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Physiological Basis of Cytokinin induced Drought Tolerance in Wheat (*Triticum aestivum* L.)

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ABSTRACT

Understanding of physiological mechanisms that enable wheat (*Triticum aestivum* L.)plants to adapt to water deficit stress condition and maintain growth and productivity during stress period is very important.Experiments werecarried out to screen the twenty five minicore wheat germplasms for water deficit stress tolerance and to study the effect of cytokinin (BA;40 µM) on physiological traits, photosynthesis and gas exchange parameters under two different water regimes in four contrasting wheat cultivars viz., HD 2987 and DBW 44 (relatively drought tolerant), HD 2888 and HD 2733 (relatively drought susceptible),differing in their tolerance to drought. Sampling was done at vegetative growth stage (35-40DAS) of wheat development. Study revealed that there was reduction in plant water status (RWC), membrane stability index (MSI), photosynthetic efficiency (Chlorophyll and photosynthesis) during water deficit stress condition. Application of cytokinin improves the physiological traits in terms of MSI, RWC, and photosyntheticrateat the vegetative stage of wheat.

Keywords: cytokinin, wheat, drought tolerance, Net Photosynthetic Rate,Photosynthesis

INTRODUCTION

Wheat is one of the premier cereal crops of worldwide importance which is grown under a wide range of climatic conditions. India is one of the major producers of wheat and it is maintaining its second position of wheat producing nation after china (Meena *et al.*, 2013). Wheatcontributes about 30% to the food basket of the country (Habibpor *et al.*, 2011; Dadbakhsh *et al.*, 2012). Abiotic stressesarethe primary cause of crop losses worldwide, reducing average yields for major crop plants by more than 50%. Drought is the most serious environmental factor limiting the productivity of wheat crop, with devastating economical and sociological impact. Estimates indicate that 25% of the world's agricultural lands are now affected by water stress. Moreover, the faster-than-predicted changes in global climate (IPCC, 2007) indicated that drought episodes will become more frequent because of the long-term effects of global warming.Mitigation of drought due to imminent climate change and to enhance wheat productivityis the key to improve wheat productivity (Singh *et al.*, 2012). Plants use a combination of different strategies to avoid or tolerate drought stressof physiological mechanisms that enable plants to adapt to water deficit and maintain growth and productivity during stress period could help in screening and selection of tolerant genotypes and using this trait in breeding programs (Zaharieva *et al.*, 2001). Among the physiological processes, photosynthesis is the basic determinant of plant growth and productivity and the ability to maintain the rate of carbon assimilation under environmental stress is fundamental importance to plant production (Lawlor, 1995). Various physiological and biochemical effects of phytohormone and plant growth regulators (PGR's) on plant systems have been documented.Cytokinins (CKs) are known to regulate several aspects of plant growth and development, including the response of plants to abiotic stress (Rivero *et al.*, 2007). CKs regulate stomatalbehaviour (Hegele *et al.*, 2008), formation and protection of cellular structures (Chiappetta *et al.*, 2006), and induction and activation of protein synthesis

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(Chernyadev, 2005). Theirrole as senescence-retarding hormones has been found and exogenous application has been demonstrated to prevent the degradation of chlorophyll and photosynthetic protein.Therefore, an experiment was conducted to study the effect of CKs on drought stress responses of wheat.

MATERIALS AND METHOD

Plant Material

For screening purpose twenty five wheat genotypes (HD 2733, PBW 343, NI 5439, HI 1563, GW 322, HD 2637, HD 2781, DL 788-2, HD 2864, HD 2894, HD 2888, Chiriya 3, DBW 14, DBW 17, HD 2967, HD 2932, C 306, HD 2987, DBW 44, HW 2036, HW 4060, HW 2055, HW 2063 and HD 2687) were taken as suggested by breeders and on the basis of days of survival and membrane stability index (MSI %), 15 days after sowing, four wheat varieties were selected DBW 44 and HD 2987 (relatively drought tolerant), HD 2888 and HD 2733 (relatively drought sensitive) were selected.

Sowing was done in 30 cm earthen pots filled with clay loam soil and farmyard manure in 3:1 ratio during winter seasonand supplied with 60, 60 and 60 kg/haof N, P, K, respectively, in the form of urea, single super phosphate and muriate of potash (MOP) at the time of sowing as basal dose. Remaining 60 kg N/hawas given after 21 days of sowing. Plants were subjected to water stress after 21- 25 days after sowing byproviding water deficit stress condition during reproductive stage. PGR treatment was given by spraying 6-Benzyl amino purine (BA) 40 µM concentrations at 30 DAS.

Plant Sampling

The plants were sampled and observations were taken for growth, physiological, biochemical parameters at 35 DAS. Three replications with five pots per replication were taken for each variety. Upper most fully expanded flag leaf was used for recording physiological (RWC, MSI, chlorophyll content and Photosynthetic rate) parameters.

Relative Water Content

Leaf relative water content (RWC) was estimated by recording the turgid weight of 0.5 g fresh leaf samples by keeping in water for 4 hours, followed by drying in hot air oven at 65^{O}Ctill constant weight was achieved (Weatherley, 1950). RWC is estimated by equation 1:

RWC=[(Fresh wt.–Dry wt.)/(Turgid wt.–Dry wt.)]X100 [Eq.1]

Membrane Stability Index

Membrane stability index (MSI) was estimated by equation 2 (Sairam *et al.*, 1997). It was calculated as:

$$MSI = [1 - (C_1/C_2)] \times 100 \quad [Eq.2]$$

Total Chlorophyll Estimation

The procedure for estimation of chlorophyll content in plants is based on the absorption of light by chlorophyll extracts, prepared by incubating the leaf tissues in Dimethyl sulfoxide(DMSO). DMSO renders plasmalemma permeable thereby; causing the leaching of the pigments (Hiscox and Israelstam, 1979) was expressed by equation 3 as mg/g FW

Total chlorophyll =

$$(20.2 \times OD_{645} + 8.02 \times OD_{663}) \times (V/1000) \times w \quad [Eq.3]$$

Net Photosynthetic Rate

Rate of photosynthesis was measured on leaves using portable Infrared Gas Analyzer (IRGA LI-6400 Model). The rate of photosynthesis was measured by operating the IRGA in the closed mode. The net photosynthetic rate was expressed as µmoles m^2/s

Statistical Analysis

The data was analyzed statistically using 3 factorial CRD (physiological analysis) and CD at 5% and ANOVA were calculated. The analysis was done using OPSTAT programme available online on CCSHAU, HISAR web site.

RESULTS AND DISCUSSION

Screening of Wheat Genotypes for Drought Tolerance

For screening purpose 25 wheat genotypes were taken as suggested by breeders.Water stress was provided by withholding irrigation and on the basis of days of survival and membrane stability index (MSI; %) under water deficit stress, four wheat genotypes were selected DBW 44 and HD 2987 (relatively drought tolerant), HD 2888 and HD 2733 (relatively drought sensitive) as shown in Fig. 1 and 2.

Relative Water Content

Relative water content (RWC) was measured at vegetative (35-40 DAS) developmental stages of wheat to assess the water status of the plants (Table 1). Under control (irrigation) conditions, wheat plants mean relative water content was 86.12%. However, the mean RWC was

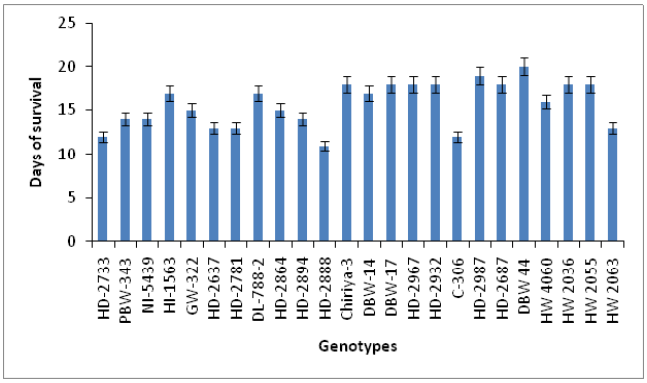

Fig.1: Screening of twenty five wheat genotypes for drought tolerance and susceptibility on the basis of days of survival under water defict stress condition.

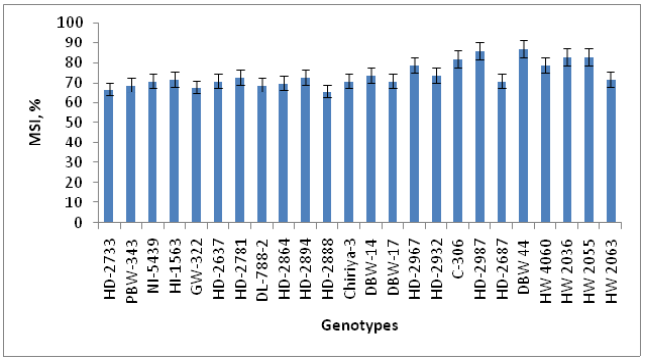

Fig. 2: Screening of twenty five wheat genotypes for drought tolerance and susceptibility on the basis of membrane stability index (MSI, %) under water defict stress condition.

Table 1: Application of BA(40 µM) on relative water content at vegetative stage in four contrasting cultivars affected by water deficit stress in wheat.

Stage	**Vegetative Stage (35-40 DAS)**				
Treatments/Variety	HD2987	DBW44	HD2888	HD2733	Mean
Control (C)	91.03	92.08	88.26	73.43	86.19
C + BA	93.17	93.22	90.54	79.35	89.07
Water Stress (WS)	73.52	74.61	72.73	63.97	71.20
WS+BA	80.72	81.35	79.24	73.47	78.69
Mean	84.61	85.31	82.92	72.75	
Factor	Variety (V)	Treatment (T)	Interaction (VxT)		
SEm ±	0.247	0.214	0.495		
CD (P = 0.05)	0.695	0.602	1.389		

Table 2: Application of BA(40 µM) on membrane stability index at vegetative stage in four contrasting cultivars affected by water deficit stress in wheat.

Stage	Vegetative Stage (35-40 DAS)				
Treatments/Variety	HD2987	DBW44	HD2888	HD2733	Mean
Control (C)	26.65	21.68	20.33	15.32	20.99
C + BA	29.31	24.67	23.34	18.34	23.91
Water Stress (WS)	21.00	16.32	14.67	10.68	15.66
WS+BA	23.68	20.34	18.33	13.66	19.00
Mean	**25.16**	**20.75**	**19.16**	**14.50**	
Factor	Variety (V)	Treatment (T)	Interaction (VxT)		
SEm ±	0.112	0.097	0.224		
CD (P = 0.05)	0.314	0.272	0.629		

declined by17% under water deficit stress condition in comparison to control wheat plants. While, water deficit plants with cytokinin increased the mean RWC by 11% in comparison to water deficit plants without cytokinin. Cultivar differences were observed more under water deficit stress as compared to the control wheat plants affected by cytokinin. Under control, wheat plants of cultivar DBW 44 and HD 2987 showed enhancement in RWC by around 1% and 3% treated by cytokinin as compared to 9%and 10%, respectively in water deficit plants treated with cytokinin. While, in HD 2888 it was 2% and 10% and in sensitive cultivar HD 2733 it was 7% and 15%, respectively.

Membrane Stability Index

Membrane Stability Index (MSI) was measured at vegetative state (35 DAS) different developmental stages of wheat (Table 2). Under control condition, wheat plants mean MSI (%) was 82.88%whereas, the mean MSI was declined by15% under water deficit stress condition in comparison to control wheat plants. While, water deficit plants with cytokinin (BA) increased the mean MSI by 5% in comparison to water deficit plants without CKs. Control wheat plants of cultivar DBW 44 and HD 2987 showed enhancement in MSI by around 1% and 2% treated by CKs as compared to 4%and 5%, respectively in water deficit plants treated with CKs. While, in HD 2888 it was 2% and 4% and in sensitive cultivar HD 2733 it was 4% and 5%, respectively.

Total Chlorophyll Content

Total chlorophyll content (mg/g/dw) is one of the important traits of senescence, was estimated under two water regimes in all the four cultivars differing in their tolerance to water deficit stress at vegetative stage of wheat (Table 3). Under controlled conditions wheat plants mean total chlorophyll content was (10.97). However, the mean total chlorophyll content was declined by24%under water stress in comparison to control wheat plants. While, water deficit plants with CKs increased the total chlorophyll content by 10% in comparison to water deficit plants without CKs. Cultivar differences were observed more under water deficit stress as compared to the control wheat plants affected by CKs. Control wheat plants of cultivar DBW 44 and HD 2987 showed enhancement in total chlorophyll by -3% and 6% when treated withCKs as compared to 7% and 12% respectively in water deficit plants treated with CKs. While, in HD 2888 it was 3% and 7% and in sensitive cultivar HD 2733 it was 6% and 9%, respectively.

Photosynthesis Rate

Photosynthesis rate (µmols $CO_2/m^2/s$) was estimated to assess the CO_2 assimilation efficiency under different treatments at vegetative developmental stage of wheat (Table 4). Under control condition, wheat plants mean photosynthesis rate was 21.1µmols $CO_2/m^2/s$. However, water deficit stress reduced mean photosynthesis rate by 26%in comparison to control wheat plants. While, water deficit plants with CKs recordedincrease inmean photosynthesis rate by 22% in comparison to water deficit plants without CKs. Control wheat plants of cultivar DBW 44 and HD 2987 showed enhancement in photosynthesis by around 14% and 10%, as compared to 24%and 12%, respectively in water deficit plants treated with CKs. While, in HD 2888 it was 15% and 25%, under control and water deficit condition respectively, treated with CKs and in sensitive cultivar HD 2733 it was 20% and 28% under treated condition.

Water scarcity creates the most significant limitations

Table 3: Application of BA(40 µM)on total chlorophyll content (mg/gdw)at vegetative stage in four contrasting affected by water deficit stress in wheat

Stage	**Vegetative Stage (35-40 DAS)**				
Treatments/Variety	HD2987	DBW44	HD2888	HD2733	Mean
Control (C)	11.50	11.78	11.36	9.25	10.97
C + BA	12.19	12.14	11.74	9.81	11.47
Water Stress (WS)	8.76	8.93	8.64	7.15	8.37
WS+BA	9.81	9.56	9.28	7.82	9.11
Mean	**10.56**	**10.60**	**10.25**	**8.50**	
Factor	Variety (V)	Treatment(T)	Interaction (VxT)		
SEm ±	0.12	0.11	0.25		
CD (P = 0.05)	0.36	0.31	NS		

Table 4: Application of BA (40 µM) on photosynthesis rate (µmoles CO2/m2/s) atvegetative stage in four contrasting affected by water deficit stress in wheat.

Stage	Vegetative Stage (35-40 DAS)				
Treatments/Variety	HD2987	DBW44	HD2888	HD2733	Mean
Control (C)	26.65	21.68	20.33	15.32	20.99
C + BA	29.31	24.67	23.34	18.34	23.91
Water Stress (WS)	21.00	16.32	14.67	10.68	15.66
WS+BA	23.68	20.34	18.33	13.66	19.00
Mean	25.16	20.75	19.16	14.50	

to crop productivity (Boyer, 1982). Detrimental effect of cell dehydration on physiological and biochemical reactions and consequently growth and productivity is well documented (Lawlor, 1995).Various observations from this experiment showed that the application of CKs in terms of foliar spray on the four cultivar of wheat enhanced their tolerance to drought. In the present study, tolerant cultivars were able to maintain greater RWC (85-90%) compared to susceptible ones (70-75%) under water deficit stress condition. Tolerant cultivars had higher values of RWC indicating their greater ability to water uptake from the soil as compared to susceptible ones. A dramatic decline was observed in leaf RWC under water stress condition in various field crops (Siddique *et al.*, 2000, Basu *et al.*, 2004). Thus, an ability to maintain high RWC under stress conditions could be an adaptive feature. It has been hypothesized that genotype which keeps their stomata open under stress condition while maintaining adequate leaf RWC can be considered as suitable for dry region (Blum *et al.*, 1996). Membrane stability is a widely used criterion to assess crop drought tolerance, since water stress caused water loss from plant tissues which seriously impairs both membrane structure and function. In the present study, tolerant cultivars were able to maintain higher MSI (85%) in comparisonto susceptible ones (75%) under water deficit stress condition. Application of CKs enhanced drought tolerance in all the cultivars but effect was more pronounced in case of susceptible cultivars. The results from electrolyte leakage measurements in this study showed that membrane integrity was more conserved intolerant genotypes than susceptiblevarieties; this is in agreement with the conclusion of Almeselmani *et al.* (2011) that electrolyte leakage was correlated with drought tolerance.

The well-known phenomenon in senescing leaves is the loss of chlorophyll content. In this study, the chlorophyll in tolerant cultivars (DBW 44 and HD 2987) was higher than sensitive one (HD 2888 and HD 2733) during leaf senescence.Leaf senescence was delayed by cytokinin application in all the four cultivars studied. The present study revealed that exogenous application of CKs *via* foliar application helped plants maintaining the chlorophyll pigments and hence mitigated the adverse effects of drought stress. These findings are in line with some earlier reports on okra (Amin *et al.*, 2009) and in wheat (Azzedine *et al.*, 2011).The final stage of leaf development is inevitably senescence with a decline in physiological activity. Senescence of flag leaves in four wheat cultivars grown in the pot was characterized by photosynthetic rate.The stability of photosynthetic components could be attributed tomaintenance of positive leaf RWC under stress as a result of osmotic adjustment (Basu *et al.*, 2004). In the present investigation, drought stress caused a marked reduction in net photosynthesisin all the four genotypes. However, the drought tolerant cv. HD 2987 and DBW 44 was superior to the drought sensitive genotypes HD 2888 and HD 2733 with respect to this gas exchange attributes (Table 4). Drought-induced reduction in photosynthesis rate has been reported earlier in a number of crops including wheat (El Hafid *et al.*, 1998), maize (Ali and Ashraf, 2011). In the present study, exogenous application of CKs mitigated the adverse effects of drought on photosynthesis in all the four wheat genotypes.

CONCLUSION

Prolong water stressleadtochanges in various morphological and physiological traits that adversely affect growth and performanceofwheat.These findings suggest the role of CKs in plant development. CKs play a significant role in plants at physiological level. CKs treatment was more effective under water stress condition. The experimental evidences suggest that it may be possible to enhance the drought tolerance by delaying drought induced leaf senescence through more synthesis of cytokinin.

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