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Alleviating harmful effects of chilling stress on rice seedling via application of spermidine as seed priming factor

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Chilling stress is a major limiting factor for rice production in many parts of the world. The study was carried out in the Seed Research Laboratory of the Department of Crop Science, Ferdowsi University of Mashhad in summer 2011. Rice (cv. Khazar) seeds were soaked in 50, 150 and 300 mg L^{-1} spermidine aerated solutions for 48 h and then dried back to the original moisture content and were sown in three temperatures (28°C as normal, 12 and 8°C as chilling stress). Chilling stress (8°C) reduced the root (39%) and shoot (52%) growth in untreated seeds, while the reduction of 11% root growth and 20% shoot growth was observed when the seeds were primed with 300 mg L^{-1} spermidine solution. The electrolyte leakage (EL) of the seedling leaves significantly increased in low temperatures as it was 67% in 8°C in compared with normal condition. Chilling stress significantly increased superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activity in rice seedlings leaves though this increase was not significant for glutathione reductase (GR). Seed priming with spermidine had a positive effect on seedlings leaves antioxidant activity in every temperature conditions. As in 8°C, the sharp increase (73%) in SOD activity was occurred at 300 mg L^{-1} spermidine solution and it was 23 and 46% for CAT and APX, respectively. In general, seed priming with spermidine alleviated the chilling effect, probably as a result of activating antioxidants production processes and membrane stabilizing in cellular structures.

Key words: Antioxidant, chilling injury, polyamine, priming, seedlings.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereals in the world feeding approximately 4 billion people, while chilling stress is a major limiting factor for its production in many parts of the world. The range of air temperature around 15 to 20°C lead to chilling damage in rice though the level of damage is related to period of chilling stress, growth stage and genotypes (Pouramir et al., 2013). In northern regions of Iran, rice is sown from early April to middle of May, when mean temperature is around 15°C which leads to rotten rice seedlings, causing heavy seed loss and a delayed in growth period (Sharifi, 2010).

Chilling stress in germination and vegetative growth growth stages causes poor germination and emergence,

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delayed in seedling growth, leaf discoloration, reduced height and tiller numbers and consequently, lose yield in rice (Takeoka et al., 1992).

A lot of research on several plants demonstrated that seed invigoration treatments have effectively improved germination and early seedling growth particularly at suboptimal conditions (Bradford, 1986; Afzal et al., 2002; Farooq et al., 2005).

Seed priming is one of the seed invigoration treatments that allows seeds to absorb water to a point where germination processes start but radicle emergence does not occur (Bradford, 1986). Shorter germination time, emergence over all seedbed environmental and broad temperature range of germination, leading to uniformity, better crop establishment and therefore improved harvest quality and yield, particularly under abnormal and stress situation in the field are the typical responses to seed priming (Farooq et al., 2007).

Polyamines including spermidine, putrescine and spermine have been recognized as a group of plant growth regulators with the great effects on plant growth and development. Plant growth rate is positively related to amount of polyamine levels in a wide range of environmental conditions (Watson and Malmberg, 1998).

Polyamines play different roles in the plant including binding with different macromolecules and stabilize their structures. In addition, they are able to play act as regulators in various fundamental cellular processes such as cell division, differentiation and proliferation, cell death, DNA and protein synthesis and gene expression (Igarashi and Kashiwagi, 2000; Childs et al., 2003). Iqbal and Ashraf (2005) demonstrated that wheat (*Triticum aestivum* L.) seed priming with spermidine, spermine and putrescine increased photosynthetic capacity and also wheat growth under saline conditions. Xu et al. (2011) revealed that tobacco (*Nicotiana tabacum* L.) seed priming with putrescine improved germination percentages, germination index, seedling length and dry weight in comparison with controls which caused chilling tolerance. Farooq et al. (2007) showed that sunflower (*Helianthus annuus* L.) seeds priming with polyamines (spermidine and putrescine) increased germination percentages, root and shoot length, early seedling growth and decreased emergence time. However, Lin and Kao (1995) revealed that with increasing salinity levels, the spermine content did not change in root and shoot tissues of the rice but spermidine and putrescine levels were increased though they were not able to ameliorate of the growth inhibition of seedlings imposed by NaCl. Pretreatment of rice seeds with putrescine caused an increase in its level in shoots but could not relieve the inhibition effect of NaCl on seedling growth. Several study have shown that exogenous application of spermidine as an important polyamine can play an important role in recovering the plasma membrane damage and electrolyte leakage (EL) in rice genotypes and cucumber in response to environmental stresses like chilling, salinity and drought (Kubis, 2008; Roy et al., 2005). Xu et al. (2011) showed that the amount of seedling leaf relative water content (RWC) improved under chilling stress via seed priming with putrescine probably by osmotic adjustment or might alter in cell wall stretchiness. Cao et al. (2008) reported that seed soaking with putrescine improves chilling tolerance of maize seeds.

The objectives of this research were firstly, to investigate the physiological and biochemical changes in rice seedlings during chilling stress and secondary, the possibility of induce chilling tolerance in early seedling growth of rice by seed priming with spermidine.

MATERIALS AND METHODS

Seed

Rice seeds were obtained in October, 2010 from the Rice Research Institute, Rasht, Iran. All seeds were harvested in the same season based on seed uniformity and without any spots of diseases. They were transferred to Seed Research Laboratory of the Department of Crop Science, Ferdowsi University of Mashhad and were placed in air-tight container and kept at 4°C for further use. Germination test using between papers on 4 replicates of 25 seeds revealed the germination of 94%.

Seed priming

Seeds were soaked 48 h in three spermidine solutions (50, 150, 300 mg L^{-1}), distilled water and unprimed seed as control. Distilled water treatment was used to eliminate the possible effect of water on seed priming. Then seeds were washed six times by distilled water and dried back to the original seed moisture content at 28 ± 2°C.

Emergence in sand

Seeds were sown in 1 cm depth of moist sand in trays (45 × 25 × 10 cm). Trays were put in controlled conditions in a growth chamber at 28°C and 15/9 h light/dark for 19 days for normal temperature conditions. In order to induce chilling stress conditions, another two sets were put in the same above conditions for 15 days , before moving at 8 and 12°C for 4 days, respectively.

The experiment was carried out in a completely randomized design with 4 replicates of 25 seeds. Emergence was counted daily for 15 days.

Time taken to 50% emergence (E50) was calculated using the following equation (Equation 1) of Coolbear et al. (1984) modified by Farooq et al. (2005):

$$E50 = ti + \frac{\left[\left(\frac{N}{2}\right) - ni\right](tj - ti)}{nj - ni} \quad (1)$$

Where N is the final number of emerged seeds, and ni and nj the cumulative number of seeds emerged by adjacent counts at times ti and tj when ni < N/2 < nj.

Mean emergence time (MET) was calculated according to the equation (Equation 2) of Khajeh-Hosseini et al. (2009):

$$MET = \frac{\sum nt}{\sum n} \quad (2)$$

Where n is the number of seeds newly emerged at time t; t = number of days from sowing.

Then on the final day, seedlings from each replicate were carefully removed from the seed bed and washed before measuring the shoot and root length.

Biochemical analysis

Samples (second uppermost leaf from main stem) were taken from control and chilling stressed plants on 19th day after sowing.

Membrane permeability

In order to estimate the amount of leaf membrane damage, the amount of EL was determined (Blum and Ebercon, 1981). Six leaves samples were washed and then soaked in tubes containing 40 ml distilled water for 12 h. Afterward, the first electrolyte conductivity (C1) of the solutions was determined by a conductivity meter (4510, Jenway, manufactured in Camlab House, Norman Way Industrial Estate, Over, Cambridge CB24 5WE, United Kingdom). After all, samples were sealed in boiling water for 20 mi and then, the second electrolyte conductivity (C2) of the solutions was determined after equilibration at 25°C. Membrane permeability (EL) was defined as follows (Equation 3):

$$EL\ (\%) = (C_1/C_2) \times 100 \quad (3)$$

Relative water content (RWC)

To evaluate the leaf RWC, 0.5 g fresh weight of leaf samples (fw) was soaked 24 h in tubes including 40 ml of distilled water. Then leaves were quickly weighed (tw) and were kept in oven at 70°C for 48 h to calculate dry weight (dw). RWC was calculated by the following equation (Equation 4):

$$RWC\ (\%) = \frac{fw - dw}{tw - dw} \times 100 \quad (4)$$

Enzyme assays

Leaf fresh samples (0.1 g) was powdered in liquid nitrogen and homogenized in 1 ml of 0.1 M potassium phosphate buffer of pH 7.8 containing 1 mM ethylene diamine tetra acetic acid (EDTA) by a homogenizer into microtubes. Insoluble materials was removed by refrigerated centrifuge (Beckman Coulter headquarters in Brea, California, USA) at 12000 g for 20 min at 4°C and the supernatant used as the source of enzyme extraction. To determine the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), 100 µl of supernatant were taken and all steps of antioxidants determination carried out at 4°C. SOD activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium, according to Yu and Rengel (1999). CAT activity was assayed by measuring the initial rate of hydrogen peroxide disappearance according to Velikova et al. (2000). APX and GR activities were determined according to Yamaguchi et al. (1995) and Lee and Lee (2000), respectively.

RESULTS

Temperature and priming had no significant effect on final emergence percentages (E%), E50 and MET (Table 1) since counting of emerged seeds was finished in Day 15, while chilling stress treatment was induced in Day 16. Although, seed priming with spermidine solutions had higher E% and lower E50 and MET than unprimed seeds in all temperatures (Table 1).

Although chilling stress considerably decreased the root (39%) length, seed priming with spermidine improved the root length in both chilling stress conditions and normal temperature. Under optimum temperature conditions seedlings raised from primed seeds with 50 mg L^{-1} spermidine solution had the highest root length (90 mm) though in 12 and 8°C stress conditions the highest root length (68 and 66 mm) was obtain from primed seeds with 300 mg L^{-1} spermidine solution (Table 1).

Chilling stress significantly reduced shoot length (52%) compare with control (Table 1). Although, seed priming improved the shoot growth in all temperatures particularly at 8°C where shoot length increased (67%) from 61 mm in untreated seeds to 102 mm in primed seeds with 300 mg L^{1} spermidine solution (Table 1).

Chilling stress reduced the growth of both root and shoot. But its effect on shoot was stronger than the root hence, the root shoot length ratio increased (28%). Seed priming decreased the root shoot length ratio in all temperatures particularly at 8°C (Table 1).

The RWC of the seedling leaves decreased in both untreated and treated seeds in chilling stress conditions. Seed priming slightly increased RWC in all temperature though these differences were not significant (Table 2).

The EL of the seedling leaves significantly increased in low temperatures. This increase was 67% in 8°C compared with normal condition. However, rice seed priming by spermidine solutions decreased the leakages in every temperature conditions (Table 2). For instance in 8°C, seed priming with spermidine 300 mg L^{-1} decreased (26%) the leakages from 25.2 in untreated seeds to 18.6 in treated seeds.

Chilling stress significantly increased SOD, CAT and APX activity in rice seedlings leaves though this increase was not significant for GR (Table 2). The highest increase in antioxidant activity due to chilling stress with 54% belongs to SOD and this amount was 38 and 25% for CAT and APX, respectively. Seed priming with spermidine had a positive effect on seedlings leaves antioxidant activity in every temperature conditions (Table 2). As in 8°C, the sharp increase (73%) in SOD activity occurred at 300 mg L^{-1} spermidine solution and it was 23 and 46% for CAT and APX, respectively.

DISCUSSION

Rice seed priming with spermidine improved emergence and speed of emergence though the differences were not significant. Chilling stresses (8°C) reduced the root (39%) and shoot (52%) growth in untreated seeds, while the reduction of 11% root growth and 20% shoot growth was observed when the seeds were primed with spermidine

Table 1. The effects of chilling stress and seed priming of rice with spermidine on final emergence percentages (E%), time taken to fifty percent emergence (E50), mean emergence time (MET), root length (RL), shoot length (SL) and ratio of root/shoot length (RL/SL) of the seedlings.

Treatment		E (%)	E50 (days)	MET (days)	RL (mm)	SL (mm)	RL/SL
	P1	78	7.81	5.12	74	127	0.58
	P2	79	7.77	5.16	79	136	0.58
T1	P3	83	7.53	4.83	90	152	0.59
	P4	85	7.24	4.45	89	150	0.59
	P5	85	6.98	4.51	81	149	0.54
	P1	80	7.67	4.98	51	91	0.56
	P2	79	7.74	5.10	50	92	0.54
T2	P3	83	7.11	4.62	65	102	0.64
	P4	84	6.93	4.58	67	110	0.61
	P5	87	6.85	4.39	68	112	0.61
	P1	79	7.79	5.01	45	61	0.74
	P2	78	7.58	4.81	47	69	0.68
T3	P3	82	7.41	4.75	50	76	0.65
	P4	84	7.17	4.55	62	98	0.63
	P5	87	7.12	4.62	66	102	0.65
LSD		9.79	1.05	0.94	10.56	15.68	0.02

P1, Untreated seeds (control); P2, distilled water; P3, spermidine 50 mg L^{-1}; P4, spermidine 150 mg L^{-1}; P5, spermidine 300 mg L^{-1}; T1, temperature of the whole period was 28°C; T2, 15 days at 28°C and then 4 days at 12°C; T3, 15 days at 28°C and then 4 days at 8°C.

Table 2. The effects of chilling stress and seed priming with spermidine on relative water contents (RWC), electrolyte leakages (EL) and enzyme assays of the seedling leaves of the rice.

Treatment		RWC (%)	EL (%)	SOD (unit.g^{-1} fw)	CAT (µmol.g^{-1} fw min^{-1})	APX (unit.g^{-1}fw)	GR (µmol. g^{-1} fw)
	P1	85	15.1	11.0	90	18.5	13.04
	P2	86	13.8	11.1	89	17.8	12.41
T1	P3	87	12.4	12.2	98	19.3	14.11
	P4	87	10.2	14.3	110	23.2	14.5
	P5	89	10.6	14.2	112	26.8	15.88
	P1	82	16.7	16.6	111	21.2	14.04
	P2	81	17.1	16.1	110	22.4	15.42
T2	P3	83	15.9	20.2	120	25.9	17.15
	P4	85	13.2	25.6	125	29.2	16.05
	P5	86	13.5	24.5	135	30.1	17.33
	P1	78	25.2	16.9	124	23.2	16.21
	P2	78	25.5	18.1	126	23.1	15.47
T3	P3	79	22.7	24.2	136	28.9	18.03
	P4	83	19.2	28.3	138	30.6	18.61
	P5	82	18.6	29.2	153	33.8	19.42
LSD		12.06	4.07	5.24	16.36	4.61	8.71

P1, Untreated seeds (control); P2, distilled water; P3, spermidine 50 mg L^{-1}; P4, spermidine 150 mg L^{-1}; P5, spermidine 300 mg L^{-1}; T1, temperature of the whole period was 28°C; T2, 15 days at 28° C and then 4 days at 12°C; T3, 15 days at 28°C and then 4 days at 8°C.

300 mg L^{-1} (Table 1). Therefore, chilling reduced the shoot growth stronger than the root growth in early seedling growth of rice as reflected in a higher root shoot length ratio, while spermidine protected the seedlings against chilling. Decreasing shoot length due to chilling was reduced from 52 to 20% as a result of seed priming

with spermidine whereas it was reduced from 39 to 11% for root length. Therefore, protecting effect of spermidine on shoot length was stronger than that of the root length in chilling stress conditions (8°C). Chilling stress can affect root and shoot growth through inhibiting both cell division and expansion (Pahlavanian and Silk, 1988; Tardieu and Granier, 2000). The activity of the A-type cyclin dependent kinase (CDKA), a major regulator of cell cycle progression, is associated with the decrease in leaf growth rate of some species under stress conditions. Rymen et al. (2007) claimed that temperature directly affects enzyme kinetics in many biochemical reactions and thereby the growth rate of plant organs. They reported that leaf growth inhibition by low night temperature is tightly linked to the reduction of cell production which is a consequence of prolonged cell cycle duration and not of a reduced cell number in the leaf meristem.

West et al. (2004) indicated that decrease in cell production and a smaller mature cell length was the reason of growth reduction of the *Arabidopsis* stressed roots but average cell cycle duration was not affected. Thus, the reduced cell production was a consequence of smaller number of dividing cells (meristem size reduction). There are some evidences demonstrating the role of exogenous application of polyamines in cold acclimation (Groppa and Benavides, 2008). Polyamines regulate plant growth and development via several physiological processes. These compounds are thought to possess sharp effects on plant growth and development (Watson and Malmberg, 1998). Therefore, in the current study, improving root and shoot length caused by seed priming in chilling stress might be as a result of increased cell division within the apical meristem, resulting in enhanced plant growth. Polyamines play regulatory roles in various important cellular processes including cell division, differentiation and proliferation, cell death, DNA and protein synthesis and gene expression alongside the stabilizing macromolecular structures (Igarashi and Kashiwagi, 2000; Childs et al., 2003; Kusano et al., 2008). Xu et al. (2011) also indicated that germination, seedling length and dry weight of chilling-sensitive tobacco varieties enhanced by putrescine treatments under chilling conditions.

Chilling stress causes damage in plant by production of reactive oxygen species (ROS), however, plant have antioxidant enzymes playing a vital role in improving chilling tolerance (Bolkhina et al., 2003). Studies showed that polyamines are able to improve plants antioxidant system against ROS and therefore decrease the rate of cells injuries and increase plant root and shoot growth under chilling stress conditions (Bolkhina et al., 2003; Xu et al., 2011).

Temperature and priming had no significant effect on RWC though spermidine slightly improved the leaves RWC probably by osmotic adjustment or changing cell wall stretchiness.

More ELs (67%) was observed in the leaves of the rice seedlings under chilling stress (8°C), while it was 27% in seedling leaves raised from seeds primed with 300 mg L^{-1} spermidine solution. Therefore, priming relieved 40% of EL that can be related to priming positive effect on cell membrane and organs. Cell membranes are the major targets of environmental stresses. Enhanced EL was considered to be a symptom of membrane damage induced by chilling stress and deterioration (Pessarakli, 1999). Chilling stress results in several malfunctions at cellular levels including damage to membranes hence increasing their permeability (Simon, 1974; Pessarakli, 1999). Exogenous application of spermidine as an important polyamine is an effective way to recover the plasma membrane damage and to mitigate the EL in response to environmental stresses such as drought, salinity and chilling (He et al., 2002; Roy et al., 2005; Kubiś, 2008). Saeidnejad et al. (2012) showed that maize seedling EL was recovered by seed priming with spermidine in chilling conditions.

In the current study, antioxidants system effectively responded to the stress conditions and seed priming with spermidine enhanced the responses. Increase in antioxidants activity in chilling stress condition is a proper response to production of activates oxygen species (ROS) induced by chilling (Wang and Li, 2006). Therefore, the activities of antioxidative enzymes are related to chilling stress tolerance though the generation of ROS is a common event in growth and developmental processes. ROS can interrupt plant normal metabolism by oxidative damage of different cellular components, including DNA, proteins, lipids, and pigments which results in reduced photosynthetic capacity and growth inhibition (Pessarakli, 1999; Bolkhina et al., 2003; Xu et al., 2011). Antioxidants in the plants protect them against activate oxygen species. Several studies demonstrated that antioxidants such as SOD, CAT, APX and GR can play an important role in plant chilling tolerance by scavenging the activate oxygen species (Afzal et al., 2002; Blokhina et al. 2003; Wang and Li 2006). Shen et al. (1999) demonstrated that SOD and APX activity in chilling-tolerant cucumber (*Cucumis sativus* L.) cultivars was higher than chilling sensitive ones. In a study on the maize, it was showed that CAT plays an important role in plant protection against chilling stress (Prasad, 1997). Lee and Lee (2000) indicated that higher APX activity in plant leaves under chilling stress is a more efficient scavenging system which may result in stronger protection against ROS during chilling. Similarly, Saruyama and Tanida (1995) demonstrated that increase in the activity of APX and CAT leads to chilling tolerance in rice cultivars.

Polyamines accumulation in plant cell can reduce chilling injury. As putrescine accumulation reduced the chilling injury in tomato (*Solanum lycopersicum* L.) (Kim et al., 2002), chickpea (*Cicer arietinum* L.) (Nayyar, 2005)

and maize (Cao et al., 2008) under chilling stress. Many studies demonstrated that genetic modification of the polyamine biosynthetic pathway is a useful tool to recognize the function of polyamines in plant responses to abiotic stresses in both crops and model plants (Igarashi and Kashiwagi, 2000; Childs et al., 2003; Kusano et al., 2008). It was shown that antioxidant activities in tobacco plant under chilling conditions can be enhanced by seed priming with polyamine (Xu et al., 2011).

Conclusion

In this study, chilling had a negative effect on rice seedling physiological and biochemical properties that leads to reduction of the root and shoot length and also increasing in the antioxidants activities. Seed priming with spermidine was able to decrease the negative effect of chilling stress due to activating antioxidants production processes and membrane stabilizing in cellular structures. We believe that our results provide additional support to the role of spermidine as a polyamine in mitigating chilling effect on rice early growth stage. Application of other polyamines to enhance chilling tolerance of rice seedling could be the subject of future studies.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Afzal I, Basra SMA, Ahmad N, Cheema MA, Warriach EA, Khaliq A (2002). Effect of priming and growth regulator treatment on emergence and seedling growth of hybrid maize (*Zea mays* L.). Int. J. Agric. Biol. 4:302–306.

Blokhina O, Virolainen E, Fagerstedt KV (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A Rev. Annu. Bot. 91:179–194. http://dx.doi.org/10.1093/aob/mcf118

Blum A, Ebercon A (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21:43–47. http://dx.doi.org/10.2135/cropsci1981.0011183X002100010013x

Bradford KJ (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. Hortscience 21:1105–1112.

Cao D, Hu J, Gao C, Guan Y, Zhang S, Xiao J (2008). Chilling tolerance of maize (*Zea mays* L.) can be improved by seed soaking in putrescine. Seed Sci. Technol. 36:191-197.

Childs A, Mehta D, Gerner E (2003). Polyamine-dependent gene expression. Cell Mol. Life Sci. 60:1394-1406. http://dx.doi.org/10.1007/s00018-003-2332-4, PMid:12943227

Coolbear P, Francis A, Grierson D (1984). The effect of low temperature pre-sowing treatment under the germination performance and membrane integrity of artificially aged tomato seeds. J. Exp. Bot. 35:1609–1617. http://dx.doi.org/10.1093/jxb/35.11.1609

Farooq M, Basra SM, Khan MB (2007). Seed priming improves growth of nursery seedlings and yield of transplanted rice. Arch. Agron. Soil Sci. 53: 315-326. http://dx.doi.org/10.1080/03650340701226166

Farooq M, Basra SMA, Hafeez K, Ahmad N (2005). Thermal hardening: a new seed vigor enhancement tool in rice. J. Integr. Plant. Biol. 47:187–193. http://dx.doi.org/10.1111/j.1744-7909.2005.00031.x

Granier C, Inzé D, Tardieu F (2000). Spatial distribution of cell division rate can be deduced from that of p34cdc2 kinase activity in maize leaves grown at contrasting temperatures and soil water conditions. Plant. Physiol. 124:1393-1402. http://dx.doi.org/10.1104/pp.124.3.1393, PMid:11080314 PMCid:PMC59236

Groppa M, Benavides M (2008). Polyamines and abiotic stress: Recent advances. Amino. Acids. 34:35-45. http://dx.doi.org/10.1007/s00726-007-0501-8

He L, Nada K, Tachibana S (2002). Effects of spermidine pretreatment through the roots on growth and photosynthesis of chilled cucumber plants (*Cucumis sativus* L.). J. Japan. Soc. Hort. Sci. 71: 490-498. http://dx.doi.org/10.2503/jjshs.71.490

Igarashi K, Kashiwagi K (2000). Polyamines: mysterious modulators of cellular functions. Biochem. Bioph. Res. Co. 271: 559-564. http://dx.doi.org/10.1006/bbrc.2000.2601

Iqbal M, Ashraf M (2005). Changes in growth, photosynthetic capacity and ionic relations in spring wheat (*Triticum aestivum* L.) due to pre-sowing seed treatment with polyamines. Plant. Growth. Regul. 46:19-30. http://dx.doi.org/10.1007/s10725-005-5901-8

Khajeh-Hosseini M, Lomholt A, Matthews S (2009). Mean germination time in the laboratory estimates the relative vigour and field performance of commercial seed lots of maize (*Zea mays* L.). Seed. Sci. Technol. 37:446-456.

Kim TE, Kim SK, Han TJ, Lee JS, Chang SC (2002). ABA and polyamines act independently in primary leaves of cold-stressed tomato (*Lycopersicon esculentum*). Physiol. Plantarum. 115:370-376. http://dx.doi.org/10.1034/j.1399-3054.2002.1150306.x

Kubiś J (2008). Exogenous spermidine differentially alters activities of some scavenging system enzymes H_2O_2 and superoxide radical levels in water-stressed cucumber leaves. J. Plant. Physiol. 165:397-406. http://dx.doi.org/10.1016/j.jplph.2007.02.005

Kusano T, Berberich T, Tateda C, Takahashi Y (2008). Polyamines: essential factors for growth and survival. Planta 228:367-381. http://dx.doi.org/10.1007/s00425-008-0772-7

Lee DH, Lee CB (2000). Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: In gel enzyme activity assays. Plant Sci. 159: 75-85. http://dx.doi.org/10.1016/S0168-9452(00)00326-5

Lin CC, Kao CH (1995). Levels of endogenous polyamines and NaCl-inhibited growth of rice seedlings. Plant. Growth Regul. 17:15-20.

Nayyar H (2005). Putrescine increases floral retention, pod set and seed yield in cold stressed chickpea. J. Agron. Crop. Sci. 191:340-345. http://dx.doi.org/10.1111/j.1439-037X.2005.00158.x

Pahlavanian AM, Silk WK (1988). Effect of temperature on spatial and temporal aspects of growth in the primary maize root. Plant. Physiol. 87:529-532. http://dx.doi.org/10.1104/pp.87.2.529

Pessarakli M (1999). Effect of Low Temperatures on the Structure of Plant Cells. In: Hudak J, Salaj J. Hand Book of Plant and Crop Stress. New York: Marcel-Dekker Inc; P. 231-270. http://dx.doi.org/10.1201/9780824746728

Pouramir F, Khajeh-Hosseini M, Esfahani M (2013). Methods for rice genotypes cold tolerance evaluation at germination stage. Int. J. Agric. Crop Sci.18:2111-2116.

Prasad TK (1997). Role of catalase in inducing chilling tolerance in pre-emergence maize seedlings. Plant Physiol. 114:1369–1376.

Roy P, Niyogi K, Sengupta DN, Ghosh B (2005). Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H+-ATPase in salt-tolerant and salt-sensitive rice cultivars. Plant Sci.168: 583-591. http://dx.doi.org/10.1016/j.plantsci.2004.08.014

Rymen B, Fiorani F, Kartal F, Vandepoele K, Inzé D, Beemster GT (2007). Cold nights impair leaf growth and cell cycle progression in maize through transcriptional changes of cell cycle genes. Plant Physiol. 143: 1429-1438. http://dx.doi.org/10.1104/pp.106.093948

Saeidnejad AH, Pouramir F, Naghizadeh M (2012). Improving chilling tolerance of maize seedlings under cold conditions by spermine

application. Notulae. Sci. Biol. 4: 110-117.
Saruyama H, Tanida M (1995). Effect of chilling on activated oxygen-scavenging enzymes in low temperature-sensitive and -tolerant cultivars of rice (*Oryza sativa* L.). Plant Sci. 109:105–113. http://dx.doi.org/10.1016/0168-9452(95)04156-O
Sharifi P (2010). Evaluation on sixty-eight rice germplasms in cold tolerance at germination. Rice Sci. 17:77-81. http://dx.doi.org/10.1016/S1672-6308(08)60107-9
Shen W, Nada K, Tachibana S (1999). Effect of cold treatment on enzymic and nonenzymic antioxidant activities in leaves of chilling-tolerant and chilling-sensitive cucumber cultivars. J. Japan. Soc. Hort. Sci. 68:967–973. http://dx.doi.org/10.2503/jjshs.68.967
Simon E (1974). Phospholipids and plant membrane permeability. New. Phytol. 73:377-420. http://dx.doi.org/10.1111/j.1469-8137.1974.tb02118.x
Takeoka Y, Al Mamun A, Wada T, Kaufman PB (1992). Reproductive Adaptations of Rice to Environmental Stress. Japan: Japan Scientific Societies Press.
Tardieu F, Granier C (2000). Quantitative analysis of cell division in leaves: methods, developmental patterns and effects of environmental conditions. Plant Mol. Biol. 43:555-567. http://dx.doi.org/10.1023/A:1006438321386
Velikova V, Yordanov I, Edreva A (2000). Oxidative stress and some antioxidant system in acid rain treated bean plants: Protective role of exogenous polyamines. Plant. Sci. 151:59-66. http://dx.doi.org/10.1016/S0168-9452(99)00197-1
Wang LJ, Li SH (2006). Salicylic acid-induced heat or cold tolerance in relation to Ca2+ homeostasis and antioxidant systems in young grape plants. Plant. Sci. 170:685–694. http://dx.doi.org/10.1016/j.plantsci.2005.09.005
Watson MB, Emory KK, Piatak RM, Malmberg RL (1998). Arginine decarboxylase (polyamine synthesis) mutants of *Arabidopsis thaliana* exhibit altered root growth. Plant J. 13:231-239. http://dx.doi.org/10.1046/j.1365-313X.1998.00027.x
West G, Inzé D, Beemster GT (2004). Cell cycle modulation in the response of the primary root of Arabidopsis to salt stress. Plant. Physiol. 135: 1050-1058. http://dx.doi.org/10.1104/pp.104.040022, PMid:15181207 PMCid:PMC514139
Xu S, Hu J, Li Y, Ma W, Zheng Y, Zhu S (2011). Chilling tolerance in Nicotiana tabacum induced by seed priming with putrescine. Plant Growth Regul. 63:279-290. http://dx.doi.org/10.1007/s10725-010-9528-z
Yamaguchi K, Mori H, Nishimora M (1995). A novel isoenzyme of ascorbate peroxidase localized on glyoxysomal and leaf peroxisomal memberanes in pumpkin. Plant Cell Physiol. 36:1157-1162. PMid:8528608
Yu Q, Rengel Z (1999). Micronutrient deficiency influences plant growth and activities of superoxide dismutase in narrow-leafed lupines. Annu. Bot. 83:175-182. http://dx.doi.org/10.1006/anbo.1998.0811