Research Article
Protective Effect of Exogenous PGRs on Chlorophyll Fluorescence and Membrane Integrity of Rice Seedlings under Chilling Stress

[1]Farhad Mohabbati, [2]Farzad Paknejad, [2]Saeed Vazan, [2]Davood Habibi, [3]Mohammad Reza Tookallo and [4]Foad Moradi
[1]Department of Agronomy,
[2]Agricultural Research Centre, Karaj Branch, Islamic Azad University, Karaj, Iran
[3]Bojnord Branch, Islamic Azad University, Bojnord, Iran
[4]Department of Molecular Physiology, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

Abstract: The effects of chilling stress on chlorophyll content, chlorophyll fluorescence and Malondialdehyde (MDA; a biomarker for lipid peroxidation in membrane) were evaluated in rice (*Oryza sativa*) seedlings during chilling stress and subsequent recovery phase using a phytotron. The effects of Indole-3-Acetic Acid (IAA), Abscisic Acid (ABA) and Kinetin (CK) exogenously foliar applied to three rice genotypes differing in cold tolerance were probed. Under chilling stress reduced levels of photo inhibition and MDA due to hormone application in relation to no hormone treatment demonstrated the significant effect of applied hormones to induce chilling tolerance in the genotypes. There were different effects of hormones on genotypes and even on organs (root and shoot) in the case of MDA content, moreover the strategy for cold tolerance differed among the genotypes.

Keywords: Low temperature, MDA, membrane integrity, phytohormone

INTRODUCTION

Low positive temperatures often cause irreversible effects to metabolism and physiological processes in rice including damage to cell membranes, the broken chloroplast and eventually decreased photosynthetic ability and finally damage to rice growth and productivity. Photosynthesis is one of the main physiological processes, affected by low temperature. Changes in environmental conditions result in an imbalance between the light energy absorbed by PSII and the energy utilized by metabolism. The energy imbalance is sensed by alterations in PSII excitation pressure and thus the reduction state (Wingsle *et al.*, 1999). In the chloroplast, light reactions will continue while the energy consuming biochemical reactions are more limited in low temperature. When the rice seedlings are chilled, the superstructure of chloroplasts is damaged severely and the electron transfer function decreases (Guo-Li and Zhen-Fei, 2005). Several researches indicated that chlorophyll fluorescence parameters were strongly correlated with whole-plant mortality in response to environmental stresses (Pei-Guo and Ceccarelli, 2006). The way for measuring photosynthetic traits including chlorophyll fluorescence and chlorophyll content parameters might estimate influence of the environmental stress on growth and yield, since these traits were closely correlated with the rate of carbon exchange (Araus *et al.*, 1998;

Fracheboud *et al.*, 2004; Pei-Guo and Rong-Hua, 2000). Fv/Fm values are used to estimate the original photochemical efficiency in PS II. Fv/Fm decreased more severely in the cold-sensitive cultivars, indicating that the decrease of Fv/Fm was consistent with the tolerance to chilling in rice cultivars. qP is a ratio of opened reaction centers to total reaction centers in PSII. Upon chilling, qP in tolerant rice declined rather slowly, whereas in sensitive rice it declined continuously (Wang and Guo, 2005). It is a well documented fact that the function of a photosynthetic apparatus is sensitive to several environmental stresses, such as high and low temperature stress and salinity stress. PSII appears to be preferentially affected by chilling stress Chilling stress reduced the Fv/Fm and PSII, indicating that an important portion of the PSII reaction centre was damaged.

Production of O_2^-, OH, H_2O_2 and other ROS, interacting with DNA, proteins and lipids leads to the damage to cell components. The increase in production of ROS is a well known mechanism in stress defense that will cause a cascade of oxidative processes that are detrimental to lipids, due to the increased peroxidation processes (Lukatkin, 2005; Tambussi *et al.*, 2004). One of membrane lipids response action when exposed to cold would be the increase in the unsaturation of fatty acids compared to fatty acids in plant under normal conditions (De Palma *et al.*, 2008; Tasseva *et al.*, 2004). ER (Endoplasmic Reticulum) is the site for lipid

biosynthesis (Badea and Basu, 2009). It is already clear that there is one common effect of low temperature stress on membrane lipids among most plant species that is the increase in unsaturation level of the fatty acids (Badea and Basu, 2009). The exposure of chilling-sensitive plants to low above-zero temperatures enhances peroxidation of lipids, which induces damage to cell membranes and disturbs physiological functions. The extent of LP activation depends on the duration of cold stress and is correlated to the extent of chilling injury (Lukatkin, 2002). The amount of thiobarbiturate-reactive substances (malonic dialdehyde being a major component) is indicative of only terminal stages of lipid peroxidation (Lukatkin, 2003). In particular, cell disturbances are caused by activation of lipid peroxidation, which involves polyunsaturated fatty acids located predominantly in cell membranes (Lukatkin, 2003). The increasing rate of LP alters the membrane structure and elevates the membrane permeability to ions and water, which ultimately disrupts physiological processes in the cell. In the case of severe stress, these alterations may cause damage and eventual plant death (Lukatkin, 2003). Chilling-induced changes of molecular order in membrane lipids could be the cause of chilling injury Phase transitions, even in minor fractions of membrane lipids (particularly, phosphatidylglycerol featuring a high melting point), are believed to produce solid-phase domains, which would damage cell membranes and injure the cells (Lukatkin, 2005). The phase transition is related to phase separation of membrane components, characterized by the appearance of gel-like lipid regions in the plane of the bi layer. Changes in the lipid phase of membrane enhance the damage due to the decrease in the ATP level, the misbalance of metabolism and the increase in membrane permeability The nonselective permeability of membranes was found to increase upon cooling, which is considered as one of the basic elements of chilling injury (Lukatkin, 2005). Excessive excitation energy, obtained by the photosynthetic apparatus during illumination at chilling temperatures, causes photo oxidative damage to photo systems in thylakoid membranes and leads to photo inhibition of photosynthesis; the effects that are enhanced by lower temperatures and higher illumination (Lukatkin, 2005). At the initial period of chilling treatment, electron-transporting systems (in mitochondria, chloroplasts and plasma lemma) are turned blocked or temporarily uncoupled, which leads to the transfer of electronic energy to molecular oxygen, yielding ROS, in the first place. This, in turn, stimulates lipid peroxidation in membranes. An enhanced activity of lipid peroxidation leads to structural changes of membranes and elevates their permeability to ions and water, which finally breaks the normal course of physiological processes in the cell (Lukatkin, 2005). By increasing the level of low-molecular weight or enzymatic antioxidants (application of exogenous substances with antioxidant activity or super production of SOD and other antioxidant enzymes in transgenic plants), one can improve the chilling tolerance of plants or their cells. Some phytohormone analogues have similar ant oxidative effects. The pretreatment of plant prior to stress (acclimation) provides the means to increase the antioxidant protection and to lower the chilling-induced damage to physiological functions. Exogenous chemical treatments (including the treatments with phytohormone analogues) at sublethal cooling can facilitate the recovery after stress. This is related to a great reparative capacity of plant. There is some evidence that various biologically active substances can alleviate the negative effects of environmental cues. Low-toxic synthetic phytohormones applied at extremely low concentrations e.g., cytokinin analogs are particularly interesting in this respect. The activity of some recently synthesized preparations is by several orders of magnitude higher than that of natural phytohormones. Thidiazuron is one of such substances with defoliant activity. This synthetic growth regulator exhibits cytokinin activity at low concentrations and elevates cold resistance of cucumber leaves (Lukatkin, 2005).

Oxidation of unsaturated fatty acids by singlet oxygen produces distinctly different products such as MDA (Bradley and Min, 1992). MDA is a common product of lipid peroxidation and a sensitive diagnostic index of oxidative injury (Janero, 1990). The object of this study was to determine the effects of plant growth regulators for improving chilling tolerance in rice seedlings by means of enhancing antioxidative defense or by protecting effects on membrane integrity.

MATERIALS AND METHODS

Plant growth and treatment conditions: Three rice cultivars including IR72944-1-2-2 and IR73688-57-2 (abbreviated as IR33 and IR34, based on nomenclature in data sheath, respectively), IRRI breeding cold-tolerant lines and Hoveize (cold-sensitive originated from Iran) through the seedling stage were selected for this investigation which was carried out in Agricultural Biotechnology Research Institute of Iran (ABRII) during 2010. Genotypes were hydroponically cultured in 18 L plastic containers with Styrofoam sheets as bonnet in a growth cabinet (Phytotron) with light intensity of about 700 mmoL/m^2/s and 12 h duration, 70% relative humidity and 29/22°C day/night temperature. Pre-germinated seeds of each cultivar were sown in holes made on Styrofoam sheets, with 20 holes per sheet. Three seeds were sown within each hole. The sheets were first floated on distilled water in

plastic trays for 3 days then a nutrient solution (Yoshida, 1981) was used until the plants were 14-day-old. Then ABA (Abscisic acid), IAA (Indole-3-Acetic Acid, Sigma) and CK (Kinetin, Sigma) were foliar sprayed on the plant thoroughly. Each hormone (5×10^{-5} M) was sprayed separately with 0.5% (v/v) Teepol as the surfactant. Plants sprayed with the same volume of 0.5% Teepol solution were used as no-hormone treatments. Thereafter to impose cold stress, the phytotron temperature lowered to 15/13°C day/night. The control was set under normal temperature (29/22°C day/night) in another phytotron with the same light intensity and relative humidity. After chilling treatment for 14 days, temperature was reset to the control conditions for two additional weeks as recovery stage. The culture solution was renewed once a day with the pH adjusted to 5.2 by adding either NaOH or HCl. The rice leaf for determination was randomly sampled every day after exposed to chilling and hormone application.

Measurements of chlorophyll fluorescence: In vivo Chl. fluorescence was measured in fully expanded attached leaves in the phytotron for control and chilled plants pre-darkened for 1 h using a PAM-2000 portable fluorometer (Walz, Effeltrich, Germany) connected to a notebook computer. Three parameters of fluorescence including Fv/Fm (maximal Photosystem II quantum yield of dark-adapted samples), Y (quantum yield) and qP (photochemical quenching) were calculated online by PAM fluorometry and the saturation pulse method. The ratio between variable and maximal fluorescence (Fv/Fm) was measured in dark-adapted leaves. The ratio of variable to maximum fluorescence (Fv/Fm) derived from the measurement was used as a measure of the maximum photochemical efficiency of Photosystem II (PS II). The quantum yield of electron transport through PS II (Y) was calculated according to Genty *et al.* (1989).

Chlorophyll content: Leaf samples were taken and immediately frozen in liquid nitrogen and stored at -80ºC until analysis. (0.01 g) of plant materials were pulverized in a mortar and liquid nitrogen then grinded materials were placed in a 2.5 mL tubes and added 2 mL ice-cold 80% acetone then shacked and remained in a cold room for 1 h. The homogenate centrifuged at $13000 \times g$ for 10 min at 4ºC. The extracts were assayed in a spectrophotometry method at 663 and 645 nm specific for Chl. a and Chl. b, respectively. Arnon's equation was used to obtain total Chl. content (Arnon, 1949).

Determination of MDA: Lipid peroxidation was measured as the accumulation of its end product (MDA). For the measurements of LP in shoots and roots, the Thiobarbituric Acid (TBA) test, (Heath and Parker, 1968), was used by a slight modifications. An aliquot (0.07 g) of control treatments and of treatments exposed to chilling was homogenized in 5 mL of 0.1% (w/v) TCA solution. The homogenate was centrifuged at $15000 \times g$ for 15 min and 0.5 mL of the supernatant was added to 1 mL of 0.5% (w/v) TBA in 20% TCA. The mixture was incubated in boiling water for watchfully 30 min and the reaction was stopped by placing the reaction tubes immediately in an ice bath for 10 min. Then the samples were centrifuged at $15000 \times g$ for 5 min and the optical density of supernatant was measured at 532 nm, subtracting the value for non-specific absorption at 600 nm using a spectrophotometer (VARIAN, Carry 300). The amount of MDA-TBA complex, colored product of lipid oxidation (red pigment), was calculated from the extinction coefficient 155 mm^{-1}/cm and the MDA concentration was expressed in moL/1 g of leaf fresh weight. MDA concentrations were calculated by means of an extinction coefficient of 156 mM/cm and the following formula: MDA (μmoL/g fresh wt.) = $[(A532-600)/156] \times 10^3 \times$ dilution factor (Kuk *et al.*, 2003).

Statistical analysis: A factorial trial based on Completely Randomized Design (CRD) was used with three replications. The data were analyzed through an analysis of variance using the Generalized Linear Model (GLM) procedure of SAS (version 9.1). Additionally, Least Significant Different (LSD) multiple range comparison tests were used to determine differences between means (p≤0.05). Excel, Minitab and Sigma plot were used to auxiliary statistical works and drawing the plots.

RESULTS

Chlorophyll content: In all genotypes hormone application showed no significant effect on chlorophyll content at S1 stage whereas at Rec. stage the responses of genotypes were different to hormones. All hormone treatments raised chlorophyll content of IR34 genotype comparing with no hormone treatment but in IR33 genotype only ABA increased it. In Hoveize as a sensitive genotype, at Rec. stage ABA and IAA were more effective than others and could enhance chlorophyll level (Fig. 1).

Chlorophyll fluorescence: Chilling stress caused a decrease in quantum Yield (Y) of all genotypes (Fig. 2) but the rate of this reduction was severe in sensitive genotype, Hoveize (Fig. 2g). Hormone application suppressed drastic reduction of this parameter under
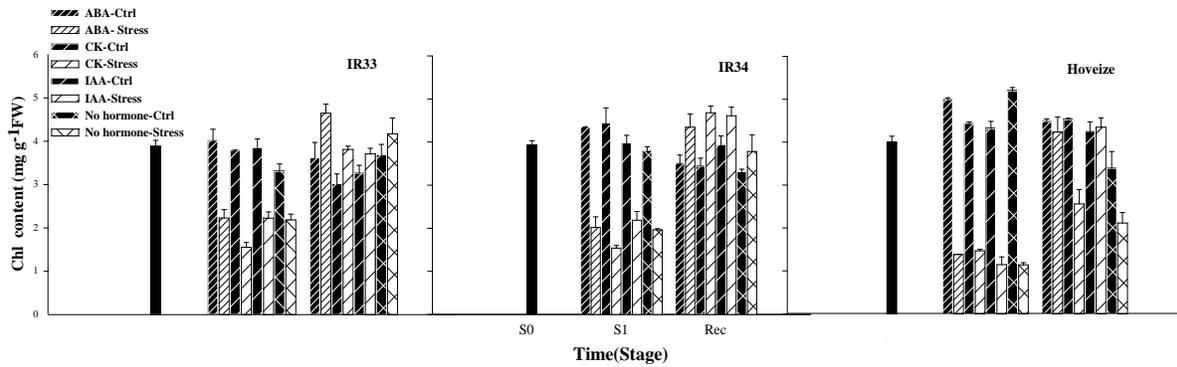
Fig. 1: Effects of hormone application on shoot total chlorophyll content of genotypes under control and stress temperatures
S0: The stage without hormone application under control temperature; S1: The stage in which hormone applied plants were exposed to chilling stress; Rec: Recovery from chilling (n = 3; p≤0.05)
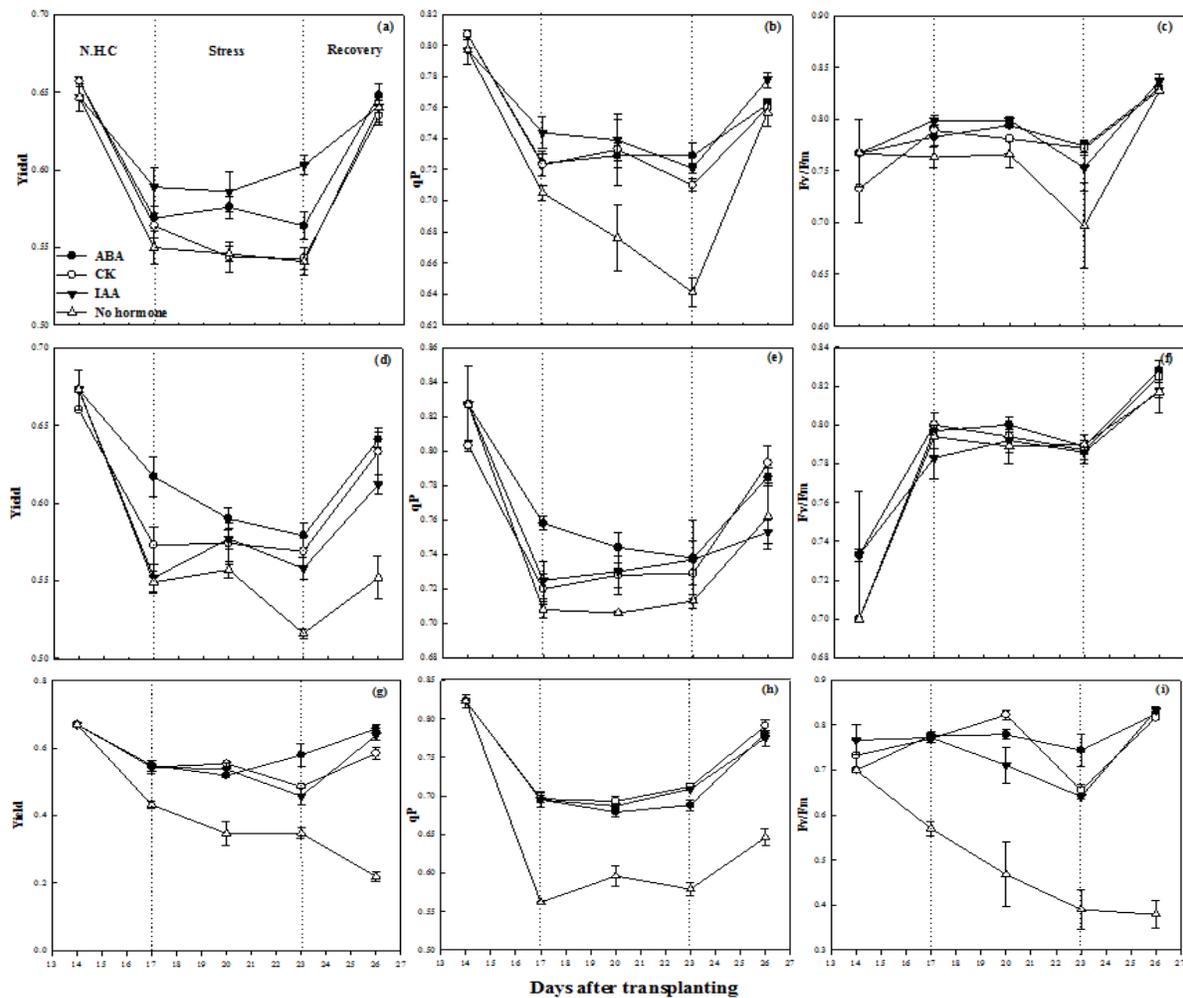
Fig. 2: Effects of hormones on fluorescence parameters of genotypes under chilling temperature. (a, d, g) Yield, (b, e, h) qP, (c, f, i) Fv/Fm, each in IR33, IR34 and Hoveize genotypes respectively (n = 3; p≤0.05), N.H.C means no hormone applied plants under control temperature conditions
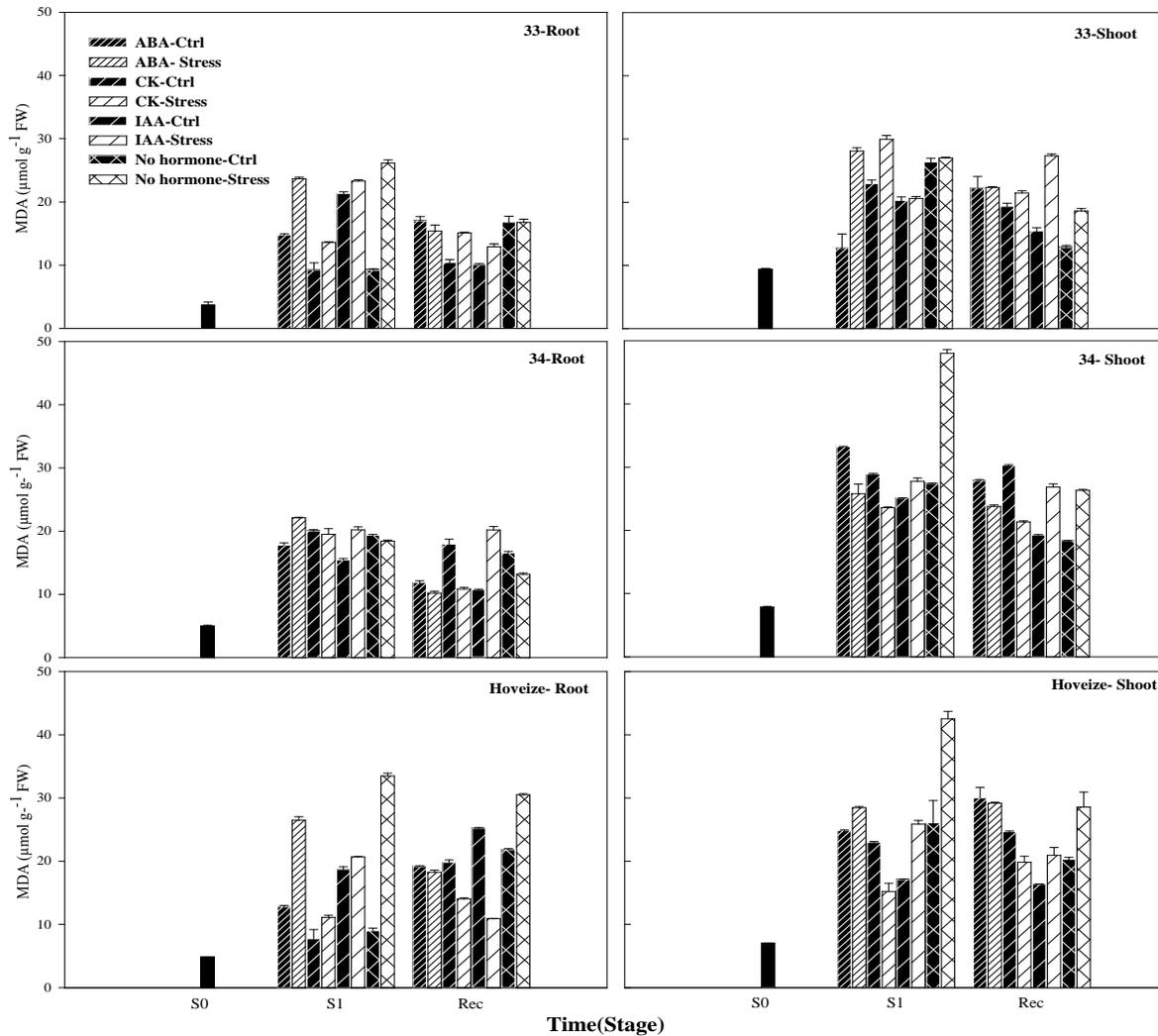
Fig. 3: Effects of hormone application on the shoot and root MDA content of genotypes under control and stress temperatures
S0: The stage without hormone application under control temperature; S1: The stage in which hormone applied plants were exposed to chilling stress; Rec: Means recovery from chilling (n = 3; p≤0.05)

low temperature stress, whereas no hormone treatment showed intensive decrease of Y particularly in IR34 and Hoveize genotypes with rapid returning to initial value at recovery stage in IR33 and IR34 (because of their innate tolerance) and further drop in Hoveize (because of its susceptibility). At recovery stage in which temperature upturned, hormonal treatments enabled plants to recover as soon but not in no-hormone treatment, in which the rate of Y remained at reduced state. ABA and IAA had highest effect (Fig. 2d and 2g). Chilling reduced qP. No application of hormone caused a significant decrease of qP in all genotypes but in tolerant genotypes it returned to initial values during recovery period (Fig. 2b and 2e). Hoveize was unable

to recover without hormone application but in hormone applied treatments it recovered very well with no significant drop during stress period (Fig. 2h). Fv/Fm decreased under low temperature stress in no-hormone-applied sensitive genotype (Hoveize) and retained at this situation (Fig. 2i). Similar to Y, qP and Fv/Fm of Hoveize dropped rapidly during the chilling period and were not restored during recovery. However, their rates in hormone applied leaves were reduced slowly during chilling, compared to no-applied leaves and were restored to the original level after 3 days of recovery.

MDA content: Applied hormones showed different affects among the genotypes and even in root and shoot

of each genotype during chilling and recovery periods. In the root of IR33 IAA and CK were more effective to limit the level of MDA to rise whereas its content rose up to 184% without hormone application in relation to control temperature. Hormones had no significant effect in the root of IR34 but in the shoot, hormone application showed a restriction effect to increase MDA content and no hormone treatment demonstrated an increase of 119% in comparison with control condition. MDA content significantly increased under low temperature, which is indication of membrane damage. As expected the sensitive genotype (Hoveize) showed the highest amount of root and shoot MDA content under chilling stress (S1) in no-hormone treatment (271 and 63% in root and shoot respectively); IAA (only 10% increase) in root and CK (remarkable 51% decrease) in shoot were effective. As indicated by the level of MDA production, lipid peroxidation was clearly noticeable in hormone application. In no-hormone treatments, however, LP occurred during chilling stress and the level of LP continued to increase in the recovery period in sensitive genotype. LP was negligible in hormone applied treatments (Fig. 3).

DISCUSSION

In our study, we found lesser damage to the Chl. content in the ABA-treated plants (as well as other hormone-treated ones) that is in conformity with the findings of Kumar *et al*. (2008) in chilling-stressed chickpea plants treated with ABA, which possibly maintained the photosynthesis in the stressed plants. Protective role of exogenously applied ABA against cold stress was also shown in chickpea during early vegetative growth (Bakht *et al*., 2006).

Applied hormones could protect chlorophyll from degradation or reduced synthesis caused by chilling stress through delayed senescence. Our results are in consistence with the results obtained in experiments with wheat seedlings under water deficiency conditions by Monakhova and Chernyad'ev (2007) showing the protective effect of cytokinin on photosynthesis under adverse environmental conditions. There are plenty of unknowns about the mechanisms are triggered when exogenous hormones apply to plants. Based on the Lukatkin (2005) statements presumably, phase transitions of minor lipids in membranes (mitochondrial and chloroplast membranes in particular) play a certain role in induction of chilling injury in chilling-sensitive plants (Lukatkin, 2005). One of the features of cytokinins is to delay senescence of plant parts to which they were added, or in which they are synthesized at higher rates. Cytokinins delay senescence by slowing down macromolecules decomposition, principally those

that are components of photosynthetic apparatus (Stopari and Maksimovi, 2008). Higher levels of MDA were found in no-hormone treatments under chilling stress with respect to hormone applied seedlings. This demonstrates that LP is enhanced when hormones are absent and indicates that hormones may effectively protect membranes against denaturation. We found a decrease in qP (photochemical quenching of fluorescence) associated to MDA increase. It is possible that hormones successfully act as a defensive agent in most of the cases. It is pertinent to suggest that hormone may quench chilling injury by reacting as membrane stabilizer reducing the damaging effect of cold products and their oxidative pressure over the membranes. Anyhow our experiments cannot prove whether hormones are acting as a compound to react with chilling imposes on the membranes. The results obtained indicate that the chilling of sensitive plants induced oxidative stress in their cells, which manifested itself in enhanced LP intensification and MDA generation. However, the temporal pattern of these processes differed. The MDA concentration was increased during the period of chilling (S1) and even day after plant returning to control conditions. Stress-induced disturbances in membrane mechanisms and the reparation of the damage due to hormone application ensure us to recommend that some PGRs are applicable in most stresses and that by gene manipulating it is possible to produce transgenic plants to cope with all types of stresses. This was substantiated by the results of plant treatments with exogenous hormones in our study. Based on the findings of Popov *et al*. (2008) on transgenic plants, the *desC* gene activity results in an increase in the relative content of polyunsaturated Fatty Acids (FA) in the membrane lipids ensuring the liquid state of membranes during chilling. In this way cellular homeostasis is preserved and the steady synthesis of antioxidant enzymes is maintained under hypothermia (Popov *et al*., 2008); accordingly, chilling tolerance is enhanced in hormone applied rice seedlings likely by induction of cold regulating genes. For rice our data (data not shown) suggested that hormone treatment would improve growth of seedlings at low temperatures by enhancing membrane stability.

CONCLUSION

According to our results showing the low content of MDA in CK-applied treatments, presumable antioxidative effect of CK may be a reason for cold acclimation in cold susceptible Hoveize. In the case of other effective hormones it can be attributed to their protective role on membranes or on its fluidity and maintaining the integrity of membranes and its-related

mechanisms including ATP-ase activity to retain cellular energy, unsaturation membranes and etc., to cope with the chilling stress. Since under chilling conditions the oxidation potential becomes high, hormones (in the case of CK) themselves or by mediating the enzymatic or non-enzymatic systems, can quench cold-induced ROS and reduce the damage at the membrane level. Summing up our data, we conclude that in contrast to the control seedlings, the hormone applied seedlings were able to maintain the integrity of membrane under chilling stress, which enabled them to efficiently decrease the rate of LP by preventing the accumulation of ROS. MDA content reflects the level of the membrane LP resulting from oxidative stress; its accumulation in hormone applied plants was very low and in some cases lower than that in control temperature, whereas, in no hormone applied plants, these values reached to 271%. In the roots of cold-tolerant IR33, the rate of LP did not change due to hormones. In this connection, we can conclude that LP changes are related to plant cold tolerance and its organs.

ABBREVIATIONS

Chl   : Chlorophyll
LP   : Lipid peroxidation
MDA : Malondialdehyde

ACKNOWLEDGMENT

REFERENCES

Araus, J., T. Amaro, J. Voltas, H. Nakkoul and M. Nachit, 1998. Chlorophyll fluorescence as a selection criterion for grain yield in durum wheat under mediterranean conditions. Field Crop. Res., 55(3): 209-223.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris. Plant Physiol., 24(1): 1-15.

Badea, C. and S.K. Basu, 2009. The effect of low temperature on metabolism of membrane lipids in plants and associated gene expression. Plant Omics J., 2(2): 78-84.

Bakht, J., A. Bano and P. Dominy, 2006. The role of abscisic acid and low temperature in chickpea (*Cicer arietinum*) cold tolerance II: Effects on plasma membrane structure and function. J. Exp. Bot., 57(14): 3707.

Bradley, D.G. and D.B. Min, 1992. Singlet oxygen oxidation of foods. Cr. Rev. Food Sci. Nutr., 31: 211-236.

De Palma, M., S. Grillo, I. Massarelli, A. Costa, G. Balogh, L. Vigh and A. Leone, 2008. Regulation of desaturase gene expression, changes in membrane lipid composition and freezing tolerance in potato plants. Mol. Breeding, 21(1): 15-26.

Fracheboud, Y., C. Jompuk, J. Ribaut, P. Stamp and J. Leipner, 2004. Genetic analysis of cold-tolerance of photosynthesis in maize. Plant Mol. Bio., 56(2): 241-253.

Genty, B., J. Briantais and N. Baker, 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA)-General Subjects 990(1): 87-92.

Guo-Li, W. and G. Zhen-Fei, 2005. Effects of Chilling stress on photosynthetic rate and chlorophyll fluorescence parameter in seedlings of two rice cultivars differing in cold tolerance. Rice Sci., 12(3): 187-191.

Heath, R.L. and L. Parker, 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys., 125: 407-408.

Janero, D.R., 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Bio. Med., 9(6): 515-540.

Kuk, Y.I., J. Shin, N.R. Burgos, T.E. Hwang, O. Han, B.H. Cho, S. Jung and J.O. Guh, 2003. Antioxidative enzymes offer protection from chilling damage in rice plants. Crop. Sci., 43(6): 2109-2117.

Kumar, S., G. Kaur and H. Nayyar, 2008. Exogenous Application of abscisic acid improves cold tolerance in chickpea (*Cicer arietinum* L.). J. Agron. Crop Sci., 194(6): 449-456.

Lukatkin, A.S., 2002. Contribution of oxidative stress to the development of cold-induced damage to leaves of chilling-sensitive plants 2: The activity of antioxidant enzymes during plant chilling. Russ. J. Plant Physiol., 49(6): 878-885.

Lukatkin, A.S., 2003. Contribution of oxidative stress to the development of cold-induced damage to leaves of chilling-sensitive plants 3: Injury of cell membranes by chilling temperatures. Russ. J. Plant Physiol., 50(2): 243-246.

Lukatkin, A.S., 2005. Initiation and development of chilling injury in leaves of chilling-sensitive plants. Russ. J. Plant Physiol., 52(4): 542-546.

Monakhova, O. and I. Chernyad'ev, 2007. A protector effect of cytokinin preparations on the photosynthetic apparatus of wheat plants under water deficiency conditions. Appl. Biochem. Microbiol., 43(6): 641-649.

Pei-Guo, G. and L. Rong_Hua, 2000. Effects of high nocturnal temperature on photosynthetic organization in rice leaves [J]. Acta Bot. Sin., 7.

Pei-Guo, L. and M. Ceccarelli, 2006. Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. Agr. Sci. China, 5(10): 751-757.

Popov, V., O. Antipina and T. Trunova, 2008. Oxidative Stress in the Tobacco Plants at Hypothermia. Scientific Works of the Lithuanian, Institute of Horticulture and Lithuanian University of Agriculture Sodininkyste Ir Daržininkyste, 27(2): 121-127.

Stopari, G. and I. Maksimovi, 2008. The effect of cytokinins on the concentration of hydroxyl radicals and the intensity of lipid peroxidation in nitrogen deficient wheat. Cereal Res. Commun., 36(4): 601-609.

Tambussi, E., C. Bartoli, J. Guiamet, J. Beltrano and J. Araus, 2004. Oxidative stress and photodamage at low temperatures in soybean (*Glycine max* L.) Merr. leaves. Plant Sci., 167(1): 19-26.

Tasseva, G., J. Davy de Virville, C. Cantrel, F. Moreau and A. Zachowski, 2004. Changes in the endoplasmic reticulum lipid properties in response to low temperature in Brassica napus. Plant Physiol. Biochem., 42(10): 811-822.

Wang, G. and Z. Guo, 2005. Effects of chilling stress on photosynthetic rate and chlorophyll fluorescence parameter in seedlings of two rice cultivars differing in cold tolerance. Rice Sci., 12(3): 187-191.

Wingsle, G., S. Karpinski and J. Hällgren, 1999. Low temperature, high light stress and antioxidant defence mechanisms in higher plants. Phyton-Horn, 39: 253-268.

Yoshida, S., 1981. Climatic Environment and its Influence. International Rice Research Institute (Los Baños, Filipinas) Fundamentals of Rice Crop Science Los Baños, pp: 65-110.