Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

IN-BUILT ANTIOXIDATION CAPACITY OF *sub1A* QTL IN RICE (*Oryza sativa* L.) AND ITS MODULATION BY EXOGENOUS APPLICATION OF POLYAMINE AND NITRIC OXIDE

Indraneel Saha, Debabrata Dolui, Arijit Ghosh, Malay Kumar Adak*

Plant Physiology and Plant Molecular Biology Research Unit, Department of Botany, University of Kalyani, Kalyani, 74 1235, W.B., India

Received – April 07, 2020; Revision – May 23, 2020; Accepted – June 01, 2020

Available Online – June 25, 2020

DOI: [http://dx.doi.org/10.18006/2020.8\(3\).210.223](http://dx.doi.org/10.18006/2020.8(3).210.223)

KEYWORDS

sub1A QTL

Inbuilt antioxidation capacity

Put

SNP

Physiological parameters

ABSTRACT

In rice submergence or flooding stress is secondarily manifested into an oxidative stress. *sub1A* quantitative trait locus (QTL), an ethylene response element is the key to submergence tolerance in resistant rice genotypes. In this paper the induction of two elicitors: putrescine (Put) and sodium nitroprusside (SNP) have been tested for their efficacy in antioxidation paths in cv. Swarna and Swarna Sub1. Initially, total polyamines (PAs) pool was over expressed under Put for both the cultivars but SNP was insensitive to response. On the contrary, SNP recorded more inducing for free amino acids and proline content, however, elaborately in cv. Swarna Sub1. Through path analysis SNP established more contributory on cv. Swarna Sub1 in moderation of glutathione. SNP was more effective than Put irrespective of cultivars with phenolics, flavonoids and anthocyanin content. Responses of SNP were more promoting to DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] and ferric chelation except the phosphomolybdenum assay in cv. Swarna Sub1. Both in histochemical analysis and content of superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) were differed irrespective of cultivars. Finally, phenylalanine ammonia lyase (PAL) activity ensured the efficacy of Put and SNP in compatible manner possibly to induce the non enzymatic antioxidation pathways. Therefore, the extent elicitation through inbuilt antioxidation capacity in rice offered by *sub1A* QTL recorded significantly varied and that may be the key to better adaptability to submergence induced oxidative stress as discussed.

* Corresponding author

E-mail: mkadak09@gmail.com (Malay Kumar Adak)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
(<http://www.horizonpublisherindia.in/>).
All rights reserved.

All the articles published by [Journal of Experimental Biology and Agricultural Sciences](#) are licensed under a [Creative Commons Attribution-NonCommercial 4.0 International License](#) Based on a work at www.jebas.org.

1 Introduction

Cereals like rice suffer from wider ranges of abiotic stress sensitivity mostly focused to limitation of water (Larkunthod et al., 2018). The cellular dehydration, a key factor for perception of any abiotic stress may also be available even under water excess. Rice could be illustrated as suffering from osmotic deficit under prolong period of submergence (Locke et al., 2018). Under submergence and post submergence period rice cultivars are significantly varied with root hydraulic conductance following curtail of water relation. This is also complemented with development of deficit of aerobic status of the tissues perpetuating the changes in redox (Saha et al., 2018a). This is typified with rice plants where on recede of water from submergence induces another mode of abiotic stress. The aerial shoot on rapid expose to high oxygen tension with intense illumination develops reactive oxygen species (ROS). Consequently, plants are prone to oxidative damages by over expressed peroxidation reactions resulting tissue lysis. It becomes the critical for maintenance of redox with stored oxygen under submergence (Bui et al., 2019). Rice genotypes are significantly varied with regards to aerobic sustenance under submergence either as susceptible or tolerant. Therefore, submergence stress in rice is inherently concurrent to oxidative stress. Tolerant genotypes ought to possess with improved antioxidation while the plants facing an oxidative exposure at post submergence.

In antioxidation paths a number of signalling residues are commonly found in stress perception and its downstream reactions. Few such residues in plant species are freely diffusing across the membrane, easy to conjugate with organic residues, compatible to cellular pH (Sadiq et al., 2018). Polyamines (PAs) are simple straight chain hydrocarbon residues, free or in glycosides are frequently found in moderation of abiotic stresses. Amino acids (ornithine/methionine) are precursor of PAs biosynthesis and can be diverged into intermediate paths leading nitric oxide (NO) generation. NO has been less frequent as plants' signalling residue under submergence mediated oxidative stress. It is still reported that there is an inverse relationship with biosynthesis of PAs and NO according to cellular demand to meet the water stress. Still, this would often be a question for any direct involvement of PAs or NO either independently or in combination (Mattoo & Sobieszczuk-Nowicka, 2019). NO has often being implicated in guard cell regulation along with abscisic acid (ABA) functioning pathways. NO is also referred with equivalence of signal compounds similarly others like hydrogen peroxide (Domingos et al., 2015). Under soil moisture deficit roots are over produced with ABA even with exogenous NO donation. This draws attention for NO in a synergistic action with signalling molecules like PAs. PAs mediated plants' responses may match with application of NO in few mutants of *Arabidopsis* (Sánchez-Rangel et al., 2016). This work indicated that oxidative stress inducing ROS production

could alleviate by NO interaction. Therefore, either PAs or NO has been quite a distinct approach in stress reactions and its resultant into tolerant or susceptible genotypes.

In the present study selected rice genotypes differing in submergence tolerance with possession of *sub1A* QTL. This is well understood that *sub1A* QTL is accomplished with tolerance to submergence induced anaerobic stress. This is undergone another mode of tolerance when post submergence period establishes an oxidative burst in the tissues. Rice genotypes with *sub1A* QTL mediated functioning appears to be a good tolerant to such an oxidative stress. Therefore, any chemical signalling with PAs and NO for *sub1A* QTL inducing antioxidation mechanism is expected to be relevant. Thus, current study exercised rice genotypes cv. Swarna and Swarna Sub1 (as submergence susceptible and tolerant respectively) with application of Put (a di-amine) and SNP (NO donor residue) to monitor their inbuilt antioxidation capacity. The information is expected to contribute the selection pressures for rice genotypes with possibility of PAs and NO dependent mechanism in submergence tolerance.

2 Materials and Methods

2.1 Development of rice seedlings, treatments and sampling for analysis

The present experiment was materialized in the experimental garden and laboratory of Plant Physiology in the department of Botany, University of Kalyani, Kalyani (22°58'30"N, 88°26'04"E), West Bengal, India. Viable seeds of rice (*Oryza sativa* L.) cultivar were used for this study as experimental material and collected from farmers' field with proper identification and authentication for cv. Swarna (submergence susceptible) and Swarna Sub1 (submergence tolerant). Germinated seedlings were grown in 1/4th Murashige and Skoog medium (Murashige & Skoog, 1962). This was maintained for 7 days under the condition of appropriate light and dark cycle (16L: 8D), light intensity of 900-1000 $\mu\text{Em}^{-2}\text{S}^{-1}$ of photon flux density, 85% of relative humidity, 28 $\pm$ 5 °C of temperature. 15 days old seedlings were transplanted in 1/4th strength of Murashige and Skoog medium supplemented with different treatments of Put (2 mM) and SNP (1 mM) along with a control set (0 mM Put and SNP) in complete randomized block design (RBD) and kept for 3 days to acclimatize all the treatments. After completion of the treatment plants were washed repeatedly with de-ionized water and freezed in liquid nitrogen following transfer to cold storage (-80 °C) for different assays.

2.2 Separation and quantification of PAs by thin-layer chromatography (TLC)

Fresh plant sample was homogenized in 5% (v/v) perchloric acid (HClO₄) following centrifugation at 12,000 Xg for 15 min at 4 °C.

Extracts were separated on TLC plate (Whatman LK6D) in solvent system of cyclohexane/ethylacetate (5:4, v/v) and standards of PAs were run in parallel. For quantification of PAs, bands were scraped into 2 ml of ethylacetate and fluorescence was read at 500 nm (excitation) and 350 nm (emission) with the help of a spectrophotofluorometer (Young & Galston, 1983).

2.3 Determination of amino acids and peptides

Free amino acids content was determined with the help of 80% aqueous ethanol. In a reaction mixture of saturated ninhydrin (20 g ninhydrin in 500 ml of methyl cellosolve) the concentrate fraction of amino acids was incubated using equal volume of water and n-propanol as diluents. The absorbance was read at 570 nm by UV/VIS spectrophotometer (Cecil, CE7200) and expressed on fresh weight basis with standard as leucine (Showler, 2002).

For determination of proline fresh plant tissue was homogenized in 3% aqueous sulphosalicylic acid following centrifugation at 10,000 Xg for 15 min at 4 °C. The aqueous phase was reacted with ninhydrin solution (1.25 g ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid) following boiling in water bath and transferred to ice bath for cooling the upper phase with saturated toluene. From the clear solution the amount of proline was calculated by reading the absorbance at 520 nm by UV/VIS spectrophotometer (Bates et al., 1973). L-proline was used for calibration within range of 0.01- 0.1 µM per ml.

For assay of reduced glutathione or GSH (EC: 1.8.1.7) plant sample was homogenized in trichloroacetic acid (TCA) solution under cold condition following centrifugation at 15,000 Xg at 4 °C. The supernatant was taken in 0.1 mM phosphate buffer (pH 8.0). An aliquot of supernatant with the assay mixture [phosphate buffer and 0.1% (w/v) *o*-phthalaldehyde] was incubated at room temperature and fluorescence intensity was read at 420 nm (excitation) and 350 nm (emission) (Kocsy et al., 2001). For oxidized glutathione or GSSG (EC: 1.8.1.7), the supernatant was incubated with 0.4 M N-ethylmaleimide (NEM) for 30 min following diluting with 0.1 N sodium hydroxide (NaOH) and pH was adjusted to 12.0. Thereafter, an aliquot of the mixture was added to the same buffer as taken for GSH except 0.1 N NaOH and fluorescence intensity was read at 420 nm (excitation) and 350 nm (emission). With the help of standard glutathione the content of GSH and GSSG were measured using same reference.

2.4 Determination of total phenolics, flavonoids and anthocyanin content

Total phenolics were extracted from ethanolic extract of the plant sample by centrifugation at 10,000 Xg for 15 min at 4 °C. An aliquot of supernatant (0.5 ml) was reacted with Folin-Ciocalteu reagent. Thereafter, 20% sodium carbonate was added to it and the

mixture was subjected to incubation in a boiling water bath for 1 min. Finally, the mixture was cooled and the absorbance was read at 650 nm by UV/VIS spectrophotometer. To determine the total phenolics content, gallic acid was used as standard (Mohsen & Ammar, 2009).

From the ethanolic extract of the plant sample the supernatant was re-extracted with the help of n-butanol-water (1:1) and concentrated under vacuum at 50 °C. Thereafter, the residue was dissolved in 50% ethanol following addition of 5% sodium nitrite (NaNO₂) and 10% aluminium nitrite [Al(NO₂)₃]. Then the reaction was stopped with 1 M NaOH solution. Absorbance was read at 510 nm by UV/VIS spectrophotometer after diluting the supernatant with 30% ethanol. To determine total flavonoid content, quercetin was used as standard (Basu et al., 2010).

Alcoholic leaf extract of the plant sample was diluted with potassium chloride (KCl) buffer (pH 1.0) and the other with sodium acetate (CH₃COONa) buffer (pH 4.5). Absorbance was read at 510 and 700 nm against a blank. Then the monomeric anthocyanin pigment was calculated taking cyanidine-3-glucoside as standard (Jansen & Flamme, 2006).

2.5 Assay of DPPH, ABTS, phosphomolybdenum and ferric chelation

For DPPH scavenging assay, alcoholic plant extract was incubated with 25 mg/l DPPH solution for 30 min at room temperature. The absorbance was read at 517 nm by UV/VIS spectrophotometer with butylated hydroxytoluene (BHT) as synthetic scavenger (Dey et al., 2012).

ABTS⁺ radical cation scavenging assay was performed with the alcoholic plant extract of plant sample. 0.1 ml of extract was reacted with 3 ml of ABTS solution in 2.4 mM potassium persulphate (K₂S₂O₈) buffer. The absorbance was read at 734 nm by UV/VIS spectrophotometer using BHT as synthetic standard (Re et al., 1999).

For phosphomolybdenum assay, alcoholic plant extract was incubated with 1 ml of reagent solution containing 0.6 M sulphuric acid (H₂SO₄), 28 mM sodium phosphate (Na₃PO₄) and 4 mM ammonium molybdate [(NH₄)₂MoO₄] following agitation on thermal block for 90 min at 95 °C and cooled to room temperature. The absorbance of upper aqueous phase was read at 820 nm by UV/VIS spectrophotometer (Ferreira et al., 2007).

Leaf extract was incubated with 0.1 ml of 2 mM iron chloride (FeCl₂) for 3 min followed by addition of 0.2 ml of 5 mM ferrozine solution. On equilibrium for 10 min at room temperature, absorbance was read at 562 nm by UV/VIS spectrophotometer (Dinis et al., 1994).

2.6 Histochemical detection and content of O_2^- and H_2O_2

Histochemical detection of O_2^- was performed by thoroughly washing of leaves and roots with de-ionized water and incubated with 50 mM phosphate buffer containing 6 mM Nitroblue tetrazolium (NBT) (pH 4.8) for overnight. After that, the leaves were made de-chlorophyllous by the help of lactic acid-glycerol-ethanol solution (1:1:4 v/v/v) and boiled in water bath for 10-15 min. The dark blue patches of formazan compound on leaves and roots were captured by digital camera (Dewinter) (Meng et al., 2012).

For determination of O_2^- generation, fresh plant sample was homogenized in 65 mM phosphate buffer (pH 7.8) under cold condition following centrifugation at 8,000 Xg for 30 min at 4 °C. An aliquot of the supernatant (100 μ l) was added to 3 ml of assay mixture containing 65 mM phosphate buffer (pH 6.8), 10 mM hydroxylamine and subjected to incubation for 30 min at room temperature. To this solution, 10 mM sulphanilamide with 7 mM α -naphthylamine was added and kept under dark for sufficient time. Absorbance was recorded at 530 nm by UV/VIS spectrophotometer. To determine O_2^- generation, NO_2^- was used as standard (Eltner, 1976).

Histochemical detection of H_2O_2 was performed by incubating leaves and roots with freshly prepared 5 mM 3,3'-Diaminobenzidine (DAB) solution in phosphate buffer for overnight at dark and pH was adjusted to 3.8. After that, the leaves were made de-chlorophyllous with lactic acid-glycerol-ethanol solution (1:1:4 v/v/v) and boiled in water bath for 10-15 min. The distributions of H_2O_2 were captured by digital camera (Dewinter) (Li et al., 2010).

For determination of H_2O_2 accumulation, fresh plant sample was homogenized in 2 ml of 1% (w/v) TCA solution following centrifugation at 10,000 Xg at 4 °C. The supernatant was treated with 10 mM potassium phosphate buffer (pH 7) and 1 mM potassium iodide (KI) following incubation in dark at room temperature. Finally, the absorbance was read at 390 nm by UV/VIS spectrophotometer using H_2O_2 as standard (Kumar et al., 2014).

2.7 Assay of phenylalanine ammonia lyase activity

Plant sample was extracted in phosphate buffer (pH 7.8) following centrifugation at 12,000 Xg for 15 min at 4 °C. An aliquot (0.5 ml) of the supernatant was reacted with 2.5 ml of a 0.2 % solution of L-phenylalanine in 50 mM tris-HCL. The incubation of the reaction mixture was set for 24 hrs at room temperature and the absorbance was read at 290 nm by UV/VIS spectrophotometer (Peltonen & Karjalainen, 1995).

2.8 Statistical analysis

The data was represented with mean value $\pm$ SE for 3 replications of each parameter in this experimental work and the statistical analysis was done through student's *t*-test (SPSS software IBM, USA) and analysis of variance (ANOVA). Significant variation was indicated by as * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$).

3 Results

3.1 TLC of PAs and their quantifications

The separation and detection of PAs profile through chromatography separation has revealed its quantification following specific spectrophotometric methods (Figure 1A). Result of study revealed that accumulation of PAs in both cv. Swarna and Swarna Sub1 are dependent to application of external elicitors. Likewise, Put when applied in the rice seedlings, a significant ($P \leq 0.001$) up regulation by 2.65 and 2.84 fold over control was recorded for cv. Swarna and Swarna Sub1 respectively (Figure 1B). On the contrary, SNP acted as inhibitor when both the cultivars had a non significant change as compared to control. Therefore, the opposing effects of two elicitors (Put and SNP) may not be any selection pressure for total PAs accumulation in these rice cultivars.

3.2 Amino acids and peptides

To check the possible changes for Put and SNP mediated cellular responses, initially plants recorded a significant variation ($P \leq 0.05$, 0.01) in amino acids content. This was monitored in the concentrations of total free amino acids pool (Figure 2A) and proline, a stressed induced amino acid (Figure 2B). For Put and SNP, it showed that the two rice cultivars had their maximum accumulation for proline especially in cv. Swarna Sub1. The maximum accumulations of proline were 1.21 and 1.65 fold than control under Put and SNP. However, free amino acids content had the peak values with 1.14 and 1.42 fold than control for the same in cv. Swarna Sub1 when amino acids extracted from Put and SNP treatment. Considering the inter-conversion of amino acids into their downstream peptides glutathione was considered. The cellular concentration of glutathione was computed reduced (GSH) to oxidized (GSSG) and it had the maximum reduction (Figure 2C). The subdue reduction of GSH/GSSG were in the order of 23.07 and 63.63% less over the control in cv. Swarna. Still, cv. Swarna Sub1 had been less sensitive to effect GSH:GSSG than the other variety by 9.09 and 46.15% reduction under Put and SNP as compared to control. Therefore, the rice cultivars were markedly varied as an induction to chemical elicitors to establish their sensitivity in downstream activities.

Figure 1 Total PAs content in two rice cultivars (cv. Swarna and Swarna Sub1) under Put (2 mM) and SNP (1 mM) treatments for 72 hrs. Data presented in bars with $\pm$ SE (n=3) from independent experimental sets and the significant difference between two cultivars under each treatment was represented as * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) by student t- test. (A) Separation and distribution of Pas (Put- Putrescine, Spd- Spermidine, Spm- Spermine) from plant extract through TLC in solvent system of cyclohexane/ethylacetate against standard of PAs. Distinct spot may locate the individual PA as marked within the plate and (B) changes of total PAs content

Figure 2 Amino acids and peptides content in two rice cultivars (cv. Swarna and Swarna Sub1) under Put (2 mM) and SNP (1 mM) treatments for 72 hrs. Data presented in bars with $\pm$ SE (n=3) from independent experimental sets and the significant difference between two cultivars under each treatment was represented as * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) by student t- test. (A) Changes of free amino acids content (B) Changes of proline content and (C) Changes of GSH:GSSG activity

Figure 3 Different potential antioxidants content in two rice cultivars (cv. Swarna and Swarna Sub1) under Put (2 mM) and SNP (1 mM) treatments for 72 hrs. Data presented in bars with $\pm$ SE ($n=3$) from independent experimental sets and the significant difference between two cultivars under each treatment was represented as * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) by student t- test (A) Changes of total phenolics content (B) Changes of total flavonoids content and (C) Changes of total anthocyanin content

3.3 Total phenolics, flavonoids and anthocyanin content

Total phenolics and flavonoids content recorded a significant variation ($P \leq 0.05$, 0.01) between two rice cultivars, however, under Put and SNP treatment except anthocyanin content where it showed no such significant variation under the same treatments. It was the cv. Swarna Sub1 where total phenolics, flavonoids as well as anthocyanin content were greater than the other cultivar. Moreover, it was SNP treatment where all these contents were more than Put. In case of total phenolics and flavonoids content as shown in Figure 3A and Figure 3B there recorded increase of 1.18 and 1.69 fold under Put and 1.30 and 1.84 fold under SNP in cv. Swarna respectively. Likewise, in cv. Swarna Sub1 there recorded 1.19 and 1.68 fold increase under Put and 1.35 and 2.17 fold increase under SNP for the same. Anthocyanin content was varied but not significant even under control condition where cv. Swarna had the maximum accumulation by 1.14 fold than cv. Swarna Sub1 (Figure 3C). Interestingly, the responses of plants received some marked changes when elicited by Put and SNP where cv. Swarna Sub1 had a better induction for anthocyanin by 1.5 and 1.8 fold respectively against control than the other cultivar.

3.4 DPPH scavenging, ABTS scavenging, phosphomolybdenum complex and ferric chelation

For both DPPH and ABTS scavenging assay, there was a gradual decrease throughout the treatments however, more in cv. Swarna Sub1. It showed a significant variation ($P \leq 0.001$) between the cultivars under Put treatment only for DPPH assay. There recorded gradual decrease in two cultivars by same per cent under Put treatment over control (Figure 4A). On the other hand there recorded no any significant variation in case of ABTS scavenging assay for both the rice cultivars (Figure 4B). In case of total phosphomolybdenum complex assay, it was only SNP treatment that maintained same trends as control for both cultivars. In this assay a significant variation ($P \leq 0.01$, 0.05 , 0.001) was recorded between cv. Swarna and Swarna Sub1 all through the treatments (Figure 4C). On contrary, same trends as control were followed under Put and SNP treatment in ferric chelation assay and there recorded a significant variation ($P \leq 0.05$, 0.01) only under control and SNP treatment between two cultivars (Figure 4D). An increase of 1.44 and 1.62 fold under SNP treatment over control were documented in cv. Swarna and Swarna Sub1 respectively.

Figure 4 Different antioxidative assays in two rice cultivars (cv. Swarna and Swarna Sub1) under Put (2 mM) and SNP (1 mM) treatments for 72 hrs. Data presented in bars with $\pm$ SE ($n=3$) from independent experimental sets and the significant difference between two cultivars under each treatment was represented as * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) by student t- test. (A) Changes of DPPH radical scavenging (B) Changes of ABTS radical scavenging (C) Changes of total phosphomolybdenum content and (D) Changes of ferric chelation

3.5 Histochemical analysis and production of ROS

Whether the production ROS would be constitutive or any variation of those through the chemical elicitation has also been checked both in vitro and in vivo level. As shown in Figure 5A, B, D and E, a clear tissue specific distribution of $O_2^{\cdot-}$ and H_2O_2 were the features to indicate possible involvement of chemical elicitation. The lamina of the leaves and tap roots were stained with respective histological dyes to locate probable accumulation of ROS. Interestingly, SNP was most effective moderators to $O_2^{\cdot-}$. Still, the relief of oxidative damages was more significantly distinguish for leaflet than root. On quantification of $O_2^{\cdot-}$ generation there recorded a significant variation ($P \leq 0.01, 0.05$) under Put and SNP treatment between two rice cultivars (Figure 5C). But in case of H_2O_2 accumulation it was only the SNP treatment where a significant variation ($P \leq 0.05$) was documented between the cultivars (Figure 5F).

3.6 PAL activity

Rate limiting biosynthetic enzyme (PAL) recorded no any significant variation between two rice cultivars, however, under

Put and SNP treatment. The response of enzyme activity induced through Put and SNP made it more complicated pathways. Regarding PAL activity the most sensitive treatment appear to be SNP with its maximum rate regardless of rice cultivars. As compared to control that variations were 2.27 and 2.58 fold in excess in cv. Swarna and Swarna Sub1 (Figure 6). This is indicative of the fact that the maximum concentration would be differing when the cultivars received the elicitation only through SNP but not with Put.

On critical analysis of variations (ANOVA) of the different antioxidative attributes it clearly indicates those were significantly varied among them in different probability level, however, in each cultivar (Table 1). Moreover, all the parameters were significantly varied ($P \leq 0.05$) in cv. Swarna Sub1 than the other. ROS whether its accumulation by constitutive or inductive pathways would be adjusted by inbuilt antioxidation cascades. This was more evident that out of the antioxidation modules all were most contributory except for the phosphomolybdenum complex mechanism. This held true with reference to cv. Swarna Sub1 as elicited from statistical modules, not in cv. Swarna. May be these cultivars would

Figure 5 Histochemical analysis by different staining and content of ROS in two rice cultivars (cv. Swarna and Swarna Sub1) under Put (2 mM) and SNP (1 mM) treatments for 72 hrs. Data presented in bars with $\pm$ SE (n=3) from independent experimental sets and the significant difference between two cultivars under each treatment was represented as * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) by student t- test. (A) NBT staining in leaves (B) NBT staining in roots (C) Changes of $O_2^{\cdot -}$ generation (D) DAB staining in leaves (E) DAB staining in roots and (F) Changes of H_2O_2 accumulation

Figure 6 Changes of PAL activity in two rice cultivars (cv. Swarna and Swarna Sub1) under Put (2 mM) and SNP (1 mM) treatments for 72 hrs. Data presented in bars with $\pm$ SE (n=3) from independent experimental sets and the significant difference between two cultivars under each treatment was represented as * ($P \leq 0.05$), ** ($P \leq 0.01$) and *** ($P \leq 0.001$) by student t- test

.be defying in tolerance to antioxidation, through constitutive by such a single reaction. Therefore, the other exercised parameters might be reliable for screening of oxidative stress tolerance for rice cultivars if subjected to any oxidative stress. This may also be illustrated further that cv. Swarna Sub1, a submergence tolerant pathway has more constitutive efficiency (even not being exposed to submergence stress) than the other insensitive one like cv. Swarna, herein.

4 Discussion

PAs the most ubiquitous aminated organic residues have been well exercised in relation to abiotic stress tolerance in almost the crop species. PAs are mostly contributed for the stress tolerance with its polycationic nature for binding of negative domain of cell membranes, proteins, nucleic acids and others. This activity is offered in many physiological processes including

Table 1 ANOVA of different biochemical attributes in two rice cultivars (cv. Swarna and Swarna Sub1) under Put (2 mM) and SNP (1 mM) treatment for 72 hrs.

Parameters	cv. Swarna			cv. Swarna Sub1		
	$P \leq 0.05$	$P \leq 0.01$	$P \leq 0.001$	$P \leq 0.05$	$P \leq 0.01$	$P \leq 0.001$
Total polyamine content						
Total free amino acid content						
Proline content						
GSH:GSSG activity						
Total phenolics content						
Total flavonoids content						
Total anthocyanin content						
DPPH scavenging assay						
ABTS scavenging assay						
Phosphomolybdenum content						
Ferric chelation assay						
O ₂ ⁻ generation						
H ₂ O ₂ accumulation						
PAL activity						

Data presented with mean $\pm$ SE (n=3) from independent experimental sets and the significant values within three different probability level were represented as three different colors ($P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$). Uncolored boxes are with insignificant values

developmental organogenesis, flowering initiation, embryogenesis, fruit setting and extended up to senescence as well as abscission phenomenon (Kushad et al., 1990). However, all these when face any abiotic stressors they may have some common tendency to be exposed with ROS. In fact, ROS induced peroxidation of macromolecules are protected by PAs as a shielding material (Goyal et al., 2019). NO being highly diffusible in nature has been a linked with PAs metabolism as reported in many crop species. In rice also it has been demonstrated where application of NO synthesis inhibitor may significantly be down regulated the activity of PAs for its antioxidation activities (Recalde et al., 2018). In biosynthetic pathways arginine happens to be a common precursor for both PAs and NO (Hsieh et al., 2014). Thus, arginine linked nitrate synthase and nitrate reductase activity are modulated by biosynthesis of PAs but in opposite manner. This is typified in the present experiment where SNP application had significantly reduced the total PAs content irrespective of cv. Swarna and Swarna Sub1 as compared to control and most significantly with Put application. This is quite in agreement where NO induced PAs deactivation was described, however, in *Arabidopsis* where nitrate reductase is mutated (Chen et al., 2016). This may be evident that reactions with nitrate reductase may not be the soul path for NO production. These cross talk is often meets with other internal factors adhered to PAs biosynthesis in plant species. In the present experiment the reversal effect of Put and SNP would be supportive for that cross talk where rice seedlings may not be justified for selection for PAs abundance in terms of NO metabolism. NO has been more involved as a key signalling residue involving in series of physiological and cellular functionals. Almost the cases in higher plants are customized with all physiological responses with a fair touch of ROS, however, within threshold values. NO itself behaves as a nitrogen reactive species (RNS) to maintain that optimum level of ROS. In few cases investigation has observed any inhibition of NO synthase could repress the NO signalling. This is perpetuated in its downstream involvement in physiological responses.

This is an aspiring to understand a definite role of chemical elicitors like Put and SNP in induction for antioxidation capacities. We took into account the *sub1A* QTL which has been a definite in response efficiently under anaerobic stress. The later stress essentially becomes severe for rice under complete or partial submergence (Saha et al., 2018b). A secondary module of submergence tolerance is exercised by elevation of antioxidation pathways. Contextually, oxidative stress is a manifestation of higher oxidized redox under direct exposure to higher partial oxygen tension. This is more aggravated under intense illumination of the shoots under submerged tissues. PAs and NO have already proved their ability in moderation of stress tolerance (Hasanuzzaman et al., 2019). For PAs the affinity of amino group

to bind with negatively charged residues like cellular membrane, nucleic acids may shield the tissues from ROS peroxidation (Lenis et al., 2017). Therefore, application of PAs in the form of Put would be justified in deciphering the antioxidant capacity. It refers the potential of cellular mechanism with some special organic residues without involving enzymatic cascades. NO basically a RNS which has the equal chances either to accept or donate an electron and thereby, can alter the redox of over oxidized biomolecules. In plant biological system involvement in oxidation pathways with few others substrates like arginine, PAs etc. are quite responsive. Those are reduced to produce NO by few anaplerotic reactions in cellular environment trailing their normal oxidation (Robinson et al., 2018). This has been well documented in physiological processes of PAs and NO. Therefore, NO in biological system may be regarded as cellular sensor in regulation under oxidative stress (Boehm et al., 2019).

Plants may accommodate different biomolecules which have the diverse roles including antioxidation capacities. Undoubtedly, oxidative stress is ensured under water deficit either through dehydration or/and acquisition of salts in excess (Saha et al., 2020). Therefore, free amino acids and proline accumulation as evident regardless of the rice cultivars may be thought as an adaptive measure. Still, proline is also behaved as an antioxidant as revealed from other studies even in rice also (Anafjeh et al., 2017). The application of Put and SNP undoubtedly established as an effective inducer for free amino acids and proline and that may be exercised for both osmoticum as well as antioxidants. In earlier communication NO in plant tissue may be functioning in protein hydrolysis into free amino acids that including the proline also. On the other hand PAs has its established role as self protecting to oxidative stress, however, shielding the biomolecules with its polycationic back bone. So, for both the cases, PAs and NO would be a reliever as well as evocation of antioxidation against oxidative redox. The cv. Swarna Sub1 was more utilizing in reduced thiol residues consistently than cv. Swarna in the form of GSH to GSSG ratio. This is a proven fact that NO has been a redox sensor for cultivar metabolites under oxidative stress. This is maintained through NADPH dependent thiol reductase in cytosol and cell organelle. The requirement of more reduced non thiol residues like glutathione (GSH) carries the potential for plants under depleted redox (Kart et al., 2016). In the present experiment a fall in GSH to GSSG ratio maximally through SNP treatment regardless of rice cultivars also supports the flow of GSH into ascorbate pathway. The later is the main path for enzymatic antioxidation cascades where reduced glutathione appears to be an efficient electron donor to ascorbate. In ascorbate pathway few peroxidase enzymes including ascorbate peroxidase are integrally bearers in GSH/GSSG redox. The later becomes an essential moiety in redox homeostasis in plant species under abiotic stresses (Lee et al., 2016). Therefore, Put and SNP would be marked as the chemical

elicitors not only to conserve the reduced redox but also to exercise those into succeeding antioxidative cascades.

On account of stable antioxidant in plants in the present experiment were significantly varied with the influence of Put and SNP. Initially, total phenolics including flavonoids also were markedly up regulated and influenced by Put and SNP. On comparative basis cv. Swarna Sub1 had the better ability to accumulate the phenolics and flavonoids than cv. Swarna. This cultivar has been a standard check, particularly, under submergence mediated oxidative stress to induce better antioxidant capacity. In the present case it also holds true that Put and SNP had their up regulatory roles on both of the antioxidants as documented elsewhere (Tang et al., 2017). PAs being the precursor of enzymatic biosynthesis of NO is quite apprehended to induce any of physiological effects common to SNP induction. Thus, another antioxidant, the anthocyanin with ability of ROS quenching is quite significant between the cultivars as a function of chemical inducer in the present experiment. In an epoxide mediated inter-conversion of anthocyanin and other xanthophyll, PAs reported to supplement the quenching phenomenon. Anthocyanin with its unsaturated backbones can easily be reduced by ROS and thereby the role in antioxidation is accomplished (Song et al., 2019). For the NO induction as found in the present experiment with SNP application similar trend of activities of anthocyanin directly reflects the co-linearity of antioxidation capacity.

It is the inbuilt capacity of antioxidation where plant extract is evaluated for the lysis of free radicals/ROS. The simulated oxidative stresses by exercise of different chemical residues are the base lines to react with different secondary metabolites. This is for either to conjugate or reduced the ROS in the system. DPPH and ABTS are the standard ROS inducing free radical recorded equal potential for cv. Swarna and Swarna Sub1. It is interesting to note that PAs and NO might induce the antioxidation residues for both the rice varieties. This caused the values for IC_{50} more decreased vis-à-vis more antioxidation reactions. PAs have been frequently cited as elicitors in quenching ROS as compared to NO in plant system. NO are basically redox homeostatic residues not directly involved in any nucleophilic reaction to reduce the ROS (Uchida et al., 2002). As a whole, the antioxidation capacities of PAs and NO are quite helpful as evident from the present experiment. In a similar way the complex reaction with multivalent metal derivatives (like phosphomolybdenum) also scores the ROS sequestering ability (Banda et al., 2016). Both the rice varieties differed in content of phosphomolybdenum complex with PAs and NO but significantly increased as compared to control. These discrepancies may be due to affinity based reactions with molybdenum to PAs and NO in complex formation by plant extract (Gupta & Igamberdiev, 2015). Similar interpretation could also be applicable with other such moieties like ferric chelators in ROS

sequestering. Fe (iron) with its variable valences is reliable source in chelate reaction differentially to reduce the oxidative power of ROS. The plant extract has a number of Fe derivatives or residues where the chelation is frequent and that prevents the sequestering of the complex within the cytosolic or non cellular spaces. This is to invade the further ROS induced lysis of biomolecules establishing an indexed for plants' total antioxidation capacities (Saha et al., 2019). In the present experiment PAs and NO have their confirmatory roles to sustain the antioxidation capacities regardless of rice cultivars.

In close observation the accumulated O_2^- and H_2O_2 would be a good indicator for plants responding to Put and SNP as a source of PAs and NO. It is quite logical that NO being a RNS must have the affinity to donate its free electron to molecular oxygen producing O_2^- . This got relevance from earlier studies that intermediates of nitrate metabolism would be an authentic source on NO generation both by enzymatic as well as non enzymatic paths (Cánovas et al., 2016). Put like other PAs related residues have the equal compatibility to induce NO over accumulation in results of antioxidation defence against salinity stress. Therefore, the compatibility of PAs and NO must have equal probability to induce ROS. However, the accumulated ROS may either be crossing the threshold level could induce oxidative damages of biomolecules within the optimum level to motivate other cellular responses. The co-linearity with NO and H_2O_2 would be a factor for stress has been well clarified. In application of inhibitor for H_2O_2 and NO had the implementation on rice seedlings and induce the sensitivity under salinity (Mostofa et al., 2015).

As a precursor as well as the key enzyme for biosynthesis of phenolics, PAL is most important as rate limiting. In the present experiment PAL activities were over expressed, however, not significantly varied between the cultivars. Still, Put and SNP had influences more on initiation of phenolics biosynthesis by over expression of PAL activity as compared to control. PAL is thus, a target protein to assume antioxidation stress tolerance for concern species regardless of abiotic stressors (Ren et al., 2019).

Conclusion

The antioxidation pathways including both enzymatic and non enzymatic systems were exhibited variable in those cultivars, however, maximum in few cases for cv. Swarna Sub1. The second line of investigation established the efficiency of two chemical elicitors (viz. Put and sodium SNP), however, also variable through different parameters. SNP appears to be most convincing regardless of cultivars to retain cellular integrity by lowering the concentration of ROS. Probably SNP may regulate any concern steps for H_2O_2 biosynthesis and it imparts on downstream paths. So, SNP also demands its worth of priming agent for stress tolerance like H_2O_2 . Understanding and anticipating the subdued

mechanism plant species could effectively regulate the inbuilt antioxidation with optimum ROS. The non enzymatic antioxidant mostly the phenolics were proportionally induced for both Put and SNP mentioning their recognition and function. In vitro antioxidation assays, however, varied for two cultivars, still more induced in cv. Swarna Sub1 than others under Put and SNP. According to observation of present studies few inbuilt cellular pathways would effectively be acted as bio-indicators for positive modulation to oxidative stress and thus, might also serve selection pressure for rice cultivars. Moreover, the identifications of aligned biochemical pathways and aligned gene expression for over expression of PAs and NO metabolism in those cultivars are for better responses. On very outset the cv. Swarna Sub1 would be defensible for its improved antioxidation due to its inbuilt *sub1A* QTL regulated machineries.

Acknowledgements

The experiment was financially supported by Department of Science and Technology (DST), Govt. of India in the form of DST-INSPIRE (Innovation of Science Pursuit for Inspired Research fellowship [grant number: IF170249].

Conflict of interest

No potential conflict of interest was reported by the authors.

References

- Anafjeh E, Salehi Salmi M, Daneshvar M, Meratan A (2017) Effect of Salinity Stress on Growth, Proline content and Antioxidant Enzymes Activity in the halophyte *Sesuvium portulacastrum* L. Journal of Plant Process and Function 6: 267-278.
- Banda NR, Singh G, Markam V (2016) Evaluation of total antioxidant level of saliva in modulation of caries occurrence and progression in children. Journal of Indian Society of Pedodontics and Preventive Dentistry 34: 227-232.
- Basu S, Roychoudhury A, Saha PP, Sengupta DN (2010) Differential antioxidative responses of indica rice cultivars to drought stress. Plant Growth Regulation 60: 51-59.
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant and Soil 39: 205-207.
- Boehm M, Novoyatleva T, Kojonazarov B, Veit F, Weissmann N, Ghofrani HA, Seeger W, Schermuly RT (2019) Nitric oxide synthase 2 induction promotes right ventricular fibrosis. American Journal of Respiratory Cell and Molecular Biology 60: 346-356.
- Bui LT, Ella ES, Dionisio ML, Ismail AM (2019) Morpho-Physiological Changes in Roots of Rice Seedling upon Submergence. Rice Science 26: 167-177.
- Cánovas D, Marcos JF, Marcos AT, Strauss J (2016) Nitric oxide in fungi: is there NO light at the end of the tunnel?. Current Genetics 62: 513-518.
- Chen ZH, Wang Y, Wang JW, Babla M, Zhao C, García Mata C, Sani E, Differ C, Mak M, Hills A, Amtmann A (2016) Nitrate reductase mutation alters potassium nutrition as well as nitric oxide-mediated control of guard cell ion channels in Arabidopsis. New Phytologist 209: 1456-1469.
- Dey P, Chaudhuri D, Chaudhuri TK, Mandal N (2012) Comparative assessment of the antioxidant activity and free radical scavenging potential of different parts of *Nerium indicum*. International Journal of Phytomedicine 4: 54.
- Dinis TC, Madeira VM, Almeida LM (1994) Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics 315: 161-169.
- Domingos P, Prado AM, Wong A, Gehring C, Feijo JA (2015) Nitric oxide: a multitasked signaling gas in plants. Molecular Plant 8: 506-520.
- Elstner EF (1976) Inhibition of nitrite formation from hydroxylammonium-chloride: a simple assay for superoxide dismutase. Analytical Biochemistry 70: 616-620.
- Ferreira IC, Baptista P, Vilas M, Barros L (2007) Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. Food Chemistry 100: 1511-1516.
- Goyal V, Devi S, Munjal R (2019) Role of polyamines in protecting plants from oxidative stress. In: Hasanuzzaman M, Fotopoulos V, Nahar K, Fujita M (Eds.) Reactive Oxygen, Nitrogen and Sulfur Species in Plants: Production, Metabolism, Signaling and Defense Mechanisms Wiley online Publication 18: 143-157. <https://doi.org/10.1002/9781119468677.ch6>.
- Gupta KJ, Igamberdiev AU (2015) Compartmentalization of reactive oxygen species and nitric oxide production in plant cells: an overview. In: Gupta KJ, Igamberdiev AU (Eds) Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants, Springer International Publishing Switzerland Pp.1-14. DOI: 10.1007/978-3-319-10079-1.
- Hasanuzzaman M, Alhathloul HAS, Parvin K, Bhuyan MHM, Tanveer M, Mohsin SM, Nahar K, Soliman MH, Mahmud JA, Fujita M (2019) Polyamine action under metal/metalloid stress: Regulation of biosynthesis, metabolism, and molecular interactions. International Journal of Molecular Sciences 20: 3215.

- Hsieh HJ, Liu CA, Huang B, Tseng AH, Wang DL (2014) Shear-induced endothelial mechanotransduction: the interplay between reactive oxygen species (ROS) and nitric oxide (NO) and the pathophysiological implications. *Journal of Biomedical Science* 21: 3.
- Jansen G, Flamme W (2006) Coloured potatoes (*Solanum tuberosum* L.)-anthocyanin content and tuber quality. *Genetic Resources and Crop Evolution* 53: 1321.
- Kart A, Koc E, Dalginli KY, Gulmez C, Sertcelik M, Atakisi O (2016) The therapeutic role of glutathione in oxidative stress and oxidative DNA damage caused by hexavalent chromium. *Biological Trace Element Research* 174: 387-391.
- Kocsy G, Galiba G, Brunold C (2001) Role of glutathione in adaptation and signalling during chilling and cold acclimation in plants. *Physiologia Plantarum* 113: 158-164.
- Kumar D, Yusuf MA, Singh P, Sardar M, Sarin NB, Biosciences, JMI (2014) Histochemical detection of superoxide and H₂O₂ accumulation in *Brassica juncea* seedlings. *Bio Protocol* 4: 1108.
- Kushad MM, Orvos AR, Yelenosky G (1990) Relative changes in polyamines during citrus flower development. *Horticultural Science* 25: 946-948.
- Larkunthod P, Nounjan N, Siangliw JL, Toojinda T, Sanitchon J, Jongdee B, Theerakulpisut P (2018) Physiological responses under drought stress of improved drought-tolerant rice lines and their parents. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 46: 679-687.
- Lee SG, Yim J, Lim Y, Kim JH (2016) Validation of a liquid chromatography tandem mass spectrometry method to measure oxidized and reduced forms of glutathione in whole blood and verification in a mouse model as an indicator of oxidative stress. *Journal of Chromatography B* 1019: 45-50.
- Lenis YY, Elmetwally MA, Maldonado JG, Bazer FW (2017) Physiological importance of polyamines. *Zygote* 25: 244-255.
- Li J, Pandeya D, Nath K, Zulfugarov IS, Yoo SC, Zhang H, Yoo, JH, Cho SH, Koh HJ, Kim DS, Seo HS (2010) ZEBRA-NECROSIS, a thylakoid-bound protein, is critical for the photoprotection of developing chloroplasts during early leaf development. *The Plant Journal* 62: 713-725.
- Locke AM, Barding Jr GA, Sathnur S, Larive CK, Bailey J (2018) Rice SUB1A constrains remodelling of the transcriptome and metabolome during submergence to facilitate post-submergence recovery. *Plant, Cell & Environment* 41: 721-736.
- Mattoo AK, Sobieszczuk E (2019) Polyamine as signaling molecules and leaf senescence. *Senescence Signalling and Control in Plants* 10: 125-138
- Meng X, Wang M, Jiang N, Zhang D, Wang L, Liu C (2012) Regulation of both the reactive oxygen species level and antioxidant enzyme activity in drought-stressed rice organs by Benzimidazole-Based SOD1 Mimics. *Journal of Agricultural and Food Chemistry* 60: 11211-11221.
- Mohsen SM, Ammar AS (2009) Total phenolic contents and antioxidant activity of corn tassel extracts. *Food Chemistry* 112: 595-598.
- Mostofa MG, Fujita M, Tran LSP (2015) Nitric oxide mediates hydrogen peroxide-and salicylic acid-induced salt tolerance in rice (*Oryza sativa* L.) seedlings. *Plant Growth Regulation* 77: 265-277.
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473-497.
- Peltonen S, Karjalainen R (1995) Phenylalanine Ammonia-lyase Activity in Barley After Infection with *Bipolaris sorokiniana* or Treatment with its Purified Xylanase. *Journal of Phytopathology* 143: 239-245.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 26: 1231-1237.
- Recalde L, Vázquez A, Groppa MD, Benavides MP (2018) Reactive oxygen species and nitric oxide are involved in polyamine-induced growth inhibition in wheat plants. *Protoplasma* 255: 1295-1307.
- Ren W, Wang Y, Xu A, Zhao Y (2019) Genome-wide identification and characterization of the Phenylalanine Ammonia-lyase (PAL) gene family in *Medicago truncatula*. *Legume Research: An International Journal* 42: 461-466.
- Robinson JL, Smith VA, Stoll B, Agarwal U, Premkumar MH, Lau P, Cruz SM, Manjarin R, Olutoye O, Burrin DG, Marini JC (2018) Prematurity reduces citrulline-arginine-nitric oxide production and precedes the onset of necrotizing enterocolitis in piglets. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 315: 638-649.
- Sadiq I, Zulfiqar M, Maryam A, Rehman K, Noreen S, Mursalin M, Rauf SA, Amjad S, Siddiqi AR (2018) In-silico studies of CBL-CIPK signaling in rice: response against hypoxia is triggered

- by interaction of *cb110* with *CIPK15*. Pakistan Journal of Botany 50: 1407-1413.
- Saha I, De AK, Ghosh A, Sarkar B, Dey N, Adak MK (2018a) Preliminary variations in physiological modules when *sub1A* QTL is under soil-moisture deficit stress. American Journal of Plant Sciences 9: 732-744.
- Saha I, De AK, Sarkar B, Ghosh A, Dey N, Adak MK (2018b) Cellular response of oxidative stress when *sub1A* QTL of rice receives water deficit stress. Plant Science Today 5: 84-94.
- Saha I, Sarkar B, Ghosh A, De AK, Adak MK (2019) Absciscic acid induced cellular responses of *sub1A* QTL to aluminium toxicity in rice (*Oryza sativa* L.). Ecotoxicology and Environmental Safety 183: 109600.
- Saha I, Sarkar B, Ghosh A, De AK, Adak MK (2020) Physiological responses of *sub1A* QTL under induced dehydration stress for varying days in rice. Plant Science Today 7: 112-121.
- Sánchez D, Chávez AI, Rodríguez AA, Maruri I, Urano K, Shinozaki K, Jiménez JF (2016) Simultaneous silencing of two arginine decarboxylase genes alters development in Arabidopsis. Frontiers in Plant Science 7: 300.
- Showler AT (2002) Effects of water deficit stress, shade, weed competition, and kaolin particle film on selected foliar free amino acid accumulations in cotton, *Gossypium hirsutum* (L.). Journal of Chemical Ecology 28: 631-651.
- Song T, Li K, Wu T, Wang Y, Zhang X, Xu X, Yao Y, Han Z (2019) Identification of new regulators through transcriptome analysis that regulate anthocyanin biosynthesis in apple leaves at low temperatures. PloS One 14: 0210672.
- Tang C, Song J, Hu X, Hu X, Zhao Y, Li, B, Ou D, Peng L (2017) Exogenous spermidine enhanced Pb tolerance in *Salix matsudana* by promoting Pb accumulation in roots and spermidine, nitric oxide, and antioxidant system levels in leaves. Ecological Engineering 107: 41-48.
- Uchida A, Jagendorf AT, Hibino T, Takabe T, Takabe T (2002) Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Science 163: 515-523.
- Young ND, Galston AW (1983) Are polyamines transported in etiolated peas?. Plant Physiology 73: 912-914.