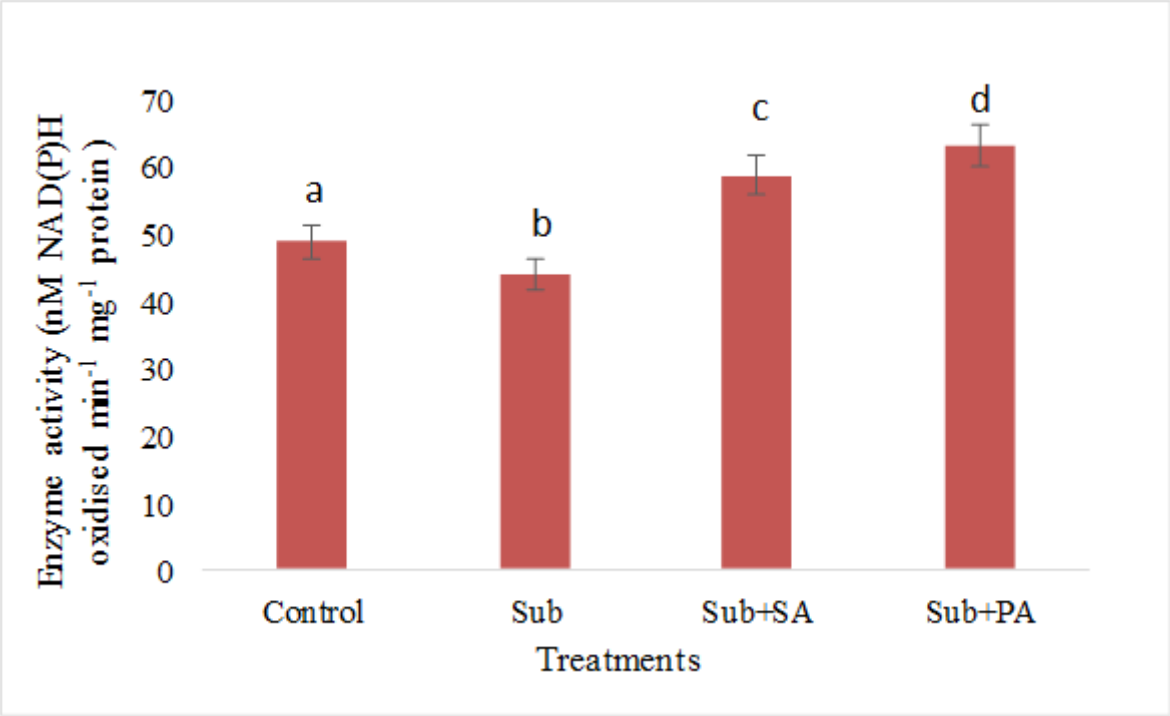

Fig. 13: Assay of GR activity in Swarna Sub1A under Normal (control), Submerged, Submerged+Salicylic acid (1mM) and Submerged+Putrescine (2mM) treatment for 5 days. Data represented as mean of three replicates (n=3) ±SE. SE is represented as vertical bar on each column. Bars showing different letters indicate significant differences according to Duncan's test at $p<0.05$.

extract the soluble peroxidase was recovered and in guaiacol mediated reaction it was recorded some discriminatory results. Submergence had not scored any significant changes in GPX activity. Thus, shows the sensitivity to ROS development under submergence (Fig. 11). However, two elicitors PA and SA were not much variable in there over expression between them, still 1.52-fold and 1.43-fold over expression as compared to submergence was distinguishing. The validity of nature for both PA and SA was also reflected when catalase (CAT) activity was concern. Plants responded in lysis of H_2O_2 by catalase was not promising under submergence. The escape from ROS as stress was also evident for CAT when PA and SA were registered. The degrees of changes in CAT activity were record by 1.39-fold and 1.31-fold for PA and SA (Fig. 12). On the contrary, the maintenance of redox in terms of glutathione metabolism was most striking in the present experiment. The fall of glutathione was evident under submergence by 10.03% as compared to control but recovered by PA and SA in the order of 1.44-fold and 1.33-fold respectively (Fig. 13).

Discussion

From the background of submergence sensitivity of rice plant, two major adaptations are observed: tolerance to water stress under submergence and reduction of oxidative damages of de-submergence/aeriation (20). However, irrespective of rice varieties prone to submergence either quiescence or escape strategies are followed. Swarna Sub1A, the present experimental material is well understood with its quiescence strategy where ethylene mediates suppression of stem elongation factors is the key factor. Thus, either degradation of bioactive gibberellins (GA) or its down-regulation of synthesis or both may be expected. Therefore, a suppression of growth is the result and that must be affected through different physiological components. In the present study, few related physiological responses are highlighted which could emphasize the different induced cellular anomaly under submergence. Initially, the suppression of the growth which is related to utilization of total photosynthates (carbohydrates) under water is much effected. A significant falling of total carbohydrate was documented as compared to control during 5 days submergence. All the replicate sets of the variety scored their maximum tolerance within 5 days (data not presented herein). Interestingly PA and SA were quite satisfactory to relive the loss of total carbohydrates as compared to submergence. The efficiency of PA and SA more likely are related to increase the stomatal conductance for CO_2 and down-stream conversion into carbohydrates. Moreover, the induction of membrane permeability to induce the PA to reach the reaction center of photosystem to shield against developing ROS has been documented and thereby, retention of photosynthetic activities. PA and SA, both owing to their negative charge domain may be a shielding capacity over the positively charged membrane and other macro-molecules (21). The roles of these elicitors have almost a similar promotive effect when alpha-amylase activity was recorded. In quiescence strategy of plants,

particularly, the rice varieties having SUB1 allele are characterized to induce ethylene for down-regulation of few genes, α-amylase being the predominant of those (22). Undoubtedly, less exhaustion of carbohydrates through solubilization for respiratory flask is the prime determinant for quiescence strategy. PA and SA are substantially complemented to induce anaerobic metabolism by over expressed amylase activity as found in present experiment. This is quite corroborated with a consumption of carbon most efficiently under submergence in SUB1 possessing variety (23). The enrichment of photosynthetic activation as good as non-submerged conditions and its sustainability may be another factor for submergence tolerant line. Swarna Sub1A is no exception of this where the synthesis of accessory pigments like carotenoid and lycopene with chlorophyll were maximally bothered. Therefore, alternative pigment system must be approachable to protect the loss of carotenoids and lycopene in a way that photo oxidation might be moderated (24). In present experiment, PA and SA are most approachable to sustain both the pigments, however, not comparable but compatible to control plants.

In the second module of submergence stress rice plants are very often encounter with an elevated oxygen tension ensuring the oxidative stress. Development of the ROS occurs, particularly, when the tissues are under de-submergence with a high oxygen partial pressure complemented with high irradiance. Multiple changes in tissues integrity and function are the results of such oxidative stress (25). In the present experiment, the accumulation of ROS in the form of $O_2^{\cdot-}$ and H_2O_2 are the results of such varietal performances for Swarna Sub1A. In a complication of few factors concerning oxidative stress, dehydration, photo inhibition, nutrient deficiency, specific ion toxicity etc cause almost complete collapse of cellular functionales to pre-mature senescence in rice land races (26). However, for Swarna Sub1A, it is not ambiguous that plants would be more sensitised with $O_2^{\cdot-}$ and H_2O_2 accumulation and thereby, a significant loss of membrane permeability might be obscured (27). The loss of membrane permeability might be accounted with an inherent phenomenon of lipid and protein oxidation under submergence (28). The Swarna Sub1A has earlier been nicely characterized with its cellular integrity for growth and development both under submergence as well as de-submergence. Still, the degenerative process leading to membrane functioning in tolerant line in the present experiment may undoubtedly confirm the roles of ROS. More so, SUB1 allele has also been influencing to the responses of ethylene sensitivity under situation of ROS activation. The later becomes an enhancer in down regulation of genes related to inter-nodal elongation rendering a suppressed growth (29). Besides the $O_2^{\cdot-}$, the other one, H_2O_2 not a free radical has more been related to SUB1 QTL (30). Ethylene signaling pathway under conditions of hypoxia following aeration results in up and/or down-regulation of stress-related genes. Lipid and protein profile and its modification as elucidated in the present experiment with PA and SA are contextual indeed. The peroxidation reaction in Swarna Sub1A with its significant variation from control might be granted as a cellular bio-marker for extent of oxidative stress. Both the moieties in cellular pH undergo dissociation to positively charged residues and establish electrophilic interaction with negatively charged domain of cellular membranes (31). The low yielding land races having low to moderate phrase of SUB1 allele might be more accomplished with their lesser membrane oxidation by PAs and SA. Therefore, the signalling of ROS to elicitor molecules (e.g. PA and SA) would also be relevant in submergence tolerance paths. In relation to ROS, the present experiment realised a distinct variation in antioxidation cascade are also over expressed with changes of submergence to re-aeration of the rice variety.

PA induced enhancement of antioxidative enzymes like SOD, GPX, CAT and GR have earlier been corroborated with anoxia related stress in crop plants (32). Still there is a question whether the SUB1 allele is constitutive or induces the anti-oxidation gene expression in nature. There is a distinct pattern and variation for the land races of rice with SUB1 or SNORKEL elements varied under submergence and de-submergence (re-aeration). Whatever the cases might be whether anaerobic signal could induce the set of gene expression (including carbohydrate catabolism) are varied with anti-oxidation genes as the plants recede the water level. Therefore, as biomarkers anti-oxidative proteins like SOD, GPX, CAT and GR would typify the changes of redox (oxidised: reduced) under high oxygen tension coupled with high re-aeration on de-submergence. In the present study, this is no exception of the fact that Swarna Sub1A may possess the secondary traits for anti-oxidation expression along with suppression of the growth under water. In general, there is a common observation that exogenous application of SA is manifested into total PA pull in plants (33). SA is interacted in a more complex pathway, particularly under stressful condition with other hormonal or growth regulators. In fact, SA with its glycosides has also been reported to down-regulate ethylene biosynthesis. In optimal concentration of ethylene with the salt induction is over expressed by its precursor 1-amino cyclopropane (ACC). On the other hand, ACC is the downstream moieties of S-adenosyl L-methionine (SAM). The later is shared with PA and ethylene as a common precursor. Therefore, the biosynthesis of PA and ethylene is considered to be competitive under abiotic stress. So, a line of involvement for ethylene mediated quiescence strategy found in SUB1 locus in rice may be justified with SA and PA

(34). Moreover, the modulation of enzyme activities by PA and SA interference also demands the recognition of regulatory step(s). This may have the possibility to over-express that might circumvent the oxidative loss of plants under submergence to de-submergence.

Conclusion

The present study is dealt with physiological and cellular responses of rice cultivar possessing the SUB1 QTL. The inherent activity of SUB1 though based on a suppression of the growth of rice under water, its post submergence oxygenic stress may not be avoided. It is the ROS, the factor of submergence mediate loss of tissue and could be reversed by application of Polyamine (PA) and Salicylic acid (SA). This also conceive the probability of introduce polyamine and jasmonic acid metabolism interference to moderate the submergence stress in the genotypes. Therefore, the biochemical paths leading the anti-oxidation may be deciphered from de-submergence mediated oxidative shock and could be compared with other moderate to high yielding, however, submergence sensitive land races. Likewise, the regulatory elements for quiescence strategies compounded with mechanism of oxidative tolerance would be the key selection pressure under submergence of rice cultivars.

Conflict of Interest

The authors have no conflict of interest.

Authors' Contribution

BS conducted the whole experiment following collection of data. AKD done statistical analysis and drafted the manuscript with collection of references. MKA hypothesised, designed the experiment, discussed the findings with scientific justification and over all written the whole manuscript.

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