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Effect of Paclobutrazol (PBZ) and Salicylic Acid (SA) on Leaf Area Relative Water Content and Yield of Maize (*Zea Mays* L.) under Water Stress

Key words: *Leaf area, Paclobutrazol (PBZ), Relative water content, Salicylic acid (SA) and Yield.*

Maize is grown both for human consumption and for other uses such as animal feed and biofuels. Since the crop is rich in vitamin C and other vitamins and minerals, as well as carbohydrates and dietary fibre. Currently, protection of plants from abiotic stresses through application of Plant Growth Regulators (PGR) attracts more attention. Paclobutrazol (PBZ), a triazole compound induces a variety of responses including decreased inter-nodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissues, increased root to shoot ratio, elevated levels of epicuticular wax formation, increased cytokinin synthesis and a transient rise in ABA, as well as conferring protection from various environmental stresses including water stress (Jaleel *et al.,* 2007).

Salicylic acid (SA) besides its role in biotic stresses induces tolerance to several abiotic stress conditions by regulating the stomatal opening and water relations, increasing photosynthetic pigments, rubisco activity and in turn photosynthesis and growth. It is known as an important signal molecule for modulating plant responses to environmental stresses (Shakirova *et al.,* 2003). Keeping this in view the present investigation was carried out with the objective to study the effect of Paclobutrazol and Salicyclic acid applications on leaf area, Relative water content and Yield of maize under water stress.

The physiological parameters of leaf area and Relative water content (RWC) and Yield are influenced by the Paclobutrazol (PBZ) and Salicylic acid (SA) were studied in the year 2018, to identify the effect of Paclobutrazol and Salicyclic acid on amelioration of Water Stress effects in Maize (*Zea mays* L.) pioneer var. 3396 in split plot design with 3 main plots and 7 sub plots were comprised which are M_1: Control (No water stress); M_2: Water stress at knee high stage (V_6 stage); M_3: Water stress at cob development stage (V_T-R_1 stage) and S_1: control; S_2: 50 ppm PBZ spray at V_6 stage; S_3: 0.5 mM SA spray at V_6 stage; S_4: 50 ppm PBZ spray at V_T-R_1 stage; S_5: 0.5 mM SA spray at V_T-R_1 stage; S_6: 50 ppm PBZ spray at V_6 and V_T-R_1 stage; S_7: 0.5 mM SA spray at V_6 and V_T-R_1 stage. Foliar sprays of PBZ and SA at V_6 and V_T -R_1 stages were given at 38 DAE and to 70 DAE respectively. The methodology was described for leaf area as, remove the leaves from the five adjacent plants sampled from each treatment in three replications were separated and leaf area was measured by using Leaf Area Meter (Model No.LP-80) and the average was expressed as leaf area plant^{-1} in cm^2. Relative water content of leaf samples collected for different treatments was determined by following the method described by slatyer and mcilory (1967). Fully expanded and matured leaves were excised (mostly third or fourth leaf from the top) and the fresh weight was recorded (w_f g). The leaves were then immersed in water for attaining maximum turgidity and then gently blotted before taking turgid weight (w_t g). Afterwards leaves were oven dried at 75^{0}C for two days and weights of these leaves were recorded (w_d g).

a. Cob yield

The cobs harvested from five tagged plants were dried, weighed and expressed as cob yield plant^{-1} in grams.

b. Kernel yield

Harvested cobs were kept for drying for 15 days. After drying the cobs were subjected to shelling plot wise and the resulted kernel yield was calculated for net plot area and it was computed to hectare and expressed as kg ha^{-1}.

Leaf area

The effects of PBZ and SA sprays on leaf area of maize under water stress the effect of main plot and sub plot treatment were found to be significant. At 38DAE, the plants subjected to water stress at V_6 (knee high) stage (M_2) recorded significantly less leaf area plant^{-1}(1593) than unstressed plants M_1 and M_3 (1868 and 1838 cm^2 plant^{-1} respectively). The leaf area recorded in sub plot treatments did not differ. The interaction effect was found significant. At 48DAE, leaf area of maize crop varied from 3585 to 4163 cm^2 plant^{-1}. The plants

subjected to water stress (at knee high) stage (M_2) recorded high leaf area (3976) compared to M_1 and M_3. Among the sub plot treatments S_3, S_7 showed significantly higher leaf area i.e., (3867 and 3863 cm^2 $plant^{-1}$ respectively) and remaining treatments were on par with control. The interaction effect was found non significant. At 70 DAE, leaf area of maize varied from 5002 to 6310 cm^2 $plant^{-1}$. The plants subjected to water stress at (V_T-R_1) cob development stage (M_3) recorded significantly lesser leaf area (5088 cm^2 $plant^{-1}$) compared to M_1 and M_2 (5601 and 5872 cm^2 $plant^{-1}$ respectively). Among the sub plot treatments SA spray (S_3, S_7) resulted in significantly higher leaf area i.e.,(5706 and 5701 cm^2 $plant^{-1}$ respectively) and remaining treatments were on par with control. The interaction between the main plots and sub plots was found non significant. At 80DAE, leaf area recorded stood in the range of 5982 to 6834 cm^2 $plant^{-1}$.Among main treatments, plants subjected to water stress at cob development stage (M_3) had significantly lesser leaf area (6305.) than plants in M_1 and M_2 (6388 and 6525 cm^2 $plant^{-1}$ respectively). Among the sub plot treatments SA spray (S_5, S_7) showed significantly higher leaf area (6692 and 6668 cm^2 $plant^{-1}$ respectively) than control and remaining treatments were on par. The interaction effect was found non significant. Water stress imposed at V_6 stage resulted in 17.2% decline in leaf area compared to unstressed plants. At 48DAE, leaf area of water stressed plants M_2 was 1.1 folds higher over control, which might be due to PBZ and SA spray. Plants treated with SA sprays S_3 and S_7 produced 1.1 times more leaf area over control. Water stress imposed at V_T-R_1 stage resulted in 10.7% decline in leaf area compared to unstressed plants. SA spray (S_3, S_7) enhanced the leaf area to an extent of 1.1 times more over control. At 80 DAE, 1.2 folds increase in leaf area of water stressed plants M_3 could be due to PBZ and SA spray. Plants treated with SA (S_5, S_7) produced 1.1 times more leaf area over control. These findings indicated that the impact of SA spray on leaf area under water stress was marginal. Bideshki and Arvin (2010) Zammaninejad in maize (2013), Ghai *et al.* (2014) in mash bean and Khan et al. (2003) in soyabean reported increase in leaf area in SA treated plants under water stress.

Relative Water Content (%)

The influence of PBZ and SA sprays on relative water content of maize leaves under water stress in main treatments and sub treatments were found to be significant. At 38DAE, the plants subjected to water stress at V_6 (knee high) stage (M_2) had less relative water content (71.8%) compared to unstressed plants M_1 and M_3 (78.7 and 77.5% respectively). No difference was observed among sub plot treatments. The interaction effect was found significant for RWC values. At 48DAE, the plants in M_2 recorded less RWC value (75.0%) compared to unstressed plants M_1 and M_3 (78.9 and 77.9% respectively). The sprays of PBZ and SA resulted in improvement of leaf water status over control, but only S_3 and S_7 showed significantly higher RWC (79.3, and 79.7% respectively) than control and remaining treatments were on par with control. The interaction effect was found non significant.

At 70DAE, (Table 2) plants subjected to water stress at (V_T-R_1) cob development stage (M_3) possessed less RWC value (71.9) compared to M_1 and M_2 (79.1 and 80.4% respectively). Among the PBZ and SA spray treatments S_3 and S_7 i.e., SA spray at V_6 stage showed significantly higher RWC (79.2 and 79.8%, respectively) than control and remaining treatments were on par. The interaction between the main plots and sub plots was found significant, high RWC value was observed in M_2S_1 (83.7) and low value (69.7) in M_3S_1. At 80DAE, among main treatments, plants subjected to water stress at cob development stage recorded lesser RWC value (75.5) than plants in M_1 and M_2 (80.1 and 81.2% respectively). Sprays of PBZ and SA sprays resulted in improvement of leaf water status, but only S_5 and S_7 showed significantly higher values (81.0 and 82.9% respectively) and remaining treatments were on par with each other and control. The interaction effect was found non significant. Water stress imposed at V_6 stage resulted in 9.6 per cent decline in RWC value. At 48 DAE, the rise (4.5%) in RWC value indicates the stress amelioration effect caused by PB and SA sprays. Plants sprayed with SA at V_6 stage enhanced the RWC value by 5.5 to 6.1 per cent.

Water stress imposed at cob development stage resulted in more (10.10%) decline in RWC value, which could be due to advancement in age of the crop. At 80DAE, the rise (5.1%) in RWC value is an evidence for stress amelioration effect. Plants sprayed with SA at V_6 stage enhanced the RWC value by 5.9 to 6.7%. Spray of SA (S_5, S_7) enhanced the RWC by 6.2 to 8.6%. The maintenance of high RWC under stress conditions is a desirable trait, that depends on high root shoot ratio, absisic acid induced reduction in stomatal opening, chlorophyll content and photosynthesis. The increase in RWC with application of 0.5mM SA might be due to regulation of stomatal openings and reduction of water loss under water stress conditions which enable the plants to maintain turgor. He *et al.* (2005) and Sakhabutdinova (2003) postulated that SA increases the production of photosynthetic apperatus which inturn produce more photo synthates. This enhanced photosynthetic activity increases sap

Table 1. Effect of PBZ and SA on leaf area under water stress effect in maize at 38 DAE,48 DAE,70 DAE and 80 DAE

TREATMENTS	LEAF AREA (cm^2 $plant^{-1}$)															
SUB PLOTS / MAIN PLOT	38DAE				48DAE				70DAE				80DAE			
	M_1	M_2	M_3	MEAN	M_1	M_2	M_3	MEAN	M_1	M_2	M_3	MEAN	M_1	M_2	M_3	MEAN
S_1– control (no spray)	1881	1611	1752	1748	3611	3803	3589	3668	5605	5592	5002	5400	6185	6246	6032	6155
S_2 – 50 ppm PBZ spray at V_6 stage	1815	1633	1815	1754	3620	4033	3516	3723	5697	5853	5182	5578	6264	6430	6024	6239
S_3 – 0.5 mM SA spray at V_6 stage	1891	1544	1891	1775	3732	4159	3710	3867	5717	6310	5092	5706	6220	6409	5982	6204
S_4 – 50 ppm PBZ spray at V_T-R_1 stage	1842	1550	1797	1730	3778	3790	3821	3797	5420	5549	5036	5335	6320	6510	6503	6444
S_5 – 0.5 mM SA spray at V_T-R_1 stage	1912	1619	1875	1802	3585	3841	3848	3758	5405	5649	5111	5388	6708	6834	6534	6692
S_6 –50 ppm PBZ spray at V_6 and V_T-R_1 stage	1876	1653	1876	1802	3609	4045	3676	3777	5600	5884	5116	5533	6297	6517	6508	6441
S_7 – 0.5 mM SA spray at V_6 and V_T-R_1 stage	1861	1539	1861	1754	3702	4163	3724	3863	5760	6265	5076	5701	6720	6731	6554	6668
Mean	1868	1593	1838		3662	3976	3698		5601	5872	5088		6388	6525	6305	
	SEm ±	CD (p=0.05)	CV (%)		SEm ±	CD (p=0.05)	CV (%)		SEm ±	CD (p=0.05)	CV (%)		SEm ±	CD (p=0.05)	CV (%)	
MAIN TREATMENTS	39.9	156.9	10.3		70.3	210.7	11.7		120.2	396.3	12.6		44.2	175.3	8.4	
SUB TREATMENTS	45.2	139.6	7.6		40.5	135.3	9.4		89.3	280.6	8.2		135.6	422.3	10.6	
INTERACTION	4.3	14.2			70.5	NS			58.6	NS			80.5	NS		

Table 2. Effect of PBZ and SA on RWC under water stress effect in maize at 38DAE,48 DAE,70 DAE and 80 DAE

TREATMENTS		RELATIVE WATER CONTENT (%)															
SUB PLOTS		38DAE				48DAE				70DAE				80DAE			
	MAIN PLOT	M_1	M_2	M_3	MEAN	M_1	M_2	M_3	MEAN	M_1	M_2	M_3	MEAN	M_1	M_2	M_3	MEAN
S_1– control (no spray)		76	70	76	74	77	71	76	75	78	76	69	74	78	79	71	76
S_2 – 50 ppm PBZ spray at V_6 stage		78	72	78	76	78	76	77	77	77	80	70	76	80	83	72	78
S_3 – 0.5 mM SA spray at V_6 stage		80	73	79	77	80	78	79	79	81	83	72	79	80	85	71	72
S_4 – 50 ppm PBZ spray at V_T-R_1 stage		77	69	75	73	78	71	76	75	78	78	71	75	78	77	77	77
S_5 – 0.5 mM SA spray at V_T-R_1 stage		79	71	80	76	76	73	77	75	77	79	72	76	82	80	80	81
S_6 –50 ppm PBZ spray at V_6 and V_T-R_1 stage		78	72	74	75	79	75	78	77	78	81	72	77	77	82	77	78
S_7 – 0.5 mM SA spray at V_6 and V_T-R_1 stage		79	73	79	77	80	78	80	79	82	83	73	79	83	85	79	82
Mean		78	71	77		78	75	77		79	80	71		80	81	75	
		SEm ±	CD (p=0.05)	CV (%)		SEm ±	CD (p=0.05)	CV (%)		SEm ±	CD (p=0.05)	CV (%)		SEm ±	CD (p=0.05)	CV (%)	
MAIN TREATMENTS		1.3	5.3	8.2		0.8	3.2	8.7		1.1	5.6	8.5		1.4	4.5	8.4	
SUB TREATMENTS		1.8	5.1	7.6		1.4	4	9.3		1.4	4.3	7.7		1.2	3.8	7.2	
INTERACTION		2.3	7.3			1.2	NS			3	10			1.6	NS		

Table 3. Effect of PBZ and SA on cob yield and kernel yield under water stress in maize

TREATMENTS		Cob yield (kg ha^{-1})				Kernel yield (kg ha^{-1})			
SUB PLOTS	MAIN PLOT	M_1	M_2	M_3	MEAN	M_1	M_2	M_3	MEAN
S_1– control (no spray)		4019	4067	4000	4230	3200	3192	3241	3211
S_2 – 50 ppm PBZ spray at V_6 stage		4068	4437	4308	4271	3183	3348	3398	3310
S_3 – 0.5 mM SA spray at V_6 stage		4218	5329	5365	4971	3188	4107	4330	3875
S_4 – 50 ppm PBZ spray at V_T-R_1 stage		4083	4383	4361	4276	3143	3388	3435	3322
S_5 – 0.5 mM SA spray at V_T-R_1 stage		4541	5350	5365	5085	3206	4322	4336	3955
S_6 –50 ppm PBZ spray at V_6 and V_T-R_1 stage		4090	4463	4366	4307	3140	3387	3394	3307
S_7 – 0.5 mM SA spray at V_6 and V_T-R_1 stage		4335	5561	5530	5142	3166	4434	4404	4001
Mean		4194	4798	4843		3175	3740	3791	
		SEm ±	CD (p=0.05)	CV (%)		SEm ±	CD (p=0.05)	CV (%)	
MAIN TREATMENTS		68.6	269.5	11.5		62	243.3	8.6	
SUB TREATMENTS		88.4	253.3	13.2		69.2	201.3	9.2	
INTERACTION		8.1	23.4			8.3	25.8		

production in leaf lamella which ultimately results in maintenance of RWC in leaf and better growth in plants. Similar results with SA application were also reported by Kordi *et al*. (2013) in sweet Basil under drought and Agarwal *et al.* (2005) in wheat.

Yield

The data pertaining to effect of PBZ and SA sprays on cob yield (kg ha^{-1}) in maize under water stress were furnished in Table 3. Significant differences were noticed in main treatments and sub treatments.

Among the main plot treatments M_1 recorded the significantly low cob yield (4194.0 kg ha^{-1}) compared to plants subjected to water stress M_2 and M_3 (4798.9 and 4843.2 kg ha^{-1}). Among the sub plot treatments S_3, S_5 and S_7 showed significantly higher cob yield i.e., 4971.5, 5085.6 and 5142.3 kg ha^{-1}respectively and remaining were on par with control. The interaction effect was found significant, cob yield was low in M_1S_1 and high in M_2S_7. Under no water stress as well as water stress conditions, the spray of both PBZ and SA increased the cob yield, but the impact was more with SA spray.

Application of 0.5 mM SA (S_3, S_5 and S_7) to water stressed plants increased the cob yield by 17.5, 20.2 and 21.6 % respectively. It might be due to the improvement in plant water status, enhanced photosynthetic rate which contributes to increase the cob yield. These results were in agreement with Orabi *et al.* (2010) who reported highest yield in *cucumis sativus* with application of SA at 4mM concentration. Zamaningad *et al.* (2013) who also found that SA increased cob yield in maize.

Kernel yield

The data on influence of PBZ and SA sprays on kernel yield (kg ha^{-1}) in maize under water stress were presented in Table 3.

Among the main plot treatments, M_1 recorded significantly low kernel yield (3175.6 kg ha^{-1}) compared to the plants subjected to water stress M_2 and M_3 (3740.2 and 3791.7 kg ha^{-1}). Among the sub plot treatments S_3, S_5 and S_7 showed significantly higher kernel yield i.e., 3785.4, 3955.4 and 4001.8 kg ha^{-1}respectively and remaining were on par. The interaction between the main plots and sub plots was found significant, minimum kernel yield was observed in M_1S_6 and maximum in M_3S_7. Under no water stress condition SA spray resulted in increase of kernel yield, while PBZ resulted in decrease of kernel yield. Under water stress conditions both PBZ and SA sprays increased the kernel yield, but the impact was more with SA spray.

Application of 0.5 mM SA (S_3, S_5 and S_7) to water stressed plants increased the cob yield by 20.6, 23.2 and 24.6 % respectively. This might be due to the effect of physiological and biochemical process that were led to ameliorate in vegetative growth and active assimilation, translocation from sourse to sink (Dowood *et al.* (2012). Bekheta and Iman (2009) found that application of SA @ 15 mg L^{-1} significantly increased the yield in mungbean. Somayyeh and Ali (2012) reported in maize crop that exogenous SA application was advantageous under three irrigation levels (40, 70 and 100% of full irrigation), significantly increased the grain yield by 15.85, 20.81 and 29.74%.

The effect of Paclobutrazol (PBZ) and Salicylic acid on physiological parameters of leaf area and relative water content and yield maize under water stress in main plot and sub plot treatments were found to be significant. Among the treatments S_3: 0.5 mM SA spray at V_6 stage; S_5: 0.5 mM SA spray at V_T-R_1 stage and S_7: 0.5 mM SA spray at V_6 and V_T-R_1 stage treatments showed the best results on leaf area, relative water content and yield.

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