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OPTIMIZATION OF GERMINATION INHIBITORS FOR CONTROLLING PRE-HARVEST SPROUTING IN HYBRID RICE

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The present study was conducted to screen different growth inhibitors i.e. ethephon (125 mM), methyl jasmonate (1 mM MeJA) and their combinations (125mM ethephon + 1mM MeJA) for minimizing the harmful effects of pre-harvest sprouting during hybrid rice seed production. The combination of both growth inhibitors significantly reduced germination and growth attributes with a more prominent decrease in cultivar Zhu Liang You 06 (ZY) as compared to cultivar Qian You No.1 (QY). Moreover, the activities of α-amylase, peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and malonaldahyde (MDA) contents were significantly increased with the combination of both growth inhibitors as compared to the control. CAT activity was significantly increased with 1mM of MeJA as compared to the control. The combination of both growth inhibitors resulted in the increase of abscisic acid (ABA) contents and the decrease of gibberellins (GA) contents as compared with the control. Gene expression analysis showed that the transcription levels of ABA genes (*OsNCED1, OsNCED3* and *OsABA80x1*) were up-regulated with the combination of both growth inhibitors. While, GA genes (*OsGA3ox1, OsGA20ox1* and *OsGA2oox2*) were down-regulated by seed soaking with both growth inhibitors and their combination.

Keywords: Pre-harvest, sprouting resistance, inhibitory mechanism, ABA, GA, gene expression.

INTRODUCTION

Seed dormancy is an important agronomic trait, as low levels can cause premature germination, while too much can inhibit uniform germination. Several crops such as rice, wheat and barley lack a sufficient level of seed dormancy, and are thus vulnerable to pre-harvest sprouting (PHS) of mature grains. The PHS becomes a serious problem in recent years resulting in reduced grain weight and deterioration in the quality of kernels.

Environmental stresses can cause a significant rise in ABA level during seed maturation. The transition of the hormone content from the dormant to the germinating seed is characterized by a decline and rise in the sensitivity of ABA and GA, respectively Chiwocha *et al*. (2005). The robust involvement of ABA in the process of dormancy is a well-established fact but the mechanism is yet to be uncovered completely. The level of ABA is reported to be low at embryogenesis, which rises during maturation and again declines during seed desiccation phase. Further, the study on ABA mutants of Arabidopsis and maize revealed the involvement of ABA in a variety of physiological processes during seed development like deposit of reserve food and initiation of primary dormancy Holdsworth *et al*. (1999).

Owing to its ubiquitous presence and pleiotropic effects on plant growth and developmental processes, MeJA is considered as an important endogenous plant growth regulator Cheong and Choi (2003). Exogenous application of jasmonates at higher concentrations can cause stem and root growth inhibition Staswick *et al*. (1992), inculcate pericarp and leaf senescence Yeh *et al*. (1995) and reduce both photosynthetic and respiratory activity Maslenkova *et al*. (1990). Role of exogenous jasmonate on seed germination of non-dormant seeds was investigated in sunflower, amaranth, rape and flax seeds respectively. Jasmonates have also been found to play an important role in senescence and stress responses of plants. Tsai *et al.* (1996) reported a decline in ethylene synthesis in seedlings and detached leaves of rice, in response to jasmonate. MeJA, as growth retardant, has been successfully used for the induction of dormancy thus reducing the pre-harvest sprouting of sugarbeet, potato, onion, rice and carrot seeds.

Ethylene, a gaseous plant hormone, is reported to modulate a range of physiological processes and growth responses of plants to various environmental stimulators. Generally, ethylene is considered as growth retardant, however its role as plant growth promoting hormone has also been reported Pierik *et al.* (2006). Ethephon (2-chloroethyl phosphonic

acid), an ethylene-producing substance, is widely employed as plant growth regulator and generally recognized to have inhibitory effects. However, ethylene is involved in the regulation of various plants processes, with both stimulatory and inhibitory effects. The impact of exogenously applied gaseous ethylene or ethephone is dependent upon plant species and concentration of the growth regulator. Basra (2000) has reported the use of ethephon to delay the onset of flowering, reduce etiolation and strengthen the stem in ornamental plants. In daffodils and tulips, soil application of ethephon delayed the onset of flower initiation for 1-3 days when the bulbs were given low temperature treatment for a short period of time Moe (1980).

PHS is very common phenomenon in rice and other cereals all over the world which not only reduces the crop yield, but also deteriorates the grain quality, subsequently causing significant economic losses. Occurrence of PHS is frequently noticeable in rice as its growth period coincides with long spells of rainy season during early summer and autumn Wan *et al*. (2006). Most of the Asian countries, especially China, depend upon rice to meet the food requirements of their population. Owing to ever growing population, it is expected that the demand for rice will keep on increasing in future. Therefore, increase in rice production, through exploiting molecular mechanism and employing exogenous plant growth regulators, is inevitable. It was observed that ethephon and MeJA combination significantly inhibited seed germination and α-amylase activity in the cocklebur and maize seeds. In this study we evaluated the influence of exogenously applied ethephon, MeJA and their combination on germination, antioxidants enzymes, metabolic changes, ABA and GA levels in seeds of two rice hybrids and the expression of genes involved in this process.

MATERIALS AND METHODS

***Plant materials and growth conditions*:** Two rice hybrids (QY and ZY) were obtained from Zhejiang Nongke Seed Industry Co., Ltd, China. Methyl Jasmonate (MeJA) and ethephon were obtained from Jiangsu Sword Agrochemicals. Seeds were soaked in 1mM MeJA (T_1), 125 mM ethephon (T_2) and combination of 1mM MeJA and 125 mM ethephon (T_3) in petri dishes for one hour. Seeds soaked in tape water were considered as control (ck). After respective treatments, petri dishes were kept in controlled environment at 25°C under alternating cycle of 16 h light and 8 h dark period for 14 days. The treatments were replicated thrice and each petri dish contained 50 seeds. Seeds were considered germinated when a 2 mm long radicle protruded through the seed coat.

Final seed germination percentage was calculated after 14 days of sowing. Germination index was calculated ($GI=\sum \frac{Gt}{Tt}$), (Hu *et al*., 2006) where Gt means number of seeds germinated in the time t in days and Tt expresses the time corresponding to Gt in days. Two weeks old seedlings were sampled and rinsed with deionized water. The energy of germination was recorded on the 4th day after sowing. It represents the percentage of germinating seeds at 4th day after sowing relative to the total number of tested seeds (Ruan *et al*., 2002). The harvested seedlings were used for the measurement of root and shoot length, and dry and fresh weight. For determination of dry weight, seedlings were oven-dried at 65°C for 48 h.

***Measurement of α-amylase activities*:** Treated seeds were germinated for two days, quickly frozen with liquid nitrogen followed by storage at -80ºC temperature. Seeds were hulled then ground into fine powder, followed by homogenization with 10 mL distilled water. The mixture was centrifuged at 5000×g for 10 min. Supernatant was collected in 10 mL centrifuge tube for chromogenic reaction. The activity of α-amylase was determined by 3, 5-dinitrosalicylic acid colorimetric (DNS) method as described by Li (2000). Maltose content was quantified according to the standard curve: $M= 8.246A + 0.084$ ($R^2 = 0.999$), where "A" means absorption value of subtracting tube 2 and tube 1. α-amylase activity was defined as: enzyme activity = M*T/[R*W*t], M is maltose content (mg), T is total extracting volume, R is extracting volume used for reaction, W is weight of seed sample, t is reaction time.

***Measurement of antioxidant enzyme and MDA contents*:** The activity of catalase (CAT) was measured by reduction in absorbance at 240 nm due to the decline of extinction H_2O_2 Cakmak and Marschner, (1992). Peroxidase (POD) activity was measured with guaiacol as the substrate in a total volume of 3 ml Zhang, (1992).

Superoxide dismutase (SOD) activity was assayed by determining its ability to hamper the photochemical reduction of nitrobluetetrazolium (NBT) Rao and Sresty (2000). Reaction mixture contained 50 mM phosphate buffer (pH 7.8) reaction was started with the addition of 2 M riboflavin and reaction mixture was placed under 15 W fluorescent lamps for 15 min. The reaction mixture without enzyme extract was considered as control. The photo reduction of NBT was measured with spectrophotometer at 560 nm. APX activity was assayed as described by Nakano and Asada (1981). The assay depends upon the reduction in absorbance at 290 nm due tooxidation of ascorbate. Lipid peroxidation was quantified as malondialdehyde (MDA) content as narrated by Zhou and Leul (1998).

Glutathione reductase (GR) was analyzed using the method given by Schaedle and Bassham (1977). Fresh shoot samples (200 mg) were ground and homogenized in 5 ml of 50 mM Tris/HCl buffer (pH 7.6) solution using pestle and mortar. Then, the homogenate was centrifuged at 22000×g for 30 min at 48°C and the supernatant was utilized for enzyme analysis. The absorbance was measured with spectrophotometer at 340 nm. Glutathione (GSH) and oxidized glutathione (GSSG) were measured using the

procedure described by Law *et al*. (1983). Fresh shoot samples (0.3 g) were homogenized using 5 mL of 10% (w/v) TCA solution, followed by centrifugation of the homogenate at 15000×g for 15 minutes. The reaction mixture consisted of 100 µL of 6 mM dithionitrobenzoate (DTNB), 50 µL of glutathione reductase (10 units mL^{-1}), and 700 µL of 0.3 mM NADPH. To determine the total glutathione, 150 µL supernatant was mixed with reaction mixture. Total glutathione content was determined by the standard curve. For measuring GSSG, 120 µL of supernatant was mixed with 10 µL of 2-vinylpyridine. Then, 20 µL 50% (v/v) triethanolamine was added to the mixture. The solution was thoroughly mixed using a vortex for 30s, followed by incubation for 25 minutes at 25°C. The solution was analyzed as described above. GSSG samples, treated as mentioned above, were used to develope a calibration curve and GSH was measured by subtracting GSSG contents from the total glutathione Law *et al*. (1983).

***Determination of ABA and GA₃ contents*:** The ABA and GA contents were extracted from the seed with cold acetonitrile. The extracting solution was injected through HPLC system having C_{18} column and ultraviolet detector. The mobile phase, methanol/water (50:50, v/v), was run at a flow rate of 0.8 mL min^{-1}. Retention time of ABA and GA_3peaks was used to identify the peaks in the samples. Qin *et al*. (2013).

Leaf and root samples (100 mg each) stored at -80 °C were fine-ground using pestle and mortar with a small amount of liquid nitrogen. Total RNA content was extracted from the shoot and root samples of the 0, 1, 2.5 and 5mM MeJAexposed seedlings using an 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 RNA in a 20 µL reaction tube, followed by 4 times dilution with RNAs-free water. Primers used for quantitative real-time PCR (QRT-PCR) experiments are shown in Table 1.

Table 1. Sequences of oligonucleotide primers used in QRT-PCR

Locus	Primer name	Sequence (5'-3')
Actin	Ay212324-F	GGTATTGTTAGCAACTGGGATG
	Ay212324-R	GATGAAAGAGGGCTGGAAGA
OsNCED1	Ay 838897-F	CTCACCATGAAGTCCATGAGGCTT
	Ay 838899-R	GTTCTCGTAGTCTTGGTCTTGGCT
OsNCED3	Ay 838899-F	CCCCTCCCAAACCATCCAAACCGA
	Ay 838897-R	TGTGAGCATATCCTGGCGTCGTGA
OsABA80x1	Ak 120757-F	CAGACGAGGAGCATGACACTCA
	Ak 120757-R	GTTCCTGAACAGAGGCATCACC
OsGA3ox1	AB O56519-F	CGGACTCGGGCTTCTTCACCT
	AB O56519-R	CGAGGAAGTAGCCGAGCGAGAC
OsGA20ox1	Ak 099111-F	CCGTGGAAGGAGACGCTGTC
	Ak 099111-R	GCGGCTCATCTCGTGGCAGT
OsGA2oox2	ABO 77025-F	CGCCGACTACTTCTCCAGCACC
	ABO 77025-R	GCTGTCCGCGAAGAACTCCCT

QRT-PCR was carried out using SYBR premix EX Taq (Takara, Japan) following the manufacturer's protocol. ACT1 was used as a reference gene for endogenous control. 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.

***Transmission electron microscopy study*:** 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. After that the leaf and root samples were fixed for one hour in 1% osmium tetroxide (OsO_4), washed thrice with the same PBS by keeping 10 min gap between each washing. Afterwards, the dehydration of the samples was carried out using graded series of ethyl alcohol (50, 60, 70, 80, 90, 95 and 100%) with 15-20-min intervals, followed by final washing with absolute acetone for 20 min. The dehydrated specimens were infiltrated and kept overnight in spurr's resin mixture. The samples were heated for 9 h at 70°C and ultrathin sections were excised 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 collected data were statistically analyzed by analysis of variance (ANOVA) technique using statistical analysis system software (SAS version 9.1). The percentage data were transformed using y= arcsin [sqr (x/100)] before analysis. The difference between treatment means was calculated at 5% probability level (α=0.05, LSD).

RESULTS AND DISCUSSION

Present study indicated that germination percentage (GP), germination index (GI) and germination energy (GE) of both hybrid were significantly decreased when treated with growth inhibitors and their combination in comparison with their respective controls (Table 2). The lowest GP, GI and GE were recorded for ZY hybrid compared to QY hybrid at T_3 (1mM MeJA + 125 mM ethephon), compared with the control treatment. Similarly, seedling vigor in terms of root/shoot length and seedling weight significantly decreased after treatment with combination of growth inhibitors. In case of amylase activity, it was significantly higher in control as compared with T_1 (1mM MeJA) and T_2 (125 mM ethephon) whereas it was lowest in T_3 (1mM MeJA + 125 mM ethephon) as described in Table. 1.

The enzyme, α-amylase plays a pivotal role in germination of cereal seeds. It is responsible for degradation of insoluble starch granules to soluble sugar moieties, which are then translocated towards the embryonic axis. It can be inferred that the decline in germination percentage, germination index, germination energy, and reduced root length could be attributed to the limited synthesis of respiratory substrate

Table 2. Effect of seed soaking in growth inhibitors 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	Ck	90.0±3.10a	78.6±0.192a	70.7±4.62a	5.8±0.61a	7.75±0.67a	0.11±0.01a
No. 1	T1	77.3±5.43b	61.3±2.32b	66.7±7.02ab	4.17±0.60b	6.93±0.28ab	0.09±0.08b
	T2	71.3±3.23b	55.1±3.423c	57.3±3.45bc	3.29±0.36c	6.24±0.22bc	0.08±0.03bc
	T3	69.3±4.15b	48.6±2.241d	52.0±5.29c	3.89±0.33bc	6.00±0.59b	0.07±0.03c
Zhu Liang	Ck	89.3±2.31a	70.6±5.88a	79.3±1.15a	6.74±0.37a	8.09±0.21a	0.10±0.01a
You 06	T1	79.3±5.03b	58.4±4.373b	62.7±3.06b	4.7±0.41ab	7.00±0.43b	0.08±0.01b
	T2	82.0±3.56b	61.4±2.194b	64.7±5.03b	3.84±0.12bc	6.45±0.48bc	0.09±0.01b
	T3	64.0±2.75c	47.9±2.06c	52.7±4.16c	3.83±0.72bc	6.48±0.28b	0.08±0.02b

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

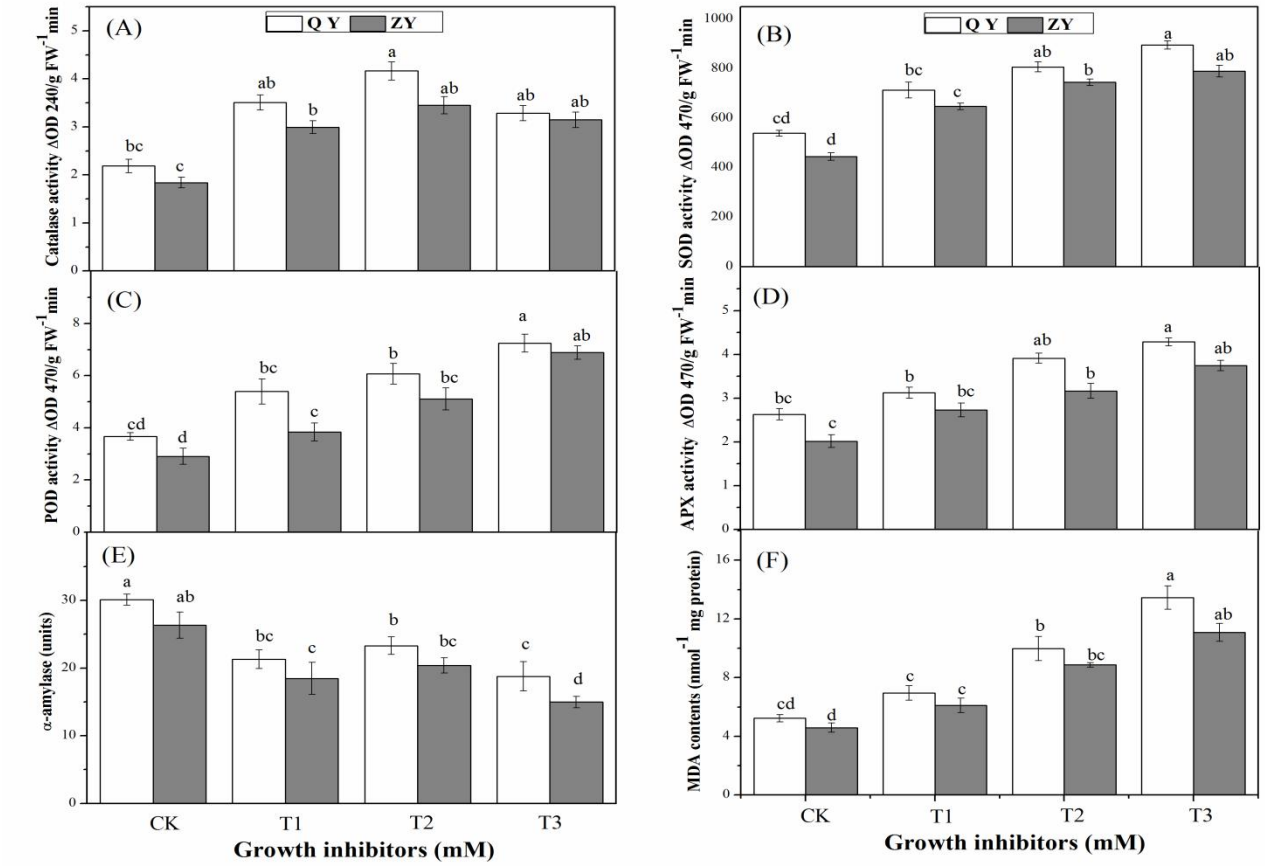

Figure 1. Effect of seed soaking with growth inhibitors on (A) Catalase (CAT), (B) Superoxide dismutase (SOD), (C) Peroxidase (POD), (D) Ascorbate peroxidase (APX) activities, (E) α-amylase and (F) Malondialdehyde (MDA) contents in two rice cultivars seedlings.

and ultimately limited energy production. In this respect, the findings of the present study are in conformity with those reported by Kepczynsk and Bialecka (1994). The inhibition of gibberellin could be the possible reason for diminution of α-amylase activity owing to its limited synthesis Kepczynski and Bialecka (1994)

The results indicated that activities of CAT, POD, SOD, APX and MDA contents in the leaves of both hybrids significantly increased after soaking with growth inhibitors and their combination (Fig. 1). Soaking seeds with inhibitors imposed stress and resulted decrease in CAT, POD, SOD and APX activities in comparison with their respective control. These results are in harmony with those obtained

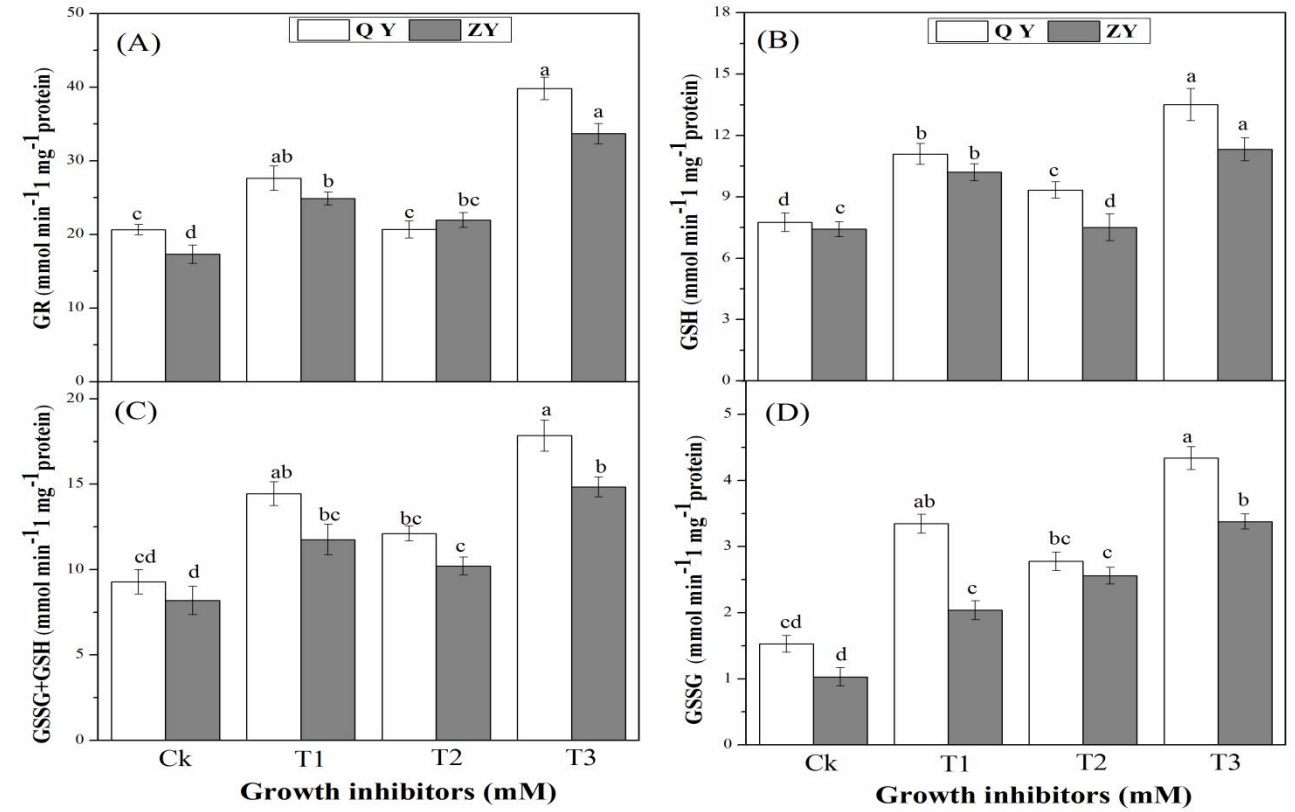

Figure 2. Effect of seed soaking with growth inhibitors on (A) glutathione reductase (GR), (B) reduced glutathione (GSH), (C) total glutathione (GSSG+GSH)and (D) (GSSG) of two rice cultivars.

that methyl jasmonate seed treatment had triggered several antioxidative enzymes. Previously, it has been reported that about 50 µmol MeJA does not hamper signal transduction but, causes inhibitory effect at concentration above 100 µmol Norastehnia *et al*. (2007).

The GR contents were increased after application of growth inhibitors; there were observed highest in case of T_3 (1mM MeJA + 125 mM ethephon) followed by T_1 (1mM MeJA) treatment, similar trend was recorded in case of GSH and GSSG (Fig. 2). Significant difference was found in total glutathione contents (GSH+GSSG) in seedlings with the maximum in T_3 followed by T_1, T_2 and control, respectively in both hybrids. An important finding of this study was the significant increase of total glutathione contents in QY hybrid as compared to ZY, irrespective of the effect of growth inhibitors.

The plant antioxidant defense system is comprised of SOD, POD, CAT, APX and GR enzymes, which regulate the redox homeostasis within the cell and prevent the generation of OH^- radical Ruciska-Sobkowiak and Pukacki (2006). The enhanced SOD activity in response to heavy metal treatment ameliorated the stress by curbing the production of ROS. In order to maintain the cellular redox state, APX and GR are important constituents of ascorbate/glutathione pathways, required for H_2O_2 scavenging Asada, (1922). The ameliorative effect of some non-enzymatic antioxidants like glutathione (GSH), proline and carbon monoxide against abiotic stresses in plants, is a well known phenomenon Sharma and Dietz (2009). In the present study, growth inhibitor treatments T_1, T_2 and T_3 induced a significant increase in the activities of non-enzymatic antioxidants like GR, GSSG, GSH and total glutathione in the seedling of both rice genotypes (Fig. 2). Phytochelatins and GSH are reported to play a crucial role in plant stress tolerance. Phytochelatins and GSH play a key role in stress tolerance to improve plant defense mechanism under stressful conditions Masood *et al*. (2012). It could be deduced that plants might depend upon GSH to combat the detrimental effects of environmental stresses and comparatively higher contents were found to be more protective against ROS production under the application of growth inhibitors individually, or in combination.

Plant hormones play a pivotal role in regulation of plant growth; GA_3 and ABA content in both rice hybrids were measured. During germination test, ABA contents were increased in T_1, T_2 and T_3 respectively, comparing with their

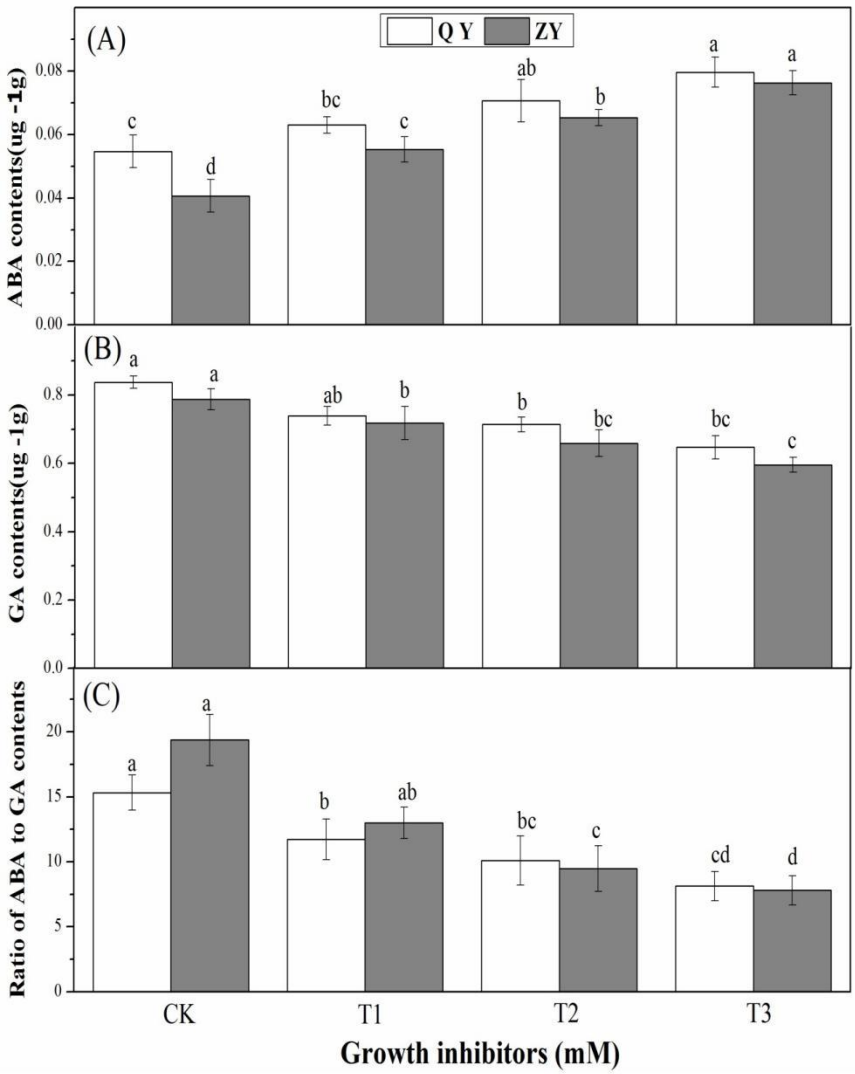

Figure 3. Effect of seed soaking with growth inhibitors on (A) ABA contents (B) GA contents and (C) ABA/GA ratio of two rice cultivars.

respective control in both hybrids (Fig. 3A). On the other hand, the contents of GA were higher in the control as compared to other treatments (Fig. 3B). The ratio of ABA to GA_3 for both rice varieties gradually decreased and the highest values were recorded with control followed by, T_1, T_2 and T_3, respectively (Fig. 3C).

Abscisic acid and GA have been reported to regulate seed dormancy and germination with different ratio Liu *et al*. (2011). The equilibrium between seed germination and dormancy is maintained by a dynamic balance between synthesis and catabolism of ABA and Gas Gutierrez *et al*. (2007). The molecular study revealed that the ABA and GA control each other antagonistically through reciprocal transcription regulating of their corresponding metabolic genes Seo *et al*. (2006); Toh *et al*. (2008). Up regulation of ABA related genes i.e *OsNCED1, OsNCED3* and *OsABA80x1* were observed in both hybrids under different concentrations of inhibitors (Fig. 4). Likewise, the GA genes including *OsGA3ox1, OsGA20ox1* and *OsGA2oox2* were also up-regulated with respect to their respective controls. Recently, Toh *et al*. (2008) suggested a model that extends the existing knowledge base about ABA/GA balance to address the changes function and expression of corresponding genes. The model suggests that the variation in the dormancy status, exhibited by different hybrids of rice, is regulated by differential expression of ABA and GA related metabolic genes. Environmental variables, like temperature and light intensity can boost up the ABA specific biosynthesis (*OsNCED* family) and GA catabolic genes, which ultimately result in seed dormancy via induced

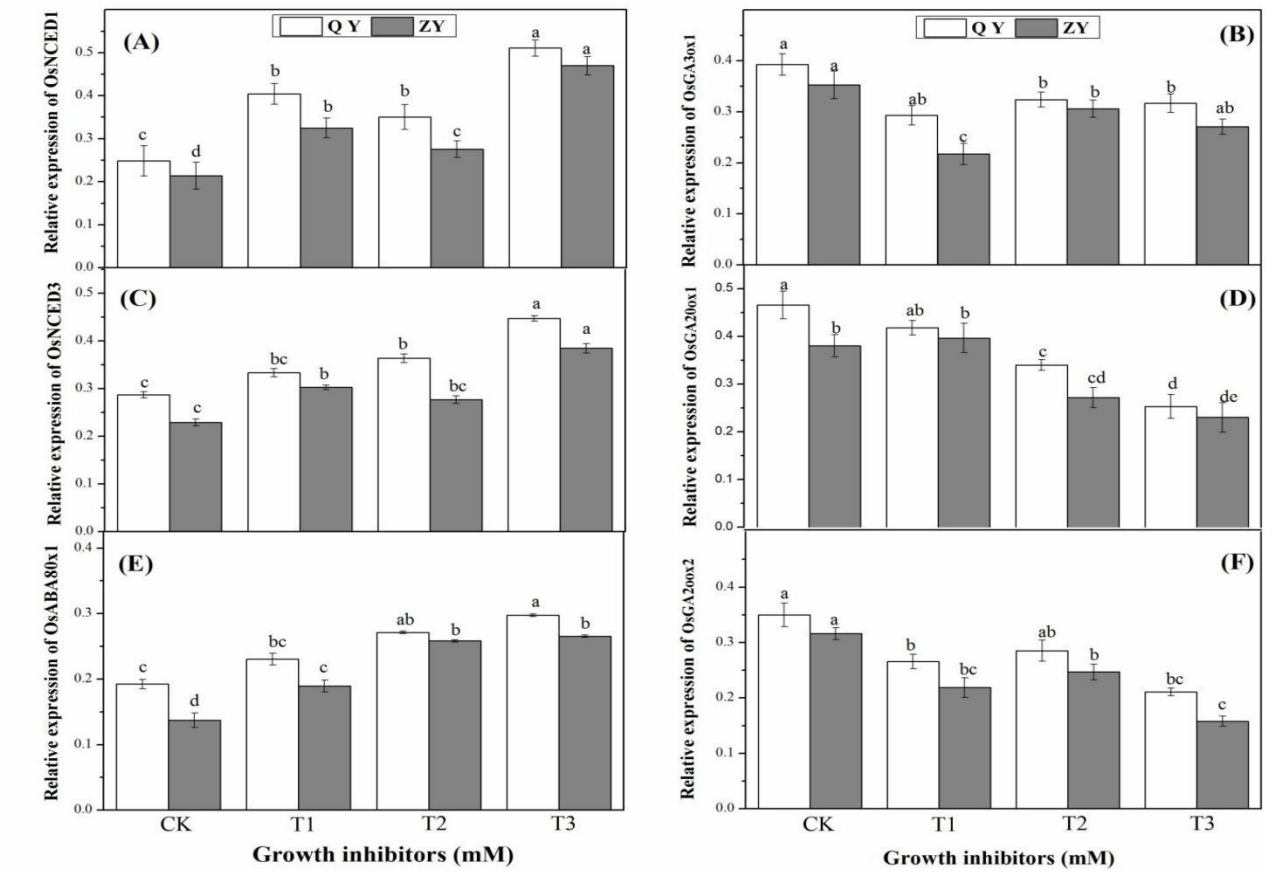

Figure 4. Effect of seed soaking with growth inhibitors on gene expressions of genes (A) *OsNCED1,* (B) *OsNCED3* (C) *OsABA80x1,* (D) *OsGA3ox1,*(E)*OsGA20ox1* and (F) *OsGA2oox2* in seedlings of two rice cultivars Qian You No.1(QY) and Zhu Liang You 06(ZY).

ABA dominance Toh *et al*. (2008). The expression level of GA biosynthesis genes (*OsGA20ox* and *OsGA3ox*) and ABA catabolic genes in also enhanced under low temperature treatment Finch-Savage *et al*. (2007).

The correlation analysis showed that CAT, POD, SOD, APX, MDA, ABA, *OsNCED1*, *OsNCED3*, *OsABA80x*, *OsGA3ox1*, *OsGA20ox1* and *OsGA2oox2* have a negative significant correlation with seed germination and germination index in both rice hybrids (Table 3). It indicates that GA and ABA/GA ratio have a positive significant correlation with seed germination and germination index of ZY hybrid. The correlation analysis reported that GA and ABA/GA insignificant correlated with seed germination of QY hybrid.

The ultrastructural changes in leaf cells under control and the combination effect of ethephon and methyl jasmonate showed thin cell and clean walls, well-developed chloroplast as compared with those of controls (Fig. 5 A-D).The TEM analysis showed that there were no significant changes in the leaf mesophyll structure among the two hybrids. At combination treatment of ethephon and methyl jasmonate, microscopic analysis showed that root tip cells of both hybrids presented clear cell walls, the cell had normal typical oval shaped mitochondria, and a well developed nucleus as compared to the control treatment (Figure 5 E-H).

Table 3. Correlation analysis between molecular and biochemical parameters with seed germination and germination index of tow cultivars of *Oryza sativa* treated with ethephon, methyl jasmonate and their combination.

Parameters	GP		GI	
	V1	V2	V1	V2
CAT	–0.836	–0.519	–0.786	–0.686
POD	–0.969**	–0.906*	–0.987**	–0.900*
SOD	–0.985**	–0.760	–0.993**	–0.861
APX	–0.946*	–0.880*	–0.950**	–0.923*
MDA	–0.899*	–0.851	–0.920*	–0.859
ABA	–0.937*	–0.864	–0.956*	–0.911*
GA	0.965**	0.870	0.985**	0.903*
ABA/GA	0.982**	0.770	0.993**	0.863
OsNCED1	–0.831	–0.995**	–0.879*	–0.983**
OsNCED3	–0.875	–0.989**	–0.909*	–0.994**
OsABA80x1	–0.963**	–0.708	–0.970**	–0.780

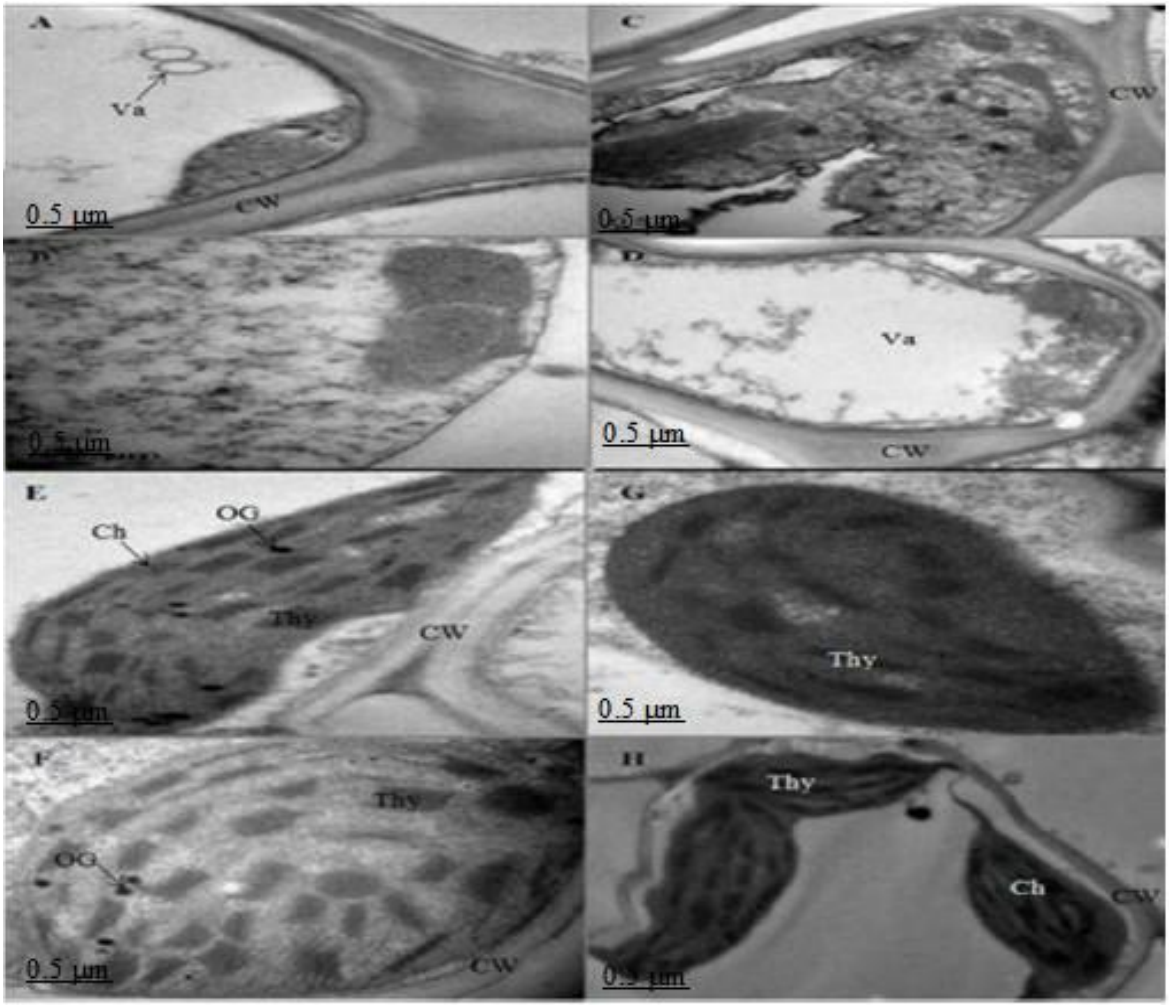

Figure 5. Transmission electron micrograph of leaf mesophyll cells (A, B, C, D) and roots meristem cells (E, F, G, H) of rice seedlings.

OsGA3ox1	–0.980**	–0.897*	–0.989**	–0.951**
OsGA20ox1	–0.986**	–0.905*	–0.990**	–0.875
OsGA2oox2	–0.946**	–0.927*	–0.971**	–0.951**

The * and ** indicate correlation significance at the 0.05 and 0.01 probability levels, respectively.

Conclusions: Soaking rice seeds in different inhibitors (MeJA and ethephon) and their combination had modulated physiological, antioxidant enzymes and molecular mechanisms of rice seedlings in both hybrids. Present study clearly shows that seed soaking with inhibitor combination inhibited germination attributes as well as roots and shoots lengths of the seedling of both hybrids. The results of present study are of great significance to minimize the problem of pre-harvest sprouting in hybrid rice.

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