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Photosynthetic Pigments and Hill Reaction Activity of Wheat Seedlings under Drought Stress as Affected by Exogenously Applied Cytokinins

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ABSTRACT

Water deficit arises due to both; insufficient rainfall and soil water. Plants experiencing it during growing season and are unable to sustain high crop yield. Cytokinins are growth hormones that regulate formative procedures such as apical dominance, chloroplast biogenesis and nutrient mobilization etc; the diminished level of these compounds during stress point towards this constraint. Presently exogenous application of natural cytokinin (Kn @ 10, 20 and 40 mg/L) and synthetic (BA @ 25 and 50 mg/L) was studied on photosynthetic parameters of wheat cultivars under water deficit conditions. PEG-induced stress significantly decreased the content of total chlorophylls in wheat cvs (HD2967, PBW660, WH1105 and PBW658). Exogenous application of both cytokinins (Kn and BA) significantly increased the accumulation of chlorophyll and hill reaction activity in leaves of wheat seedlings. The content of carotenoids was lesser in control of all selected cultivars and increased significantly under stress and also when cytokinins were supplied exogenously. From among the tested levels Kn @ 40 mg/L and BA @ 50 mg/L was found more promotary. The accumulation of more photosynthetic pigments and increase in Hill reaction activity led to increased vigour of seedlings under water deficit conditions.

Key words: photosynthetic pigments, Hill reaction, cytokinins, wheat.

Introduction

World wheat utilization is seen to decline slightly mostly because of reduced feed use (FAO 2016). It has been found that wheat contain the higher value of vegetable protein in the food of human beings, as it contains higher content of proteins and amino acids than the other major cereals like maize rice (NPCS board, 2012).

Abiotic stresses caused by environmental factors could adversely affect the growth and development of crops (Mittler*et al* 2006). Crops respond to the abiotic stresses with various modifications on morphological, cellular, physiological, biochemical and molecular level (Zhou *et al* 2015). In the last decade, lots of studies focused on the response of crops to a single stress (Siddiqui*et al* 2013). However, several abiotic stresses usually occur concurrently and crops are always subjected to a combination of different abiotic stresses in the field (Suzuki *et al* 2014). Among the abiotic stresses, drought and heat stress are two critical threats to crop growth and sustainable agriculture worldwide (Awasthi*et al* 2014). Drought stress as a consequence of insufficient rainfall or deficient soil moisture might induce various biochemical, physiological and genetic responses in plants, which severely restricted crop growth (Seki *etal* 2007 and Vadez*et al* 2012).

Photosystem II (PSII) is a sensitive component to heat stress (Cajanek*et al* 1998). Chlorophyll fluorescence is an efficient and non-destructive technique to measure the photochemical efficiency of PSII and thereby detect the damage of stress in PSII (Baker *et al* 2004). The maximum potential quantum efficiency of PSII (Fv/Fm) provides an estimate of the maximum quantum efficiency of PSII, which is primarily affected by stress (Sharma *et al* 2012). The cytokinins have been implicated as a limiting factor in the establishment of sink number and sink size in legumes, cereals and Arabidopsis. Ectopic expression of an IPT gene has been shown to increase seed yield in a variety of plants (Guo and Gan 2014). The rapid changes in endogenous levels of cytokinins in wheat have been linked to the expression of specific members of the cytokinin biosynthesis (IPT), degradation (CKX), O-glucosylation and ß-glucosidase gene families (Song *et al* 2012).

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MATERIAL AND METHODS

Four wheat (*Triticumaestivum* L.) genotypes viz. HD 2967, WH1105, PBW660 and PBW658 were obtained from Department of Plant Breeding and Genetics. Only healthy seeds were selected for the present investigation. 20 seeds were sown in each petri-plate using the distilled water and incubated at the room temperature (25±2°C), relative humidity and light was maintained in incubator. Water deficit was maintained by shifting the seedlings to the petri-plates supplemented with the PEG-6000 (-0.4MPa) solution and petri-plates treated as control was maintained as such and different treatments (mentioned in tables) of Cytokinins (Kinetin and Benzyl adenine) were given to each petri-plate.

Hill reaction activity

Hill reaction activitywas estimated by method as given by Cherry (1973).

Extraction

Leaf samples (100mg) were taken and gently groung in 5ml extraction medium (0.067 M Phosphate buffer, pH 7.5 containing 0.35M sucrose). During extraction the temperature was maintained at 0-4°C.

Estimation

Potassium ferricyanide solution was prepared by dissolving sodium chloride (1.02g) and potassium ferricyanide (13mg) in phosphate buffer (25 ml). The reaction was started by mixing 0.5ml of supernatant from above extract with 2.5ml of ferricyanide solution. The tubes were kept in light (approx.5000 lux) for 10 min. and another similar set of experiment was kept in dark. The reaction was stopped by adding 20% TCA (0.3ml). The absorbance was recorded at 420 nm on spectrometer. Hill reaction activity was expressed as decrease in absorbance mg^{-1}Chl h^{-1}.

Chlorophyll content and carotenoid content

Chlorophyll content and carotenoid contentwas estimated by method as given by Hiscox andIsrealstam (1979).

The photosynthetic pigments were extracted from the wheat leaves by placing the 100g of fresh leaves used in the photosynthesis and reflectance measurements in 5ml of the dimethylsulfoxide (DMSO) and extracting for 12 h in the dark. The concentration of the extracted pigments was calculated from the absorbance values at 665,645 and 480 nm.

Chl a = 12.19 (OD 665)-3.45(OD 645) x V/1000xW

Chl b= 21.99(OD 645)-5.32(OD665) x V/1000xW

Total Chl = 20.2(OD 480) + 8.02(OD665) x V/1000 x W

Carotenoids = (OD480) + 0.114(OD665) – 0.638(OD645)

where,

OD_{663} = OD at 663 nm

OD_{645} = OD at 645 nm

OD_{480} = OD at 480 nm

V = Total volume of solution made

W = Weight of sample (g) taken

The chlorophyll contents were expressed as mg chl g^{-1} fresh weight.

Vigour index

Vigour index of seeds were calculated as suggested by Abdul Baki and Anderson (1973).

Vigour index = Germination (%) x epicotyls length (cm)on third day after sowing number of seeds germinated were counted from each petriplate and percentage germination was calculated.

Percent germination =No. of seeds germinated /total number of seeds x 100

Epicotyls length of seedlings was measured with the help of centimeter scale.

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RESULTS AND DISCUSSION

Chlorophyll content

Table 1 depicted the chlorophyll content of the studied wheat genotypes under control, stress conditions and as effected by different concentrations of Kn and BA. The PEG stimulated drought stress significantly decreased the chlorophyll content of all genotypes. The maximum decrease in chlorophyll content was recorded in HD2967 and lesser decrease in chlorophyll content was observed in WH1105. The maximum percentage decrease (over control) was recorded in HD2967 (22.35%) followed by PBW658 (15.43%) and lesser percentage decrease was recorded in WH1105 (12.37%). Yasmeen*et al* (2013) studied drought resistance in wheat and observed that the wheat genotypes under the drought stress significantly resulted in loss of chlorophyll content.

With the different concentrations of Kn there was significant increase in chlorophyll content of all wheat genotypes. The higher chlorophyll was recorded in WH1105 followed by PBW660 and lesser chlorophyll content was observed in PBW658 with the application of Kn @ 10mg/L. The maximum percentage increase over drought was recorded in HD2967 (10.69%) followed by WH1105 (7.32%) and lesser percentage increase was recorded in PBW658 (6.08%). With the application of Kn @ 20mg/L there was further increase in chlorophyll content of studied wheat genotypes. WH1105 accumulated higher chlorophyll content and PBW658 had lesser chlorophyll content with that concentration of Kn. On the other hand the genotype HD2967 followed by PBW660 had higher percentage increase i.e 20.07% and 13.28% respectively. With the further increase in Kn concentration i.ekn @ 40mg/L the chlorophyll content of all genotypes increased further and the genotype WH1105 had higher chlorophyll content whereas PBW658 had lesser chlorophyll content. The maximum percentage increase was observed in HD2967 (22.59%) followed by WH1105 (18.29%) and least percentage decrease was recorded in PBW658 (12.04%). The application of Kn under water stress cause significant ($p<0.05$) increase in leaf chlorophyll content in rice genotypes (Khan *et al* 2016).

The chlorophyll content of different studied wheat genotypes was significantly increased by different concentrations of BA. With the application of BA @ 25mg/L there was significant increase in chlorophyll content and genotype WH1105 followed by HD2967 attained the higher chlorophyll content. The maximum percentage increase (over drought) was recorded in HD2967 (15.18%) and lesser percentage increase was recorded in WH1105 (7.42%). With the application of BA @ 50mg/L, genotype WH1105 had higher chlorophyll content and PBW658 had lesser chlorophyll as compared to other genotypes but the maximum percentage increase was recorded in HD2967 (19.59%) followed by PBW660 and lesser percentage increase was observed in PBW658 (9.47%). BAP application significantly resulted in an increased in chlorophyll content of other wheat genotypes under drought stress (Yasmeen*et al* 2013).

Hill Reaction Activity (HRA)

PEG induced drought stress significantly reduced the HRA of all genotypes and more pronounced effect was observed in PBW658 (Table 2). The genotype HD2967 and WH1105 maintained higher HRA even under the drought stress conditions. The maximum percentage decrease (over control) was recorded in PBW658 (36.37%) followed by PBW660 (33.81%) and WH1105 (23.82%) had lesser percentage decrease. Similarly, Radhika*et al* 2013 found that there was reduction in the Hill reaction activity of other wheat genotypes under drought stress.

Different concentrations of Kn tested significantly reduced the adverse effect of PEG and significantly increased the HRA of all seedlings. With kn 10mg/L, the HD2967 attained higher HRA. The percentage increase over drought was recorded high in PBW658 (11.62%) followed by PBW660 (10.44%) and HD2967 (8.93%). With foliar spray of Kn @ 20mg/L there was further increase in HRA of all genotypes and maximum value was recorded in HD2967 and PBW658 had lower value. The maximum percentage increase was observed again in PBW658 (20.09%) and lesser was recorded in WH1105 (13.98%). The higher concentration of Kni.eKn @ 40mg/L significantly increased the HRA of wheat genotypes and maximum HRA was recorded in HD2967 followed by PBW660. Maximum percentage increase was recorded in PBW658 (38.70%) and lesser percentage increase was observed in WH1105 (25.48%).

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BA application significantly increased the HRA of the studied genotypes. At BA @ 25mg/L the genotype HD2967 showed higher HRA as compared to other genotypes. The maximum percentage increase over drought was recorded in PBW658 (19.76%) and lesser was observed in WH1105 (11.08%). BA @ 50mg/L further increased the HRA and maximum value was recorded in HD2967 and least was recorded in PBW658. Maximum percentage increase was recorded in PBW658 (34.71%) followed by PBW660 (32.36%). There was significant increase in Hill reaction activity with the application of BAP similar observations were recorded earlier by Radhika and Thind (2013).

Carotenoid content

The carotenoid content of all studied genotypes significantly increased with the PEG induced drought stress (Table 3). The genotype PBW658 followed by HD2967 had higher carotenoid content under the controlled conditions. The PEG treated seedlings had further increase in carotenoid content but the maximum carotenoid content was observed in PBW658 followed by PBW660. On the other hand the maximum percentage increase (over control) was recorded in PBW660 (19.74%) followed by PBW658 (19.25%). Earlier, Abdel-Motagally*et al* (2016) recorded that the carotenoids content values were higher (11.40 and 11.37 mg/g of FW) in drought stressed wheat genotypes.

The carotenoids are accessory pigments that protect the photosynthetic apparatus during stress conditions and Kn application further increased formation of these accessory molecules. The maximum increase was recorded in PBW658 followed by PBW660 but the higher percentage increase (over drought) was observed in HD2967 (17.45%) followed by PBW658 (17.18%) and lesser percentage increase was recorded in PBW660 (11.34%). With the application of Kn @ 20mg/L there was further increase in carotenoid content of all genotypes and maximum increase was observed in PBW658 and lesser increase was recorded in WH1105. On the other hand the maximum percentage increase was recorded in genotype WH1105 (30.68%). The genotype HD2967 followed by PBW660 attained the maximum carotenoid content with the application of Kn 40 mg/L but the maximum percentage increase was recorded in HD2967 (57.45%) and lesser percentage increase was recorded in PBW658 (35.76%). Previously, it was observed that the phytohormones like Kn increased the carotenoid content under drought stress conditions in maize genotypes (Shaddad*et al* 2011).

BA application significantly increased the carotenoid content of all seedlings. With @ BA 25mg/L PBW658 showed the maximum carotenoid content as compared to other genotypes and WH1105 had lesser increase in carotenoid content but the maximum percentage increase over drought was observed in WH1105 (39.84%) followed by HD2967 (39.01%). There was further increase in carotenoid content of all the wheat genotypes with the application of BA @ 50mg/L and genotype PBW658 again attained the maximum value of carotenoid content but the maximum percentage increase was recorded in WH1105 (41.83%) followed by HD 2967 (41.37%) and lesser percentage increase was observed in PBW660 (33.34%). It was observed by Hussein *et al* 2009 that application of BA promoted carotenoid content.

Vigour index

PEG induced drought stress significantly reduced the vigour of all the selected genotypes (Table 4). Presently, more VI during controlled conditions but PEG significantly reduced the VI of all the genotypes. The different PEG concentrations reduced the daily mean germination, germination index and mean germination time in wheat (Almaghrabi*et al.* 2012). Different concentrations of Kn significantly increased the VI of the studied wheat genotypes. With the application of Kn @ 10mg/L maximum value was recorded in PBW658 followed by PBW660. On the other hand with the application of Kn @ 20mg/L VI of all the genotypes increased further and the maximum increase was recorded in PBW658. The application of Kn @ 40mg/L further, maximum value was recorded in PBW658 followed by PBW660. On the other hand the maximum percentage increase was recorded in PBW660 (23.91%) followed by PBW658 (18.09%) and least percentage increase was recorded in WH1105 (15.08%). BA also ameliorated the negative effect of drought stress; BA tends to increase the VI of all selected genotype but with the application of BA @ 25mg/L the highest vigour index was recorded in genotype PBW658 followed by PBW660. With the higher concentration of BA i.e BA@ 50mg/L there is more increase in VI of all genotypes. But the higher increase was observed again in PBW658.

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Earlier, BA pre-treatment could overcome the negative effect of salt stress on percentage germination, radical elongation and fresh weight in barley (Cavusoglu*et al.* 2008).

Table 1. Effect of different concentrations of Kn (10, 20 and 40mg/L) and BA (25 and 50mg/L) on chlorophyll content at 10 DAS in wheat under PEG induced drought stress.

Treatments / Genotypes	HD2967	PBW660	WH1105	PBW658
T1-Control	1.018	0.999	1.044	0.987
T2- Stress (PEG)	0.832 (22.35)	0.873 (14.43)	0.929 (12.37)	0.855 (15.43)
CD 5%	**0.00358**	**0.00156**	**0.00221**	**0.00358**
T3-PEG+Kn(10)	0.921 (10.69)	0.934 (6.98)	0.997 (7.32)	0.907 (6.08)
T4- PEG+Kn(20)	0.999 (20.07)	0.989 (13.28)	1.032 (11.08)	0.933 (9.12)
T5-PEG+Kn(40)	1.020 (22.59)	1.009 (15.57)	1.099 (18.29)	0.958 (12.04)
CD 5%	**0.00951**	**0.00184**	**0.0136**	**0.00684**
T6-PEG+BA(25)	0.960 (15.18)	0.956 (9.50)	0.998 (7.42)	0.921 (7.72)
T7- PEG+BA(50)	0.995 (19.59)	0.999 (14.43)	1.024 (10.22)	0.936 (9.47)
CD 5%	**0.00529**	**0.00328**	**0.00282**	**0.00276**

Table 2. Effect of different concentrations of Kn (10, 20 and 40mg/L) and BA (25 and 50mg/L) on Hill reaction activity at 10DAS in wheat under PEG induced drought stress.

Treatments / Genotypes	HD2967	PBW660	WH1105	PBW658
T1-Control	0.956	0.922	0.894	0.821
T2- Stress (PEG)	0.761 (25.62)	0.689 (33.81)	0.722 (23.82)	0.602 (36.37)
CD 5%	**0.0167**	**0.0162**	**0.0045**	**0.0162**
T3-PEG+Kn(10)	0.829 (8.93)	0.761 (10.44)	0.789 (9.27)	0.672 (11.62)
T4- PEG+Kn(20)	0.868 (14.06)	0.811 (17.70)	0.823 (13.98)	0.723 (20.09)
T5-PEG+Kn(40)	0.974 (27.98)	0.922 (33.81)	0.906 (25.48)	0.835 (38.70)
CD 5%	**0.0134**	**0.0969**	**0.00962**	**0.00247**
T6-PEG+BA(25)	0.866 (13.79)	0.801 (16.25)	0.802 (11.08)	0.721 (19.76)
T7- PEG+BA(50)	0.967 (27.06)	0.912 (32.36)	0.899 (24.51)	0.811 (34.71)
CD 5%	**0.0116**	**0.0117**	**0.00159**	**0.00287**

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Table 3. Effect of different concentrations of Kn (10, 20 and 40mg/L) and BA (25 and 50mg/L) on carotenoid content at 10DAS in wheat under PEG induced drought stress.

Treatments / Genotypes	HD2967	PBW660	WH1105	PBW658
T1-Control	0.0473	0.0471	0.0421	0.0483
T2- Stress (PEG)	0.0510 (7.87)	0.0564 (19.74)	0.0502 (19.23)	0.0576 (19.25)
CD 5%	**0.0453**	**0.0654**	**0.0211**	**0.0443**
T3-PEG+Kn(10)	0.0599 (17.45)	0.0628 (11.34)	0.0578 (15.13)	0.0675 (17.18)
T4- PEG+Kn(20)	0.0659 (29.21)	0.0699 (23.93)	0.0656 (30.68)	0.0701 (21.70)
T5-PEG+Kn(40)	0.0803 (57.45)	0.0789 (39.89)	0.0725 (44.42)	0.0782 (35.76)
CD 5%	**0.650**	**0.0653**	**0.0231**	**0.0543**
T6-PEG+BA(25)	0.0709 (39.01)	0.0721 (27.83)	0.0702 (39.84)	0.0743 (28.99)
T7- PEG+BA(50)	0.0721 (41.37)	0.0752 (33.34)	0.0712 (41.83)	0.0780 (35.41)
CD 5%	**0.231**	**0.0453**	**0.0343**	**0.0221**

Table 4. Effect of different concentrations of Kn (10, 20 and 40mg/L) and BA (25 and 50mg/L) on vigour index in wheat under PEG induced drought stress.

Treatments / Genotypes	HD2967	PBW660	WH1105	PBW658
T1-Control	275.5	290.0	256.5	300.0
T2- Stress (PEG)	199.8 (38.88)	207.0 (40.09)	195.5 (31.20)	221.0 (35.75)
CD 5%	**0.664**	**0.530**	**0.347**	**0.283**
T3-PEG+Kn(10)	218.0 (9.11)	220.0 (6.28)	209.0 (6.96)	231.0 (4.52)
T4- PEG+Kn(20)	221.5 (10.61)	232.0 (12.07)	216.0 (10.48)	237.0 (7.23)
T5-PEG+Kn(40)	231.0 (15.61)	256.5 (23.91)	225.0 (15.08)	261.0 (18.09)
CD 5%	**0.235**	**0.021**	**0.166**	**0.167**
T6-PEG+BA(25)	209.0 (4.60)	216.0 (4.34)	204.0 (4.34)	237.0 (7.24)
T7- PEG+BA(50)	228.0 (14.11)	231.0 (11.59)	221.5 (13.04)	254.0 (14.93)
CD 5%	**0.144**	**0.204**	**0.353**	**0.203**

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