



Effect of proline and salicylic acid on germination and antioxidant enzymes at different temperatures in Muskmelon (*Cucumis melo* L.) seeds

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Abstract: Effect of different seed treatments hydration, warm water, proline (10mM and 20mM) and salicylic acid (0.1mM and 0.5mM) was investigated on percent germination and activity of antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) at different temperatures in Muskmelon (*Cucumis melo* L.) seeds. It was observed that lower temperature (20°C) had decreased seed germination and activity of various antioxidant enzymes. Various seed treatments increased percent germination and activity of these enzymes as compared to control at both the temperatures. Proline 20mM (96.6) and SA 0.1mM (91.6) showed better results as compared to proline 10mM (95.0) and salicylic acid 0.5mM (86.6) respectively. Thus proline and salicylic acid play an ameliorating role on low temperature stress by enhancing the activities of antioxidant enzymes and scavenging the free radicals.

Keywords: Antioxidant enzymes, Low temperature, Muskmelon, Proline, Salicylic acid

INTRODUCTION

Muskmelon (*Cucumis melo* L.) is one of the major vegetable crop in the world and belongs to the family Cucurbitaceae (Sensory *et al.*, 2007). It contains calcium, phosphorus, vitamin A and C and is a famous summer fruit. Poor seed germination is a common phenomenon at sub-optimal temperature which causes a great concern for growers who grow this crop in the month of February. Temperature stress affects many physiological and biochemical processes and induces molecular mechanisms and gene expression to modulate plant responses (Siboza *et al.*, 2014). Chilling stress, defined as the temperature in a range low enough to suppress growth without ceasing cellular functions, is known to induce several abnormalities at various levels of cell organization (Balestrasse *et al.*, 2010). Low temperature induces the reduction in percent germination, vigor and the growth of seedlings and it also causes oxidative stress in tissues and generation of reactive oxygen species (ROS) and it increases the level of the non-enzymatic antioxidants (Airaki *et al.*, 2011). The adverse effect of low temperature can be mitigated by treating the seeds with different chemicals i.e. proline and salicylic acid. Proline is a proteinogenic amino acid with an exceptional conformational rigidity and is essential for primary metabolism and its exogenous application enhanced stress tolerance at low concentrations (Hayat *et al.*, 2012). Proline accumulation is important for the tolerance of certain adverse environmental conditions and it is an important regulator of cellular ROS balance. Salicylic acid is naturally

occurring phenolic compound and endogenously synthesized signaling molecule in plants. It is involved in the regulation of growth and development of plants as well as their responses to biotic and abiotic stress factors (Miura and Tada 2014). The present study was undertaken with the objective to evaluate the effect of seed treatments on percent germination and activities of antioxidant enzymes at low temperature.

MATERIALS AND METHODS

The experiment was carried out at the PG laboratory, Department of botany, Punjab agricultural university, Ludhiana. The seeds of muskmelon (*Cucumis melo* L.) c.v. Punjab Sunehri were procured from department of vegetable science, PAU Ludhiana and used for observations on percent germination and estimation of different antioxidant enzymes. There were six seed treatments viz. hydration, warm water soaking, proline 10mM, proline 20mM, salicylic acid 0.1mM and salicylic acid 0.5mM. The seeds were surface sterilized by dipping in 0.1 percent mercuric chloride and washed twice with distilled water and then dried on filter paper. Then the treatments were given by soaking the seeds in different concentrations of proline and salicylic acid. After seed treatments, seedlings were raised by placing the seeds on top of the germination paper in seed germinator for 8 days at 20°C and 25°C. Eight days old seedlings were used to calculate percent germination by following ISTA rules Anonymous (2008).

Percent germination = No. of seeds germinated / Total no. of seeds x 100

The activity of catalase (Chance and Maehly 1955), peroxidase (Shannon *et al.*, 1966) and superoxide dismutase (Marklund and Marklund, 1974) were recorded.

Catalase enzyme (Chance and Maehly, 1955): To 1.9 ml of chilled 0.1M sodium phosphate buffer (pH7.0) added 0.1 ml of the enzyme extract. The reaction was started by adding 1 ml of H₂O₂ to the reaction mixture. The absorbance was recorded at 240 nm for 1 minute at intervals of 30 second. Enzyme activity was expressed as μ moles of H₂O₂ decomposed /min/gFW using 0.0394 as extinction coefficient.

Peroxidase enzyme (Shannon *et al.*, 1966): Enzyme extract 0.1 ml was added to 3 ml of chilled guaiacol. Addition of 0.1 ml of H₂O₂, started the reaction and the rate of decrease in absorbance was read at 470 nm at 30 seconds intervals for 3 minutes. The activity is expressed as units/min/g FW.

Superoxide dismutase enzyme (Marklund and Marklund, 1974): To a cuvette, added 1.5 ml of Tris-HCl buffer (pH 8.2), 0.5 ml of 6 mM EDTA, 1ml of 6 mM pyrogallol solution and 0.1 ml of enzyme extract. Absorbance at 420 nm after an interval of 30 seconds was recorded upto 3 minutes. The activity is expressed as units/min/g FW.

Statistical analysis: The data was statistically analyzed by using CPCS software.

RESULTS AND DISCUSSION

Percent germination: During the present investigation, it was observed that percent germination was less at 20°C than 25°C (Table 1). Hydration and Warm water (45°C) treatment of seeds for 10 minutes increased the germination as compared to control. At 20°C and 25°C, the seeds treated with proline 10M and 20 mM increased percent germination compared to control. Different concentrations of salicylic acid (SA) also showed an increase in percent germination at both the temperatures. Proline 20 mM and SA 0.1mM showed better results as compared to proline 10mM and SA 0.5mM respectively.

Decrease in germination at low temperature may be attributed to increase in the production of ROS in plants (Hodgson and Raison 1991). Increase in germination in the present is also in agreement with the findings of Singh *et al.* (2016) in Pea and Espanany *et al.* (2016) in black cumin (*Nigella sativa*). Attenuation of isoproturon (IPU) toxicity by salicylic acid (SA) was observed by Singh *et al.* (2016). Salicylic acid (100mg/L) improved the germination, radicle elongation and dry weight in compared with the control under cadmium stress (Espanany *et al.*, 2016). Shrutti *et al.* (2016) reported similar increase in germination of naturally aged seeds of radish by proline and salicylic acid. Increase in percent germination by hydration, warm water, proline and salicylic acid may be due to metabolic

Table 1. Effect of seed treatments on germination of muskmelon (*Cucumis melo* L.) seeds.

Treatment	Percent germination	
	25°C	20°C
Control	85.0	75.3
Hydration	88.3	78.7
Warm water (45°C, 10min.)	86.3	79.6
Proline (10mM)	95.0	86.0
Proline (20mM)	96.6	88.9
Salicylic acid (0.1mM)	91.6	87.6
Salicylic acid (0.5mM)	86.6	83.6
CD at 5%	3.90	7.60

repair during imbibitions (Bray *et al.*, 1989), a buildup of germination-enhancing metabolites (Basra *et al.*, 2005) and osmotic adjustment under abiotic stress conditions (Hyun *et al.*, 2003). SA improved drought and chilling stress tolerance in wheat (*Triticum aestivum* L.) by protecting the structure of plant cell under low temperature (Horvath *et al.*, 2007). Proline has an important role in increasing the germination as well as resistance to various stresses by enhancing the activities of antioxidant enzymes (Agami 2014). Salicylic acid at 50 and 100mg/L when applied to seeds under low temperature (at 24°C and 19°C with day and night length of 15/9 h) conditions improved the germination rate in cucurbits (Rehman *et al.*, 2011). Salicylic acid may be involved in improving the rate of germination because it induces the activities of enzymes involved in germination of *Arabidopsis* seeds (Rajjou *et al.*, 2006). The increased percent germination in present results corroborates with the findings of Talat (2013) who reported that application of proline (100 mM) increased the percent germination in wheat. Lee and Hong (2002) suggested that salicylic acid (0.5 and 1.0mM) promotes percent germination in cucumber

Antioxidant enzymes: Antioxidant enzymes such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) have a main role in scavenging of the ROS.

Catalase activity (ΔA /min/g FW): Different treatments increased the activities of antioxidant enzymes at 25°C and 20°C (Table 2). Hydration of seeds for 6 hrs and warm water treatment increased the catalase activity over control at both the temperatures. Both the concentrations of proline enhanced the activity of catalase as compared to control at 25°C and 20°C Proline 20mM showing better results. Similarly SA 0.1 and 0.5mM also improved the catalase activity over SA 0.5mM and control at both the temperatures SA 0.1 mM being more effective.

Peroxidase (ΔA /min/g FW): Different seed treatments increased the peroxidase activity at both the temperatures. (Table 2). At 20 °C and 25°C, hydration and warm water enhanced the activity of peroxidases as compared to control. Both the concentrations of proline and salicylic acid were effective for increasing

Table 2. Effect of seed treatments on antioxidant activities of muskmelon (*Cucumis melo* L.) seedlings.

Treatments	Catalase ($\Delta A/\text{min/g FW}$)		Peroxidase ($\Delta A/\text{min/g FW}$)		Superoxide dismutase ($\Delta A/\text{min/g FW}$)	
	25°C	20°C	25°C	20°C	25°C	20°C
Control	46.83	39.79	4.41	2.53	58.2	50.8
Hydration	48.37	44.08	5.55	2.75	62.0	53.1
Warm water (45°C, 10min.)	51.25	45.79	5.67	3.63	68.2	54.8
Proline (10mM)	56.29	53.06	7.6	5.83	75.4	60.9
Proline (20mM)	59.33	54.14	8.73	6.18	79.1	63.2
Salicylic acid (0.1mM)	60.69	50.11	5.97	5.45	69.3	61.5
Salicylic acid (0.5mM)	61.01	52.78	5.64	5.03	64.2	58.4
CD at 5%	1.15	1.03	0.70	1.35	0.67	1.29

the peroxidase activity. The effect of proline 20mM and salicylic acid 0.1mM was better as compared to proline 10mM and salicylic acid 0.5mM.

Superoxide dismutase ($\Delta A/\text{min/g FW}$): Hydration and warm water treatment increased the superoxide dismutase activity and over control (Table 2). Seeds treated with proline and salicylic acid had more superoxide activity as compared to control. The effect of proline 20mM was more as compared to proline 10mM. SA 0.1 and SA 0.5mM improved the activity over control at 20°C and 25 °C. Treatment of seeds with salicylic acid also improved the superoxide dismutase activity of seedlings as SA 0.1mM increased the superoxide activity more as compared to SA 0.5mM.

It was observed that low temperature decreased the activities of antioxidant enzymes. Catalase activity was decreased in avocado fruit (Sharon and Kahn 1979) and cucumber leaves (Omran 1980) during chilling. It leads to the production of free radicals. Superoxide and hydrogen peroxide produced in plant tissues exposed to low temperature may cause the peroxidation of membrane lipids (Murugan and Iyer, 2016). Increase in the activity of CAT, POX and SOD by proline and salicylic acid may be attributed to their role in scavenging the free radicals produced by low temperature. Proline acts as a scavenger of reactive oxygen species (Signorelli *et al.*, 2013; Das and Roychoudhary, 2014). Seed priming with salicylic acid triggered the activities of superoxide dismutase, peroxidase, and catalase, and enhanced the accumulations of glutathione and free proline in rice seedlings and help to prevent the rice seedlings from chilling induced oxidative stress. (Hussain *et al.*, 2016). The exogenous application of SA significantly ($P < 0.05$, ANOVA) increased the POD and CAT activities. SA treatment eliminates the accumulation of H_2O_2 in leaves under chilling stress (Zhou *et al.* 2006). Mutlu *et al.* (2016) reported that exogenous SA (0.1mM) could play an ameliorating role on cold tolerance by regulating reactive oxygen species and the activities of antioxidative enzymes in both cold-sensitive and cold-tolerant cultivars of barley. Zhang *et al.*, (2016) reported that Chilling stress resulted in greater induction of SA levels in the chilling-tolerant cultivar Changchun mici in both

leaves and seeds compared to the chilling-sensitive one Beijing jietou, while the former one contained higher levels of SA than the latter one in the seeds under normal conditions. Moreover, exogenous application of SA increased significantly the POD and CAT activities and soluble protein content and that exogenous SA can enhance the chilling tolerance ability, which might be achieved through modulating the antioxidant system in cucumber. Chen *et al.* (2016) proposed the role of SA and enzymes CAT and POX in providing resistance against powdery mildew in wheat. It was reported by Yusuf *et al.* (2008) that the toxic effects generated by the lower concentration of NaCl (50 mmol/L) were completely overcome by the application of SA (10 μ M/l) increasing the peroxidase activity. The increased activity of POD and CAT was correlated with chilling tolerance in cucumber (Kang and Saltveit 2001). SOD can catalyze the dismutation of O_2^- into oxygen (O_2) and H_2O_2 to control lipid oxidation and protect membrane systems from being oxidized by ROS. Exogenous SA treatments enhanced the SOD activity significantly (at CD 5%) and it improves the plant growth and development, flowering, ion uptake, stomatal regulation and photosynthesis (Kadioglu *et al.*, 2011). Exogenous application of proline improves the functioning of peroxidase (Yan *et al.*, 2011) and catalase (Ali *et al.*, 2014) enzymes. Similar results were also reported by Yusuf *et al.*, (2008) in mustard, Shrutti and Gupta (2015) in radish and Kaur and Gupta (2015) in Cucumber.

Conclusion

It is evident that different seed treatments increased the germination and activities of antioxidant enzymes at both the temperatures. But among all the treatment of proline 20mM and salicylic acid 0.1mM showed better results over other seed treatments and thus provide tolerance to low temperature by scavenging the free radicals. Thus Proline 20 mM and SA 0.1mM increased percentage germination by scavenging the free radicals produced as indicated by the increased activities of the antioxidant enzymes viz. CAT, POX and SOD. Thus proline and SA can be used to reduce the adverse effects produced by low temperature stress.

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