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SHORT COMMUNICATION

EMERGENCE AND SEEDLING ESTABLISHMENT IN MAIZE UNDER LOW TEMPERATURE STRESS AND ITS AMELIORATION WITH SALICYLIC ACID

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Emergence experiments were carried out under laboratory conditions at optimum (25°C) and sub-optimum (15°C) temperatures at PPFD 450-500 µmol m^{-2} s^{-1} and photoperiod (h) of 16:8 (L:D) in two maize cultivars, *viz*. Sheetal and Paras (recommended for *rabi* and main season respectively). Sub-optimum temperature had no adverse effect on emergence percentage, while days to emergence were deleteriously affected. Biochemical parameters, *viz*. root metabolic activity, chlorophyll content, Hill reaction activity and catalase activity (estimated from 3rd leaf of seedling) decreased while total sugars, sucrose, proline content and peroxidase activity increased at 15°C in both the cultivars. Salicylic acid (SA) (10, 20 and 50 µg ml^{-1}) used as seed soaking pre-treatment improved per cent emergence, root metabolic activity, total sugars and sucrose at both 25°C and 15°C in the two cultivars. In the cultivar Sheetal, chlorophyll content and Hill reaction activity decreased at 25°C but increased at 15°C. While in Paras, there was non-significant difference in chlorophyll content and Hill reaction activity at 25°C but increased at 15°C. Proline content increased at 15°C in both the cultivars. SA increased peroxidase but decreased catalase activity in the two cultivars at both the temperatures. SA (20 µg ml^{-1}) was most effective in ameliorating chilling tolerance.

***Key words*: Antioxidant enzymes, emergence, low temperature, maize, salicylic acid**

Maize is one of the leading cereal crops in the world. It is grown during summer/rainy season in India and many other countries of the world. The growth of maize crop is restricted when exposed for a prolonged period to extreme low or high temperatures (Dass *et al.* 2005). In maize, sensitivity to low temperature stress requires attention because field sown crop can experience chilling temperatures at germination, emergence and early growth stages (Hodges *et al.* 1997a). In India, chilling injury in maize has considerable importance for cultivation during *rabi* season (Saxena *et al.* 2002). Low temperature during germination results in slow asynchronous emergence and poor stand establishment (Nykiforuk and Johnson-Flanagan 1998). Several seed pre-treatments, *viz*. priming with PEG or potassium salts or pre-treatments with growth regulators have been reported to ameliorate chilling injury in sensitive crops (Bedi and Basra 1993). In the present investigation, physiological changes in maize seedlings associated with low temperature stress and its amelioration with salicylic acid pre-treatment have been studied.

The seeds of maize (*Zea mays* L.) cultivar Sheetal (harvested during May, 2005) and Paras (harvested during September, 2005) were obtained from the department of Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana. The emergence studies were carried out in plastic trays

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filled with sand up to a height of 3.0 cm and moistened with about 350 ml of distilled water. Prior to use, the sand was sun-dried and then placed in an oven at 80°C for 24 h. Prior to sowing, seeds were surface-sterilized by soaking in an aqueous solution of $HgCl_2$ (0.1%) for 1 min followed by several rinses in distilled water. Twenty-four seeds were placed in each tray in six rows with four seeds per row. The trays were placed in growth chamber at photon flux rate of 450-500µmol $m^{-2} s^{-1}$ and photoperiod (h) of 16:8 (L:D) at low (15°C) and optimum (25°C) temperatures respectively. The experiments were repeated twice. The trays were observed daily and re-moistened as per requirement. Those seeds whose coleoptile protruded from soil surface were considered as emerged. Emergence percentage was recorded upto 20 days after sowing. Days to emergence were calculated as the average number of days necessary for a seed to achieve a 2 cm coleoptile above the soil surface (Hodges *et al.* 1997a). Prior to sowing, the seeds were soaked in salicylic acid solutions of 10, 20 and 50 µg ml^{-1} respectively for 24 h. The control seeds were soaked in water for the same duration. The root respiratory activity was determined from root tip sections (5.0 cm) following the method of Boamah and Fletcher (1983). Total chlorophyll content (Hiscox *et al.* 1979), Hill reaction activity (Cherry 1973), total soluble sugars (Dubois *et al.* 1956), sucrose (Roe *et al.* 1934), proline (Bates *et al.* 1973) and activities of peroxidase (Shannon *et al.* 1966) and catalase (Aebi 1983) were estimated from 3rd fully expanded leaf of maize seedlings.

At sub-optimum temperature (15°C), the emergence percentage of seedlings was unaffected at the end of 20 days but the days taken for seedlings to emerge were more at 15°C than at 25°C in both the cultivars (Table1). Under field conditions, delayed germination results in poor stand establishment. Pre-treatment of seeds with salicylic acid (SA) @ 10, 20 and 50 µg ml^{-1} reduced the days to emergence and improved percentage in both the cultivars at both the temperatures (Table 1) and SA @ 20 µg ml^{-1} was the most effective treatment. The root respiratory activity estimated after the emergence of 3rd

Table 1. Effect of Salicylic acid pre-treatment on emergence, root respiratory activity (OD at 486 nm), chlorophyll content (mg g^{-1} fw) and Hill reaction activity (decrease in absorbance mg^{-1} Chl h^{-1}) at 25ºC and 15ºC in maize cultivars Sheetal and Paras

Treatments	% Emergence		Days to emergence		Root respiratory activity		Total chlorophyll content		Hill reaction activity	
	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C
Sheetal										
Control	89.59	91.84	6.71	10.98	0.050	0.011	1.069	1.339	0.092	0.055
SA_{10}	95.82	96.55	4.65	7.48	0.048	0.051	0.894	1.337	0.074	0.117
SA_{20}	100.00	96.87	4.32	6.04	0.058	0.054	0.965	1.331	0.079	0.128
SA_{50}	94.55	95.82	5.37	8.04	0.054	0.057	0.928	1.343	0.081	0.117
C.D. (5%): SA	3.58	2.36	0.17	0.13	NS	0.010	0.023	NS	0.008	0.014
Temp x SA		1.64		0.884		0.009		0.033		0.011
Paras										
Control	95.44	97.33	8.03	15.65	0.067	0.026	0.904	0.776	0.160	0.074
SA_{10}	97.29	96.66	5.98	11.30	0.077	0.033	0.984	0.789	0.154	0.084
SA_{20}	100.00	100.00	5.16	9.15	0.083	0.046	0.985	0.878	0.155	0.144
SA_{50}	96.35	100.00	6.32	10.76	0.086	0.131	1.213	0.783	0.158	0.131
C.D. (5%): SA	3.09	0.89	0.38	0.36	0.011	0.009	0.079	0.008	NS	0.011
Temp x SA		2.02		1.20		0.018		0.029		0.010

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leaf was higher at 25°C than at 15°C (Table 1). SA pre-treatment effectively enhanced root respiratory activity at 15°C while at 25°C, the root respiratory activity remained unchanged in Sheetal (Table 1).

The amount of chlorophyll and Hill reaction activity was less at 15°C than at 25°C in both the cultivars (Table 1). A strong decrease in chlorophyll content and Hill reaction activity at low temperature has also been reported earlier (Takac 2004). At 25°C, the amount of total chlorophyll in 3rd leaf of maize seedlings decreased in SA pre-treated seeds in Sheetal while in Paras, it remained unchanged (Table 1). Hill reaction activity also followed the similar trend (Table 1). Further, at 25°C, SA treated seedlings showed a decrease in catalase activity (Table 2) which may lead to an increase in endogenous concentration of H_2O_2 as catalase is a H_2O_2 scavenging enzyme. An increase in endogenous levels of H_2O_2 has also been reported in SA treated banana seedlings at 30/22°C (Kang *et al.* 2003). A decrease in chlorophyll content in SA pre-treated seedlings may therefore, be due to increased concentration of H_2O_2 as reactive oxygen species (e.g. H_2O_2) are known to cause degradative reactions including chlorophyll quenching (Elstner 1982). At 15°C, the total chlorophyll content and Hill reaction activity in 3rd leaf of Sheetal increased in SA treated seeds (Table 1). In Paras, this increase was significant for 10 and 20 µg ml^{-1}. It has been reported earlier also that at low temperature, SA treated seedlings had higher total chlorophyll content as compared to control (Agarwal *et al.* 2005).

The total soluble sugar content was higher at 15°C than at 25°C in both Sheetal and Paras (Table 2). However, this increase was more in case of Sheetal (*rabi season*) in comparison to Paras. In seedlings raised at 15°C from SA treated seeds, total soluble sugars increased significantly with all the three concentrations of SA in both the cultivars (Table 2). Maximum increase in sugar content was observed with 20 µg ml^{-1} SA pre-treatment. However at 25°C, the amount of total soluble sugars remained unchanged in Sheetal but increased significantly in Paras (Table 2). Likewise sucrose contents were higher at 15ºC as compared to those at

Table 2. Effect of Salicylic acid pre-treatment on total soluble sugars (mg g^{-1} dw), sucrose content (mg g^{-1} dw), proline content (mg g^{-1} fw), peroxidase and catalase activity (change in absorbance min^{-1} g^{-1} fw) at 25ºC and 15ºC in maize cultivars Sheetal and Paras

Treatments	Total soluble sugars		Sucrose		Proline		Peroxidase		Catalase	
	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C
Sheetal										
Control	1.892	5.344	0.233	1.887	0.080	0.149	87.91	144.83	1.437	1.394
SA_{10}	2.234	6.684	0.268	4.027	0.094	0.493	102.61	149.57	0.754	0.985
SA_{20}	2.282	8.543	0.389	3.419	0.124	0.637	96.26	176.59	1.013	1.213
SA_{50}	2.231	8.167	0.298	4.760	0.106	0.724	94.39	165.81	1.452	1.386
C.D. (5%): SA	NS	0.206	NS	0.367	0.008	0.034	2.85	1.24	0.035	0.019
Temp. x SA		0.224		0.011		0.014		3.98		0.027
Paras										
Control	1.954	3.321	0.150	2.140	0.060	0.113	60.19	65.85	1.206	0.840
SA_{10}	3.310	7.813	0.393	3.790	0.067	0.317	74.32	109.70	1.043	0.705
SA_{20}	4.230	9.795	0.439	4.493	0.077	0.492	97.59	170.99	0.859	0.644
SA_{50}	3.167	7.221	0.328	3.168	0.078	0.554	79.61	138.59	0.922	0.822
C.D. (5%): SA	0.150	0.190	0.055	0.293	NS	0.033	0.66	1.43	0.156	0.021
Temp x SA		0.077		0.013		0.008		4.45		0.018

25ºC in both Sheetal and Paras (Table 2). In the seedlings raised from SA treated seeds at 25ºC, sucrose content remained unaffected by SA treatment in Sheetal but in Paras, SA was effective in increasing the sucrose content (Table 2). At 15ºC, SA pre-treatment increased the sucrose content in both the cultivars and maximum increase was recorded with 20 µg ml^{-1} SA (Table 2). In a previous study, Hodges *et al.* (1997b) have observed accumulation of total sugars in four maize lines differing in sensitivity to chilling. Sugar accumulation was more in chilling sensitive line during short-term exposure (1-8 days), and this may be due to a decrease in rate of utilization of carbohydrates in chilling sensitive cultivars (Farrar 1988). The soluble sugars confer chilling tolerance by acting as primary cryoprotectants.

Proline content was more at 15ºC than that at 25ºC (Table 2). Free proline is known to accumulate in response to biotic and abiotic stresses and has been shown to protect plants against free-radical induced damage (Matysik *et al.* 2002). At both 25ºC and 15ºC, proline content increased significantly in SA pre-treated seedlings of Sheetal as compared to control, but in Paras, there was non-significant improvement at 25°C (Table 2). Maximum increase in proline content was observed in Sheetal at 15ºC following 50 µg ml^{-1} SA treatment. Exogenous SA increased proline content in the leaves of treated seedlings of *Prunus* at low temperature (Lin *et al.* 2004).

The activity of peroxidase was higher at 15ºC than at 25ºC for both Sheetal and Paras but the activity of catalase followed the reverse trend (Table 2). An increase in peroxidase activity due to low temperature stress has also been reported in wheat seedlings (Berova *et al.* 2002). Chilling-related decrease in catalase activity has also been reported by various other workers and there is evidence that it can become photo-inactivated (Feierbend *et al.* 1992). Peroxidase and catalase activity was higher in Sheetal as compared to Paras at both temperatures. Several studies comparing different species reported that chilling-resistant species have a greater antioxidant capacity than other species that are chilling sensitive (Gossett *et al.* 1994). With SA pre-treatment, the activity of peroxidase increased in both the cultivars at both the temperatures (Table 2). In maize, addition of SA to hydroponic growth solution provided protection against low temperature stress and there was increase in peroxidase and other antioxidant enzymes (Janda *et al.* 1999). However, the activity of catalase in seedlings raised from SA treated seeds decreased over control in both Sheetal and Paras at both 25 and 15ºC (Table 2). With 50 µg ml^{-1} SA treatment the activity was at par with the control (Table 2). A decrease in activity of catalase under low temperature has also been observed in rice, wheat and cucumber seedlings. This decrease was correlated with increase in endogenous salicylic acid content in plants (Shim *et al.* 2003). While, pre-treatment with SA reduced catalase activity at 30/22ºC but at 5ºC, there was an increase in catalase activity in banana seedlings (Kang *et al.* 2003).

It may be concluded that SA pre-treatment helped to improve emergence, seedling growth and biochemical parameters, but SA was relatively more effective at sub-optimum (15ºC) than at optimum temperature.

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