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Alleviating role of methyl jasmonate and zinc on morpho-physiological and biochemical attributes in chickpea (*Cicer arietinum* L.) under salinity stress

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Abstract

A pot-culture experiment was undertaken to investigate the possible role of putative phytohormone Methyl jasmonate (MeJA) (10µM and 15µM) through seed hardening and Zinc (15mg/Kg soil) as basal dose in extenuating the salinity effects in chickpea genotype BG-362 on the earlier vegetative growth stage subjected to 100mM salinity stress. The results on evaluation of the effects of methyl jasmonate (MeJA) and Zinc on physio-biochemical attributes, antioxidant enzyme activity were observed significant. The application of both MeJA and Zn was found to improve the chlorophyll content, length of shoot, shoot dry weight, relative water content and antioxidant enzyme activity like catalase and minimizes the accumulation of Hydrogen Peroxide (H_2O_2) in salt-fed seedlings. However, all these parameters were significantly reduced under alone treatment of 100mM salinity. Observations on different parameters were recorded at four intervals of 30, 60, 90 and 120 days after salt exposure**.** From the above parameters we summarize our results that MeJA and Zn showed mitigating effects by ROS scavenging through antioxidant defense machinery, increase in measure of plant water solution i.e., relative water content, chlorophyll content and growth parameters under salinity stress.

Keywords: Salinity stress, methyl Jasmonates, antioxidant enzyme catalase, chlorophyll content, shoot length, relative water content, H_2O_2 content

Introduction

Pulses forms its significant position in vegetarian diet due to its high protein content in its grains. Among pulses chickpea shows its imperative role in sustainable agriculture by maintaining soil fertility through biological nitrogen fixation and its outstanding nutritive properties. The instability in productivity of chickpea in previous years was due to abiotic stresses. Abiotic stress like salinity is known to disturb the physiological balance and create osmotic stress in cells leading to production of Reactive oxygen species (ROS).

Salinity stress resulted in heavy flower drop, pod shedding, poor seed set which ultimately lowered the productivity of chickpea in country. About 45 million ha of irrigated land predictable to about 20% producing one-third of the world's food, is salt-affected (srivastava *et al* 2015). Ion cytotoxicity and osmotic stress due to excessive ion concentration (Na^+, Cl^-, SO_4) are the adverse effects of salinity on plant growth (Zhu, 2002) [39]. Metabolic imbalances caused by ion toxicity, osmotic stress and nutritional deficiency under saline conditions may also lead to oxidative stress (Zhu, 2002) [39]. A high NaCl concentration in tissues is toxic for growth of glycophytes (Glenn *et al*., 1999) [14]. The tolerance or susceptibility of plant to salinity stress may depend upon the alterations in the levels of endogenous phytohormones such as abscisic acid (Jin *et al*. 2000) [20], salicylic acid (Hoyos and Zhang 2000) [18], and jasmonates (Chao *et al*. 1999; Thaler 1999) [7, 35] which ultimately induced many proteins of tolerance or susceptibility. To alleviate the salinity stress certain novel phyto hormones technology or tools are used in biotechnological, plant breeding and plant physiology fields.

Phyto hormones have a leading role in various physiological and developmental processes in plants (Rohwer and Erwin, 2008; Ashraf *et al*., 2010; Kumar *et al*., 2014) [33, 3, 22] An important phytohormone methyl jasmonate ubiquitously found in the plant kingdom are derivatives of the fatty acid metabolism (Kupper *et al*., 2009; Gao *et al*., 2011; Jalalpour *et al*., 2014) [23, 13, 19] MeJA are believed to play an active role in a variety of JA-induced plant growth, senescence developmental and physiological activities have been reported including fertility, biotic and abiotic stress tolerance, sex determination, storage organ formation, reproductive processes, root elongation, fruit ripening and senescence, oxidative defense, and interaction with other hormones.

Nutrient balance and nutrient uptake is a matter of great concern in salinity affected soils the uptake is greatly affected in saline soils result in deficiency of significant nutrients in plants the increase in sodium and chloride ions in soil solution may lead to depletion of major and minor nutrients like phosphorous, zinc, potassium, calcium etc. Pulses are the heavy feeder of minor nutrients like Zn, Ca, Mo, Co, B, and Cu. Deficiency of any element may show severe symptoms under salinity stress. Zinc is found to be an important nutrient involved in activation of several enzymes and is directly involved in the biosynthesis of growth regulators such as auxin, which promotes production of more plant cells and biomass that will be stored in the plant organs especially in seeds besides this zinc was found to be the component of several antioxidant enzymes role in reducing ROS accumulation.

The current study was undertaken to evaluate the role of exogenously applied MeJA in counteracting salinity stress in chickpea genotype BG-362. We examined the effect of exogenous MeJA on growth parameters, chlorophyll content, relative water content, catalase activity, and H_2O_2 content in salt-stressed chickpea plants.

Materials and Methods

The present-investigation was undertaken during 2016-2017 in polyhouse of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Seeds of chickpea genotype BG-362 were procured from Pulse Research Laboratory, I.A.R.I, New Delhi. Seeds were surface sterilized with 0.1% $HgCI_2$ treatment for 5 min and then washed with sterile distilled water followed by seed hardening treatment. Plastic pots of uniform size (30x30cm) were filled with 10 kg of air-dried soil and farmyard manure in 6:1 ratio. Salinity stress was induced after plants were established well in pots in polyhouse. Potassium sulfate was evenly added @10mM for inducing salinity stress with NaCl. Methyl jasmonate ($C_{13}H_{20}O_3$) was applied at the rate of 10 and 15µM in combination with or without zinc under salinity stress. Potassium sulfate was evenly added @10mM for inducing salinity stress with NaCl. The growth parameters were examined after 30, 60, 90 and 120 days of salt exposure. The experiment was arranged in a Completely Randomized Design (CRD) with three replicates.

Total Chlorophyll content

The chlorophyll content was estimated as per protocol of Hiscox and Isrealstam (1979) by non-maceration method using dimethyl sulphoxide (DMSO). Total chlorophyll was calculated according to Arnon (1949)

Chl a = {(12.7 x A 663) - (2.69 x A 645)} x V/1000 W (mg/g fresh wt.)

Chl b = {(22.9 x A 645) - (4.68 x A 663)} x V/1000 W (mg/g fresh wt.)

Total chl = {(20.2 x A 645) - (8.02 x A 663)} x V/1000 W (mg/g fresh wt.)

Where,
V = volume of solvent,
W = weight of sample

Hydrogen peroxide

Hydrogen peroxide was assayed as per the protocol of Mukherjee and Choudhary (1983) [28].

Catalase activity

Catalase was assayed by measuring the disapperance of H_2O_2 as per protocol of Teranishi *et al.* (1974) [34].

Relative water content (RWC)

Relative water content was estimated in the leaves of plants grown under optimum controlled and salinity stressed plants. The RWC was calculated using the formula proposed by Weatherly (1950) [37]

$$RWC = \frac{\text{Fresh weight- Dry weight}}{\text{Turgid weight- Dry weight}} \text{ X } 100$$

Results and Discussion

Plant Height

Table 1: Effect of Methyl jasmonates and zinc on plant height (cm) in chickpea genotype BG-362 under induced salinity at different stages of growth

Treatments	30 DAS		60 DAS		90 DAS		120 DAS	
	2016	2017	2016	2017	2016	2017	2016	2017
T0	12.99	13.91	26.42	27.20	39.33	40.75	43.37	44.83
T1	6.82	8.58	14.73	15.32	22.14	22.94	24.41	25.23
T2	13.65	14.56	27.68	28.63	41.65	45.56	48.16	50.11
T3	12.75	13.77	25.98	26.88	39.12	39.83	43.81	43.82
T4	15.99	16.61	32.12	33.21	50.82	52.65	56.04	57.92
T5	9.12	10.56	19.10	19.72	29.63	30.70	32.67	33.77
T6	7.78	9.47	16.55	17.29	24.44	25.32	28.28	27.86
T7	15.98	16.60	32.10	33.19	48.38	50.46	53.68	55.51
T8	15.91	16.45	31.96	32.84	47.21	48.91	52.06	53.81
T9	11.41	12.68	23.43	24.45	35.93	38.97	41.29	42.87
T10	9.19	10.64	19.22	19.91	30.48	32.62	34.28	35.68
SEm±	0.68	0.62	1.28	1.39	1.73	2.04	2.01	2.39
LSD (p=0.05)	1.98	1.83	3.75	4.07	5.08	5.97	5.90	7.00

T0: Control; T1: 100mM; T2: 10µM MeJA; T3: 15µM MeJA; T4: Zn; T5: 100mM NaCl+10µM MeJA; T6: 100mM NaCl+10µM MeJA; T7: Zn+10µM MeJA; T8: Zn+15µM MeJA; T9: Zn+10µM MeJA+100mM NaCl; T10: Zn+15µM MeJA+100mM NaCl

Leaf number per plant

Table 2: Effect of Methyl jasmonates and zinc on number of leaves per plant in chickpea genotype BG-362 under induced salinity at different stages of growth

Treatments	30 DAS		60 DAS		90 DAS		120 DAS	
	2016	2017	2016	2017	2016	2017	2016	2017
T0	81.57	82.57	129.39	135.00	208.21	238.54	225.63	260.73
T1	39.93	45.31	62.67	79.58	102.78	109.08	111.38	113.19
T2	82.21	84.87	130.40	138.42	209.88	241.91	227.44	266.44
T3	76.26	82.45	120.88	134.82	198.26	235.62	214.85	259.51
T4	92.27	100.13	146.53	161.11	240.33	281.56	260.44	312.11
T5	57.10	63.89	90.17	107.21	147.89	150.37	160.27	205.37
T6	47.86	48.68	75.37	84.60	123.62	127.85	125.96	129.25
T7	90.91	98.34	144.35	158.45	236.75	276.92	256.56	305.00
T8	84.70	90.81	134.40	147.25	220.44	257.35	238.88	283.45
T9	69.74	69.62	110.43	115.73	185.11	197.26	195.60	217.27
T10	60.46	65.98	95.57	110.33	156.74	157.81	161.86	171.81
SEm±	3.03	4.53	4.85	6.74	8.69	12.78	10.24	15.00
LSD (p=0.05)	8.88	13.30	14.23	19.78	25.48	37.49	30.03	43.99

T0: Control; T1: 100mM; T2: 10µM MeJA; T3: 15µM MeJA; T4: Zn; T5: 100mM NaCl+10µM MeJA; T6: 100mM NaCl+10µM MeJA; T7: Zn+10µM MeJA; T8: Zn+15µM MeJA; T9: Zn+10µM MeJA+100mM NaCl; T10: Zn+15µM MeJA+100mM NaCl

Data pertaining to plant height and number of leaves as influenced by salinity at four stages of plant growth i.e. vegetative, flowering and pod initiation and pod formation was observed and is presented in table 1 and 2 plant height was known to be an important index of plant growth in abiotic stress. Salinity stress adversely affects the plant height and number of leaves. Plants become stunted and rate of leaf emergence and its growth was significantly affected under salinity stress. In general, an increasing trend in plant height was observed with advancement in crop age in chickpea. Plant height was differed significantly up to maturity among different treatments. Combined treatment of 10 µM MeJA + 100mM salinity stress + Zn showed minimum reduction in plant height and number of leaves compared with alone treatment of salinity however the combined treatment of 10 µM MeJA + 100mM salinity stress was statically at par with alone treatment of salinity. The highest plant height and number of leaves was recorded in only Zn treated plants and was found at par with control and alone dose of 10 µM MeJA. A decrease in plant height and number of leaves per plant with increasing salinity has, been reported in many crop plants *viz* rice (EI-Shouny, 1976) [10], cotton (Malofeev *et al.*, 1979) [24], wheat, barley (Bhardwaj, 1960) [5], brassica (Ansari, 1972) [1] and cowpea (Gomes *et al.*, 1983) [15]

Total Chlorophyll content

Table 3: Effect of Methyl jasmonates and Zinc on total chlorophyll content (mg^{-1} g^{-1} fresh weight) in chickpea genotype BG-362 under induced salinity at different stages of growth

Treatments	30 DAS		60 DAS		90 DAS		120 DAS	
	2016	2017	2016	2017	2016	2017	2016	2017
T0	4.235	4.555	4.614	4.858	5.622	5.793	4.814	5.039
T1	2.672	3.118	2.986	3.220	3.722	3.703	2.875	2.842
T2	4.244	4.622	4.623	4.934	6.016	5.984	4.957	5.154
T3	4.224	4.603	4.602	4.911	5.528	5.699	4.797	4.898
T4	4.848	5.364	5.252	5.778	6.282	6.249	5.274	5.353
T5	3.763	4.153	4.122	4.399	5.322	5.361	4.468	4.562
T6	3.438	3.836	3.784	4.038	5.023	4.997	4.092	4.178
T7	4.570	5.175	4.963	5.563	6.182	6.216	5.190	5.299
T8	4.439	4.813	4.826	5.150	6.022	5.990	5.087	5.194
T9	4.157	4.537	4.532	4.837	5.512	5.650	4.659	4.757
T10	3.773	4.339	4.133	4.611	5.428	5.400	4.557	4.653
SEm±	0.229	0.227	0.238	0.259	0.332	0.349	0.249	0.259
LSD (p=0.05)	0.672	0.667	0.699	0.759	0.974	1.023	0.732	0.760

T0: Control; T1: 100mM; T2: 10µM MeJA; T3: 15µM MeJA; T4: Zn; T5: 100mM NaCl+10µM MeJA; T6: 100mM NaCl+10µM MeJA; T7: Zn+10µM MeJA; T8: Zn+15µM MeJA; T9: Zn+10µM MeJA+100mM NaCl; T10: Zn+15µM MeJA+100mM NaCl

Relative water content (RWC %)

Table 4: Effect of Methyl jasmonates and zinc on relative water content (RWC %) in leaves of chickpea genotype BG-362 under induced salinity at different stages of growth

Treatments	30 DAS		60 DAS		90 DAS		120 DAS	
	2016	2017	2016	2017	2016	2017	2016	2017
T0	63.38	66.42	76.19	78.61	78.36	80.41	75.42	78.83
T1	42.18	43.10	52.14	52.42	55.18	55.68	54.18	55.72
T2	63.42	66.46	76.34	78.78	78.42	80.47	75.63	79.06
T3	62.18	65.10	75.24	77.58	77.42	79.41	74.36	77.67
T4	72.38	76.32	86.34	89.67	89.43	92.22	84.42	88.62

T5	51.18	53.00	67.38	69.02	68.13	69.49	64.18	66.60
T6	42.48	43.43	52.43	52.74	55.38	55.89	54.38	55.94
T7	72.18	76.10	84.92	88.12	88.63	91.37	82.38	86.40
T8	71.48	75.33	84.56	87.73	88.28	90.99	82.18	86.18
T9	61.43	64.27	75.19	77.53	77.13	79.10	74.16	77.46
T10	51.38	53.22	67.42	69.06	68.23	69.60	64.23	66.65
SEm±	2.02	2.22	2.46	2.68	2.91	3.11	3.22	3.50
LSD (p=0.05)	5.92	6.51	7.22	7.86	8.54	9.11	9.44	10.27

T0: Control; T1: 100mM; T2: 10μM MeJA; T3: 15μM MeJA; T4: Zn; T5: 100mM NaCl+10μM MeJA; T6: 100mM NaCl+10μM MeJA; T7: Zn+10μM MeJA; T8: Zn+15μM MeJA; T9: Zn+10μM MeJA+100mM NaCl; T10: Zn+15μM MeJA+100mM NaCl

Perusal of the table 3 and 4 revealed that chlorophyll contents and Relative water content (%) are maximum in leaves of only zinc treated plants followed by combination of zinc with methyl jasmonate. Whereas the lower values were recorded in salinity treated plants compared with control. Combined treatment of methyl jasmonate (10μM) + salinity (100mM) + Zn (15mg/Kg soil) showed significantly higher values compared with only salinity stressed plants. The total chlorophyll content and Relative water content (%) decreased with increasing age of plant and recorded highest at 90DAS. Slightly higher concentration of MeJA under salinity stress (15µM MeJA + 100mM) showed inhibitory effects and was found statistically at par with alone treatment of salinity. However the 10µM MeJA dose performed better under salinity stress as compared with 15µM MeJA. Relative water content reflects the metabolic activity in tissues affected by various types of stresses as it is an alternative measure of plant water solution and also responsible for the activation of various enzymes in cells. The chlorophyll content decreased in salinity treated plants. Low chlorophyll, content causes reduction in light absorption by leaves (Evans; 1996), and consequently reduces the biosynthesis of carbohydrates. Marked reduction in chlorophyll content at 100 and 150 mM NaCI over the control in stem and leaves of green gram was observed by (Muthukumarswamy and Panneerselvam, 1997) [29], in cucumber by (Kaya and Higgs, 2002) [21] and in cotton by (Meloni *et al.,* 2003) [26].

In abiotic stress the RWC decreased with the increase in levels of stress. In present study the RWC content considerably decreased in salinity stressed plants these results are in line with the study of Tipirdamaz and Cakirlar 1990 [36] who reported decrease RWC in chickpea by salinity stress. Harinasut *et al.,* 1996) [17]. Significant decrease in relative water content (RWC) occurred in rice seedlings, subjected to salinity stress the lower dose of MeJA was comparable with control plants however the higher dose of 15µM MeJA showed similar results like the alone dose of salinity the JA induced decline in RWC and chlorophyll content has also been reported by various workers (Weidhase *et.al.,* 1987; Parthier 1990) [38, 31].

Catalase activity

Table 5: Effect of Methyl jasmonates and Zinc on catalase activity (nmol.g^{-1}fresh weight min^{-1}) in chickpea genotype BG-362 under induced salinity at different stages of growth

Treatments	**30 DAS**		**60 DAS**		**90 DAS**		**120 DAS**	
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
T0	153.39	154.68	160.18	158.47	167.33	167.23	165.12	165.39
T1	155.26	157.59	162.17	161.46	169.49	170.09	165.00	166.72
T2	153.34	154.63	160.13	158.42	167.28	167.13	165.02	165.29
T3	153.44	154.73	160.23	158.52	167.22	167.28	165.63	164.44
T4	150.79	152.03	157.42	155.74	164.34	165.08	160.14	163.26
T5	158.04	159.43	165.13	163.36	172.70	173.38	168.78	170.87
T6	156.30	159.49	163.28	163.42	170.70	170.18	165.19	168.31
T7	151.51	152.76	158.18	156.49	165.16	165.12	160.23	163.30
T8	151.70	152.95	158.38	156.69	165.38	165.32	160.42	162.20
T9	161.06	165.27	168.34	169.38	176.19	176.42	171.43	174.15
T10	158.14	161.42	165.23	165.41	172.81	174.03	169.28	171.45
SEm±	0.516	0.639	0.549	0.659	0.728	0.677	0.249	0.692
LSD (p=0.05)	1.514	1.875	1.610	1.933	2.135	1.986	0.732	2.030

T0: Control; T1: 100mM; T2: 10μM MeJA; T3: 15μM MeJA; T4: Zn; T5: 100mM NaCl+10μM MeJA; T6: 100mM NaCl+10μM MeJA; T7: Zn+10μM MeJA; T8: Zn+15μM MeJA; T9: Zn+10μM MeJA+100mM NaCl; T10: Zn+15μM MeJA+100mM NaCl

Catalase activity differed and influenced significantly by various treatments at different stages of plant growth table 5. A comparable increase in Catalase activity was recorded upto 90 DAS in salinity treated plants after that, very insignificant variations were observed at 120 DAS. The highest mean catalase activity was recorded in combination of treatments 10µM MeJA +100mM NaCl + Zn 15mg/Kg soil compared with only salinity treated plants. However the alone dose of Zinc recorded the lowest mean catalase activity compared with only salinity stressed plants. A marked stimulation of CAT activity was recorded in methyl jasmonate and zinc treated plants under salinity stress. The lower dose of MeJA (10µM MeJA) performed better than the slightly high dose i.e., 15µM MeJA. Thus, it was observed that MeJA had positive effects on CAT activity and ameliorates the adverse effect of salinity stress in chick pea genotype. ROS affects the normal functioning of cell by disturbing membranes integrity damage to protein, DNA and lipids (Apel and Hirt, 2004; Foyer and Noctor, 2005). (Hao *et al.,* 2006) [2, 12, 16]. Data of our work are in line with the findings of (Ashraf and Harris, 2004; Chawla *et al.,* 2013) [8] A burst in oxidative stress was observed due to elevated levels of Na^+ are in different plants and tissues

Chen *et al.,* 2014 [9] evinces application of JA minimizes the production of ROS,) followed by decrease in MDA activity in Kandelia obovata under Cd stress. The augmentation in CAT activity in MeJA and Zn treated plants in present investigation was in accordance with the conclusion of (Mayak *et.al*., 1983 and Naik *et.al*., 2002) Chen *et al*. (2014) [25, 30, 9] also observed that JA enhanced the CAT and APX activities in *K. obovata* seedlings subjected to Cd stress.

The current study clearly demonstrated that exogenous MeJA may not only be involved in the defense mechanism against wounding and pathogen stress, but also in alleviating the detrimental effect of salt stress, although the magnitude was comparatively lower in chickpea as compared to other plants. MeJA application before exposure of plants to salinity stress may results in alleviation of certain endogenous phytohormones and their signaling components for tolerance or susceptibility to various abiotic stresses. The outstanding role of Jasmonates in various physiological and reproductive processes in plants draws attention of researchers in fields of plant breeding, biotechnology and plant physiology to develop tolerant genotypes to stress. Various evidences cleared JAs play a role from germination up to senescence. However various genes involved in growth regulation at different stages of development, are yet to be identified. But there is limited information available in the literature on how these plant processes like activation of antioxidant defense machinery, activation of genes related to endogenous phytohormones vary from species to species with JA application.

Conclusion

From aforesaid discussion we concluded that salinity stress affects the certain important traits, manifested by decline in chlorophyll content, RWC, plant height, catalase activity and increase in H_2O_2 accumulation in chickpea genotype due to its susceptibility to salinity. Both salinity and slightly higher dose of MeJA treatment significantly affected the indices of vegetative growth. The results suggest that Methyl jasmonate worked effectively in alleviating the negative influences of salinity stress at concentration 10 μM in chickpea genotype and this 10 μM concentration could be utilized for the induction of plant defensive system that will enable the plant to withstand many biotic and abiotic stresses. Further investigations on methyl jasmonate threshold concentrations on other crops need to be studied to recognize the further role at molecular levels. it is therefore appears from above investigation that MeJA can generally be used as a growth regulator to enhance germination and plant growth.

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