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Ameliorating effect of seed priming by salicylic acid on biochemical traits in *Rabi* maize (*Zea Mays* L.) genotypes under normal and delayed sowing

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Abstract
A field experiment was carried out during the winter (*Rabi*) season (2013-14) at Agricultural Research Farm, Banaras Hindu University, Varanasi to study the ameliorating effect of seed priming by salicylic acid on biochemical traits in maize genotypes i.e., HUZM-185 and HUZM-80-1 under normal and delayed sowing. The experiment was laid out in split plot design comprising eight treatment combinations in three replications. Seeds were primed with salicylic acid (SA) @ 20 µg mL^{-1} and 40 µg mL^{-1} along with hydro priming (distilled water) for overnight and non-primed seeds as control before both sowings i.e., normal and delayed sowing. Observations were recorded at 20, 40 and 60 days after sowing for total chlorophyll, total soluble sugar, protein and proline contents in leaves. It was found that for the studied biochemical traits, genotype HUZM-185 primed with 20 µg mL^{-1} salicylic acid significantly increased in delayed sowing as compared to normal sowing. This finding suggests that maize genotypes were found to differ in their ability to respond to delayed sowing under influence of seed priming with salicylic acid.

Keywords: delayed sowing, maize, priming, salicylic acid

Introduction
Maize (*Zea mays* L.), an important monocotyledonous cereal crop belongs to the tribe Tripsaceae (Maydeae) of family Poaceae and often regarded as “queen of cereal”. In India, maize is the 3rd most important food crops after rice and wheat. Its importance lies in the fact that it is not only used for human food and animal feed but at the same time it is also widely used for corn starch industry, corn oil production, baby corn etc. *Rabi* maize is grown on an area of 1.49 Mha with a production of 6.40 MT and its productivity is 4,288 kg ha^{-1} (ISOPOM, 2013-14) [8]. Sowing of *rabi* maize crop at right 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 vegetative growth is very sensitive to low temperature. Seed priming has been shown to improve seed performance under sub-optimal temperature conditions (Lin and Sung, 2001) [9].
Salicylic acid (SA) is a phytohormone of phenolic nature. It is ubiquitous in plants generating a considerable impact on plant growth, development, mineral uptake and transport, photosynthesis, and transpiration. Salicylic acid and other salicylates are known to affect various physiological and biochemical activities of plants and may play a key role in regulating their growth and productivity (Arberg, 1981) [2]. Salicylic acid has been found to play a key role in the regulation of plant growth, development, interaction with other organisms and in the responses to environmental stresses (Raskin, 1992a, b; Yalpani *et al*., 1994; Senaratna *et al*., 2000) [14, 15, 21, 18]. 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 (Bedi and Dhingra, 2007) [17]. Keeping this in view, the present investigation was carried out to study the mitigating effect of salicylic acid on some of the biochemical traits in maize genotypes i.e., HUZM-185 and HUZM-80-1 under normal and delayed sowing.

Materials and Methods
A field experiment was carried out during the winter (*Rabi*) seasons of 2013-14 at Agricultural Research Farm, Banaras Hindu University, and Varanasi in the north-eastern plain zone. The experiment was laid out in split plot design comprising eight treatment combinations [V1T1-Control; (HUZM-185 + non-primed seed), V1T2- hydro primed (HUZM-185 + distilled water), V1T3-(HUZM-185 + 20 µg mL^{-1} SA), V1T4- (HUZM-185 + 40 µg mL^{-1} SA), V2T1-

Control; (HUZM-80-1 + non-primed seed), V2T2- hydro primed (HUZM-80-1 + distilled water), V2T3-(HUZM-80-1 + 20 µg mL^{-1} SA), V2T4- (HUZM-80-1 + 40 µg mL^{-1} SA)] and replicated thrice. The observations were recorded in uppermost fully expanded leaf at 20, 40 and 60 days after sowing for total chlorophyll content, total soluble sugar content, protein content and proline content under both normal sowing (1^{st} fortnight of november) and delayed sowing (1^{st} fortnight of december).

Data obtained from various observations were analyzed as per the standard analysis of variance (ANOVA) procedure for split plot design given by Gomez and Gomez (1984) [7].

Biochemical traits analysis

Total chlorophyll content was determined according to method of Arnon (1949) [3] and expressed as (mg g^{-1} fresh weight). The amount of total chlorophyll was calculated as under;

Total Chlorophyll (mg g $^{-1}$ fresh weight)

$$= [20.2(A.645) =8.02(A.663)] \times V/1000xW$$

Where,

A = Absorbance of chlorophyll extract at the specified wavelength

V = Final volume of the 80% acetone chlorophyll extract

W = Fresh weight in grams of the tissue extracted

Total soluble sugar was determined by the method of Yemm and Willis (1954) [25] and expressed as mg g^{-1} fresh weight.

Total protein content was determined by the protocol of Lowry *et al*. (1951) [10] and expressed as mg g^{-1} fresh weight.

Total proline content was determined by the method of Bates *et al*. (1973) [4] and expressed as mg g^{-1} fresh weight.

Results and Discussion

During winter season, maize crop is grown in the month of December in north India plains and is exposed to cold temperature at vegetative stage in the month of January. Salicylic acid (SA) plays a regulatory role in plant metabolism and physiology and also plays a great role in plant response to abiotic stress factors of various natures, i.e., temperature, salinity, drought, and heavy metal etc. (Pal *et al*., 2013) [12]. Salicylic acid can improve tolerance to these abiotic stresses by seed priming (Farooq *et al*., 2009; Carvalho *et al*., 2011; Sayyari *et al*., 2013; Farzin Pouramir-Dashtmian *et al*., 2014) [6, 5].

Under delayed sown condition, due to effect of low temperature, total chlorophyll content [Table 1] of leaves declines due to degradation of chlorophyll molecules. Present investigation showed significant increment among treatments and genotype HUZM-185 primed with salicylic acid @ 20 µg mL^{-1} were performed better at 20, 40 and 60 DAS under delayed sown as compared to normal sown condition; such results are previously reported by Ahmad *et al*. (2015) that priming strategies improved leaf Chl a and b contents. Zhou *et al*. (1999) [23] reported that photosynthetic pigments were increased in corn with SA application. Moreover, Khan *et al.* (2003) [16] showed that SA increased photosynthetic rate in corn and soybean.

Total soluble sugar play a complex essential role in plant metabolism as substrates in biosynthetic processes and energy production as well as in a sugar sensing and signaling systems. Soluble sugars may function as a typical osmoprotectant, stabilizing cellular membranes and maintaining turgor pressure (Khan *et al*., 2012) [16]. In the present research study, there occurred accumulation of total soluble sugar content [Table 2] under low temperature stress in both genotypes of maize. Salicylic acid primed seeds by 20 µg mL^{-1} increased the soluble sugar accumulation in HUZM-185 under delayed sown as compared to normal sown condition. It is likely that SA seed soaking triggered the low temperature tolerance mechanism in HUZM-185 through enhanced sugar accumulation in delayed sown as compare to normal sown conditions. Dashtmian *et al*. (2014) reported that soluble sugar content of rice seedling leaves considerably increased as temperature decreased and seed priming with SA enhanced the responses.

In present investigation, salicylic acid seed treatment caused highest accumulation of leaf protein content [Table 3] in HUZM-185 with 20 µg mL^{-1} SA under delayed sown as compare to normal sown condition. The higher soluble proteins accumulation by HUZM-185 under low temperature stress indicates the better adaptability of this inbred line under low temperature stress. The SA was highly effective to increase the soluble protein content of maize leaves. Dashtmian *et al*. (2014) reported that chilling stress (8°C) reduced the protein content (35%) in untreated seeds while the reduction of 6% in protein content was observed when the rice seeds were primed with SA 50 mg L^{-1}. Therefore, seed priming with SA solution was able to mitigate (29%) the negative effect of chilling stress on protein content of seedling leaves.

The present study indicated that proline [Table 4] synthesis and accumulation could be induced under low temperature and seed priming with SA solution could intensify it under delayed as compare to normal sowing conditions. Genotype HUZM-185 primed with 20 µg mL^{-1} SA performed better among other treatments under delayed sowing as compared to normal sowing condition. Nowak *et al*. (2010) [11] mentioned that accumulation of proline in plant cells is a protective mechanism in which plants use to protect themselves against abiotic stresses like low temperature. They also reported that proline acts as a signal/regulatory compound affecting several physiological and biochemical processes in plants under normal and stress conditions. Aghaee *et al.* (2011) [1] demonstrated that with a decrease in temperature, proline concentration increased. Similarly, Yadegari *et al.* (2007) [20] revealed that proline concentration in leaves of soybean seedling increased as temperature decreased. Moreover, Salicylic acid treatment at 20 µg mL^{-1} showed highest significant effect in both genotypes under delayed sown as compared to normal sown conditions.

It was concluded that seed priming by 20 µg mL^{-1} of salicylic acid have positive significant effect on biochemical traits viz., total chlorophyll content, total soluble sugar content, protein content and proline content in both genotypes HUZM-185 and HUZM-80-1 at all stages studied under normal and delayed sown conditions. Nevertheless, this aspect also requires further in depth investigation.

Table 1: Effect of salicylic acid (SA) on total chlorophyll content (mg g^{-1} fresh weight) in *rabi* maize (*Zea mays* L.) leaves under normal and delayed sown conditions during 2013-2014

Genotypes	Treatments	Days after sowing								
		20			40			60		
		Normal	Delayed	Mean	Normal	Delayed	Mean	Normal	Delayed	Mean
HUZM-185	T1 (Non-primed)	1.8	1.7(-5.37)	1.7	1.9	1.9(-1.76)	1.9	2.2	2.2(-1.26)	2.2
	T2 (Hydro)	2.0	2.0(-1.34)	1.8	2.1	2.1(-1.10)	2.0	2.4	2.3(-0.80)	2.3
	T3 (20 µg mL^{-1} SA)	2.4	2.5(+3.64)	2.1	2.5	2.6(+4.28)	2.3	3.1	3.2(+4.50)	2.8
	T4 (40 µg mL^{-1} SA)	2.0	2.0(+2.87)	1.8	2.2	2.3(+3.10)	2.1	2.5	2.6(+3.34)	2.4
Mean		2.04	2.04		2.18	2.21		2.53	2.58	
HUZM 80-1	T1 (Non-primed)	1.7	1.4(-16.83)	1.6	1.8	1.5(-14.43)	1.7	2.1	1.9(-6.36)	2.1
	T2 (Hydro)	1.7	1.6(-3.91)	1.8	1.9	1.9(-2.04)	2.0	2.3	2.2(-1.76)	2.3
	T3 (20 µg mL^{-1} SA)	1.9	1.9(+3.39)	2.2	2.2	2.3(+3.67)	2.4	2.6	2.7(+4.03)	2.9
	T4 (40 µg mL^{-1} SA)	1.7	1.7(+1.75)	1.9	2.0	2.1(+2.90)	2.2	2.4	2.4(+3.32)	2.5
Mean		1.74	1.68		1.98	1.94		2.32	2.32	
Grand Mean		1.89	1.86		2.08	2.07		2.43	2.45	
Particulars		SEm±	CD at 5%		SEm±	CD at 5%		SEm±	CD at 5%	
Sowing		0.01	0.03		0.003	0.01		0.002	0.01	
Genotype		0.01	0.03		0.003	0.01		0.002	0.01	
Sowing × Genotype		0.06	0.21		0.05	0.19		0.05	0.16	
Treatment		0.01	0.03		0.01	0.02		0.003	0.01	
Sowing × Treatment		0.01	0.04		0.01	0.03		0.01	0.01	
Genotype × Treatment		0.01	0.04		0.01	0.03		0.01	0.01	
Sowing × Genotype × Treatment		0.02	0.06		0.01	0.04		0.01	0.02	

Values in parentheses indicate per cent increase or decrease under delayed sowing over normal sowing

Table 2: Effect of salicylic acid (SA) on total soluble sugar content (mg g^{-1} fresh weight) in *rabi* maize (*Zea mays* L.) leaves under normal and delayed sown conditions during 2013-2014

Genotypes	Treatments	Days after sowing								
		20			40			60		
		Normal	Delayed	Mean	Normal	Delayed	Mean	Normal	Delayed	Mean
HUZM-185	T1 (Non-primed)	26.0	24.8(-4.69)	23.5	31.0	30.3(-2.15)	30.0	39.3	38.7(-1.59)	37.1
	T2 (Hydro)	28.7	28.0(-2.33)	27.2	35.7	35.0(-1.87)	33.7	45.2	45.0(+0.54)	42.0
	T3 (20 µg mL^{-1} SA)	34.8	36.3(+4.88)	31.1	45.9	48.0(+4.60)	40.9	59.1	63.1(+6.78)	57.6
	T4 (40 µg mL^{-1} SA)	31.6	32.2(+2.10)	28.8	40.0	41.0(+2.50)	36.5	52.0	53.4(+2.76)	48.5
Mean		30.2	30.3		38.1	38.6		48.9	50.1	
HUZM 80-1	T1 (Non-primed)	21.0	19.0(-9.52)	21.9	29.0	26.7(-8.05)	28.5	35.0	33.0(-5.71)	35.8
	T2 (Hydro)	25.7	24.7(-3.93)	26.4	31.7	30.7(-3.16)	32.8	38.7	38.0(-1.72)	41.5
	T3 (20 µg mL^{-1} SA)	27.3	28.3(+3.46)	32.3	36.0	37.3(+3.70)	42.7	56.0	59.0(+5.36)	61.1
	T4 (40 µg mL^{-1} SA)	26.0	26.3(+1.28)	29.3	33.0	33.7(+2.02)	37.3	45.0	46.2(+2.72)	49.8
Mean		25.2	24.6		32.4	32.1		43.7	44.1	
Grand Mean		27.6	27.5		35.3	35.3		46.3	47.1	
Particulars		SEm±	CD at 5%		SEm±	CD at 5%		SEm±	CD at 5%	
Sowing		0.2	NS		0.1	NS		0.4	NS	
Genotype		0.2	0.6		0.1	0.5		0.4	1.3	
Sowing × Genotype		0.6	NS		0.7	NS		1.1	NS	
Treatment		0.2	0.6		0.4	1.1		0.4	1.2	
Sowing × Treatment		0.3	0.8		0.5	1.5		0.6	1.7	
Genotype × Treatment		0.3	0.8		0.5	1.5		0.6	1.7	
Sowing × Genotype × Treatment		0.4	NS		0.7	NS		0.8	NS	

Values in parentheses indicate per cent increase or decrease under delayed sowing over normal sowing

Table 3: Effect of salicylic acid (SA) on protein content (mg g^{-1} fresh weight) in *rabi* maize (*Zea mays* L.) leaves under normal and delayed sown conditions during 2013-2014

Genotypes	Treatments	Days after sowing								
		20			40			60		
		Normal	Delayed	Mean	Normal	Delayed	Mean	Normal	Delayed	Mean
HUZM-185	T1 (Non-primed)	9.1	8.3(-8.76)	7.8	11.4	10.7(-6.43)	10.1	13.4	12.7(-5.48)	12.1
	T2 (Hydro)	10.8	10.2(-5.43)	9.2	12.9	12.3(-4.64)	11.3	16.2	16.0(-1.35)	13.9
	T3 (20 µg mL^{-1} SA)	15.0	16.3(+9.20)	12.3	18.7	20.5(+9.82)	15.7	26.5	29.3(+10.7)	20.7
	T4 (40 µg mL^{-1} SA)	11.5	12.3(+6.94)	10.4	14.0	15.1(+7.62)	12.4	19.7	21.3(+8.12)	16.0
Mean		11.6	11.8		14.3	14.6		19.0	19.8	
HUZM 80-1	T1 (Non-primed)	6.5	5.7(-12.80)	7.0	8.7	7.9(-9.20)	9.3	10.7	10.0(-6.54)	11.3
	T2 (Hydro)	7.6	7.0(-8.41)	8.6	9.7	9.0(-7.25)	10.7	11.6	11.3(-2.30)	13.7
	T3 (20 µg mL^{-1} SA)	9.7	10.3(+6.51)	13.3	12.8	13.7(+7.29)	17.1	14.8	16.0(+8.11)	22.7
	T4 (40 µg mL^{-1} SA)	9.3	9.7(+4.32)	11.0	10.8	11.2(+4.33)	13.2	12.3	13.0(+5.69)	17.2

Mean	8.3	8.2		10.5	10.5		12.4	12.6	
Grand Mean	9.9	10.0		12.4	12.6		15.7	16.2	
Particulars	SEm±	CD at 5%		SEm±	CD at 5%		SEm±	CD at 5%	
Sowing	0.2	NS		0.2	NS		0.3	NS	
Genotype	0.2	0.6		0.2	0.7		0.3	1.2	
Sowing × Genotype	0.4	NS		0.5	NS		0.6	NS	
Treatment	0.2	0.7		0.2	0.5		0.2	0.7	
Sowing × Treatment	0.4	1.0		0.3	0.7		0.3	1.0	
Genotype × Treatment	0.4	1.0		0.3	0.7		0.3	1.0	
Sowing × Genotype × Treatment	0.5	NS		0.4	NS		0.5	NS	

Values in parentheses indicate per cent increase or decrease under delayed sowing over normal sowing

Table 4: Effect of salicylic acid (SA) on proline content (μg g^{-1} fresh weight) in *rabi* maize (*Zea mays* L.) leaves under normal and delayed sown conditions during 2013-2014

Genotypes	Treatments	Days after sowing								
		20			40			60		
		Normal	Delayed	Mean	Normal	Delayed	Mean	Normal	Delayed	Mean
HUZM-185	T1 (Non-primed)	55.60	54.67(-1.68)	50.82	68.47	67.67(-1.17)	63.48	87.83	87.00(-0.95)	83.18
	T2 (Hydro)	63.09	62.33(-1.20)	58.74	75.67	75.33(-0.44)	71.55	99.56	99.33(-0.22)	93.63
	T3 (20 μg mL^{-1} SA)	93.96	119.20(+26.86)	84.85	101.62	123.67(+21.70)	95.06	127.69	151.67(+18.77)	117.51
	T4 (40 μg mL^{-1} SA)	78.10	93.15(+19.28)	68.68	92.18	107.33(+16.44)	82.46	112.07	128.33(+14.51)	102.87
Mean		72.69	82.34		84.48	93.50		106.79	116.58	
HUZM 80-1	T1 (Non-primed)	46.03	44.33(-3.70)	49.50	58.50	57.33(-1.99)	62.50	78.53	77.67(-1.10)	82.33
	T2 (Hydro)	54.40	53.33(-1.96)	57.83	67.43	66.67(-1.14)	71.00	87.70	87.00(-0.80)	93.17
	T3 (20 μg mL^{-1} SA)	75.73	91.69(+21.07)	105.44	88.50	102.75(+16.10)	113.21	107.33	124.36(+15.86)	138.01
	T4 (40 μg mL^{-1} SA)	59.26	68.71(+15.95)	80.93	72.74	82.45(+13.34)	94.89	93.67	106.11(+13.28)	117.22
Mean		58.86	64.52		71.79	77.30		91.81	98.78	
Grand Mean		65.77	73.43		78.14	85.40		99.30	107.68	
Particulars		SEm±	CD at 5%		SEm±	CD at 5%		SEm±	CD at 5%	
Sowing		0.56	1.94		0.83	2.86		0.92	3.19	
Genotype		0.56	1.94		0.83	2.86		0.92	3.19	
Sowing × Genotype		5.34	18.49		4.78	NS		5.37	NS	
Treatment		0.60	1.76		0.97	2.83		0.97	2.84	
Sowing × Treatment		0.85	2.48		1.37	4.00		1.37	4.01	
Genotype × Treatment		0.85	2.48		1.37	4.00		1.37	4.01	
Sowing × Genotype × Treatment		1.20	3.51		1.94	NS		1.94	NS	

Values in parentheses indicate per cent increase or decrease under delayed sowing over normal sowing

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