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RESEARCH ARTICLE

Exploring the Mechanism of Exogenous Applied Methyl Jasmonate for Germination Inhibition in Hybrid Rice

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ABSTRACT

The present study was conducted to evaluate the role of different levels of Methyl jasmonate (MeJA) i.e. control, 1, 2.5 and 5 mM to minimize pre-harvest sprouting in rice. The results suggested that MeJA (5 mM) significantly reduced germination percentage, germination energy, germination index, shoot height, root length and seedling biomass in comparison with the control treatment. This reduction was more obvious in cultivar Zhu Liang You 06 (ZY) than in Qian You No.1 (QY). The activity of antioxidative enzymes i.e. catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and malondialdehyde (MDA) contents were significantly increased after treating with 1 mM and 2.5 mM concentrations of MeJA. However, higher concentration of MeJA (5 mM) significantly reduced the activities of antioxidant enzymes, seedling biomass and increased ROS generation. The α-amylase activity was significantly decreased with seed soaking in 5 mM of MeJA as compared to the control. Gene expression analysis suggested a significant up regulation of root *Amy2A* and *Amy3A* mRNA in comparison with the shoot, by seed soaking in MeJA. Higher concentration of MeJA (5 mM) improved resistance to pre-harvest sprouting in both rice cultivars. However, little cell damage and higher ROS accumulation were also observed.

INTRODUCTION

Germination of physiologically mature cereal seeds in the ear or panicle under humid environment before harvesting is called as pre-harvest sprouting (PHS). It is a common phenomenon in several cereal crops such as wheat, maize, rice and barley in most regions of the world (Fang and Chu, 2008). Besides reduction in grain yield, PHS also deteriorates the quality of produce, consequently causing significant economic losses. Due to long rainy period in early summer and autumn in Southern China, frequent occurrence of PHS has been reported in hybrid rice (Wan et al., 2006). In southern China, PHS affects more than 6% of rice area, which could rise up to 20% in case of hybrid rice (Guo et al., 2004). Rice, being a staple food in China, is important to the rest of the world as well. The alarming rate of increase in human population, rapid urbanization and rising per capita income, will increase the demand for rice and rice products. Therefore, to enhance the yield of rice through exogenous application of plant growth regulators and novel genetic approaches is essential for the agricultural industry.

Owing to the pleiotropic effects of Jasmonic acid (JA) and its derivatives on plants' growth and developmental processes, it is considered as an important endogenous plant growth regulators (Cheong and Choi, 2003). Plants possess inducible defense systems to combat pathogens and herbivores (Memelink, 2009). In addition, JA plays a defensive role in signaling pathways. Exogenously applied JA inhibited stem and root growth, induced pericarp or leaf senescence and reduced photosynthetic and respiratory activity in plants (Maslenkova et al., 1990). The investigations on novel

jasmonate-induced proteins have confirmed the role of endogenous jasmonates in defense system and responses of plants to stressful environments. Moreover, the role of exogenous jasmonate on germination of non-dormant seeds was investigated by different researchers on sunflower, amaranth (Bialecka and Kepczynski, 2003), flax and rape seeds (Wilen et al., 1991). Tsai et al. (1996) have reported a pivotal role of Jasmonates in senescence and stress response by inhibiting ethylene production in detached leaves of rice. Nonetheless, extremely scant information exists about the utilization of methyl jasmonate as a growth inhibitor to minimize the incidence of PHS in hybrid rice.

The α-amylase is an important enzyme synthesized during germination, which helps to mobilize the starch reserves in the endosperm. As a result of α-amylase synthesis, breakdown of starch takes place, which degrades the quality of grains (Lenton, 2001). During PHS, α-amylase provides a source of energy for radicle emergence by converting endospermic starch into simples sugars (Tangphatsornruang et al., 2005). It activates the stored carbohydrates by breaking down α-1, 4 linkage of polyglucans. During germination and seedling establishment of cereals, the level of α-amylase related mRNA was higher expressed by gibberellins in the endosperm while down-regulated by sugars in the embryo. Up- and down-regulation of α-amylase related genes is triggered by sugar starvation and abundance, respectively, in the embryo of germinating rice seedling by controlling transcription rates and mRNA stability. In the process of cereal grain germination, the transition from a dormant tissue to high metabolic activity involves starch mobilization to meet the energy demands for growth.

Pre-harvest sprouting is a problematic issue in rice seed production under warm and humid environments. Up to now, there is no clear information in the available literature about any mechanism to minimize PHS of cereal seeds. The current study was carried out to probe the effect of varying concentration of methyl jasmonate as growth inhibitor to reduce the occurrence of pre-harvest sprouting in rice cultivars.

MATERIALS AND METHODS

Two hybrid rice (*Oryza sativa* L.) cultivars Qian You No.1 (QY) and Zhu Liang You 06 (ZY) were obtained from Zhejiang Nongke Seed Industry Co., Ltd. Different concentrations of Methyl Jasmonate (1mM, 2.5mM and 5mM) were used for seed soaking for 1 hr. Distilled water was used for control treatment. Fifty seeds per treatment were germinated in incubation chamber, using plastic trays, at 25°C under alternate cycle of 8 h light and 16 h dark period for two weeks. All the treatments were replicated three times. Finally, the percentage of germination was determined after 14 days of sowing. Germination index (GI) was calculated using the formula GI= $\sum \frac{Gt}{Tt}$. Where Gt represents number of germinated seeds in the time t (day) and Tt stands for time (days) corresponding to Gt (Hu et al., 2006). Root length, shoot length, fresh and dry weight was recorded by sampling 10 randomly selected seedlings.

Measurement of antioxidant enzyme and α-amylase activities

Fourteen days old rice seedlings were sampled to measure the activity of anti-oxidative enzymes. CAT activity was measured by observing the absorbance at 240 nm, which corresponds to the remaining H_2O_2. The reaction mixture was prepared with 25 mM phosphate buffer (pH 7.0) and 0.2 mL enzyme extract. The reaction started with the addition of 0.4% H_2O_2 (Cakmak and Marschner, 1992). POD activity was measured with guaiacol as the substrate in a total volume of 3 mL (Zhang, 1992). The reaction mixture consisted of 25 mM phosphate buffer (pH 7.0), 1.5% guaiacol, 0.4% H_2O_2 and 0.2 mL of enzyme extract. The absorbance due to oxidzed guaiacol (E = 25.5 mM^{-1} cm^{-1}) was measured at 470 nm.

SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Rao and Sresty, 2000). The photo reduction of NBT was measured at 560 nm and one unit of SOD was defined as being present in the volume of extract that caused inhibition of the photo- reduction of NBT by 50%. APX activity was measured according to the methods of Nakano and Asada (1981). The assay measured the absorbance at 290 nm as ascorbate was oxidized. The MDA content in seedlings was measured as described by Zhou and Leul (1998).

To measure α-amylase activity, seeds treated with different concentrations of MeJA (control, 1, 2.5, 5 mM), were allowed to germinate for two days, frozen in liquid nitrogen, and stored in a -80ºC freezer. Thereafter, the α-amylase activity was measured by a 3, 5-dinitrosalicylic acid colorimetric (DNS) method as described by Li (2000).

Determination of reactive oxygen species (ROS) and histochemical analysis

To determine the hydrogen peroxide (H_2O_2) contents, leaf and root samples (0.5g) were extracted with 5.0 mL of trichloroacetic acid (TCA, 0.1%) in an ice bath, and the homogenate was centrifuged at 12,000g for 15 min (Velikova et al., 2000). For determination of extra-cellular hydroxyl radicals ($^{\cdot}OH$), leaf and root samples (0.5 g) were incubated in 1 mL of 10 mM Na-phosphate buffer (pH 7.4), consisting of 15 mM 2-deoxy-D-ribose (SRL, Mumbai), at 37°C for 2 hr (Halliwell et al., 1987). Superoxide radical ($^{\cdot}O_2$) was determined according to the method of Jiang and Zhang (2001).

Analysis of gene expression

Frozen leaf and root tissues (100 mg each) were ground thoroughly in a pestle and mortar with a small amount of liquid nitrogen. Total RNA content was isolated from the shoot and root samples using RNA isolation protocol (Takara, Japan). The RNA purity was checked spectrophotometrically by means of the 260/280 (OD) nm ratio optical density. cDNA was synthesized using Primer Script RT reagent Kit (Takara, Japan) from 1 µg of total RNA in a 20 µl reaction, and diluted 4-times with water. The primers used for quantitative real-time PCR (QRT-PCR) experiments were as follows; *Amy2A* (F: 5'CACCTCAATGGTGTCGTCCA'3, R: 3'AGCCA ACCTCGTCAGACTTG'5) *Amy3A* (F: 5'ACGTGTTC GACTGGAACCTC'3, R: 3'TTTATCCCGTTGCGCT TCCT).

QRT-PCR was performed using SYBR premix EX Taq (Takara, Japan). ACT1 was used as an endogenous control gene to normalize the expression of the other genes. The PCR program was as follows: 30 s at 95 °C, followed by 40 cycles of 10 s at 95 °C, 30 s at 60 °C. The relative expression level was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Transmission electron microscopy observation

After 14 days of treatment, Fresh leaf segments and root tips (8-10 samples per treatment) were excised from seedlings selected at random, followed by overnight fixation in 2.5% solution of glutaraldehyde (v/v) formulated in 0.1 M PBS (sodium phosphate buffer, pH 7.4) and washed thrice with the same buffer solution. The samples were then fixed in 1% osmium tetroxide (OsO_4) for 1 h and washed thrice with the same PBS by keeping 10 minutes gap between each washing. Afterwards, the dehydration of the samples was carried out using 50, 60, 70, 80, 90, 95 and 100% ethanol with 15–20-min intervals, followed by final washing with absolute acetone for 20 minutes. The dehydrated specimens were kept overnight in spurr's resin mixture. The samples were heated for 9 h at 70°C, cut into ultra thin sections and loaded on copper grids to visualize under transmission electron microscopy (JEOLTEM- 1230EX) at 60.00 Kv accelerating voltage (Salah et al., 2015).

Statistical analysis

The treatments were arranged in a factorial experiment with completely randomized design. All data were analyzed using Statistical Analysis System software (SAS version 9.1). Before analyzing, the percentage data were transformed as y= arcsin [sqr (x/100)].

RESULTS

Germination and seedling vigor evaluation

Different concentrations of methyl jasmonate significantly reduced the germination percentage, germination energy and germination index of both rice genotypes, compared to the control groups. Lowest GP, GI and GE were recorded in the seeds of ZY as compared to QY treated with 5 mM MeJA concentration (Table 1). The results clearly showed that root length, shoot length and seedling dry weight were significantly decreased after treatment with methyl jasmonate (5 mM) as compared to respective controls. Exposure of plants to different levels of MeJA caused clear phenotypic modifications as visualized by reduced plant height and root length of both cultivars (Fig. 7).

Antioxidant enzymes, MDA, Histochemical Staining and α-amylase activity

The antioxidant enzyme activities (CAT, POD, SOD and APX) and MDA as affected by MeJA concentrations are presented in Fig. 1. Highest activity of SOD, POD and catalase was recorded with MeJA at 1 mM concentration as compared to control (Fig. 1a-c). While, 2.5mM and 5 mM MeJA concentration resulted in decrease of SOD, POD and catalase activities as compared to 1mM MeJA. Regarding APX and MDA, the results showed that MeJA at 2.5 mM concentration increased their activity in both genotypes over their respective control treatments (Fig.1d, e). In contrast, at higher concentration of MeJA (5 mM) decreased APX and MDA activities in both rice cultivars. The results clearly indicated that antioxidant enzyme activity was lower in ZY than QY irrespective of MeJA concen-trations. The activity of α-amylase decreased gradually with increasing MeJA concentration, as compared to control. The lowest α-amylase activity was recorded with higher concentration of 5mM MeJA (Fig.1f).

The data regarding reduced glutathione (GSH), glutathione reductase (GR), oxidized glutathione (GSSG) and total glutathione (GSH+GSSG) are shown in Fig. 2. Methyl jasmonate at higher concentration (5 mM) significantly increased GR, GSH, GSSG and GSH+GSSG as compared to the control. The results illustrated a non-significant variation between two rice cultivars (Fig. 2a, c, e, g). Significant increase in ROS (H_2O_2, O_2^- and ^-OH) was recorded with increase of MeJA concentration. Highest values of ROS accumulation were recorded with higher concentration of MeJA (5 mM) in both rice cultivars, compared with the control treatment (Fig. 2b, d, f). However, the increase in ROS was more prominent in cultivar QY as compared to ZY. GSH contents increased gradually in the leaves; maximum GSH contents were recorded at 5 mM MeJA, whereas GSH contents were at par at 1 and 2.5 mM MeJA in both cultivars. A significant change was observed in GSSG contents of seedlings under varying levels of MeJA. Maximum GSSG contents were found at 5 mM MeJA concentration in both genotypes. The results depicted that total glutathione (GSH+GSSG) content was clearly enhanced irrespective of MeJA concentrations, in comparison with their respective control treatments.

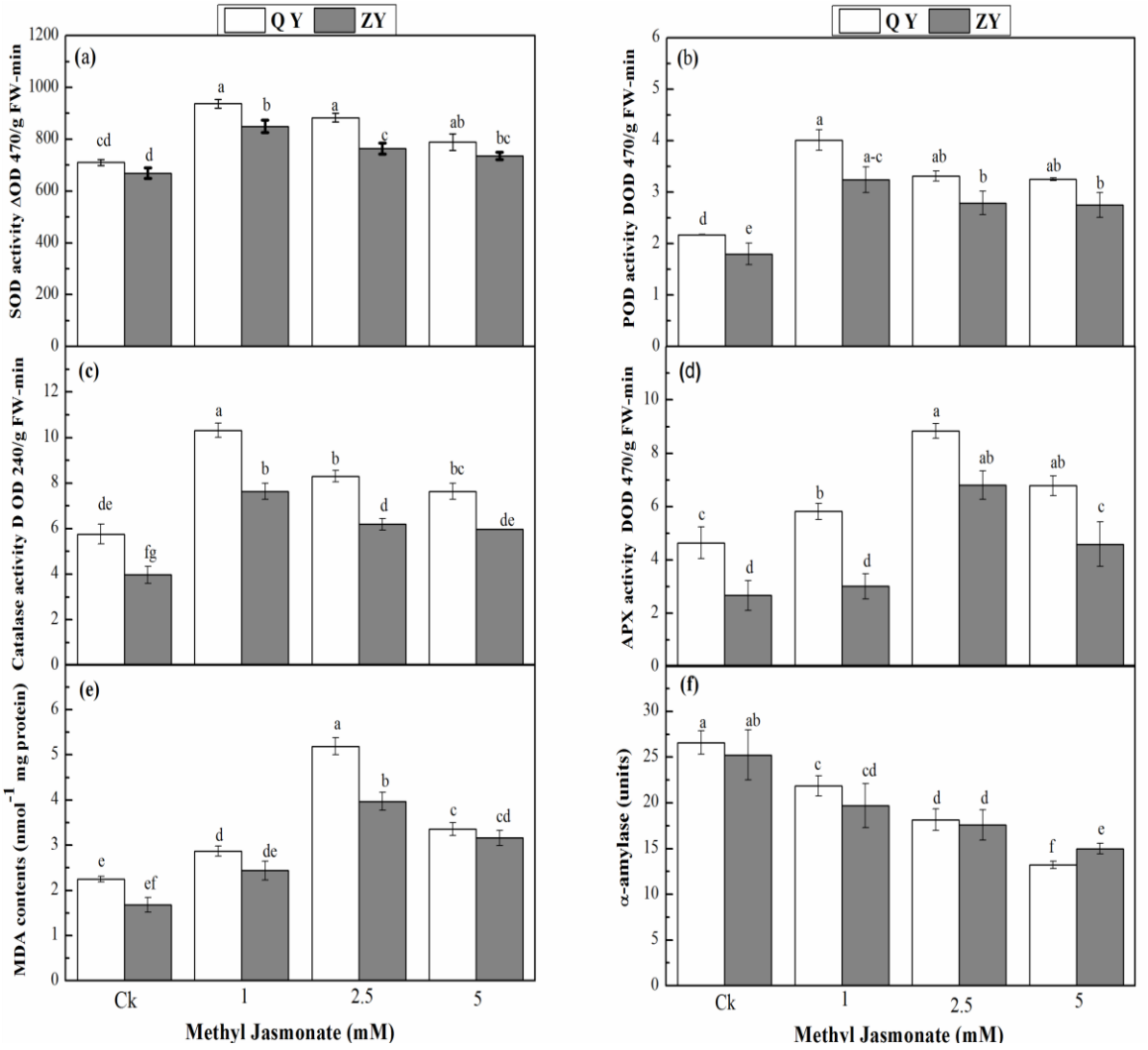

Fig. 1: Effect of exogenous methyl jasmonate on (a) Superoxide dismutase (SOD), (b) Peroxidase (POD), (c) Catalase (CAT), (d) Ascorbate peroxidase (APX) activities (e) Malondialdehyde (MDA) contents in leaves and (f) α-amylase in seed of two rice cultivars Qian You No.1 (QY) and Zhu Liang You 06 (ZY).

Minimum H_2O_2 contents were recorded in control plants whereas H_2O_2 contents increased with increasing concentration of MeJA, however, highest H_2O_2 contents were observed in cultivar QY as compared to ZY. An obvious difference in superoxide radical (O_2^-) and OH contents was noted in both genotypes when seeds were soaked in varying concentrations of MeJA (Fig. 2b, d, f). The results of DAB and NBT staining of roots revealed that histochemically detected contents of H_2O_2 and O_2 are in line with the results of ROS measurement. Further, the colored spots showed the evidence of physical presence of ROS. The colored spots were more noticeable in genotype ZY than in QY at 5 mM MeJA compared to control as shown in Fig. 3A, B.

Methyl Jasmonate seed soaking and gene expression

The transcription levels of two important amylase genes i.e. *Amy2A* and *Amy 3A* were determined in root and shoot of two rice cultivars (Fig. 4). The expression of *Amy2A* was up-regulated both in root and shoot under varying MeJA concentrations. An increase in the expression of *Amy2A* was observed in shoots after exposure to 5mM MeJA in both cultivars and the highest transcript level of *Amy2A* was observed upon exposure to 5 mM MeJA (Fig. 4). But, results showed decreasing trend in shoot and root of both cultivars when treated with 2.5 mM concentration of MeJA (Fig. 4a, b). Increase in *Amy2A* expression was found in roots upon exposure to MeJA in both rice genotypes and this enhancement was more prominent in mRNA of *Amy2A* in QY as compared to ZY (Fig. 4a, b). The results indicated that seed soaking with MeJA induced reduction in transcription levels of *Amy2A* in shoot as compared to roots in both genotypes (Fig. 4a, b). Up-regulation of *Amy3A* expression was recorded in both

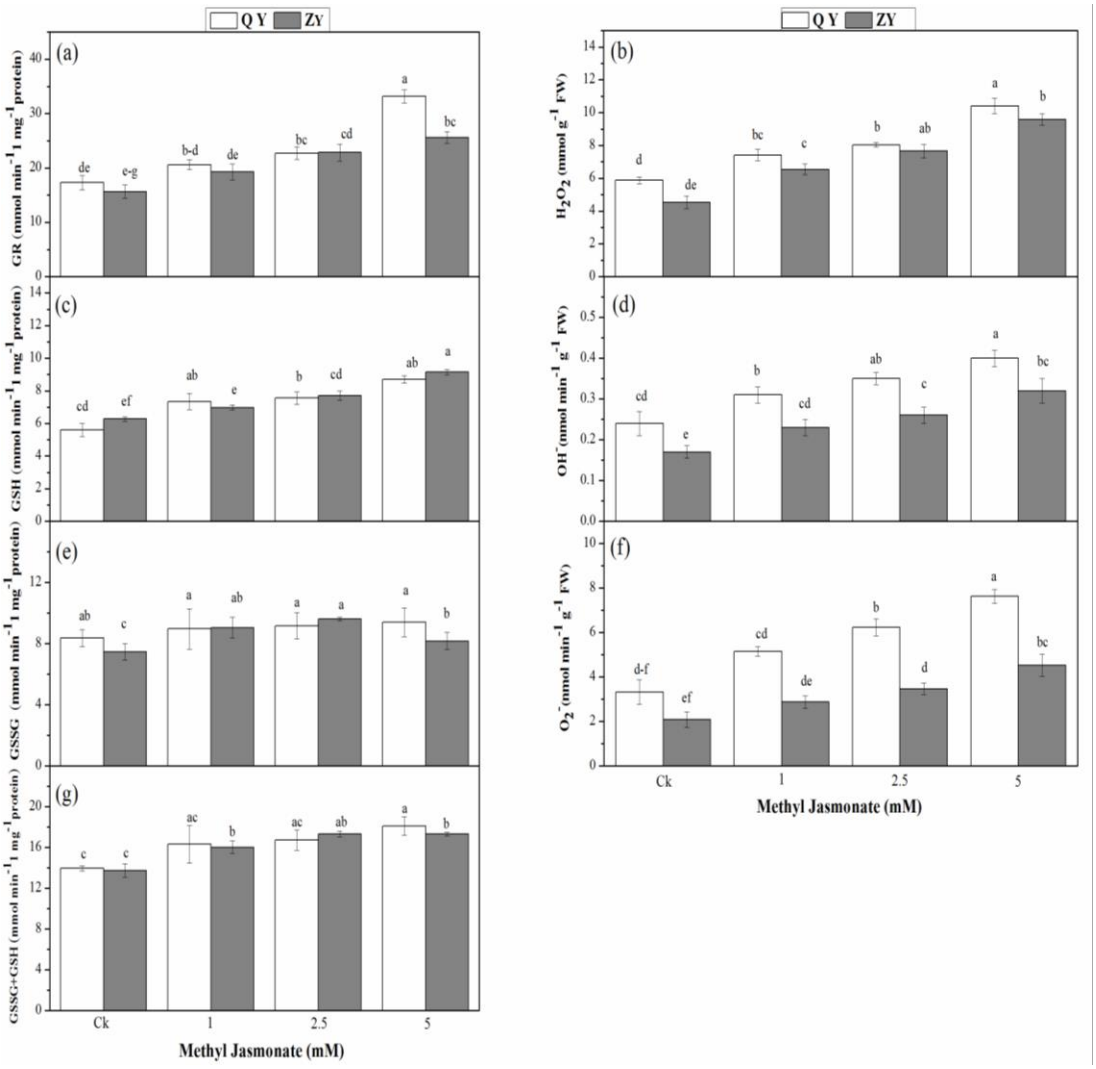

Fig. 2: Effect of seed soaking with methyl jasmonate on (a) glutathione reductase (GR), (b) hydrogen peroxide (H_2O_2), (c) reduced glutathione (GSH), (d) hydroxyl ion (^{-}OH) (e) oxidized glutathione (GSSG) (f) superoxide radical (O_2^-) and (g) total glutathione (GSSG+GSH) of two rice cultivars Qian You No.1 (QY) and Zhu Liang You 06 (ZY).

root and shoot upon exposure to different concentrations (1 and 2.5 mM) of MeJA in comparison with control plants. The results for shoot and root of QY cultivar exhibited a declining trend when treated with 5 mM concentration of MeJA (Fig. 4c). Moreover, seed soaking with MeJA induced significant reduction in transcription levels of *Amy3A* in shoot as compared to roots in both cultivars (Fig. 4c, d).

Transmission electron micrographs of leaf cell of both varieties showed a distinct cell wall (CW), well composed chloroplast (Ch), granule thylakoid (GT), mitochondria (M) with a good cristae and osmiumphobilic granule (OG) (Fig. 5A, D). Exposure to lower concentration of MeJA (2.5 mM) resulted in clear cell wall (CW), thylakoid (GT), osmiumphobilic granule (OG) and developed chloroplast (Ch) in leaf cell of Qian You No.1 (Fig. 5B). While, at higher concentration of MeJA (5mM), TEM micrographs of leaf cell of both cultivars showed ruptured cell wall (CW), granule thylakoid (GT) and osmiumphobilic granule (OG), however the damage was more prominent in Zhu Liang You 06 cultivar (Fig. 5C, F).

Transmission electron microscopy

The TEM micrographs of root cell of both cultivars under control showed a distinct cell wall, well composed chloroplast, granule thylakoid, mitochondria with a good cristae and osmiophilic granules (Fig. 6A, D). In contrast, TEM micrographs of root cell of Qian You No.1 showed destroyed cell wall, granule thylakoid and osmiophilic granules (Fig. 6C). While, in Zhu Liang You 06 cultivar the damage was more obvious at higher concentration of MeJA since a lot of vacuoles were observed (Fig. 6F).

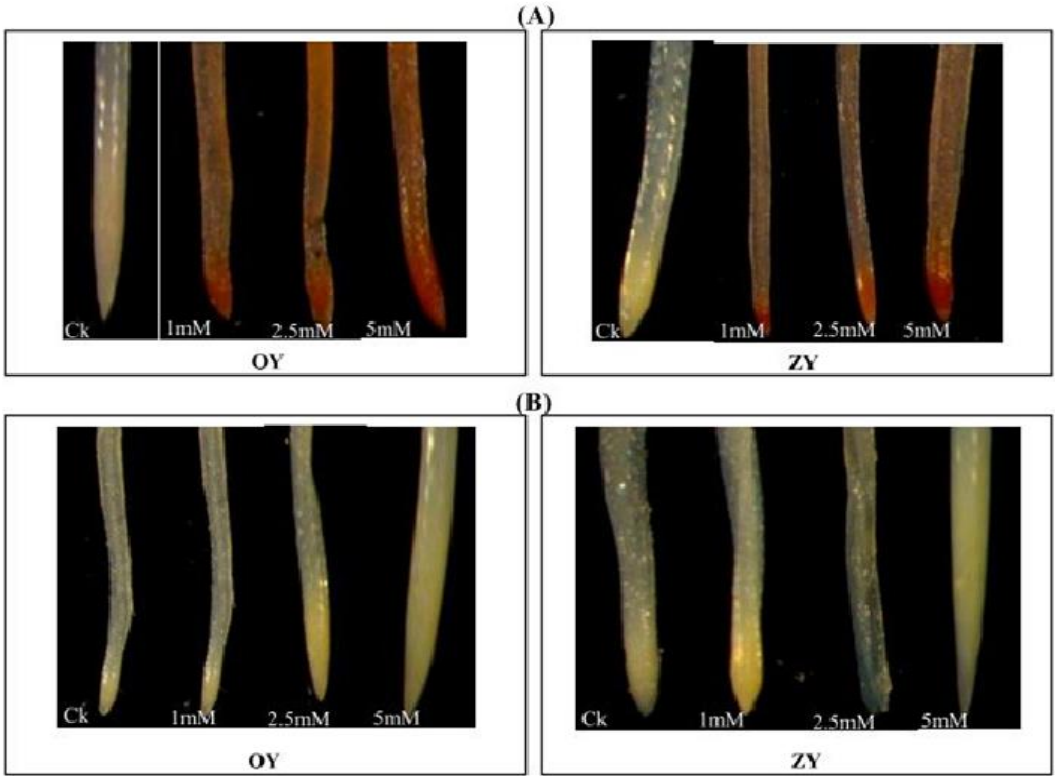

Fig. 3: Effect of different treatments of Methyl Jasmonate (Ck, 1, 2.5 and 5 mM on root tip of QY and ZY cultivars treated with (A) 3, 3-diaminobenzidine (DAB) and (B) Nitro-blue tetrazolium (NBT). Strength of color showed the negative effect of H_2O_2 and O_2 respectively.

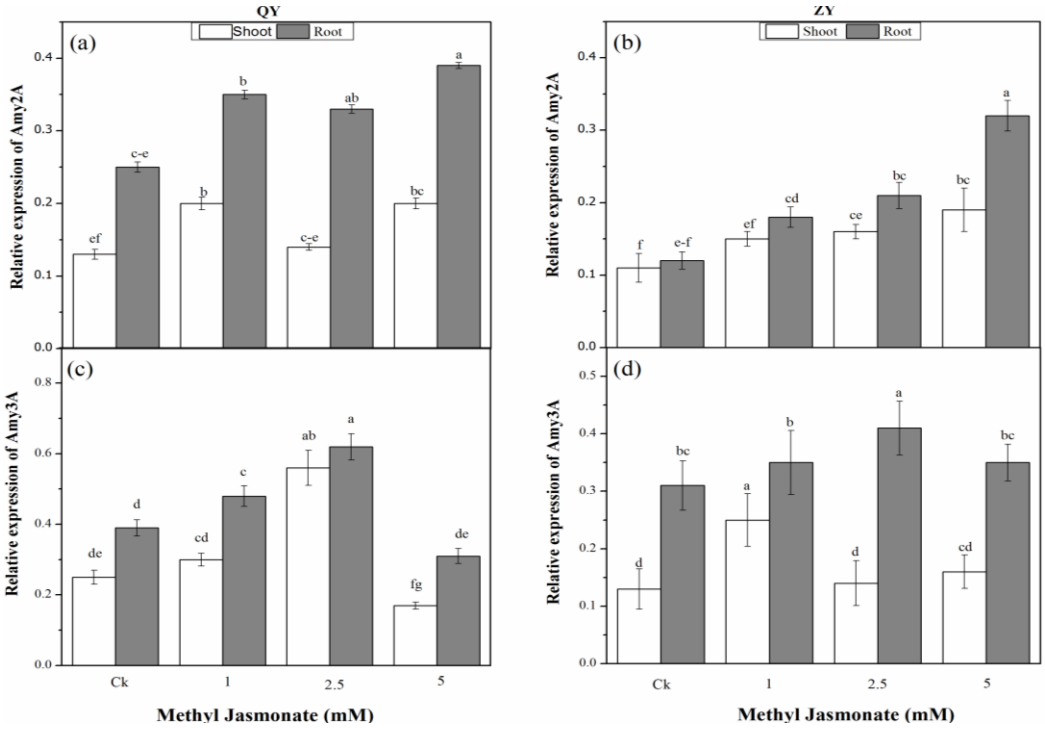

Fig. 4: Effect of seed soaking with methyl jasmonate on gene expressions of *Amy2A* and *Amy3A* in shoots and roots of two rice cultivars Qian You No.1 (QY) and Zhu Liang You 06 (ZY).

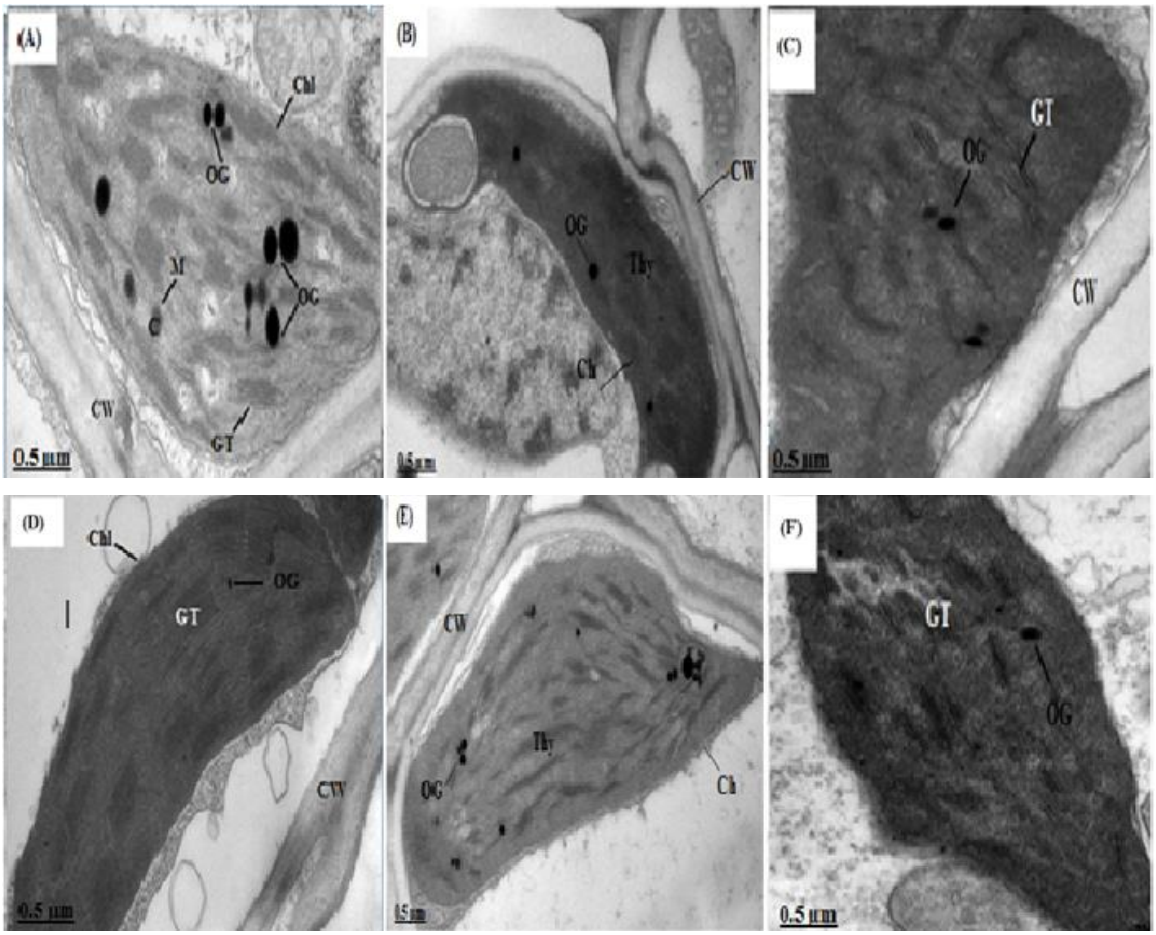

Fig. 5: Electron micrographs of leaf cell of two cultivars of *Oryza sativa* (cvs. Qian You No.1 and Zhu Liang You 06) grown under control, 2.5 and 5mM methyl jasmonate (MeJA). (A) TEM micrographs of leaf cell of Qian You No.1 under control show a clear cell wall (CW), well developed chloroplast (Ch), granum thylakoid (GT), mitochondria (M) with a good cristae and osmiophilic granule (OG). (B) TEM micrographs of leaf cell of Qian You No.1 exposed to 2.5 mM of MeJA show clear cell wall (CW), granum thylakoid (GT), osmiophilic granule (OG) and developed chloroplast (Ch). (C) TEM micrographs of leaf cell of Qian You No.1 exposed to high concentration of methyl jasmonate (5mM) show destroyed cell wall (CW), granum thylakoid (GT) and osmiophilic granule (OG). (D) TEM micrographs of leaf cell of Zhu Liang You 06 under control show clear cell wall (CW), developed chloroplast (Ch), granum thylakoid (GT) and osmiophilic granule (OG). (E) TEM micrographs of leaf cell of Zhu Liang You 06 exposed to 2.5 mM of MeJA show cell wall (CW), osmiophilic granule (OG), granum thylakoid (GT) and developed chloroplast (Ch).(F) TEM micrographs of leaf cell of Zhu Liang You 06 exposed to high concentration of methyl jasmonate (5mM) show granum thylakoid (GT) and osmiophilic granule (OG).

Table 1: Effect of seed soaking in MeJA on germination percentage (GP %), germination index (GI), germination energy (GE %), root length (RL), shoot length (SL) and seedling dry weight (SDW) of two rice cultivars.

Cultivars	MeJA (mM)	GP (%)	GI	GE (%)	RL (cm)	SL(cm)	SDW (g)
Qian You No. 1	CK	92.67±1.15 a	81.21±3.32 a	92.66±1.15 a	5.40±0.59 a	8.72±0.29 a	0.13±0.01 a
	1	87.33±3.61ab	61.48±4.62 b	74.01±4.73 ab	4.96±0.39 ab	7.31±0.72 b	0.12±0.02 ab
	2.5	80.68±2.31 b	52.53±5.24 c	44.66±3.81 bc	3.96±0.29 c	6.55±0.65 b	0.11±0.01 bc
	5	69.31±3.06 c	42.58±5.16 d	35.33±3.21 c	4.40±0.43 bc	6.33±0.68 b	0.10±0.01 c
Zhu Liang You 06	CK	91.33±1.15 a	80.18±1.33 a	90.67±1.16 a	5.23±0.22 a	10.09±0.91 a	0.14±0.02 a
	1	78.67±1.19 b	56.09±1.42 b	71.32±3.06 b	4.47±0.73 ab	8.93±0.32 ab	0.12±0.01 b
	2.5	77.33±2.25 b	52.37±2.65 b	46.01±4.16 c	4.13±0.18 bc	7.37±0.78 bc	0.11±0.01 b
	5	59.31±3.06 c	37.93±2.41 c	26.67±5.03 d	3.50±0.47 c	6.06±0.65 c	0.09±0.02 c

*Significant difference (α=0.05, LSD) among treatments within the same cultivar.

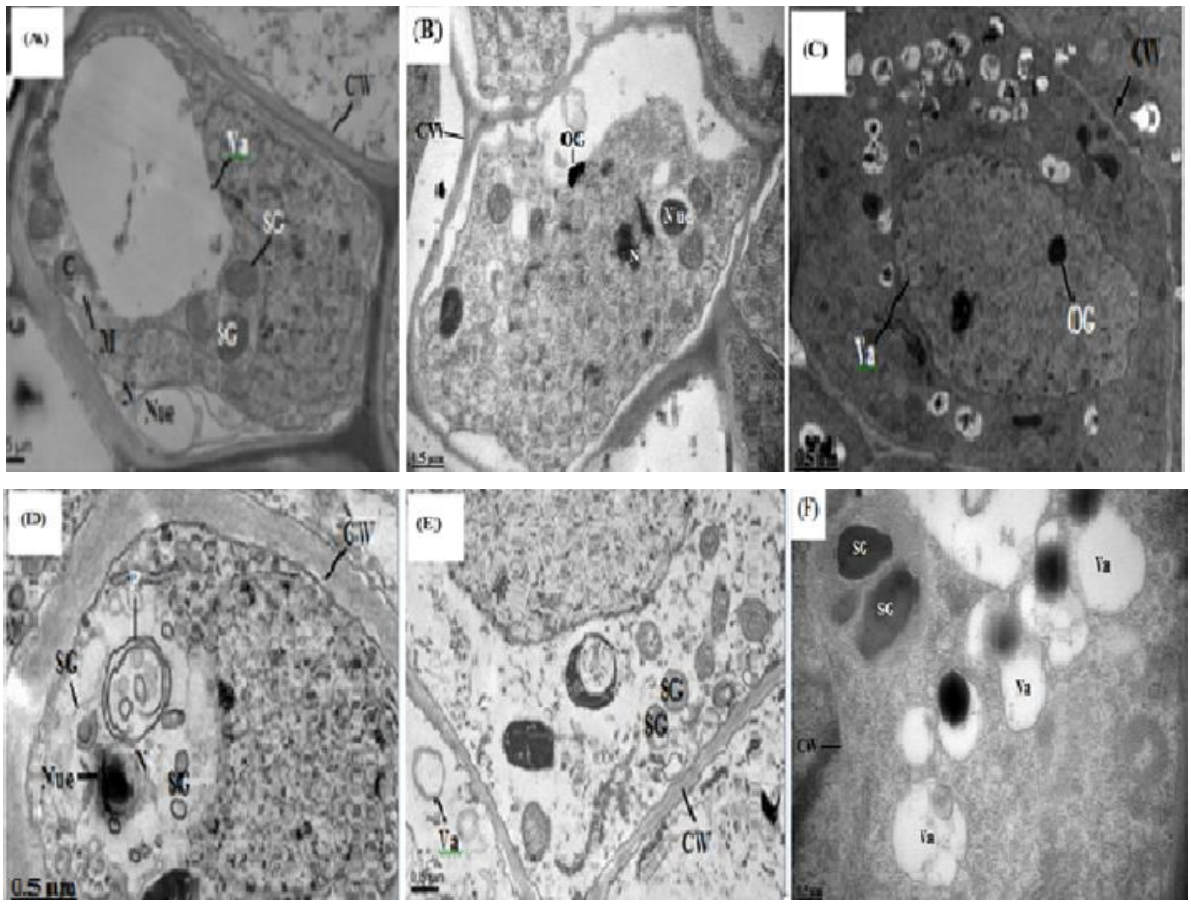

Fig. 6: Electron micrographs of root cell of two cultivars of *Oryza sativa* (cvs. Qian You No.1 and Zhu Liang You 06) grown under control, 2.5 and 5mM (MeJA). (A) TEM micrographs of root cell of Qian You No.1 under control show a clear cell wall (CW), well developed chloroplast (Ch), granum thylakoid (GT), mitochondria (M) with a good cristae and osmiophilic granule (OG). (B) TEM micrographs of root cell of Qian You No. 1 exposed to 2.5 mM of methyl jasmonate show clear cell wall (CW), large size elongated nucleus (N) with roundish nucleoli (Nue) and osmiophilic granule (OG). (C) TEM micrographs of root cell of Qian You No. 1 exposed to high concentration of MeJA (5mM) show destroyed cell wall (CW), granum thylakoid (GT) and osmiophilic granule (OG). (D) TEM micrographs of root cell of Zhu Liang You 06 under control show clear cell wall (CW), developed chloroplast (Ch), granum thylakoid (GT) and osmiophilic granule (OG). (E) TEM micrographs of root cell of Zhu Liang You 06 exposed to 2.5 mM of MeJA show granum thylakoid (GT) and osmiophilic granule (OG). (F) TEM micrographs of root cell of Zhu Liang You 06 exposed to high concentration of MeJA (5mM) show destroyed cell wall (CW), starch granule (SG) and Vacuole (Va).

DISCUSSION

In the present study different doses of MeJA inhibited seed germination percentage, germination index, germination energy, as well as plant height, root length and dry biomass of the seedlings of both rice cultivars (Table. 1). The findings of the current investigation showed a decline in the activity of α-amylase with increase in Me JA concentration (Fig. 2e). Enzyme α-amylase plays a pivotal role in cereal seed germination. It is responsible for the degradation of insoluble starch molecules to metabolizable simple sugar moieties, which are then translocated to the growing embryo. It can be inferred that the decrease in germination percentage, germination energy, germination index and root length might be due to scarcity of respiratory substrate, ultimately leading to reduced energy availability that results in decline of plasma membrane proton-pumping-ATPase activity and reduction in cell wall acidification. In this respect, the findings of the present study are in conformity with the previous results (Bialecka and Kepczynski, 2003). The reduced activity of α-amylase might be the consequence of the inhibited synthesis of gibberellin caused by MeJA.

Further results indicated that activities of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX) and malondialdehyde (MDA, used as oxidative stress marker) contents in the

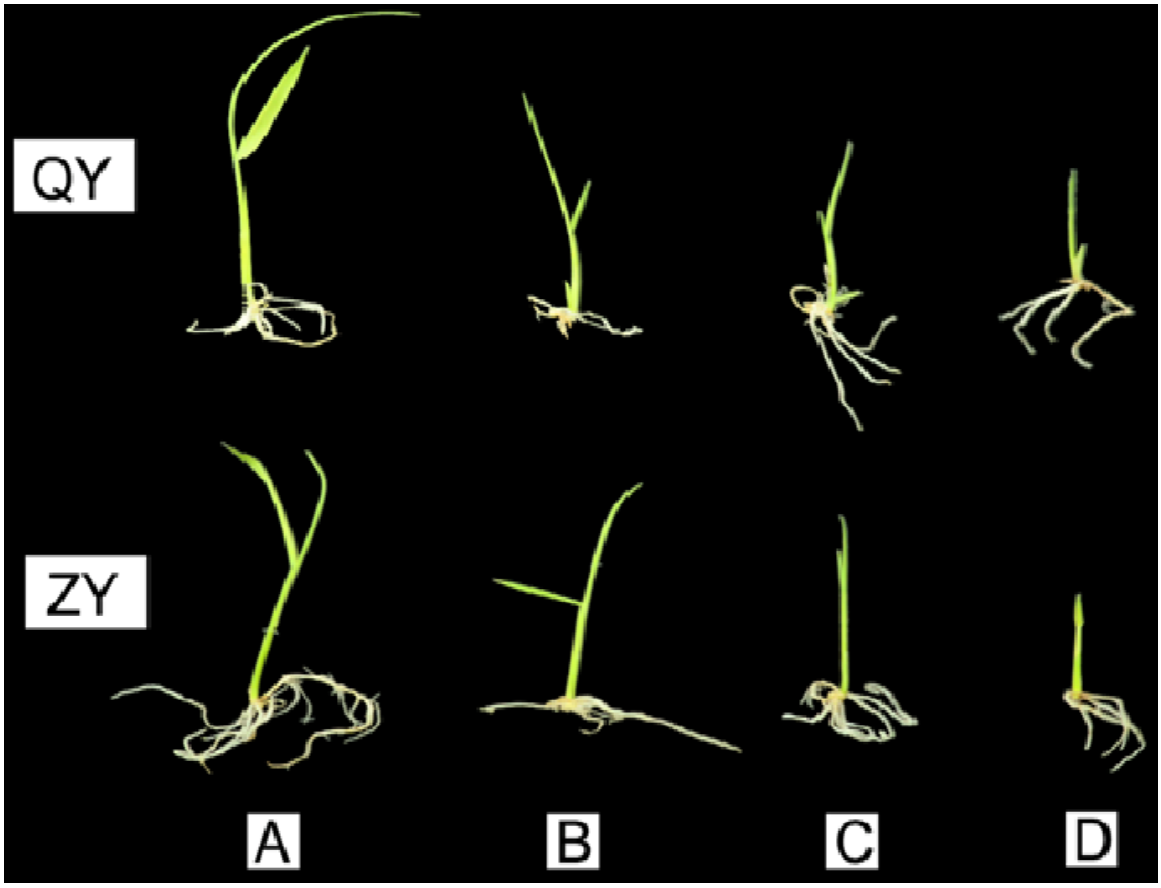

Fig. 7: Phenotypes of fourteen days old seedlings of Qian You No.1 (QY) and Zhu Liang You 06 (ZY) (A) Control, seeds treated with water; (B) Seeds treated with 1 mM of MeJA; (C) Seeds treated with 2.5 mM of MeJA; (D) Seeds treated with 5mM of MeJA).

leaves of two cultivars of *Oryza sativa* significantly increased at 1mM and 2.5 mM concentrations as compared to control and decreased significantly at 5 mM of MeJA as compared to its lower concentration (Fig. 1a-e). The present study showed that pre-soaking of seeds with MeJA had higher CAT, POD, SOD, APX activities and MDA content as compared with the control. These results are in harmony withF those obtained by Abdelgawad et al. (2014) who found that 50 µM of MeJA promoted production of several antioxidative enzymes. The results of present study are in line with those obtained by Qiu et al. (2014) stating that at higher concentrations of MeJA the activities of antioxidant enzymes were significantly increased. Methyl jasmonate plays a vital role in signal transduction pathways under stressed conditions by increasing CAT, POD, SOD and APX activity. Previously, It has been reported that MeJA caused signal transduction around 50 µmol while exerts inhibitory effects at higher concentration i.e. above 100 µmol (Nojavan-Asghari and Norastehnia, 2006). So, at higher concentrations it can be used as growth retardant.

Generally, ROS are accumulated in the cells in response to different kinds of stresses. Data showed that seedlings treated with higher MeJA concentration (5 mM) showed higher contents of ROS like H_2O_2, ^{-}OH and $^{-}O_2$. The contents of ROS were found to be enhanced significantly in the seedlings of both cultivars with increasing levels of methyl jasmonate. However, the cultivar QY was more severely influenced in comparison with ZY. Reactive oxygen species, when produced in excess, react with proteins, lipids and nucleic acids, causing a rise in lipid peroxidation, plasma membrane leakage and DNA degradation which consequently causes a severe malfunction of the cell (Halliwell and Gutteridge, 1990). In response to various environmental stresses, ROS may play important roles, as secondary messengers, to modulate the genes expression and biosynthesis of proteins involved in plant defense mechanism (Bukhari et al., 2015). However, at higher concentrations, they have been reported to be cyto-toxic (Zhang and Xing, 2008). The ROS may cause damage to the cells as a result of reduced protein and RNA concentration, deteriorated gas exchange capacity and loss of pigments. In the

present research, the higher concentrations of MeJA increased ROS generation and MDA content that could be the possible explanation for the damage of plant cells and tissues.

The main components of antioxidant defense system are SOD, POD, CAT, APX and GR which regulate the homeostasis of $\bar{O}_2$ and H_2O_2 within the cell and prevent the formation of OH^-(Ruciska and Pukacki, 2006). The elevated level of SOD activity, in response to heavy metal stress, exhibited ameliorative effect of antioxidant enzyme system on plants' physiological mechanisms under stressful environmental conditions, by regulating ROS homeostasis (Bukhari et al., 2016). To maintain the redox state of the cell, APX and GR are vital constituents of APX/GR pathways required for the scavenging of H_2O_2 mainly generated in the chloroplasts and other cell organelles (Asada, 1992). Increase in the GR activity has been ascribed as de-novo synthesis of the enzyme protein (Baisak et al., 1994). Besides, the results of histochemical staining also favored the QY by depicting a higher number of colored spots in roots of ZY cultivar. The results of DAB and NBT staining confirmed the concentration dependent increase in ROS production in the roots of MeJA treated rice seedlings, which is a sign of oxidative stress to plants in response to MeJA exposure. Similarly, detection of H_2O_2 through DAB staining further supports high accumulation of H_2O_2 in the leaves of rice seedlings under nano-CuO stress.

It is well known that some non-enzymatic antioxidants like GSH, carbon monoxide and proline play critical roles in plant responses to abiotic stresses (Sharma and Dietz, 2009). In the present study, different MeJA concentrations (1, 2.5 and 5 mM) enhanced the activities of non-enzymatic antioxidants like GSH, GSSG and total glutathione in the seedlings of both rice cultivars (Fig. 2). Phytochelatins and GSH have been considered to play a pivotal role in stress tolerance mechanism; the differential regulation of GSH and GR is of major concern in plants' responses to environmental stresses (Gill et al., 2015). In *Pteris vitatta*, GSH contents were reported to be increased under arsenic stress (Sun and Henson, 1991). It can be deduced that plants might depend upon GSH to combat detrimental effects of stress and relatively higher content was considered to be more ameliorative against ROS generation under exposure to MeJAs.

For starch degradation, a set of enzymes is needed. However, only alpha-amylase is considered to play a major role in the process (Sun and Henson, 1991). After seed imbibition, sugars are rapidly consumed in the embryo, leading to a transient sugar depletion that acts as a signal for rice α-amylase gene expression. In cereals, the expression of α-amylase genes during seed germination and seedling growth is negatively regulated by sugars in the embryo and positively regulated by gibberellins in endosperm (Hong et al., 2012). The QRT-PCR results demonstrated the higher expression levels of *Amy2A* and *Amy3A* genes under exposure to different MeJA concentrations (Fig. 4a, b, c, d). Up regulation in mRNA level of *Amy* genes was found, but the response of all genes in roots and shoots was not similar. In response to oxidative stress inducing chemicals, an elevated expression of CAT genes was reported in arabidopsis and rice (Smeets et al., 2008). Moreover, in rice and barley, the expression of α-amylase gene in the embryo is negatively regulated by sugars (Perata et al., 1997) and positively regulated by gibberellins (GA) in the endosperm (Loreti et al., 2000). Similarly, Rubio et al. (2014) observed up-regulation of Vvα-AMY genes in grapevine buds in response to hypoxia, suggesting a mediatory role of soluble sugars under limited oxygen supply. A robust enhancement in the expression level of plastidic *FeSOD* in grapevine buds has also been observed under metal stress. The regulatory mechanisms of MeJA biogenesis and the activation of related genes at molecular level are not well explored (Cheong and Choi, 2003).

In the present study, the TEM revealed a dose dependent effect of MeJA on root and shoot ultrastructure of both ricer cultivars. A clear damage was observed in the leaf mesophyll cells under exposure to 5mM MeJA in both cultivars, as compared to control (Fig. 1). Irrespective of cultivars, an increase in the number of starch grains and plastoglobuli was observed under higher MeJA concentration, showing a clear indication of stress (Daud et al., 2013). Bukhari et al. (2015a) reported the similar observation under heavy metal stress in tobacco. Compared to the control treatment, an obvious anatomical deformation, alongwith ruptured cell structure and disappearance of various organelles, was noted in the root tip cells of both rice cultivars in response to higher MeJA concentration. Previously, Panda (2007) has observed a deterioration in the root tip cells of *O. sativa* under heavy metal toxicity.

In conclusion, the seed soaking of rice seeds in different MeJA concentrations had affected antioxidant enzyme system, physiological, and molecular mechanisms of rice seedlings in both cultivars under study. Data suggested that seed soaking with MeJA (5mM) inhibited the germination percentage, germination energy, germination index as well as root and shoot length of the seedling of both cultivars. Moreover, this study depicted increased activity of antioxidant enzymes (CAT, POD, SOD, and APX) at 1mM and 2.5mM MeJA concentration, but at higher level i.e. 5 mM the trend was on lower side. The α-amylase activity decreased with increasing levels of MeJA; the *Amy2A* and *Amy3A* were more up-regulated in roots as compared to shoots of both cultivars. The outcome of present investigation would be of great importance to

the scientific fraternity engaged in exploring the role of MeJA in reducing pre-harvest sprouting in rice as well as other cereal crops.

Authors' contributions

JH conceived the idea. AN and SMK designed the project. AN, MSH, YG, SAHB, MD performed the experiment. AN and SAHB wrote the manuscript. All he author read and approved the manuscript.

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