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INFLUENCE OF PLANT GROWTH PROMOTER AND ABIOTIC STRESS TREATMENT ON GAS EXCHANGE PARAMETERS IN INDIAN MUSTARD (*BRASSICA JUNCEA* (L.) CZERN & COSS.)

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ABSTRACT

An experiment was conducted to assess the interactive action of water logging (WL), high temperature (HT) as well as water logging + high temperature (WL + HT) on different gas exchange parameters at 80 DAS in Indian mustard. A marked reduction in photosynthetic rate (8.60), carboxylation efficiency (2.99), stomatal conductance (131.33) has been observed under water logging condition in comparison to HT and WL+ HT, whereas water logged crop showed ameliorative effect after foliar application of IAA 100, 500, Kinetin 05 and 50 μM. Significant ameliorative effect has been observed by application of Kinetin 50 μM in respect to photosynthetic rate (11.83), stomatal conductance (227.66) and carboxylation efficiency. Internal carbon (286.66) and water potential (0.92) observed highest under WL condition in comparision to HT and WL+HT under control environment whereas internal carbon (273.66) and water potential (0.69) reduced significantly in kinetin 50 μM sprayed plants. Transpiration rate showing decreasing trends 1.61 and 1.67 in WL+HT and WL respectively and increasing trends 2.83 and 2.55 in WL+HT and WL respectively observed after application of plant growth promoters.

KEY WORDS
Brassica juncea
Plant Growth Promoters
water logging
High temperature, gas exchange

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INTRODUCTION

Brassica is one of the most important oilseeds cultivated in tropical and subtropical region of the world and is the second most important oilseed in India, contributing about 30% of the total edible oil production of the country (Chakraborty and Uprety, 2012). Waterlogging is one of the most widespread abiotic determinants for crop growth, leading to the depletion of oxygen, which is vital to plants (Subbaiah and Sachs, 2003). The depletion of oxygen is a major feature of waterlogging because the diffusion of oxygen in water is 10-4 times slower than that in air (Dat *et al.*, 2004). The imbalance between the slow diffusion of gases and the rate that oxygen is consumed by micro-organisms and plant roots drastically reduces the supply of oxygen (Jackson and colmer 2005), which is vital to the roots of plant. The O_2 deficiency in the root system forces plants to switch their respiration from aerobic to anaerobic mode, resulting inevitably in low yield of ATP, accumulation of toxic end products of anaerobic respiration, rapid depletion of organic compounds and may limit crop growth due to alteration in metabolism (Drew, 1992).

Decrease in rate of photosynthesis as a result of water logging is due to (I) Stomatal closure which interferes with diffusion of CO_2 and water vapours (II) Reduced leaf area and chlorophyll content as a result of enhanced senescence and leaf abscission (III) Reduction of the key enzyme, RuBP Carboxylase, etc. In addition, flooding inhibits translocation of photosynthates. Kinetin application ameliorated the deleterious effects of heat stress and improved the water status of plants under both aerobic and anaerobic conditions (Gadallah, 1999). High temperature and high photon flux density (PFD) in *B. juncea*, reduces leaf photosynthesis and also the activity of Ribulose-bis-phosphate Carboxylase (RuBPcase) (Desiraju and Ajay, 1998). An experiment was therefore carried out to see the interactive effect various hormones and abiotic stress treatment i.e., water logging (WL), high temperature (HT) as well as WL + HT on gas exchange and water relation parameters in Indian mustard.

MATERIALS AND METHODS

The present investigations were conducted on mustard (*Brassica juncea* cv. RH-30) under screen house conditions. Seeds obtained from Oil seeds section, Chaudhary Charan Singh Haryana Agriculture University, Hisar were sown in pots lined with polythene bags filled with six kg field soil. After 20 days of sowing Hoagland solution was applied. Plants were exposed to various stress treatments *viz.*, water logging (WL) (stagnation of water 2cm above the soil surface), high

temperature (HT) (covering with polythene enclosures between 10 a.m. to 3.30 p.m.) and water logging + high temperature (WL + HT) at 80 DAS for five days each along with control plants which were maintained at field capacity (FC) and ambient temperature conditions. Observations were recorded 5 days after stress treatment (6th day) and all the treatments were replicated thrice. Different hormones *viz.*, IAA 100mM, IAA 500mM, Kin 5mM and Kin 50mM were sprayed 24 hour before imposition of various stresses, with the objective to mitigate the stress effects, along with control plants, which were sprayed with water.

Gas exchange studies were conducted five days after treatment (DAT). These observations were taken during 10:00 to 11:30 a.m. The CO_2 exchange rate (CER or A, m mol m^{-2} s^{-1}), transpiration rate (E, m mol m^{-2} s^{-1}) and stomatal conductance (SC, m mol $m^{-2}s^{-1}$) of third fully expanded leaf from top were determined by using portable IRGA (PP system model CIRAS-1 manufactured by PP system USA). Leaf cuvette was clamped on upper surface of the leaf (in this position PAR sensor faces sunlight and was upward) and then its position was shifted in such a way that maximum PAR was obtained and minimum differences in CO_2 and water vapours between reference and analytical cell were achieved.

At this stage exchange switch was pressed and when the reading was stable, values were recorded. Whole sequence of steps was repeated by inverting the leaf cuvette and clamping the sensor on lower surface of leaf. Values of Sc, E and A were added while for temperature mean value was used. Printouts were obtained using PC-interface. Following indices were calculated from the readings taken and were analysed without any consideration of their sign. WUE and CE were determined using the method of Rensburg and Kriiger (1993).

Water Use Efficiency (WUE) = A/E (m mol $m^{-2}s^{-1}$/m mol m^{-2} s^{-1})

Carboxylation Efficiency (CE) = A/Ci (m mol m^{-2} s^{-1}/m mol mol^{-1}) $x10^2$

Fresh weight of fix number of discs taken from third fully expanded leaf from top was recorded. It was then floated on de-ionized water for 6 h at constant temperature in diffused light. It was gently cleaned with tissue paper and take turgid weight. Then it was dried in oven at 70ºC for 72 hrs and dry weight recorded. RWC was calculated using formula (Weatherlay, 1965).

$$RWC\ (\%) = \frac{\text{Fresh weight - Dry weight}}{\text{Fully turgid weight – Dry weight}} \times 100$$

Osmotic potential of third fully expanded leaf from top was measured by freezing and thawing of leaf sample. Sap was extracted by crushing the sample. Filter paper discs soaked with sap were used for observing osmotic potential using vapour pressure osmometer (Model 5100B Wescor, Inc. Logan, Utah, USA). Standard curve was prepared with the help of sodium chloride solution using 0.1, 0.2, 0.3, ...1.0 molal solution. Values were calculated using standard curve and expressed in - MPa.

Leaf water potential of the third fully expanded leaf from top was determined by using Pressure Chamber and the values were expressed in - MPa. (Scholander *et al.*, 1965).

RESULTS AND DISCUSSION

Photosynthetic rate decreased markedly (Table 1) in stressed plants (9.89, 10.61, 9.14 µ mol m^{-2} s^{-1} in WL, HT and WL + HT respectively) as compared to those of control (11.75). The reduction was maximum in WL + HT (22.21% reduction over control) followed by WL (15.82%) and HT (9.70%). Besides, a spray of IAA100 (9.86), IAA500 (11.33) or Kin50 (11.46) successfully mitigated the adverse effect of various stresses on photosynthetic rate. Per cent increase over water spray being maximum in Kin50 (12.79%) followed by IAA500 (11.51%). In water spray, it was 10.16 µ mol m^{-2} s^{-1}. On the contrary,

Table 1: Interactive effect of various hormonal concentrations (µM) and stress treatment at stage II (80 DAS) on photosynthetic rate (µ mol m^{-2} s^{-1}), internal carbon concentration (µ mol mol^{-1}) and carboxylation efficiency (µ mol m^{-2} s^{-1}/µ mol mol^{-1})

	Stress	Water	IAA 100	IAA 500	Kin 5	Kin 50	Mean
	Control	12.78	10.8	12.25	10.43	12.48	11.75
	Water logging (WL)	8.6	9.46	11.1	8.46	11.83	9.89
	High temperature (HT)	10.43	10.7	11.16	9.46	11.3	10.61
Photo-synthetic rate	WL + HT	8.83	8.5	10.8	7.36	10.23	9.14
	Mean	10.16	9.86	11.33	8.93	11.46	
	CD (P=0.05)	Stress = 0.70 Hormone = 0.78					
		Stress x Hormone = N.S.					
	Control	220.33	219.33	212.33	221.33	212.33	217.13
	Water logging (WL)	286.66	286	272.66	283.33	273.66	280.46
	High temperature (HT)	241.66	243.33	236.66	243.33	234.33	239.86
Internal carbon	WL + HT	274	273.33	229.33	276.33	263.66	263.33
	Mean	255.66	255.5	237.75	256.08	246	
	CD (P=0.05)	Stress = 9.83 Hormone = 10.99					
		Stress x Hormone = N.S.					
	Control	5.79	4.92	5.76	4.71	5.87	5.41
	Water logging (WL)	2.99	3.3	4.06	2.98	4.32	3.53
	High temperature (HT)	4.31	4.39	4.71	3.88	4.82	4.42
Carboxy-lation efficiency	WL + HT	3.21	3.1	4.1	2.66	3.87	3.39
	Mean	4.08	3.93	4.66	3.56	4.72	
	CD (P=0.05)	Stress = 0.28 Hormone = 0.32					
		Stress x Hormone = N.S.					

Table 2: Interactive effect of various hormonal concentrations (µM) and stress treatment at stage II (80 DAS) on stomatal conductance, transpiration rate (µ mol m^{-2} s^{-1}) and water use efficiency (µ mol m^{-2} s^{-1} /µ mol m^{-2} s^{-1})

	Stress	Water	IAA 100	IAA 500	Kin 5	Kin 50	Mean
	Control	245.33	250.33	269.66	226.33	282.66	254.86
	Water logging (WL)	131.33	142	203	162	227.66	173.2
	High temperature (HT)	177.66	130	212.66	146.66	217.66	176.93
Stomatal conduc-tance	WL + HT	151.66	146	207.33	162.33	216	176.66
	Mean	176.5	167.08	223.16	174.33	236	
	CD (P=0.05)	Stress = 18.83 Hormone = 9.31					
		Stress x Hormone = N.S.					
	Control	2.62	2.94	3.34	2.29	3.03	2.84
	Water logging (WL)	1.67	1.99	2.8	1.33	2.55	2.07
	High temperature (HT)	2.01	2.22	2.72	1.94	2.49	2.27
Trans-piration rate	WL + HT	1.61	1.9	2.41	1.12	2.83	1.97
	Mean	1.98	2.26	2.82	1.67	2.72	
	CD (P=0.05)	Stress = 0.22 Hormone = 0.25					
		Stress x Hormone = N.S.					
	Control	4.89	3.67	3.65	4.57	4.11	4.18
	Water logging (WL)	4.94	4.79	4.08	6.87	4.65	5.06
Water use efficiency	High temperature (HT)	5.24	4.86	4.05	4.91	4.59	4.73
	WL + HT	5.7	4.5	4.6	6.56	3.66	5
	Mean	5.19	4.45	4.09	5.73	4.25	
	CD (P=0.05)	Stress = 0.47 Hormone = 0.53					
		Stress x Hormone = 1.63					

Table 3: Interactive effect of various hormonal concentrations (µM) and stress treatment at stage II (80 DAS) on relative water content (%), osmotic and water potential (-Mpa)

	Stress	Water	IAA 100	IAA 500	Kin 5	Kin 50	Mean
	Control	78.93	81.82	83.05	82.22	84.76	82.16
	Water logging (WL)	72.02	81.62	85.39	80.38	85.88	81.06
Relative water content	High temperature (HT)	70.78	70.49	83.92	84.54	87.28	79.4
	WL + HT	74.87	83.17	91.46	82	88.65	84.03
	Mean	74.15	79.28	85.95	82.29	86.65	
	CD (P=0.05)	Stress = N.S. Hormone = 3.89					
		Stress x Hormone = N.S.					
	Control	1.14	1.11	1.33	1.77	1.13	1.33
	Water logging (WL)	1.82	1.55	1.15	1.86	1.2	1.51
	High temperature (HT)	1.59	1.22	1.034	1.73	1.16	1.35
Osmotic potential	WL + HT	1.87	1.91	1.49	1.91	1.35	1.7
	Mean	1.6	1.45	1.25	1.82	1.25	
	CD (P=0.05)	Stress = 0.15 Hormone = 0.16					
		Stress x Hormone = 0.32					
	Control	0.52	0.45	0.48	0.52	0.48	0.49
	Water logging (WL)	0.92	0.9	0.77	0.86	0.69	0.83
Water potential	High temperature (HT)	0.79	0.71	0.63	0.82	0.66	0.72
	WL + HT	0.79	0.78	0.58	0.85	0.65	0.72
	Mean	0.8	0.71	0.62	0.76	0.62	
	CD (P=0.05)	Stress = 0.06 Hormone = 0.07					
		Stress x Hormone = 0.13					

internal carbon concentration increased in stressed plants (Table 1) the increase being more in WL (280.46 µ mol mol^{-1}; 29.16% increase over control) followed by WL+HT (263.33; 21.27% reduction) and HT (239.86; 10.46% reduction) with respect to control (217.13). Hormonal concentrations at higher level alleviated the stress effect (237.75 and 246.00 in IAA500 and Kin50 respectively; the per cent reduction over water spray being 7.00 and 3.77 respectively in IAA500 and Kin50). A significant decrease in carboxylation efficiency of stressed plants (Table 1) was observed (3.53, 4.42 and 3.39 µ mol m^{-2} s^{-1} /µ mol mol^{-1} respectively in WL, HT and WL+HT) as compared to control plants (5.41). Further, the higher level of hormones mitigated the adverse effect of various stresses (4.66 and 4.72 respectively in IAA500 and Kin50).

Likewise, the stomatal conductance (Table 2) also reduced in the plants exposed to various stresses (173.20, 176.93, 176.66 and 254.86 m mol m^{-2} s^{-1} in WL, HT, WL+HT and control respectively). IAA500 as well as Kin50 spray were effective in maintaining the adverse effect of various abiotic stresses on stomatal conductance. Accordingly, a significant decrease in

transpiration rate (Table 2) was noticed in the stressed plants, the reduction being maximum in WL + HT (1.97) and WL (2.07) followed by HT (2.27) as compared to control (2.84). The deleterious effect of various stresses was successfully overcome by the application of IAA500 as well as Kin50 if sprayed one day prior to stress treatment. Water use efficiency prominently increased in stressed plants (Table 2), the increase being maximum in WL (5.06) and WL + HT (5.00) followed by HT (4.73), while in control it was 4.18 μ mol m^{-2} s^{-1} /μ mol mol^{-1}. Here again, IAA100 (4.45), IAA500 (4.09) as well as Kin50 (4.25) spray mitigated the stress effect. However, application of Kin5 was unable to reduce the deleterious effect of WL (6.87) and WL + HT (6.56) as evident from the increase in water use efficiency in comparison to control.

Photosynthetic rate, carboxylation efficiency (Table 1), stomatal conductance and transpiration rate (Table 2) decreased in response to various stresses, while internal carbon content (Table 1) and water use efficiency (WUE) (Table 2) increased. IAA500 as well as Kin50 spray ameliorated the deleterious effects of WL and HT. Further, IAA100 was also effective in increasing the transpiration rate and decreasing WUE in stressed plants. Our results correspond to those of Chatterjee *et al.* (1976), who noticed 14.33 % increases in photosynthetic CO_2 fixation due to foliar application of 20 ppm IAA in rice varieties. Moreover, the foliar spray of BA was also found to counteract the deleterious effects of water logging on stomatal conductance and photosynthesis in tomato (Bradford, 1983) and maize (Goswami and Jaidyal, 1987). Zeatin also increased photosynthesis as well as transpiration in leaves of sunflower (Goswami and Srivastava, 1985) and ameliorated the deleterious effects of water logging in wheat plants (Gadallah, 1999). Cytokinin also played a major role in formation of photosynthetic and generative apparatus of plants, which can promote a higher level of CO_2 fixation (Likholat *et al.*, 1984). Verma et al. (2008 and 2009) have reported the foliar application of aliphatic alcohals had stimulated the mobilization of photosynthates to the kernels. Sharma and sadhna, 2012 also reported that the foliar sprays of growth regulating substances (IAA and ethrel) altered the source - sink relationship by diverting the photo assimilates to the desirable sinks in groundnut. Under water logging stress plants showing symptoms like water and nutrients stress and it has also been observed by Dalal and Nandkar, 2011 that the yield of mustard can be increased by the proper management of water supply and the required quantity of nutrient supply.

Various abiotic stresses did not affect the relative water content (RWC) of plants significantly (Table 3). Hormonal application at both lower as well as higher concentrations was effective in reducing the adverse effect of stresses (79.28, 85.95, 82.29, 86.65 and 74.15% respectively in IAA100, IAA500, Kin5, Kin50 and water). The application at higher concentrations (IAA500 and Kin50) was however more effective. WL + HT and WL showed deleterious effect on leaf osmotic potential, however exposed to high temperature (-1.35MPa) had no deleterious effect (-1.70 and -1.51Mpa respectively) compared to control (-1.33Mpa). Moreover, the application of hormones mitigated the stress effects as indicated from Table 3 (-1.45, -1.25, -1.25 and -1.60 MPa in IAA100, IAA500, Kin50 and water respectively). Kin5 spray was unable to reduce the deleterious effect of stresses. Further, IAA100 spray was unable to mitigate the effect of WL and WL + HT, while IAA500 was not able to reduce the WL + HT effect as clear from the increase in leaf osmotic potential when compared to those of control.

All the stresses markedly affected (Table 3) the leaf water potential (-0.83, -0.72 and -0.72 MPa respectively in WL, HT and WL + HT) as compared to control (0.49). Hormones at lower concentrations were ineffective however at higher concentrations (IAA500, Kin50) they mitigated the deleterious effect of HT as well as WL + HT, but not of WL. Leaf osmotic potential as well as water potential decreased as a result of stress conditions, however the relative water content (RWC) was not affected in response to stresses (Table 3). Osmotic potential was the lowest in WL + HT followed by WL and HT, while water potential was the least in WL followed by HT and WL + HT respectively. However, the deleterious effect was ameliorated on application of IAA500 or Kin50, but at lower concentrations, they were at par with water spray. Our result is in vicinity of *Kuser et al.,* 2006 that water stress significantly reduced (more negative values) the leaf osmotic potential in canola cultivars.

REFERENCES

Bradford, K. J. 1983. Involvement of plant growth substances in relation to leaf gas exchange of flooded tomato plants. *Plant Physiol.* **73:** 480-483.

Chakraborty, K. and Uprety, D. C. 2012. Elevated Co_2 alters seed composition and quality of *Brassica*. *Indian J. Plant Physiol.* **17(1):** 84-87.

Chatterjee, A., Mondel, R. K. and Sircar, S. M. 1976. Effects of growth substances on productivity, photosynthesis and translocation of rice varieties. *Indian J. Plant Physiol.* **19:** 131-138.

Dalal, L. P. and Nandkar, P. B. 2011. Effect of npk fertilizers in relation to seed yield in Brassica juncea (l) var. Pusa bold. *The Bioscan.* **6(1):** 59-60.

Dat, J. F., Capelli, N., Folzer, H., Bourgeade, P. and Badot, P. M. 2004. Sensing and signalling during plant flooding. *Plant Physiol. Biochem.* **42:** 273-282.

Desiraju, S. and Ajay, S. D. 1998. Effect of temperature on photo inhibition and recovery in Indian mustard (*Brassica juncea*). *Ann. Plant Physiol.* **12(1):** 56-61.

Drew, M. C. 1992. Soil aeration and plant root metabolism. *Soil Sci.* **154:** 259-268.

Gadallah, M. A. A. 1999. Effect of kinetin on growth, grain yield and mineral elements in wheat plants growing under excess salinity and oxygen deficiency. *Plant Growth Regulation.* **27(2):** 63-74.

Goswami, B. K. and Srivastava, G. C. 1985. Effect of ascorbic acid and zeatin on water stress induced photosynthesis and respiration in sunflower leaves. *Indian. J. Plant Physiol.* **28:** 350-357.

Goswami, C. L. and Jaidyal 1987. Effect of BA and GA_3 on stomatal resistance of maize leaf under waterlogging. *Indian J. Plant Physiol.* **30:** 317-319.

Jackson, M. B. and Colmer, T. D. 2005. Response and adaptation by plants to flooding stress. *Ann. Bot.* **96:** 501-505.

Kauser, R., Athar, H. U. R and Ashraf, M. 2006. Chlorophyll luorescence: a potential indicator for rapid assessment of water stress tolerance in Canola (B*rassica napus* l.) *Pak. J. Bot.* **38(5):** 1501-1509.

Likholat, T. V., Nilovskaya, N. T., Pomelov, A. V. and Morozova, E. W. 1984. Effect of kinetin treatment on productivity and certain

physiological indices of wheat under different conditions of irradiance. *Soviet Plant Physiol*. **31:** 13-18.

Rensburg, L. V. and Kriiger, G. H. J. 1993. Comparative analysis of differential drought stress induced suppression of and recovery in carbon dioxide fixation: Stomatal and non stomatal limitation in *Nicotiana tabaccum*. *J. Plant Physiol*. **142:** 296-306.

Scholander, P. F., Hammel, H. T., Bradstreet, E. D. and Hemmingsen, E. A. 1965. Sap pressure in vascular plants *Sci*. **148:** 339-346.

Sharma, P. and Sardana, V. 2012. Effect of growth regulating substances on the Chlorophyll, nitrate reductase, leghaemoglobin Content and yield in groundnut (*Arachis hypogaea*). *The Bioscan*. **7(1):** 13-17.

Subbaiah, C. C. and Sachs, M. M. 2003. Molecular and cellular adaptations of maize to flooding stress. *Ann. Bot*. **91:** 119-127.

Verma, A., Kaur, B., Malik, C. P., Sinsinwar, Y. K. and Gupta, V. K. 2009. Recent evelopment in Groundnut: Carbon assimilation ,Pathology, Molecular Biology .In Crop Breeding and Biotechnology Ed. Malik, C. P., Wadhwani, C. and Kaur, B. Pointer Publishers, Jaipur, pp. 161-191.

Verma, A., Malik, C. P., Sinsinwar, Y. K. and Gupta, V. K. 2008. Role of some growth regulators on crop physiology parameters influencing productivity in peanut. *J. Pl. Sci. Res*. **24:** 167-170.

Weatherlay, P. E. 1965. The state and movement of water in living organism. In: *Crop and Environment*. Fogged, G.E. (ed.) Cambridge Uni. Press, London and New York. pp. 157-184.

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