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Mitigating Effect of Salicylic Acid on Biochemical and Antioxidant Enzymes in Maize (*Zea mays* L.) Genotypes under Low Temperature Stress

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A field experiment was carried out during the winter (*Rabi*) seasons of 2013-2014 and 2014- 2015 at Agricultural Research Farm, Banaras Hindu University, Varanasi to study the mitigating effect of salicylic acid on biochemical and antioxidant enzymes in maize genotypes i.e., HUZM-185 (tolerant) and HUZM-80-1 (sensitive) under low temperature stress. The experiment was laid out in split plot design comprised eight treatment combinations in three replications. Seeds were primed with salicylic acid (SA) @ 20ppm and 40ppm along with hydro (distilled water) for overnight and dry seeds as control before both sowing i.e., normal and delay sowing conditions. Observations were recorded at 20, 40 and 60 days after sowing viz., malondialdehyde content (MDA), hydrogen peroxide content (H_2O_2), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) activities in both normal and delay sowing. It was found that tolerant genotype with 20ppm salicylic acid significantly reduced MDA and H_2O_2 production and enhanced antioxidants (SOD, CAT and POX) levels as compared to sensitive genotype on normal and delay sowing. The deleterious effect of low temperature stress was cope up by enhancing SOD, CAT and POX activities in delay sowing as compare to normal sowing condition.

Key words: Low temperature stress, Maize, Salicylic acid.

Maize contributes maximum among the food cereal crops i.e. 40 % annually (> 800 million tonnes) in the global food production. In India, maize is the 3rd most important food crops after rice and wheat. *Rabi* maize is grown on an area of 1.49 Mha with a production of 6.40 MT and yield of 4,288 kg/ha (ISOPOM, 2013-2014). Sowing of *rabi* maize crop at appropriate time is essential for growth, development and better grain yield and it is also required that the *rabi* maize crop exhibit low temperature tolerance as the early growth stages are sensitive to low temperature. Maize seeds sown when the soil temperature are 10^0C and lower are often injured by cold water imbibition stress which results in poor and erratic germination and chilling injury leads to poor seedling establishment through production of reactive oxygen species (ROS), which cause oxidative damage to various marco-molecules and cellular structures (Apel and Hirt, 2004). Plants are subjected to various abiotic stresses including water, high and low temperatures, salinity, and light stresses. Low temperature stress was defined as the temperature in a range low enough to suppress growth without ceasing cellular functions, which induce several abnormalities at various cell organization levels. Seed priming was defined as pre-sowing treatments in water or in an osmotic solution that allows seed to imbibe water to proceed to the first stage of germination, but prevents radicle protrusion through the seed coat. Exogenous SA could

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regulate the synthesis and activities of antioxidant enzymes and increase plant tolerance to biotic and abiotic stress (He *et al*., 2002; Fayez and Bazaid, 2014). Salicylic acid pre treatment helped to improve emergence, seedling growth and biochemical parameters, but salicylic acid was relatively more effective at sub optimum (15^0C) than at optimum temperature (Seema bedi and Madhu dhingra, 2007). Keeping this in view, the present investigation was carried out to study the mitigating effect of salicylic acid on biochemical and antioxidant enzymes in maize genotypes i.e., HUZM-185 (tolerant) and HUZM-80-1 (sensitive) under low temperature stress.

MATERIALS AND METHODS

Site Description

A field experiment was carried out during the winter (*rabi*) seasons of 2013-2014 and 2014-2015 at Agricultural Research Farm, Banaras Hindu University, Varanasi is situated at $25^0$18' North latitudes, $83^0$03' East longitudes and at an altitude of 128.93 meter above the mean sea levels in the north-eastern plains zone. The soil was sandy loam in texture, neutral in reaction (pH 7.3), low in organic carbon (0.42%) and available nitrogen (187.72 kg/ha) and medium in available phosphorus (18.50 kg/ha). The total rainfall received during crop season 2013-2014 and 2014-2015 was 23.10 and 24.00 mm. The experiment was laid out in split plot design comprised eight treatment combinations [V1T1- Control, (HUZM-185 + dry), V1T2- hydro (HUZM-185 + distilled water) priming, V1T3- (HUZM-185 + 20ppm SA), V1T4- (HUZM-185 + 40ppm SA), V2T1- Control; non-prime seed (HUZM-80-1 + dry), V2T2- hydro (HUZM-80-1 + distilled water) priming, V2T3-(HUZM-80-1 + 20ppm SA), V2T4- (HUZM-80-1 + 40ppm SA)] and replicated thrice. The observations were recorded in first fully expanded leaf at 20, 40 and 60 days after sowing viz., malondialdehyde content (MDA), hydrogen peroxide content (H_2O_2), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) activities in both normal and delay sowing.

Data obtained from various observations were analysis as per the standard analysis of variance (ANOVA) procedure for split plot design given by Gomez and Gomez (1984).

Biochemical and Antioxidant enzymes analysis

Lipid peroxidation was determined by measuring the malondialdehyde (MDA) content following the method of Dhindsa *et al*. (1982). Hydrogen peroxide was determined by the protocol of Mukherjee and Choudhary (1983).

For enzyme determination: Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed according to the method of Dhindsa *et al*. (1981). Catalase (CAT, EC 1.11.1.6) activity was assayed according to the method of Aebi (1983) by the decrease of absorbance at 240 nm for 1 min as a consequence of H_2O_2 consumption. Peroxidase (POD, EC 1.11.1.7) activity was determined according to the method described by Nakano and Asada (1981) as the increase of absorbance at 470 nm due to oxidation of guaiacol in the presence of H_2O_2 and formation of tetraguaiacol.

RESULTS AND DISCUSSION

Salicylic acid is a key signaling molecule in induction of plant defense mechanism and reduces symptoms of environmental stress as well as regulates plant growth and development (Horváth et al., 2007). The content of MDA, a product of lipid peroxidation, has been considered as an indicator of oxidative damage, while electrolyte leakage represents cell membrane injury. Hydrogen peroxide is a reactive oxygen species (ROS), which leads to disarrangement of bio-membrane and hampers its selectivity. A higher antioxidant activity in tolerant genotype minimized ROS production (H_2O_2) and overcomes on low temperature stress as compared to sensitive genotype. Tolerant genotype HUZM-185 with 20ppm salicylic acid significantly reduced MDA and H_2O_2 production decreases in the MDA (Table 1 a & b) and H_2O_2 (Table 2 a & b) than sensitive genotype HUZM-80-1 in leaf tissues under all treatments. Malondialdehyde content and Hydrogen peroxide content under delay sown condition was found minimum in HUZM-185 + T_3 (20ppm SA) followed by HUZM-185 + T_4 (40ppm SA) while maximum in HUZM-80-1 + T_1 (dry; non-primed seed).

SA treatments caused significant decreases in MDA and EL values in two tomato cultivars relative to control plants. The decrease

Table 1(a). Effect of salicylic acid on malondialdehyde content (n mol g-1 fresh weight) in maize (Zea mays L.) during low temperature stress under normal and delay sown condition in the year of 2013-14

Genotype	Treatment	Different days after sowing								
		20 DAS			40 DAS			60 DAS		
		Normal	Delay	Mean	Normal	Delay	Mean	Normal	Delay	Mean
HUZM-185	T1 (Dry)	2.03	2.93 (+43.9)	2.48	2.27	3.21 (+41.5)	2.74	2.53	3.13 (+24.0)	2.83
	T2 (Hydro)	1.66	2.33 (+40.6)	2.00	1.76	2.34 (+32.8)	2.05	2.09	2.44 (+16.5)	2.26
	T3 (20 ppm SA)	1.13	1.23 (+8.8)	1.18	1.41	1.50 (+6.1)	1.46	1.64	1.73 (+5.8)	1.68
	T4 (40 ppm SA)	1.36	1.57 (+15.2)	1.46	1.61	1.80 (+11.8)	1.70	1.88	2.10 (+11.7)	1.99
Mean		1.55	2.02		1.76	2.21		2.03	2.35	
HUZM 80-1	T1 (Dry)	2.63	4.67 (+77.7)	3.65	2.75	4.30 (+56.2)	3.53	2.95	3.79 (+28.7)	3.37
	T2 (Hydro)	2.23	3.75 (+67.9)	2.99	2.52	3.73 (+48.2)	3.13	2.56	3.23 (+26.1)	2.90
	T3 (20 ppm SA)	1.54	1.70 (+10.4)	1.62	1.76	1.97 (+11.6)	1.86	2.24	2.40 (+7.3)	2.32
	T4 (40 ppm SA)	2.13	2.57 (+20.3)	2.35	2.31	2.63 (+13.9)	2.47	2.33	2.73 (+17.1)	2.53
Mean		2.13	3.17		2.34	3.16		2.52	3.04	
Grand Mean		1.84	2.59		2.05	2.69		2.28	2.69	
Particulars		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%			
Sowing		0.03	0.16	0.10	0.64	0.12	NS			
Genotype		0.04	0.12	0.04	0.11	0.05	0.13			
Sowing × Genotype		0.06	0.17	0.05	0.16	0.07	NS			
Treatment		0.06	0.17	0.05	0.16	0.07	0.19			
Sowing × Treatment		0.08	0.24	0.08	0.22	0.09	0.26			
Genotype × Treatment		0.08	0.24	0.08	0.22	0.09	NS			
Sowing × Genotype × Treatment		0.12	0.34	0.11	NS	0.13	NS			

Where, SA-Salicylic Acid, DAS-Days After Sowing. Values in the parenthesis indicate % increase or decrease under delay sowing over normal sowing.

Table 1(b). Effect of salicylic acid on malondialdehyde content (n mol g-1 fresh weight) in maize (Zea mays L.) during low temperature stress under normal and delay sown condition in the year of 2014-15

Genotype	Treatment	Different days after sowing								
		20 DAS			40 DAS			60 DAS		
		Normal	Delay	Mean	Normal	Delay	Mean	Normal	Delay	Mean
HUZM-185	T1 (Dry)	2.33	3.46 (+48.3)	2.90	2.57	3.73 (+45.5)	3.15	2.83	3.57 (+26.2)	3.20
	T2 (Hydro)	1.97	2.87 (+45.8)	2.42	2.06	2.87 (+39.0)	2.46	2.31	2.77 (+19.9)	2.54
	T3 (20 ppm SA)	1.43	1.60 (+11.6)	1.52	1.72	1.87 (+8.8)	1.79	1.93	2.07 (+6.9)	2.00
	T4 (40 ppm SA)	1.67	1.98 (+18.8)	1.83	1.91	2.25 (+17.9)	2.08	2.18	2.47 (+13.1)	2.32
Mean		1.85	2.48		2.06	2.68		2.31	2.72	
HUZM 80-1	T1 (Dry)	2.92	5.40 (+84.7)	4.16	3.06	5.07 (+65.8)	4.06	3.25	4.37 (+34.5)	3.81
	T2 (Hydro)	2.53	4.40 (+73.7)	3.47	2.83	4.40 (+55.3)	3.62	2.87	3.73 (+30.2)	3.30
	T3 (20 ppm SA)	1.84	2.10 (+14.1)	1.97	2.07	2.40 (+16.1)	2.23	2.53	2.77 (+9.2)	2.65
	T4 (40 ppm SA)	2.43	3.00 (+23.3)	2.72	2.62	3.10 (+18.5)	2.86	2.62	3.13 (+19.7)	2.88
Mean		2.43	3.73		2.64	3.74		2.82	3.50	
Grand Mean		2.14	3.10		2.35	3.21		2.56	3.11	
Particulars		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%			
Sowing		0.12	0.74	0.08	0.47	0.11	NS			
Genotype		0.05	0.14	0.05	0.14	0.05	0.14			
Sowing × Genotype		0.07	0.19	0.07	0.20	0.07	NS			
Treatment		0.07	0.19	0.07	0.20	0.07	0.20			
Sowing × Treatment		0.09	0.27	0.10	0.28	0.10	0.29			
Genotype × Treatment		0.09	0.27	0.10	0.28	0.10	NS			
Sowing × Genotype × Treatment		0.13	0.39	0.14	NS	0.14	NS			

Where, SA-Salicylic Acid, DAS-Days After Sowing. Values in the parenthesis indicate % increase or decrease under delay sowing over normal sowing.

Table 2(a). Effect of salicylic acid on hydrogen peroxide content (µ mol g-1 fresh weight) in maize (Zea mays L.) during low temperature stress under normal and delay sown condition in the year of 2013-14

Genotype	Treatment	Different days after sowing								
		20 DAS			40 DAS			60 DAS		
		Normal	Delay	Mean	Normal	Delay	Mean	Normal	Delay	Mean
HUZM-185	T1 (Dry)	19.61	23.70 (+20.9)	21.65	24.63	29.13 (+18.2)	26.88	26.67	35.64 (+33.6)	31.15
	T2 (Hydro)	18.15	21.69 (+19.5)	19.92	23.34	26.37 (+13.0)	24.85	24.43	32.19 (+31.7)	28.31
	T3 (20 ppm SA)	12.17	13.00 (+6.9)	12.58	17.22	18.72 (+8.7)	17.97	20.00	21.33 (+6.7)	20.67
	T4 (40 ppm SA)	15.02	16.11 (+7.3)	15.56	19.85	22.27 (+12.2)	21.06	21.53	24.67 (+14.6)	23.10
Mean	16.24	18.63		21.26	24.12		23.16	28.46		
HUZM 80-1	T1 (Dry)	32.81	44.62 (+36.0)	38.72	37.40	49.67 (+32.8)	43.53	44.73	61.67 (+37.9)	53.20
	T2 (Hydro)	30.55	40.50 (+32.6)	35.53	35.55	45.50 (+28.0)	40.53	39.48	53.67 (+36.0)	46.57
	T3 (20 ppm SA)	24.82	27.48 (+10.7)	26.15	31.55	34.67 (+9.9)	33.11	37.00	40.00 (+8.1)	38.50
	T4 (40 ppm SA)	28.12	31.33 (+11.4)	29.73	32.92	37.39 (+13.6)	35.16	36.36	42.00 (+15.5)	39.18
Mean		29.07	35.98		34.36	41.81		39.39	49.33	
Grand Mean		22.65	27.31		27.81	32.96		31.27	38.89	
Particulars		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%			
Sowing		0.19	1.16	0.21	1.30	0.34	2.12			
Genotype		0.27	0.79	0.23	0.67	0.30	0.88			
Sowing × Genotype		0.38	1.11	0.33	0.95	0.43	1.25			
Treatment		0.38	1.11	0.33	0.95	0.43	1.25			
Sowing × Treatment		0.54	1.57	0.46	1.34	0.61	1.76			
Genotype × Treatment		0.54	1.57	0.46	NS	0.61	1.76			
Sowing × Genotype × Treatment		0.77	2.22	0.65	1.89	0.86	2.49			

Where, SA-Salicylic Acid, DAS-Days After Sowing. Values in the parenthesis indicate % increase or decrease under delay sowing over normal sowing.

Table 2(b). Effect of salicylic acid on hydrogen peroxide content (µ mol g-1 fresh weight) in maize (Zea mays L.) during low temperature stress under normal and delay sown condition in the year of 2014-15

Genotype	Treatment	Different days after sowing								
		20 DAS			40 DAS			60 DAS		
		Normal	Delay	Mean	Normal	Delay	Mean	Normal	Delay	Mean
HUZM-185	T1 (Dry)	22.61	28.03 (+24.0)	25.32	27.63	34.00 (+23.0)	30.82	29.67	40.67 (+37.1)	35.17
	T2 (Hydro)	21.15	26.00 (+22.9)	23.58	26.34	30.40 (+15.4)	28.37	27.43	36.67 (+33.7)	32.05
	T3 (20 ppm SA)	17.47	19.08 (+29.3)	18.28	20.22	22.45 (+11.0)	21.34	23.00	25.00 (+8.7)	24.00
	T4 (40 ppm SA)	18.84	21.11 (+12.0)	19.98	22.85	25.74 (+12.7)	24.29	24.53	29.55 (+20.5)	27.04
Mean	20.02	23.56		24.26	28.15		26.16	32.97		
HUZM 80-1	T1 (Dry)	35.15	49.33 (+40.4)	42.24	40.40	55.58 (+37.6)	47.99	47.73	67.33 (+41.1)	57.53
	T2 (Hydro)	33.55	45.63 (+36.0)	39.59	37.97	50.24 (+32.3)	44.11	42.48	59.00 (+38.9)	50.74
	T3 (20 ppm SA)	28.82	32.15 (+11.6)	30.49	34.55	38.67 (+11.9)	36.61	40.00	44.33 (+10.8)	42.17
	T4 (40 ppm SA)	31.12	35.67 (+14.6)	33.39	35.92	41.39 (+15.2)	38.66	39.36	46.33 (+17.7)	42.85
Mean		32.16	40.70		37.21	46.47		42.39	54.25	
Grand Mean		26.09	32.13		30.74	37.31		34.27	43.61	
Particulars		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%			
Sowing		0.40	2.45	0.15	0.93	0.40	2.47			
Genotype		0.30	0.87	0.26	0.75	0.46	1.32			
Sowing × Genotype		0.42	1.22	0.37	1.06	0.65	1.87			
Treatment		0.42	1.22	0.37	1.06	0.65	1.87			
Sowing × Treatment		0.60	1.73	0.52	1.50	0.91	2.65			
Genotype × Treatment		0.60	1.73	0.52	NS	0.91	2.65			
Sowing × Genotype × Treatment		0.85	2.45	0.73	2.12	1.29	NS			

Where, SA-Salicylic Acid, DAS-Days After Sowing. Values in the parenthesis indicate % increase or decrease under delay sowing over normal sowing.

Table 3(a). Effect of salicylic acid on superoxide dismutase activity (Units g-1 protein weight min-1) in maize (Zea mays L.) during low temperature stress under normal and delay sown condition in the year of 2013-14

Genotype	Treatment	Different days after sowing								
		20 DAS			40 DAS			60 DAS		
		Normal	Delay	Mean	Normal	Delay	Mean	Normal	Delay	Mean
HUZM-185	T1 (Dry)	14.11	15.00 (+6.3)	14.55	18.07	18.67 (+3.3)	18.37	21.33	22.33 (+4.7)	21.83
	T2 (Hydro)	17.87	19.33 (+8.2)	18.60	22.61	24.67 (+9.1)	23.64	23.33	24.67 (+5.7)	24.00
	T3 (20 ppm SA)	22.25	28.33 (+27.3)	25.29	23.75	30.00 (+26.3)	26.88	28.33	35.67 (+25.9)	32.00
	T4 (40 ppm SA)	19.00	22.00 (+15.8)	20.50	23.29	28.67 (+23.1)	25.98	26.67	32.67 (+22.5)	29.67
Mean	18.31	21.17		21.93	25.50		24.92	28.83		
HUZM 80-1	T1 (Dry)	10.03	10.33 (+3.0)	10.18	13.39	13.67 (+2.1)	13.53	17.22	17.67 (+2.6)	17.44
	T2 (Hydro)	11.33	12.00 (+5.9)	11.67	14.84	15.67 (+5.6)	15.25	19.87	20.67 (+4.0)	20.27
	T3 (20 ppm SA)	15.69	19.33 (+23.2)	17.51	18.73	22.33 (+19.2)	20.53	23.90	28.00 (+17.2)	25.95
	T4 (40 ppm SA)	12.31	13.83 (+12.4)	13.07	16.32	18.33 (+12.3)	17.33	21.71	24.67 (+13.6)	23.19
Mean		12.34	13.88		15.82	17.50		20.68	22.75	
Grand Mean		15.33	17.52		18.88	21.50		22.80	25.79	
Particulars		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%			
Sowing		0.31	1.90	0.28	1.70	0.44	2.73			
Genotype		0.20	0.57	0.25	0.73	0.26	0.76			
Sowing × Genotype		0.28	0.80	0.35	1.03	0.37	1.07			
Treatment		0.28	0.80	0.35	1.03	0.37	1.07			
Sowing × Treatment		0.39	1.14	0.50	1.45	0.52	1.52			
Genotype × Treatment		0.39	1.14	0.50	1.45	0.52	1.52			
Sowing × Genotype × Treatment		0.55	NS	0.71	NS	0.74	NS			

Where, SA-Salicylic Acid, DAS-Days After Sowing. Values in the parenthesis indicate % increase or decrease under delay sowing over normal sowing.

Table 3(b). Effect of salicylic acid on superoxide dismutase activity (Units mg-1 protein min-1) in maize (Zea mays L.) during low temperature stress under normal and delay sown condition in the year of 2014-15

Genotype	Treatment	Different days after sowing								
		20 DAS			40 DAS			60 DAS		
		Normal	Delay	Mean	Normal	Delay	Mean	Normal	Delay	Mean
HUZM-185	T1 (Dry)	16.03	17.17 (+7.1)	16.60	20.08	21.00 (+4.6)	20.54	23.33	25.00 (+7.1)	24.17
	T2 (Hydro)	19.95	21.83 (+9.4)	20.89	24.61	27.33 (+11.1)	25.97	25.33	27.33 (+7.9)	26.33
	T3 (20 ppm SA)	24.25	31.33 (+29.2)	27.79	25.75	33.33 (+29.4)	29.54	30.33	39.33 (+29.7)	34.83
	T4 (40 ppm SA)	21.00	24.67 (+17.5)	22.83	25.29	32.00 (+26.5)	28.65	28.67	36.33 (+26.7)	32.50
Mean	20.31	23.75		23.93	28.42		26.92	32.00		
HUZM 80-1	T1 (Dry)	12.03	12.60 (+4.7)	12.32	15.39	16.00 (+4.0)	15.69	19.40	20.23 (+4.3)	19.82
	T2 (Hydro)	13.33	14.33 (+7.5)	13.83	16.84	18.53 (+10.1)	17.69	21.87	23.50 (+7.4)	22.69
	T3 (20 ppm SA)	17.69	22.33 (+26.2)	20.01	20.73	25.47 (+22.8)	23.10	25.90	31.42 (+21.3)	28.66
	T4 (40 ppm SA)	14.31	16.33 (+14.1)	15.32	18.32	21.00 (+14.6)	19.66	23.71	27.27 (+15.0)	25.49
Mean		14.34	16.40		17.82	20.25		22.72	25.60	
Grand Mean		17.33	20.08		20.88	24.33		24.82	28.80	
Particulars		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%			
Sowing		0.68	NS	0.35	2.13	0.79	NS			
Genotype		0.21	0.61	0.27	0.77	0.28	0.80			
Sowing × Genotype		0.30	0.87	0.38	1.09	0.39	1.13			
Treatment		0.30	0.87	0.38	1.09	0.39	1.13			
Sowing × Treatment		0.42	1.23	0.53	1.54	0.55	1.60			
Genotype × Treatment		0.42	1.23	0.53	1.54	0.55	1.60			
Sowing × Genotype × Treatment		0.60	NS	0.75	NS	0.78	NS			

Where, SA-Salicylic Acid, DAS-Days After Sowing. Values in the parenthesis indicate % increase or decrease under delay sowing over normal sowing.

Table 4(a). Effect of salicylic acid on catalase activity (Units mg-1 protein min-1) in maize (Zea mays L.) during low temperature stress under normal and delay sown condition in the year of 2013-14

Genotype	Treatment	Different days after sowing								
		20 DAS			40 DAS			60 DAS		
		Normal	Delay	Mean	Normal	Delay	Mean	Normal	Delay	Mean
HUZM-185	T1 (Dry)	13.41	14.73 (+9.9)	14.07	15.11	16.33 (+8.1)	15.72	15.50	17.00 (+9.7)	16.25
	T2 (Hydro)	14.74	16.45 (+11.6)	15.59	16.38	18.00 (+9.9)	17.19	17.77	19.67 (+10.7)	18.72
	T3 (20 ppm SA)	17.10	22.82 (+33.4)	19.96	19.77	23.33 (+18.0)	21.55	20.95	26.33 (+25.7)	23.64
	T4 (40 ppm SA)	15.92	18.17 (+14.1)	17.04	16.96	19.33 (+14.0)	18.15	19.27	23.00 (+19.4)	21.13
Mean	15.29	18.04		17.06	19.25		18.37	21.50		
HUZM 80-1	T1 (Dry)	12.54	13.23 (+5.5)	12.89	13.84	14.67 (+6.0)	14.25	14.44	15.33 (+6.2)	14.89
	T2 (Hydro)	13.38	14.83 (+10.9)	14.11	14.86	16.00 (+7.7)	15.43	15.62	17.00 (+8.9)	16.31
	T3 (20 ppm SA)	15.82	18.93 (+19.7)	17.38	16.74	19.00 (+13.5)	17.87	19.06	23.00 (+20.7)	21.03
	T4 (40 ppm SA)	14.62	16.49 (+12.8)	15.55	15.84	17.45 (+10.2)	16.65	16.26	18.80 (+15.6)	17.53
Mean		14.09	15.87		15.32	16.78		16.34	18.53	
Grand Mean		14.69	16.96		16.19	18.02		17.36	20.02	
Particulars		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%			
Sowing		0.32	1.98	0.27	1.67	0.66	NS			
Genotype		0.12	0.34	0.16	0.45	0.28	0.81			
Sowing × Genotype		0.17	0.48	0.22	NS	0.40	NS			
Treatment		0.17	0.48	0.22	0.63	0.40	1.14			
Sowing × Treatment		0.24	0.68	0.31	0.90	0.56	1.62			
Genotype × Treatment		0.24	0.68	0.31	0.90	0.56	NS			
Sowing × Genotype × Treatment		0.33	NS	0.44	NS	0.79	NS			

Where, SA-Salicylic Acid, DAS-Days After Sowing. Values in the parenthesis indicate % increase or decrease under delay sowing over normal sowing.

Table 4(b). Effect of salicylic acid on catalase activity (Units mg-1 protein min-1) in maize (Zea mays L.) during low temperature stress under normal and delay sown condition in the year of 2014-15

Genotype	Treatment	Different days after sowing								
		20 DAS			40 DAS			60 DAS		
		Normal	Delay	Mean	Normal	Delay	Mean	Normal	Delay	Mean
HUZM-185	T1 (Dry)	11.41	12.71 (+11.4)	12.06	13.11	14.33 (+9.3)	13.72	13.50	15.00 (+11.1)	14.25
	T2 (Hydro)	12.99	14.78 (+13.8)	13.89	14.38	16.00 (+11.3)	15.19	14.57	16.47 (+13.0)	15.52
	T3 (20 ppm SA)	15.10	21.15 (+40.1)	18.13	17.77	21.67 (+21.9)	19.72	18.95	24.33 (+28.4)	21.64
	T4 (40 ppm SA)	13.75	17.17 (+24.8)	15.46	14.96	17.33 (+15.9)	16.15	17.27	21.33 (+23.6)	19.30
Mean	13.31	16.45		15.06	17.33		16.07	19.28		
HUZM 80-1	T1 (Dry)	10.54	11.37 (+7.8)	10.95	11.84	12.77 (+7.8)	12.30	12.44	13.43 (+8.0)	12.94
	T2 (Hydro)	11.38	12.91 (+13.4)	12.15	12.86	14.06 (+9.3)	13.46	13.62	15.00 (+10.2)	14.31
	T3 (20 ppm SA)	13.82	16.67 (+20.6)	15.25	14.74	17.67 (+19.8)	16.21	17.07	21.33 (+25.0)	19.20
	T4 (40 ppm SA)	12.62	14.82 (+17.5)	13.72	13.84	15.73 (+13.7)	14.79	14.26	16.80 (+17.8)	15.53
Mean		12.09	13.94		13.32	15.06		14.35	16.64	
Grand Mean		12.70	15.20		14.19	16.19		15.21	17.96	
Particulars		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%			
Sowing		0.28	1.72	0.12	0.71	0.88	NS			
Genotype		0.14	0.41	0.22	0.64	0.23	0.68			
Sowing × Genotype		0.20	0.58	0.31	NS	0.33	NS			
Treatment		0.20	0.58	0.31	0.91	0.33	0.96			
Sowing × Treatment		0.28	0.82	0.44	NS	0.47	1.36			
Genotype × Treatment		0.28	0.82	0.44	NS	0.47	1.36			
Sowing × Genotype × Treatment		0.40	NS	0.63	NS	0.66	NS			

Where, SA-Salicylic Acid, DAS-Days After Sowing. Values in the parenthesis indicate % increase or decrease under delay sowing over normal sowing.

Table 5(a). Effect of salicylic acid on peroxidase activity (Units mg-1 protein min-1) in maize (Zea mays L.) during low temperature stress under normal and delay sown condition in the year of 2013-14

Genotype	Treatment	Different days after sowing								
		20 DAS			40 DAS			60 DAS		
		Normal	Delay	Mean	Normal	Delay	Mean	Normal	Delay	Mean
HUZM-185	T1 (Dry)	3.65	4.00 (+9.6)	3.83	4.66	5.07 (+8.7)	4.87	5.39	6.00 (+11.3)	5.70
	T2 (Hydro)	4.24	4.83 (+14.0)	4.54	5.20	5.73 (+10.3)	5.47	6.30	7.40 (+17.4)	6.85
	T3 (20 ppm SA)	6.68	8.77 (+31.3)	7.72	7.85	11.33 (+44.4)	9.59	8.93	13.00 (+45.5)	10.97
	T4 (40 ppm SA)	5.14	6.33 (+23.1)	5.74	5.81	7.33 (+26.2)	6.57	7.20	10.17 (+41.3)	8.68
Mean	4.93	5.98		5.88	7.37		6.96	9.14		
HUZM 80-1	T1 (Dry)	2.87	3.00 (+4.4)	2.94	3.50	3.70 (+5.7)	3.60	4.60	4.97 (+8.05)	4.78
	T2 (Hydro)	3.46	3.83 (+10.7)	3.65	4.04	4.40 (+8.9)	4.22	4.95	5.73 (+15.8)	5.34
	T3 (20 ppm SA)	4.97	6.13 (+23.3)	5.55	5.87	7.50 (+27.8)	6.68	6.77	8.90 (+31.6)	7.84
	T4 (40 ppm SA)	3.74	4.43 (+18.6)	4.09	4.68	5.80 (+24.0)	5.24	5.44	6.93 (+27.5)	6.19
Mean		3.76	4.35		4.52	5.35		5.44	6.63	
Grand Mean		4.34	5.17		5.20	6.36		6.20	7.89	
Particulars		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%			
Sowing		0.02	0.09	0.02	0.10	0.20	1.25			
Genotype		0.10	0.28	0.11	0.32	0.09	0.26			
Sowing × Genotype		0.14	NS	0.16	0.45	0.13	0.36			
Treatment		0.14	0.39	0.16	0.45	0.13	0.36			
Sowing × Treatment		0.19	0.55	0.22	0.64	0.18	0.51			
Genotype × Treatment		0.19	0.55	0.22	0.64	0.18	0.51			
Sowing × Genotype × Treatment		0.27	NS	0.31	NS	0.25	NS			

Where, SA-Salicylic Acid, DAS-Days After Sowing. Values in the parenthesis indicate % increase or decrease under delay sowing over normal sowing.

Table 5(b). Effect of salicylic acid on peroxidase activity (Units mg-1 protein min-1) in maize (Zea mays L.) during low temperature stress under normal and delay sown condition in the year of 2014-15

Genotype	Treatment	Different days after sowing								
		20 DAS			40 DAS			60 DAS		
		Normal	Delay	Mean	Normal	Delay	Mean	Normal	Delay	Mean
HUZM-185	T1 (Dry)	2.75	3.03 (+10.3)	2.89	3.78	4.23 (+12.1)	4.00	4.49	5.00 (+11.4)	4.75
	T2 (Hydro)	3.34	3.87 (+15.8)	3.60	4.30	4.87 (+13.2)	4.58	5.40	6.37 (+17.9)	5.88
	T3 (20 ppm SA)	5.78	7.77 (+34.4)	6.77	6.95	10.33 (+48.7)	8.64	8.03	11.93 (+48.5)	9.98
	T4 (40 ppm SA)	4.24	5.43 (+28.0)	4.84	4.91	6.37 (+29.7)	5.64	6.30	9.03 (+43.3)	7.67
Mean	4.03	5.03		4.98	6.45		6.06	8.08		
HUZM 80-1	T1 (Dry)	1.98	2.13 (+7.6)	2.06	2.60	2.83 (+9.0)	2.72	3.71	4.07 (+9.6)	3.89
	T2 (Hydro)	2.57	2.90 (+13.0)	2.73	3.14	3.50 (+11.3)	3.32	4.05	4.73 (+16.9)	4.39
	T3 (20 ppm SA)	4.09	5.23 (+28.1)	4.66	4.97	6.50 (+30.9)	5.73	5.87	7.90 (+34.7)	6.88
	T4 (40 ppm SA)	2.83	3.47 (+22.4)	3.15	3.78	4.73 (+25.2)	4.25	4.55	5.87 (+28.9)	5.21
Mean		2.87	3.43		3.62	4.39		4.54	5.64	
Grand Mean		3.45	4.23		4.30	5.42		5.30	6.86	
Particulars		SEm±	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%			
Sowing		0.03	0.21	0.04	0.25	0.16	0.98			
Genotype		0.10	0.28	0.11	0.33	0.11	0.32			
Sowing × Genotype		0.14	NS	0.16	0.46	0.16	0.46			
Treatment		0.14	0.39	0.16	0.46	0.16	0.46			
Sowing × Treatment		0.19	0.55	0.23	0.66	0.22	0.64			
Genotype × Treatment		0.19	0.55	0.23	0.66	0.22	0.64			
Sowing × Genotype × Treatment		0.27	NS	0.32	NS	0.31	NS			

Where, SA-Salicylic Acid, DAS-Days After Sowing. Values in the parenthesis indicate % increase or decrease under delay sowing over normal sowing.

of MDA and electrolyte leakage in tomato leaf tissues under application of SA is consistent with that reported by Stevens *et al*. (2006) and Agamy *et al*. (2013) mentioned that SA application regulates and maintains the membrane functions of tomato plants. In addition, SA can diminish the injuries in cell membranes through enhancing the antioxidant potential of plant under stress conditions and partly maintained membrane permeability as well as reduced the amount of ion leakage (Tasgin *et al*., 2006; Orabi *et al*., 2010; 2013). Kabiri *et al*. (2014) mentioned that pre treatment with SA was evidenced by a reduction in the level of lipid peroxidation and leakage of electrolytes from plant tissues as well as by more intensive growth processes as compared to control plants.

Genotype HUZM-185 was characterized by higher activity of SOD (Table 3 a & b), CAT (Table 4 a & b) and POD (Table 5 a & b) than HUZM-80-1 in fresh leaf (Table 3, 4 and 5 respectively). Salicylic acid treatments caused marked significant increases in the activities of SOD, CAT and POD relative to control plants (i.e. non-primed and hydro primed seed) in fresh leaf of both maize genotypes grown under low temperature in both normal and delay sowing condition during both the year. Moreover, Salicylic acid treatment at 20ppm showed the highest significant effect in both genotypes under both normal and delay sowing conditions during both the year.

Exogenous SA could regulate the synthesis and activities of antioxidant enzymes and increase plant tolerance to biotic and abiotic stress (He *et al*., 2002; Fayez and Bazaid, 2014). SA was found to enhance the activities of antioxidant enzymes such as POD when sprayed exogenously to the drought stressed plants (Hayat *et al*., 2010). In Brassica juncea, Yusuf *et al.* (2012) reported that SA enhanced the level of antioxidant system (SOD and POD) under stress and stress-free conditions. Ahmad *et al*. (2012) stated that at suboptimal condition of low temperature, priming maize seeds with SA and H_2O_2 induced activities of scavenging enzymes where, 20 mL L^{-1} H_2O_2 and 20 mg L^{-1} salicylate seems to be suitable concentration for increasing the chilling tolerance.

CONCLUSIONS

Based on two years results, it is inferred that SA treatment (20ppm) have positive significant effect on biochemical (MDA and H_2O_2) and antioxidant enzymes (SOD, POD and PPO) activity of the two maize genotypes grown under low temperature conditions in both normal and delay sowing conditions during both the years. It is worthy to mention that, Salicylic acid treatment at 20ppm was the most pronounced treatment among other treatments.

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