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Influence of moisture stress management practices on biochemical properties of maize (*Zea mays* L.)

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Abstract
Field investigations were conducted in the Northern block, Agricultural Research Station, Bhavanisagar, Erode during rabi 2017-18 & 2018-19 and summer 2018 & 2019 to assess the influence of moisture stress management practices in different irrigation regimes in maize. The experiment was laid out in split plot design with three replications comprised of four irrigation regimes as main factor based on IW/CPE ratio of 1.0 ($I_{1.0}$), 0.8($I_{0.8}$), 0.6 ($I_{0.6}$) and 0.4 ($I_{0.4}$) and four moisture stress management treatments *viz*., foliar application of pink pigmented facultative methylobacteria 1% (F_{PPFM}), Brassinolide 0.1 ppm (F_{Br}), Silicic acid 0.2% (F_{SI}) and control (F_C) as a sub factor. Foliar application was given on 25 and 45 DAS for each treatment in the sub plot. Maize cultivar CO(H)M 6 was used as a test variety spaced with 60 x 25 cm.

Results revealed that, IW/CPE 0.4 registered significantly higher proline content (7.82, 7.60, 10.63 and 12.06 µg g^{-1}), catalase activity (5.09, 5.03, 5.02 and 5.70 µmol g^{-1} min^{-1}) and peroxidase activity (45.8, 45.2, 45.2 and 51.3 µmol g^{-1} min^{-1}) at 60 DAS respectively during *rabi* 2017-18 & 2018-19 and summer 2018 & 2019 among the irrigation regimes. Remarkably lower proline content, catalase and peroxidase activity were found in IW/CPE 1.0. In moisture stress management practices, significantly higher proline content at 60 DAS (5.57, 5.42, 7.50 and 8.40 µg g^{-1} respectively during *rabi* 2017-18 & 2018-19 and summer 2018 & 2019) was recorded in brassinolide 0.1 ppm applied plants barring control and admirably lower proline content was recorded in foliar application of PPFM 1% on 25 and 45 DAS. IW/CPE 1.0 with no moisture stress management practices (F_C) recorded significantly lower proline content and was comparable with other moisture stress management treatments in IW/CPE 1.0. Distinctly higher catalase (4.39, 4.33, 4.28 and 4.77 µmol g^{-1} min^{-1} at 60 DAS respectively during *rabi* 2017-18 & 2018-19 and summer 2018 & 2019) and peroxidase activity (39.5, 39.0, 38.5 and 42.9 µmol g^{-1} min^{-1} at 60 DAS respectively during *rabi* 2017-18 & 2018-19 and summer 2018 & 2019) was noticed in PPFM 1% foliar applied treatment. In interaction effect, foliar application of PPFM 1% on 25 and 45 DAS at IW/CPE 0.4 recorded lucidly higher catalase and peroxidase activity at 60 DAS. Significantly lower catalase activity was recorded in IW/CPE 0.8 (Mild stress) with control and was comparable with other treatments at IW/CPE 1.0 (No stress).

Keywords: Catalase, peroxidase, proline, H_2O_2, silicic acid

Introduction

World's leading food crop, cultivated over an area of about 192.2 mha with a production of about 1134.91 mt with average productivity of 5755 kg ha^{-1}. In terms of area second largest crop after wheat, and in production it stands first (FAO 2019). In India Maize is cultivated an area of 9.38 mha with production of 28.75 mt with average productivity of 3065 kg ha^{-1}. Maize is grown all over the world under a wide range of climates.

Water deficit is the major abiotic factor limiting plant growth and crop productivity around the world which is responsible for severe yield reduction in maize by 40% on a global scale (Daryanto *et al*., 2015) [4]. Water used by the crop is governed by the evaporative demand of the atmosphere hence, the irrigation scheduling based on IW/CPE ratio was adapted in this study. Furthermore timely implication of moisture stress management practice through exogenous application of substances can ameliorate the negative effects of drought.

Materials and Methods

The experiment was conducted at Agricultural Research Station, Bhavanisagar (11º29` N,77º08` E, and 256 m above the mean sea-level), Tamil Nadu, India during 2017 and 2018. The soil at experimental site was sandy loam (21.2% course sand, 33.1% fine sand, 19.8% silt and 25.9% clay) medium in organic carbon (0.43%), low in available nitrogen (191kg ha^{-1}), medium in phosphorus (11.2kg ha^{-1}) and potassium (389.8kg ha^{-1}). During the crop growth (November – Feb) of 2017 and 2018, monthly mean maximum and minimum temperature ranged between 31.4 ºC and 21.5 ºC; 30.8 ºC and 22.7ºC respectively. The experimental site received an rainfall of 54.6 mm in 3consecutive rainy days during *rabi* 2017 and 31 mm in 1 rainy days during rabi 2018. The mean evaporation was5.1 mm and 4.4 mm in *rabi* 2017 and 2018 respectively.

In summer 2018, the total rainfall of 79.3 mm was received in seven rainy days and in summer 2019, 118.3 mm of rainfall was received in 5 rainy days. The average maximum and minimum temperature are 35.6, 24.3 ºC and 37.1, 23.7ºC, respectively for summer 2018 and summer 2019. The evaporation ranged from 4.4 to 7.2 mm and 3.5 to 7.2 mm respectively during summer 2018 and summer 2019.

The experiment was laid out in split plot design comprised of four irrigation regimes as main factor based IW/CPE ratio of 1.0 ($I_{1.0}$), 0.8($I_{0.8}$), 0.6 ($I_{0.6}$) and 0.4 ($I_{0.4}$) and four moisture stress management treatments *viz*., foliar application of pink pigmented facultative methylobacteria at 1% (F_{PPFM}), Brassinolide 0.1 ppm (F_{Br}), Silicic acid 0.2% (F_{Sl}) and control (F_{cont}) as a sub factor. Foliar application was given on 25 and 45 DAS for each treatments in the sub plot. Maize cultivar CO(H)M 6 was used as a test variety with 60 cm row spacing and 25 cm between the plants. Recommended dose of NPK for maize hybrid 250:75:75 kg $ha^{-1}$was adopted as per crop management practices. Irrigation was given at the time of sowing and the life irrigation on the fifth day and following subsequent irrigations were scheduled based on the irrigation regimes of the main plot as per the IW/CPE ratio and irrigated at a depth of 50 mm measured using parshall flume.

Proline content of leaves was measured following the method suggested by Bates *et al*. (1973) [3]. Catalase activity was assayed spectrophotometrically as described Goldblith and Proctor (1950) [6] and the Assay of peroxidase was estimated by using the producer given by Hammerschmidt *et al*. (1982) [7]

Result and Discussion

Chlorophyll content

IW/CPE 1.0 recorded significantly higher chlorophyll content of 53.8, 56.5, 54.6, and 54.8 at 60 DAS during *rabi* 2017-18 & 2018-19 and summer 2018 & 2019 respectively. Significantly lower chlorophyll content was recorded in IW/CPE 0.4. This might be due to induced moisture stress that reduced the chlorophyll content in leaves and led to suppression of crop productivity through lesser CO_2 assimilation as was reported by Afifah *et al.* (2015) [1].

Chlorophyll content was significantly higher (50.4, 53.0, 51.2 and 51.1 at 60 DAS during *rabi* 2017-18 & 2018-19 and summer 2018 & 2019) in foliar application of PPFM @ 1% on 25 and 45 DAS (F_{PPFM}) and was comparable with foliar application of silicic acid @ 0.2% on 25 and 45 DAS (F_{Sl}). Barring control treatment, significantly lower chlorophyll content was recorded in foliar application of brassinolide @ 0.1ppm on 25 and 45 DAS (F_{Br}). At IW/CPE 0.6 (Moderate stress) foliar application of either PPFM @ 1% on 25 and 45 DAS produced reasonably higher and comparable level of chlorophyll content with F_C at $I_{0.8}$.

Under water deficit condition (IW/CPE 0.6 and 0.4), foliar application of PPFM recorded higher chlorophyll content (SPAD). The improvement in chlorophyll content under drought could be attributed to decreased activity of the chlorophyll degrading enzyme chlorophyllase or increase in the chlorophyll content as phytochromes interact with gibberellins. The results are in accordance to Misratia *et al.* (2013) [9]. Better response in SPAD values was observed with application of silicon as silicic acid. This might be due to the fact that silicon application could help in improving the photosynthetic efficiency of the crop through improved water use, reduced transpiration, and improved nutrient influx from the soil solution that resulted in maintenance of higher chlorophyll content (SPAD values) in the silicon applied treatments. Similar findings are reported by Ahmed *et al.* (2011) [2].

Proline content

Proline content at 45 DAS was significantly varied with irrigation regimes and moisture stress management practices. Proline content was significantly higher in IW/CPE 0.4 ($I_{0.4}$) with the values of 7.82, 7.60, 10.63 and 12.06 µg g^{-1} at 60 DAS respectively during *rabi* 2017-18 & 2018-19 and summer 2018 & 2019. Significantly lower proline content was recorded in IW/CPE 1.0 ($I_{1.0}$). Increased proline content under IW/CPE ratio of 0.4 might be due to the formation of amino acid as a result of drought induced stress. Proline is a non-protein amino acid that formed in plant tissues when subjected to moisture stress and rapidly metabolized upon recovery from drought (Singh *et al*., 2010). This indicated the specific role of accumulated free proline in plant tissues which acted as an osmolyte produced under moisture stress and played significant role in drought adaptation of plants.

Barring control treatment, significantly higher proline content (5.57, 5.42, 7.50 and 8.40 µg g^{-1} at 60 DAS respectively during *rabi* 2017-18 & 2018-19 and summer 2018 & 2019) was recorded in foliar application of brassinolide @ 0.1ppm on 25 and 45 DAS (F_{Br}). Significantly lower proline content was recorded in foliar application of PPFM @ 1% on 25 and 45 DAS (F_{PPFM}) during all the experiments. This might be due to the fact that PPFM ameliorated the water stress by maintaining leaf water potential for longer time by regulating the stomatal conductivity leading to reduction in the accumulation of proline (Uyprasert *et al*., 2004) [13].

Barring control treatment, substantially higher proline content was recorded in foliar application of brassinolide @ 0.1ppm on 25 and 45 DAS (F_{Br}) at IW/CPE 0.4 (Severe stress) and was comparable with foliar application of silicic acid @ 0.2% on 25 and 45 DAS (F_{Sl}). F_C at $I_{1.0}$ (No stress) recorded significantly lower proline content and was comparable with other moisture stress management treatments in $I_{1.0}$.

Catalase and Peroxidase activity

This enzyme was generally regarded as H_2O_2 scavenger involved in the reduction of damage by oxidation function (Reddy *et al*., 2012) [10]. The trend of H_2O_2 content revealed that, gradual increase of catalase enzyme activity with decrease in irrigation frequency. Maintaining a higher level of anti-oxidative enzyme activities might contribute to the drought tolerance against oxidative damage (Sharma *et al*., 2016). IW/CPE 0.4 ($I_{0.4}$) showed significantly higher catalase and peroxidase activity during at 60 DAS. Significantly lower catalase and peroxidase activity was registered in IW/CPE 1.0

($I_{1.0}$) during all the seasons. In the present study, the maximum catalase and peroxidase activity was noticed with lower irrigation regime of IW/CPE ratio of 0.4 which might be due to moisture deficit. Moisture stress increased the anti-oxidant enzyme by 35 per cent at vegetative stage and 30 per cent at reproductive stage (Sultana *et al*., 2001) [12].

Among the moisture stress management practices, foliar application of PPFM @ 1% (F_{PPFM}) recorded significantly higher catalase and peroxidase activity at 60 DAS invariably during all the seasons. Barring control treatment, significantly lower catalase and peroxidase activity was recorded in foliar application of brassinolide @ 0.1ppm on 25 and 45 DAS (F_{Br}).

In interaction effect, foliar application of PPFM @ 1% on 25 and 45 DAS in IW/CPE 0.4 (Severe stress) and 0.6 (Moderate stress) showed increased catalase and peroxidase activity. The activity of catalase and peroxidase enzyme was enhanced by foliar application of 2% PPFM as reported by Kannan (2017) [8].

Table 1: Effect of irrigation regimes and moisture stress management practices on chlorophyll content (SPAD value) of maize at 60 DAS

	Rabi 2017	Rabi 2018	Summer 2017	Summer 2018
Irrigation regimes				
$I_{1.0}$	53.8	56.5	54.6	54.8
$I_{0.8}$	51.2	53.8	52.0	52.2
$I_{0.6}$	46.5	48.8	47.2	47.1
$I_{0.4}$	43.1	45.2	43.7	43.4
SED	0.4	1.0	0.5	0.8
CD (p=0.05)	0.9	2.4	1.2	1.9
Moisture stress management practices				
F_{PPFM}	50.4	53.0	51.2	51.1
F_{Br}	48.0	50.5	48.8	48.8
F_{Sl}	49.2	51.6	49.9	49.9
F_C	46.9	49.3	47.6	47.6
SED	0.4	0.5	0.4	0.4
CD (p=0.05)	0.9	1.0	0.8	0.9
Interaction				
$I_{1.0}$ F_{PPFM}	54.6	57.4	55.5	55.6
$I_{1.0}$ F_{Br}	53.0	55.7	53.8	54.0
$I_{1.0}$ F_{Sl}	53.5	56.2	54.4	54.5
$I_{1.0}$ F_C	54.0	56.7	54.8	55.0
$I_{0.8}$ F_{PPFM}	52.7	55.3	53.5	53.8
$I_{0.8}$ F_{Br}	51.3	53.9	52.1	52.2
$I_{0.8}$ F_{Sl}	51.4	54.0	52.2	52.4
$I_{0.8}$ F_C	49.4	51.9	50.2	50.3
$I_{0.6}$ F_{PPFM}	48.8	51.2	49.5	49.4
$I_{0.6}$ F_{Br}	45.7	48.0	46.4	46.3
$I_{0.6}$ F_{Sl}	47.5	49.8	48.3	48.2
$I_{0.6}$ F_C	43.8	46.0	44.5	44.4
$I_{0.4}$ F_{PPFM}	45.6	47.9	46.3	45.6
$I_{0.4}$ F_{Br}	42.1	44.2	42.7	42.5
$I_{0.4}$ F_{Sl}	44.2	46.4	44.8	44.6
$I_{0.4}$ F_C	40.3	42.4	41.0	40.7
SED	0.9	0.9	0.8	0.9
CD (p=0.05)	1.8	1.9	1.8	1.9

* $I_{1.0}$, $I_{0.8}$, $I_{0.6}$ and $I_{0.4}$ are Irrigation regimes of IW/CPE ratio 1.0, 0.8, 0.6 and 0.4 respectively;
* F – Foliar application at 25 and 40 DAS; PPFM – Pink pigmented facultative methylobacteria @ 1%; Br – Brassinolide @ 0.1ppm; Sl-Silicic acid @ 0.2% and C – Control.

Table 2: Effect of irrigation regimes and moisture stress management practices on proline content (μg g^{-1}) at 60 DAS of maize

	Rabi 2017	Rabi 2018	Summer 2017	Summer 2018
Irrigation regimes				
$I_{1.0}$	3.25	3.16	4.33	4.66
$I_{0.8}$	3.72	3.62	4.95	5.33
$I_{0.6}$	7.09	6.90	9.56	10.96
$I_{0.4}$	7.82	7.61	10.63	12.06
SED	0.07	0.08	0.15	0.13
CD (p=0.05)	0.18	0.19	0.36	0.31
Moisture stress management practices				
F_{PPFM}	5.14	5.02	6.95	7.77
F_{Br}	5.57	5.42	7.50	8.41
F_{Sl}	5.41	5.26	7.28	8.15
F_C	5.76	5.60	7.75	8.68
SED	0.07	0.06	0.10	0.13
CD (p=0.05)	0.15	0.13	0.21	0.26
Interaction				
$I_{1.0}$ F_{PPFM}	3.38	3.29	4.50	4.84

$I_{1.0} F_{Br}$	3.24	3.15	4.32	4.64
$I_{1.0} F_{Sl}$	3.28	3.19	4.37	4.70
$I_{1.0} F_{C}$	3.11	3.02	4.14	4.46
$I_{0.8} F_{PPFM}$	3.51	3.41	4.68	5.03
$I_{0.8} F_{Br}$	3.68	3.58	4.91	5.28
$I_{0.8} F_{Sl}$	3.57	3.47	4.75	5.11
$I_{0.8} F_{C}$	4.11	4.00	5.47	5.89
$I_{0.6} F_{PPFM}$	6.67	6.55	9.07	10.40
$I_{0.6} F_{Br}$	7.32	7.10	9.85	11.29
$I_{0.6} F_{Sl}$	6.91	6.72	9.30	10.66
$I_{0.6} F_{C}$	7.45	7.24	10.03	11.49
$I_{0.4} F_{PPFM}$	7.01	6.82	9.53	10.82
$I_{0.4} F_{Br}$	8.04	7.84	10.93	12.41
$I_{0.4} F_{Sl}$	7.86	7.64	10.69	12.13
$I_{0.4} F_{C}$	8.35	8.12	11.36	12.89
SED	0.14	0.13	0.24	0.25
CD (p=0.05)	0.31	0.29	0.52	0.54

* $I_{1.0}$, $I_{0.8}$, $I_{0.6}$ and $I_{0.4}$ are Irrigation regimes of IW/CPE ratio 1.0, 0.8, 0.6 and 0.4 respectively;
* F – Foliar application at 25 and 40 DAS; PPFM – Pink pigmented facultative methylobacteria @ 1%; Br – Brassinolide @ 0.1ppm; Sl-Silicic acid @ 0.2% and C – Control.

Table 3: Effect of irrigation regimes and moisture stress management practices on Catalase activity (μmol H_2O_2 reduced g^{-1} min^{-1}) at 60 DAS of maize

	Rabi 2017	**Rabi 2018**	**Summer 2017**	**Summer 2018**
Irrigation regimes				
$I_{1.0}$	3.01	2.97	2.90	3.13
$I_{0.8}$	3.20	3.16	3.11	3.33
$I_{0.6}$	4.25	4.19	4.15	4.76
$I_{0.4}$	4.68	4.62	4.62	5.24
SED	0.06	0.05	0.07	0.07
CD (p=0.05)	0.15	0.12	0.17	0.18
Moisture stress management practices				
F_{PPFM}	4.06	4.00	3.96	4.41
F_{Br}	3.67	3.62	3.58	3.98
F_{Sl}	3.90	3.85	3.81	4.24
F_{C}	3.52	3.48	3.44	3.83
SED	0.06	0.04	0.05	0.06
CD (p=0.05)	0.12	0.09	0.11	0.13
Interaction				
$I_{1.0} F_{PPFM}$	3.20	3.15	3.05	3.32
$I_{1.0} F_{Br}$	2.95	2.91	2.86	3.07
$I_{1.0} F_{Sl}$	3.09	3.05	2.99	3.22
$I_{1.0} F_{C}$	2.80	2.76	2.71	2.91
$I_{0.8} F_{PPFM}$	3.42	3.37	3.34	3.55
$I_{0.8} F_{Br}$	3.21	3.17	3.10	3.34
$I_{0.8} F_{Sl}$	3.27	3.23	3.17	3.41
$I_{0.8} F_{C}$	2.90	2.87	2.81	3.02
$I_{0.6} F_{PPFM}$	4.45	4.39	4.33	4.99
$I_{0.6} F_{Br}$	4.03	3.97	3.95	4.51
$I_{0.6} F_{Sl}$	4.40	4.34	4.28	4.93
$I_{0.6} F_{C}$	4.12	4.07	4.05	4.61
$I_{0.4} F_{PPFM}$	5.15	5.08	5.10	5.77
$I_{0.4} F_{Br}$	4.47	4.42	4.40	5.01
$I_{0.4} F_{Sl}$	4.83	4.76	4.78	5.41
$I_{0.4} F_{C}$	4.25	4.20	4.18	4.76
SED	0.12	0.09	0.11	0.14
CD (p=0.05)	0.25	0.19	0.25	0.30

* $I_{1.0}$, $I_{0.8}$, $I_{0.6}$ and $I_{0.4}$ are Irrigation regimes of IW/CPE ratio 1.0, 0.8, 0.6 and 0.4 respectively;
* F – Foliar application at 25 and 40 DAS; PPFM – Pink pigmented facultative methylobacteria @ 1%; Br – Brassinolide @ 0.1ppm; Sl-Silicic acid @ 0.2% and C – Control.

Table 4: Effect of irrigation regimes and moisture stress management practices on Peroxidase activity (µmol H_2O_2 reduced g^{-1} min^{-1}) at 60 DAS of maize

	Rabi 2017	Rabi 2018	Summer 2017	Summer 2018
Irrigation regimes				
$I_{1.0}$	22.28	22.00	21.53	23.15
$I_{0.8}$	23.43	23.23	22.73	24.45
$I_{0.6}$	30.98	30.60	30.25	34.70
$I_{0.4}$	34.33	33.90	34.00	38.43
SED	0.6	0.4	0.6	0.4
CD (p=0.05)	1.5	1.0	1.5	0.9
Moisture stress management practices				
F_{PPFM}	29.63	29.25	28.90	32.20
F_{Br}	26.93	26.60	26.28	29.23
F_{Sl}	28.53	28.18	27.85	31.05
F_C	25.93	25.70	25.48	28.25
SED	0.5	0.4	0.4	0.4
CD (p=0.05)	1.0	0.8	0.8	0.9
Interaction				
$I_{1.0}$ F_{PPFM}	23.10	22.80	22.30	24.00
$I_{1.0}$ F_{Br}	21.70	21.40	21.00	22.50
$I_{1.0}$ F_{Sl}	22.50	22.20	21.70	23.40
$I_{1.0}$ F_C	21.80	21.60	21.10	22.70
$I_{0.8}$ F_{PPFM}	24.80	24.50	24.00	25.80
$I_{0.8}$ F_{Br}	23.60	23.30	22.80	24.50
$I_{0.8}$ F_{Sl}	24.10	23.80	23.30	25.10
$I_{0.8}$ F_C	21.20	21.30	20.80	22.40
$I_{0.6}$ F_{PPFM}	32.70	32.30	31.70	36.60
$I_{0.6}$ F_{Br}	29.80	29.50	29.30	33.40
$I_{0.6}$ F_{Sl}	31.90	31.50	31.00	35.80
$I_{0.6}$ F_C	29.50	29.10	29.00	33.00
$I_{0.4}$ F_{PPFM}	37.90	37.40	37.60	42.40
$I_{0.4}$ F_{Br}	32.60	32.20	32.00	36.50
$I_{0.4}$ F_{Sl}	35.60	35.20	35.40	39.90
$I_{0.4}$ F_C	31.20	30.80	31.00	34.90
SED	1.0	0.8	0.9	0.8
CD (p=0.05)	2.1	1.7	1.9	1.7

* $I_{1.0}$, $I_{0.8}$, $I_{0.6}$ and $I_{0.4}$ are Irrigation regimes of IW/CPE ratio 1.0, 0.8, 0.6 and 0.4 respectively;
* F – Foliar application at 25 and 40 DAS; PPFM – Pink pigmented facultative methylobacteria @ 1%; Br – Brassinolide @ 0.1ppm; Sl-Silicic acid @ 0.2% and C – Control.

Conclusion

Irrigating at IW/CPE 0.8 seems to be idle for plant growth and functioning in maize. Irrigating at IW/CPE 0.6 and 0.4 tends to stressed situation for the plant hence, the proline content and antioxidant contents are gets increased. Exogenous application of PPFM 1% on 25 and 45 DAS helps the plants to conserve water content and reduces the proline content and enhanced the antioxidant enzymes. From the experimental results it could be concluded that, irrigating at IW/CPE 0.6 coupled with foliar application of PPFM 1% on 25 and 45 DAS was suitable option for obtaining optimum yield with moderate stress on the plant under water scarcity condition.

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