Pak. J. Agri. Sci., Vol. 57(3), 725-733 ; 2020
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/20.8183
http://www.pakjas.com.pk

DROUGHT AMELIORATING EFFECT OF EXOGENOUS APPLIED CYTOKININ IN WHEAT

Muhammad Aown Sammar Raza[1,*], Muhammad Saqlain Zaheer[1,2], Muhammad Farrukh Saleem[3], Imran Haider Khan[4], Salman Ahmad[1] and Rashid Iqbal[1]

[1]Department of Agronomy, The Islamia University of Bahawalpur, Pakistan; [2]School of Agriculture, Food and Wine, The University of Adelaide, Australia; [3]Department of Agronomy, University of Agriculture Faisalabad, Pakistan; [4]Department of Agronomy, Nanjing Agricultural University, China
***Corresponding author's e-mail: aown_samar@yahoo.com**

Water deficit reduces crop productivity. Cytokinin (CK) is a growth regulator which is a potent coordinator between physiological traits and senescence. A study was carried out to investigate the drought ameliorating effects of exogenous applied cytokinin in wheat. Experiment was comprised of seven treatments(C= Control, DT= Drought at tillering, DT+CK= Drought at tillering + CK, DA= Drought at anthesis, DA+CK= Drought at anthesis + CK, DG= Drought at grain filling, DG+CK= Drought at grain filling + CK).Cytokinin was applied foliarly@ 25 mg L^{-1} solution of cytokinins. CK application under drought stress significantly increased growth and yield characteristics ($P < 0.05$). An increase of 4.36%, 7.21% and 5.55% in grain yield was recorded with CK application when drought was applied at tillering, anthesis and grain filling stage, respectively. Other physiological parameters increased by CK included photosynthesis rate, leaf area index, chlorophyll content, activities of ascorbate peroxidase, catalase, peroxidase, superoxide dismutase and endogenous hormonal level of kernels. It was concluded that CK improved plant growth, minimized ill impacts of reactive oxygen species and increase transfer of assimilates to grains under drought.

Keywords: Cytokinin, drought, grain yield, leaf area index, photosynthetic rate.

INTRODUCTION

Food production will have to increase to meet the demand of increasing world population. Daryanto *et al.* (2016) estimated that in the next 20 years, wheat production has to increase up to 60% to meet market demand. Water deficit stress is a major abiotic stress in the world especially in dry climates. Water deficit condition decreased plant growth and actual grain yield (Raza *et al.*, 2012a). Limited water availability for the plant negatively effect on plant water relationship that alters the phenotypic and physiological processes(Raza *et al.*, 2015). Limited crop growth under drought is reported by different scientists (Raza *et al.*, 2014, Daryanto *et al.*, 2016; Jamshaid *et al.*, 2018). Parameters reduced by drought include leaf water content, chlorophyll contents, 1000-grain weight and grain yield (Daryanto *et al.*, 2016), rate of photosynthesis (Niakan and Ahmadi, 2014) as well as soluble sugar contents (Nohong and Nompo, 2015).

Drought stress influences internal phytohormone concentrations (Hare *et al.*, 1997) and reduce photosynthesis rate through elevated levels of reactive oxygen species. Under water deficit stress, decrease in soil water potential alters the minerals uptake and decreased leaf expansion (Pospíšilová *et al.*, 2000). Crop production is adversely affected by the shortage of water. Reduction of crop yield is noticed more by drought stress as compare to other abiotic stresses (Saleem *et al.*, 2016). As water is very essential for plant, so limited water availability at any growth stage can alter the plant physiological functions. Water stress affects plant morphology, physiology and ultimately the grain yield of wheat (Raza *et al.*, 2017a). Cruz de Carvalho. (2008) reported that under drought condition ROS (reactive oxygen species) produced in the plant that causes the negative effect on the plant growth at molecular level. Enhancing the level of antioxidant enzymatic in the plant can reduce the ROS and can save the plant from severe damage. Different processes like organ development, germination, leaf expansion, photosynthesis and root growth are badly affected by water deficit stress. Therefor sustainable crop production it is very essential to control damaging effects of drought through different practices (Raza *et al.*, 2015). Limited water availability, high temperature and low rainfall during past few years produce risk to wheat crop productivity. Different strategies such as nutrients spray (Raza *et al.*, 2012b), compatible solutes, and use of mulches (Ahmad *et al.*, 2015) have been developed to control drought effects in crop growth.

Werner *et al.* (2001) reported that seed germination, leaf expansion, bud formation and chloroplast formation has been improved by Cytokinin (CK) application. CK can further enhance cell division as well as crop growth and grain yield. Antioxidant system improved by different hormones especially cytokinin is very helpful to scavenge these species (Banowetz, 1999). Chlorophyll contents decreased by water deficit stress can be improved with the foliar application of growth regulators (Zhang *et al.*, 2004). Moreover, cytokinin production in plant decreased by drought stress that might decrease cytokinin biosynthesis (Pospíšilová *et al.*, 2000; Dodd. 2003). However, drought has been shown to reduce endogenous CK concentrations (Pospíšilová *et al.*, 2000). During drought stress, CK role has been studied extensively in the past but it is still remains unclear in field condition.

The objective of present study was to notice the growth promoting effect of CK under water limited stress at different growth stages of wheat. We hypothesized cytokinin application will improve growth of wheat under drought.

MATERIALS AND METHODS

A field experiment having three replications was carried to determine drought ameliorating effects of exogenous applied cytokinin in wheat during 2015-16,atthe research area of Agronomy Department, Islamia University of Bahawalpur(latitude: 29.418, longitude: 71.670, elevation: 181 m, Average annual rainfall: 143 mm). The experiment included seven treatments (C= Control, DT= Drought at tillering, DT+CK= Drought at tillering + Cytokinin, DA= Drought at anthesis, DA+CK= Drought at anthesis + Cytokinin, DG= Drought at grain filling, DG+CK= Drought at grain filling + Cytokinin) with randomized complete block design. Approved wheat variety of Pakistan "Galaxy-2013" was used for line sowing @ 120 kg/hain different plots (3.5 m × 5 m). Galaxy-2013is suitable variety for growing under the agro ecological conditions of Bahawalpur. Recommended irrigations were applied with the rate of 3 Acre Inches and stop watering when we were applied drought. Plot to plot distance was maintained 3 ft to prevent water from the neighboring plot. To induce drought at a given stage, water was withheld. Foliar spray of different growth hormones can improve plant growth and Cytokinin is one of them and we select it for our experiment due to its characteristic of improving cell division. Cytokinin (Zeatin, >98% purity)was applied by foliar spray @25 mg L^{-1}(Zaheer *et al.*, 2019).Fertilizers were applied @ 120-80-60 Kg/ha NPK. The soil had the following properties:sand: 20%, silt:18%, clay: 62%, organic matter: 0.65%, nitrogen: 0.31 mg kg^{-1} dry soil, phosphorous: 4.7 mg kg^{-1} dry soil, potassium: 125 mg kg^{-1} dry soil, calcium: 102 mg kg^{-1} dry soil, pH: 7.6.Weather data of experimental site during 2015-16 is provided in Table 1.

Parameters recorded**:** Different growth and yield related parameters were recorded by standard procedure. Infra-Red Gas Analyzers (LI 6250-Li-COR) was used to determine rate of photosynthesis (Pn) of flag leaves between 8:00 am to 10:00 am(Raza *et al.*, 2017a). Stomatal conductance and transpiration rate was measured by Automatic porometer(MK3, DeltaT. Burwell Devices, England, Hertford-Herts, England). Portable laser leaf area meter (CI:2002-L model, CIDBio-Science, USA) was used to noticed leaf area index (LAI) and chlorophyll meter (CL01, Hansatech-Ltd. UK) was used to measured leaf chlorophyll contents. APX (ascorbate peroxidase), CAT (catalase), POD (peroxidase) and SOD (superoxide dismutase) activities in leaves were measured by procedure reported by Nakano and Asada (1981), Vanacker *et al.* (2000), Ghanati *et al.* (2002), Beyer and Fridovich (1987), respectively.

Table 1. Weather data of experimental site during 2015-16

Month	Temperature ˚C			Average rainfall (mm)	Rainy days
	Max.	Min.	Average		
November, 2015	35	25	31	0.0	0
December, 2015	31	20	26	0.0	0
January, 2016	24	14	20	4.5	4
February, 2016	30	20	26	0.6	2
March, 2016	35	25	32	0.6	2
April, 2016	43	34	40	4.2	4
May, 2016	45	39	43	9.9	12

Leaf relative water contents (RWC) were determined from fully expanded leaves. Plastic bags were used to placed excised leaves and transferred them immediately in to the lab for determination of fresh weight (FW). Leaves of the plants were soaked in distilled water at room temperature for 16 h. After that leaves were blotted dry with use of tissue paper and then weighted. Dry weight (DW) of leaves was measure after oven drying at 70 °C for 72 hours.

RWC calculated by the Barrs and Weatherley's (1962) equation:

$$\text{RWC}\ (\%) = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] \times 100$$

Fully expanded leaf,4th from the top from each plant was used to determined leaf water potential (-MPa) by using pressure chamber (600L model, C.W. Cook and sons, Ltd. England). For osmotic potential (-MPa), a proportion of leaves were used to measured water potential by frozen them for 2 weeks. Frozen sap extracted from crushed leaves by centrifugation (8000×g) for 4 min. Osmotic potential of sap was calculated by vapor pressure osmometer (Wescor: 5520 model, Logan USA).

Leaf turgor pressure (MPa) was determined as

$$\text{TP} = \text{WP-OP}$$

Where, TP =Leaf turgor pressure (jp), OP = leaf osmotic potential (js), WP = leaf water potential (jw)

Statistix 8.1 computer software was used for analysis of variance(ANOVA)and least significant difference (LSD) at 5% was used to determine the difference among treatments means(Steel et al., 1997).

RESULTS

Data regarding growth parameters shown in Table 2 indicate

Table 2. Drought ameliorating effects of exogenous applied cytokinin on growth and yield of wheat.

Treatments	Plant height (cm)	Spike Length(cm)	Number of spikelets /spike	Number of grains per spike	1000-grain weight (g)	Grain yield (kg ha^{-1})	Biologi-calyield (kg ha^{-1})
C= Control	95.90 a	13.33 a	23.33 a	46.66 a	36.00 a	4681 a	14043 a
DT	92.93 c	12.33 b	22.33 b	43.66 b	33.33 b	4377 b	13131bc
DT+CK	94.90 b	13.23 a	23.33 a	45.66 a	33.66 b	4568 a	13704ab
DA	95.33 ab	10.63 d	17.30 e	35.33 f	30.66 cd	3938 f	11814 d
DA+CK	95.40 ab	11.33 c	18.33 d	36.66 e	31.66bc	4222 e	12666 c
DG	95.56 ab	11.33 c	20.33 c	38.33 d	26.66 e	3798 d	11394 c
DG+CK	95.60 a	12.33 b	22.33 b	42.33 c	29.00 d	4009 c	12027 c

Treatment means per variable with different letters show significant difference from one another at $p \leq 0.05$ (n=3).

that plant height was highest in C (95.90 cm) followed by DG+CK (95.60 cm) and lowest in DT (92.93 cm).Spike length was greatest in C (13.33 cm) followed by DT and DG+CK (12.33 cm).Smallest spikes were found in DA+CK and DG (11.33 cm). Number of spikelets per spike was highest in C and DA (23.33) followed by DT and DG+CK (22.33) and minimum was in DA (17.30). Maximum number of grains per spike was found in C and DT+CK (46.66) followed by DT (43.66) and lowest was recorded in DA (35.33).

1000-grain weight (Table 2) was highest in C (36.00 g) followed by DT+CK (33.66 g) and it was lowest in DG (26.66 g). Grain yield was highest in C (4681 kg ha^{-1}) followed by DT+CK (4568 kg ha^{-1}) and it was lowest in DA (39.38 kg ha^{-1}). Biological yield was also significantly improved by CK application when drought applied at tillering and anthesis but effect of Ck was non-significant when drought was applied at grain filling stage. Biological yield was highest in C (14043 kg ha^{-1}) followed by DT+CK (13704 kg ha^{-1}) and lowest in DA (11814 kg ha^{-1}).

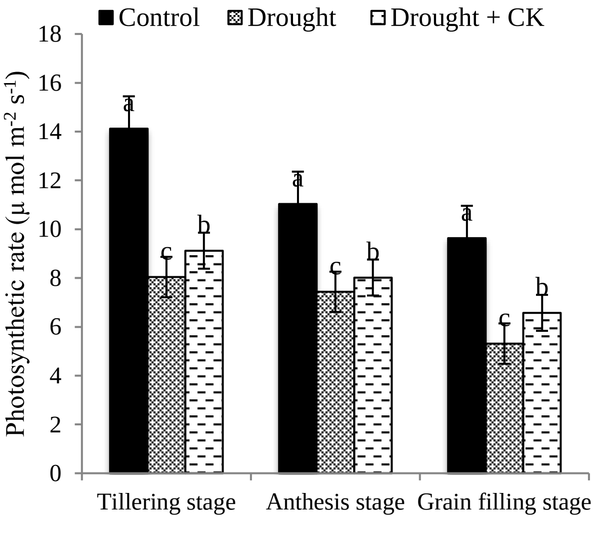

Figure 1. Photosynthetic rate of wheat affected by CK application under drought.

Data regarding photosynthetic rate (Fig. 1) and transpiration rate (Fig. 2) show that maximum photosynthesis and transpiration was noticed at tillering stage. CK application at different growth stages improved the photosynthesis and transpiration. Maximum photosynthetic and transpiration rate was observed under control treatment followed by when cytokinin was applied under drought stress and lowest was noticed under drought stress conditions without CK application at all growth stages. Non-significant difference was noticed for transpiration rate at grain filling stage between control treatment and when applied CK under drought stress at different growth stages.

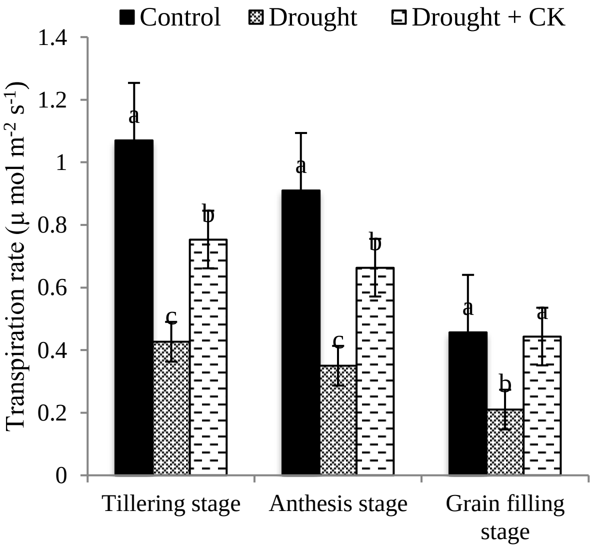

Figure 2. Transpiration rate of wheat affected by CK application under drought.

Data regarding LAI (Figure 3) show that highest LAI was noticed under control treatments (tillering: 1.9, anthesis: 5.24, grain filling: 3.56) followed by drought stress treatments having CK application (tillering: 1.7, anthesis: 4.8, grain filling: 3.19) and lowest (tillering: 1.38, anthesis: 4.8, grain filling: 3.19) and minimum was observed under drought stress treatments without CK application at all stages.

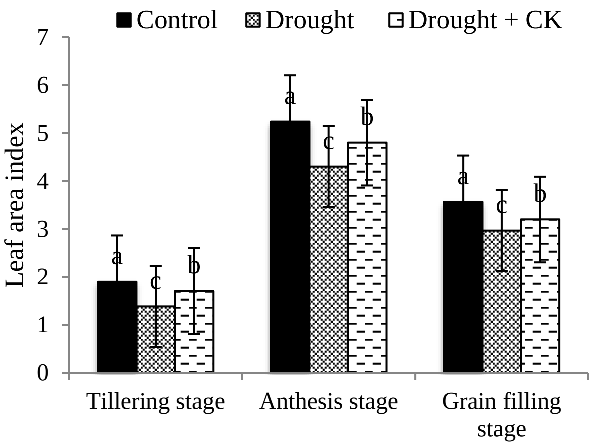

Figure 3. **LAI of wheat affected by CK application under drought.**

Chlorophyll contents significantly improved by CK application (Figure 4).Highest chlorophyll contents were observed in control treatment (tillering: 34%, anthesis: 92%, grain filling: 87%) following by when CK applied having drought stress condition (tillering: 29%, anthesis: 87%, grain filling: 81%) and lowest chlorophyll contents were observed when drought occurred without application of CK(tillering: 24%, anthesis: 82%, grain filling: 75%).

CK application was significantly improved the leaf relative water contents (RWC)under drought stress condition. Maximum RWC(tillering: 94%, anthesis: 85%, grain filling: 80%) were noticed under control treatment followed by when CK was applied under drought (tillering: 87%, anthesis: 79%, grain filling, 76%) and lowest (tillering: 84%, anthesis: 74%, grain filling: 71%) were observed under only drought treatments without CK application (Figure 4).

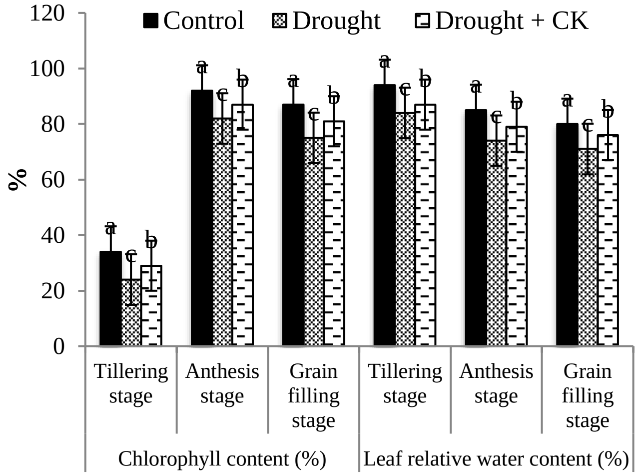

Figure 4. **Chlorophyll content (%) and relative water contents (%) of wheat affected by CK application under drought.**

Stomatal conductance data (Figure 5) show that stomatal conductance was significantly affected by the CK application. Maximum stomatal conductance (tillering: 355 mmolm^{-2}s^{-1}, anthesis: 296 mmolm^{-2}s^{-1}, grain filling: 251.33 mmolm^{-2}s^{-1}) was noticed under control treatments followed by (tillering: 327 mmolm^{-2}s^{-1}, anthesis: 272 mmolm^{-2}s^{-1}, grain filling: 225 mmolm^{-2}s^{-1}) when CK was applied under drought and lowest stomatal conductance (tillering: 309 mmolm^{-2}s^{-1}, anthesis: 251.66 mmolm^{-2}s^{-1}, grain filling: 202.33 mmolm^{-2}s^{-1}) was observed under only drought stress treatments without CK application.

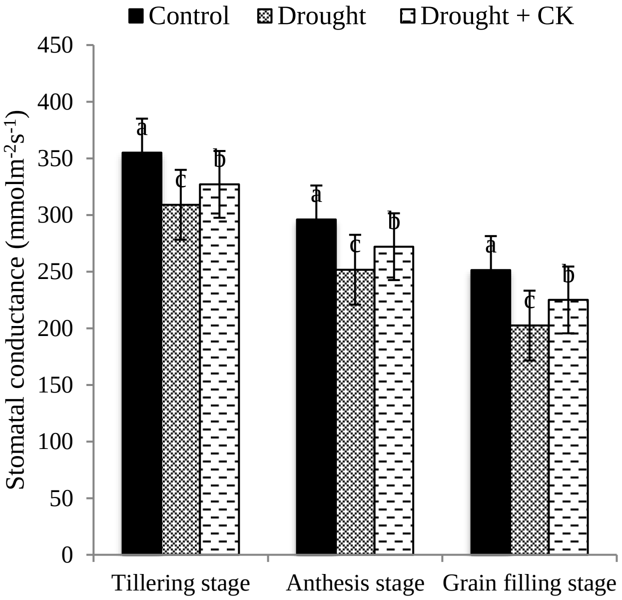

Figure 5. **Stomatal conductance of wheat affected by CK application under drought.**

Data regarding leaf water potential and osmotic potential showed that CK application under drought stress significantly improved leaf water and osmotic potential. Lowest (More negative) leaf water and osmotic potential were observed under drought stress condition followed by when CK was applied under drought and highest values were observed under control treatments (Figure 6).

CK application significantly improved leaf turgor potential. Highest leaf turgor potential was recorded under control treatment followed by when CK was applied under drought and lowest was observed when only drought occurs without CK application at all growth stages(Figure 7).

Concentration of antioxidant enzymes APX, CAT, POD and SOD (Figure 8) was highest in treatments with drought without CK, followed by drought with CK and lowest was recorded in control treatment.

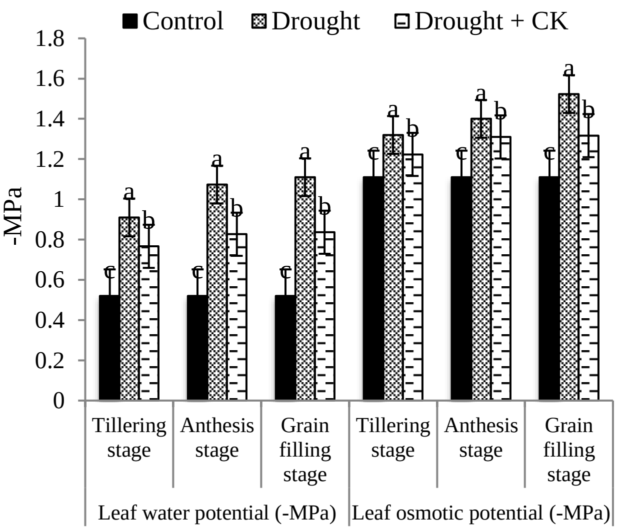

Figure 6. Leaf water potential (-MPa) and osmotic potential (-MPa) of wheat affected by CK application under drought.

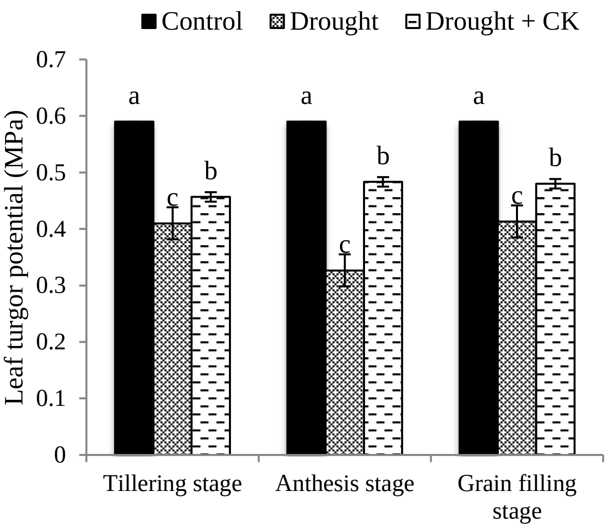

Figure7. Leaf turgor potential (MPa) of wheat affected by CK application under drought.

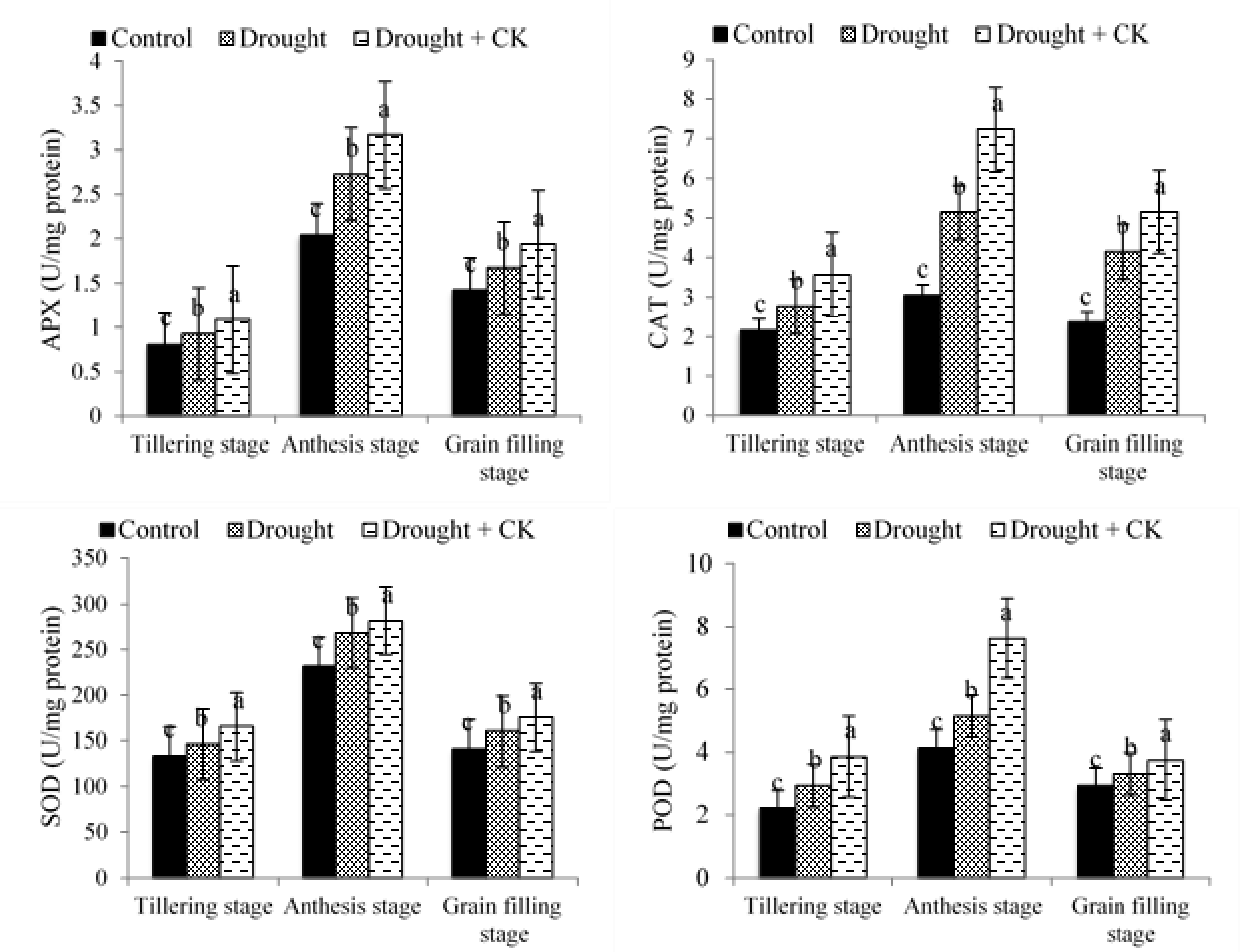

Figure 8. Antioxidant enzymatic activities of wheat affected by CK application under drought.

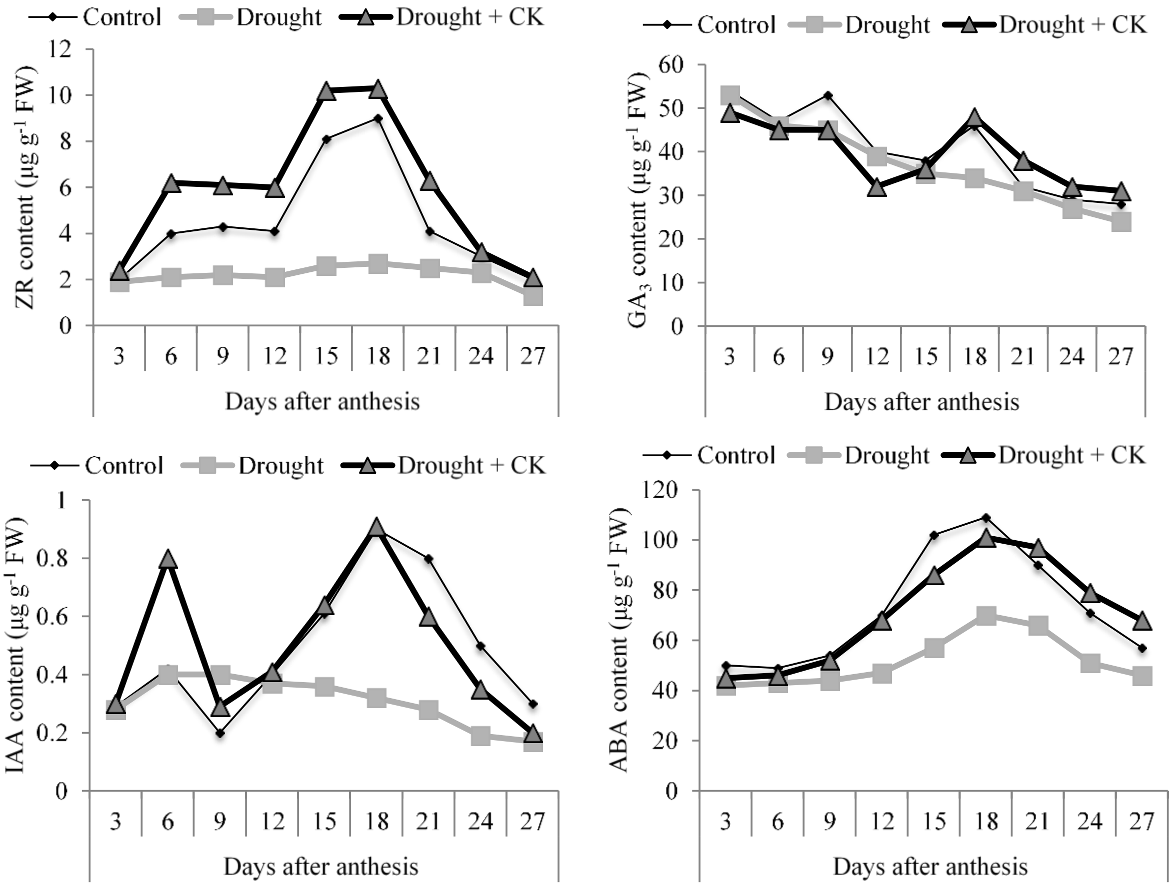

Figure 9. Zeatinriboside (ZR), gibberellin (GA_3), indole-3-acetic acid (IAA) and abscisic acid (ABA) content level of wheat affected by CK application under drought.

Zeatinriboside (ZR) levels in kernels increased between 3 to 18 days after anthesis (DAA), and were maximum at 18 DAA and then decreased suddenly with the grain filling. CK application under drought significantly increased ZR contents as compared to the only drought stress treatment. Gibberelin (GA_3) contents (Figure 9) were highest at early grain filling and then decreased.CK application had no consistent effect on GA_3. With application of CK, higher concentration of indole-3-acetic acid (IAA) was recorded on6 DAA and remained higher till 18 DAA, while remained stable in control treatment. After 2 DAA, IAA contents were similar drought treatments first increased and then decreased (Figure 9). In both drought treatments, ABA (abscisic acid) contents in kernels increased until 18 DAA and then declined.

DISCUSSION

This study showed that CK application improves wheat growth and yield under drought, irrespective of growth stage at which drought was applied. Kumari *et al.* (2018) reported that cytokinin application significantly improved growth related parameters in wheat. Drought stress significantly decreased the yield of wheat when drought occurred at tillering, but non-significant effect was noted when drought applied at anthesis and grain filling stages (Raza *et al.*, 2012b). Raza *et al.* (2017b) also reported that limited water availability negatively effect on plant water relationship that alters the phenotypic and physiological response of wheat crop. Plant growth reduction by drought is reported by many plant scientists (Raza *et al.*, 2012a; Daryanto *et al.*, 2016).The improved growth with CK can be explained by its effect on plant metabolism. CK has been shown to stimulate root shoot interaction, cell division in plant cells and nucleic acid metabolism (Hare *et al.*, 1997; Werner *et al.*, 2001; Yang *et al.*, 2016). An increase of 7%, 6% and 8%in spike length was noticed when CK was applied at tillering, anthesis and grain filling stages, respectively under drought as compared to only drought treatment with CK application. Exogenous CK application increases ratio of CK to auxins in the plant that controls branching patterns and lateral bud growth (Cline, 1991). Hare *et al.*(1997) and Yang *et al.* (2016) also noticed plant growth promotion by CK application.CK accumulate in inflorescence meristem and regulates spike development that results in an increasing number of flowers and branches(Ding *et al.*, 2014).Endospermic cell division and increase in sink capacity results in greater accumulation of assimilate and ultimately leads to more grains per spike (Yang *et al.*, 2016). The changes in endogenous hormone levels in kernels found in this study may induce greater assimilate transport into the grains, thereby improving grain weight and yield (Chen *et al.*, 2010).

CK application also increased yield related parameter such as 1000-grain weight and yield under drought. This can be explained by the effect of CK on sink strength. CK promotes endospermic cell division and sink capacity that results in greater grain weight and yield(Yang *et al.*, 2016).Further, CK improves auxins and abscisic acid production that results higher grain yield (Mazid *et al.*, 2011). Drought induces stomatal closure which causes reduction of transpiration rate and CO_2 entry into the leaves that result in lower photosynthesis (Raza *et al.*, 2012a; Bhargava *et al.*, 2013).CK application stimulates stomatal opening (Rivero *et al.*, 2009) and total number of stomata (Farber *et al.*, 2016) thereby improves photosynthesis and transpiration under drought. Due to water deficit stress soil water potential decreases and it alters the minerals uptake and leaf expansion (Pospíšilová *et al.*, 2000). LAI increased by CK application under drought stress at tillering, anthesis and rain filling stage up to 23%, 11% and 7.77%, respectively. Highest LAI was recorded at flag leaf stage and then decreased continuously at later stages. This decline might be associated with aging of leaves and leaf senescence (Dalirie *et al.*, 2010). Drought stress restrains leaf expansion and decreases leaf area due to early senescence (Fang *et al.*, 2017).Zaheer *et al.* (2019) reported that LAI decreased under drought stress due to the leaf turgidity and foliar spray of CK enhances the plant metabolic functions and increase chlorophyll synthesis that also help in maintaining LAI. Ahmad *et al.* (2015) reported that changes in mesophyll conductance lead towards physical alteration in intercellular spaces that causes leaf shrinkage and reduces leaf turgidity. Leaf relative water contents and stomatal conductance are increased by CK and thereby LAI (Rivero *et al.*, 2009).

Chlorophyll contents were decreased under water stress condition due to higher ROS that destroy chloroplasts (Chaves *et al.*, 2009; Gill and Tuteja, 2010). The higher chlorophyll contents induced by CK can be explained by stimulation of anti-oxidants. Anti-oxidants reduce reactive oxygen species (Rivero *et al.*, 2009; Diaz-Vivancos *et al.*, 2011; Bielach *et al.*, 2017). Previous studies also reported that CK reduced reactive oxygen levels (Liu and Huang, 2002; Chang *et al.*, 2016). RWC and stomatal conductance are reduced under drought due to reduced leaf size and smaller cell volume. Partial closing of stomata under drought is a main reason of less stomatal conductance (Alkhaldi *et al.*, 2012). CK increased RWC and stomatal conductance due to its stimulation of endogenous hormones level, stomatal conductance and LAI (Incoll and Jewer, 1987; Dodd, 2003; Raza *et al.*, 2017a; Kumari *et al.*, 2018). The higher RWC can explain the increase turgor potential by CK application. Osmotic potential decreased under water limited condition that causes reduction in LAI and stomatal conductance. Raza *et al.* (2017a) reported that under water limited condition macromolecules hydrolyzed in to smaller ones that increase osmolytes and ultimately more osmotic potential. By CK application more RWC and LAI obtained that are helpful to maintain water, osmotic potential and turgor potential (Kumari *et al.*, 2018).

Higher production of ROS is the reason of interruption of normal biochemical processes during water deficit condition (Chaves *et al.*, 2009). Sofo *et al.* (2015) reported that antioxidant enzymes help to decrease the effect of ROS. CAT and APX act as reducing agents and change H_2O_2 into H_2O. SOD convert superoxide radicals (more harmful) to H_2O_2(less harmful). Under drought stress POD regulate cell wall expansion. It was observed that 14.41%, 13.89%, 13.91% APX activities, 22.42%, 28.95%, 19.53% CAT activities, 23.78%, 32.72%, 11.57% POD activities, 11.69% 4.73%, 8.72% SOD activities were improved by CK application under drought stress at tillering, anthesis and grain filling stage, respectively. Significantly higher activities of antioxidants were found by CK application under drought stress. Similar results were also reported by Bielach *et al.* (2017) and Diaz-Vivancos *et al.* (2011). Deleterious effect of ROS can be minimized by developing an antioxidant defense system. Cytokinin participates in removing of ROS and increases antioxidant enzymes (Chang *et al.*, 2016). Previous studies also noticed an increase in antioxidant enzymes activities by CK application (Liu and Huang, 2002; Chang *et al.*, 2016). Leshem *et al.* (1981) reported that cytokinin inhibit the activity of xanthine oxidase that is an enzyme of ROS generators in cell. An endogenous hormones levels in kernels could change CK metabolism (Wang *et al.*, 2016) and transferred more assimilates to the grain (Chen *et al.*, 2016) and improve resistance against drought stress (Thomas *et al.*, 2000).

***Conclusion*:** Under drought stress, Cytokinin application is very useful to enhance drought tolerance potential in wheat. It was concluded that CK improved plant growth, minimized ill impacts of reactive oxygen species and increase transfer of assimilates to grains under drought So, it is recommended for the farmers to apply CK in wheat crop under limited water availability.

REFERENCES

Ahmad, S., M.A.S. Raza, M.F. Saleem, S.S. Zahra, I.H. Khan, M. Ali, A.M. Shahid, R. Iqbal, and M.S. Zaheer. 2015. Mulching strategies for weeds control and water conservation in cotton. J. Agric. Biol. Sci. 8:299-306.

Alkhaldi, A., A.N. Aldarir, M. Janat, M.A. Wahbi and A. Arslan. 2012. Effect of regulated deficit irrigation and partial root-zone drying on some quantitative indicators and the efficiency of adding nitrogen fertilizer to (*Zea mays* L.) by using n15 Isotope. American-Eurasian J. Agric. Environ. Sci. 12:1223-1235.

Banowetz, G.M., K. Ammar and D.D. Chen. 1999. Postanthesis temperatures influence cytokinin accumulation and wheat kernel weight. Plant Cell Environ. 22:309-316.

Barrs, H.D. and P.E. Weatherly. 1962. A re-examination of relative turgidity for estimating water deficits in leaves. Aust. J. Biol. Sci. 15:413-428.

Beyer, W.F. and I. Fridovich. 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161:559-566.

Bhargava, S. and K. Sawant. 2013. Drought stress adaptation: Metabolic adjustment and regulation of gene expression. Plant Breed. 132:21-32.

Bielach, A., M. Hrtyan and V.B. Tognetti. 2017. Plants under Stress: Involvement of Auxin and Cytokinin. Int. J. Mol. Sci. 18:1427.

Chang, Z., Y. Liu, H. Dong, K. Teng, L. Han and X. Zhang. 2016. Effects of cytokinin and nitrogen on drought tolerance of creeping bentgrass. PLoS ONE. 11:e0154005.

Chaves, M.M., J. Flexas and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103:551-560.

Chen, J.B., Y. Liang, X.Y. Hu, X.X. Wang, F.Q. Tan and H.Q. Zhang. 2010. Physiological characterization of ‘stay green’ wheat cultivars during the grain filling stage under field growing conditions. Acta. Physiol. Plant. 32:875-882.

Cline, M. 1991.Apical dominance. Bot. Rev. 57:318-358.

Cruz de Carvalho, M.H. 2008. Drought stress and reactive oxygen species. Plant Signal. Behav. 3:156-165.

Dalirie, M.S., R.S. Sharifi and S. Farzaneh. 2010. Evaluation of yield, dry matter accumulation and leaf area index in wheat genotypes as affected by terminal drought stress. Not. Bot. Hort. Agro. Bot. Cluj. 38: 182-186.

Daryanto, S., L. Wang and P.A. Jacinthe. 2016. Global synthesis of drought effects on maize and wheat production. PLoS ONE 11:e0156362.

Diaz-Vivancos, P., K. Majourhat, J.A. Fernández, J.A Hernandez and A. Piqueras. 2011. Study of the antioxidant enzymatic system during shoot development from cultured intercalarmeristems of saffron. Plant Growth Regul. 65:119-126.

Ding, C., J. You, L. Chen, S. Wang and Y. Ding. 2014. Nitrogen fertilizer increases spikelet number per panicle by enhancing cytokinin synthesis in rice**.** Plant Cell Reports33:363-371.

Dodd, I.C. 2003.Hormonal interactions and stomatal responses. Plant Growth.Regul.22:32-46.

Fang, Y., Y. Du, J. Wang, A. Wu, S. Qiao, B. Xu, S. Zhang, K.H.M. Siddique and Y. Chen. 2017. Moderate drought stress affected root growth and grain yield in old, modern and newly released cultivars of winter wheat. Front. Plant Sci. *8*:672.

Farber, M., Z. Attia and D. Weiss. 2016. Cytokinin activity increases stomatal density and transpiration rate in tomato. *J. Exp. Bot.* 67:6351-6362.

Ghanati, F., A. Morita and H. Yokota. 2002. Induction of suberin and increase of lignin content by excess boron in tabacco cell. Soil. Sci. Plant. Nutr. 48:357-364.

Gill, S.S. and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant. Physiol. Biochem. 48: 909-930.

Hare, P.D., W.A. Cress and J. Van Staden. 1997. The involvement of cytokinins in plant responses to environmental stress. Plant. Growth. Regul. 23:79-103.

Incoll, L.D. and P.C. Jewer. 1987. Cytokinins and stomata. In: Zeiger, E., Farquhar, G.D., Cowan, I.R. (ed.): Stomatal Function. Stanford University Press, Stanford, pp 281-292.

Jamshaid, M.U., Z.A. Zahir, H.N. Asghar and A. Jamil. 2018. Variability in stress tolerance and potential of endophytic bacteria to improve chickpea growth under water limited conditions. Pak. J. Agri. Sci. 55:531-539.

Kumari S., S. Kumar and P. Prakash. 2018. Exogenous application of cytokinin (6-BAP) ameliorates the adverse effect of combined drought and high temperature stress in wheat seedling. J. Pharmacogn. Phytochem. 7:1176-1180.

Leshem, Y.Y., J. Wurzburger, S. Grossman and A.A. Frimer. 1981. Cytokinin interaction with free radical metabolism and senescence: effects on endogenous lipoxygenase and purine oxidation. Physiol. Plant. 53:9-12.

Liu, X. and B. Huang. 2002. Cytokinin effects on Creeping Bent grass response to heat stress. Crop Science. 42:466-472.

Mazid, M., T.A. Khan and F. Mohammad. 2011. Cytokinins, a classical multifaceted hormone in plant system. J. Stress Physiol. Biochem. 7:347-368.

Nakano, Y. and K. Asada.1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant. Cell. Physiol. 22:867-880.

Niakan, M. and A. Ahmadi. 2014. 'Effects of foliar spraying kinetin on growth parameters and photosynthesis of tomato under different levels of drought stress. Iran. J. Plant Physiol. 4:939-947.

Nohong, B. and S. Nompo. 2015. Effect of water stress on growth, yield, proline and soluble sugars contents of signal grass and napier grass species. Am-Eurasian J. Sustain. 9:14-21.

Pospíšilová, J., H. Synkova and J. Rulcova. 2000. Cytokinins and water stress. Biologia. Plantarum. 43:321-328.

Raza, M.A.S, S. Ahmad, M.F. Saleem, I.H. Khan , R. Iqbal, M.S. Zaheer, I. Haider and M. Ali. 2017a. Physiological and biochemical assisted screening of wheat varieties under partial rhizosphere drying. Plant. Physiol. Biochem. 116:1-7.

Raza, M.A.S., M.F. Saleem and I.H. Khan. 2015. Combined application of glycinebetaine and potassium on the nutrient uptake performance of wheat under drought stress. Pak. J. Agri. Sci. 52:19-26.

Raza, M.A.S., M.F. Saleem, I.H. Khan, M. Jamil, M. Ijaz and M.A. Khan. 2012b. Evaluating the drought stress tolerance efficiency of wheat (*Triticum aestivum* L.) cultivars. Russian J. Agric. Socio-Econo. Sci. 12:41-46.

Raza, M.A.S., M.F. Saleem, M. Jamil and I.H. Khan. 2014. Impact of foliar applied glycinebetaine on growth and physiology of wheat (*Triticum aestivum* L.) under drought conditions. Pak. J. Agri. Sci. 51:337-344.

Raza, M.A.S., M.F. Saleem, M.Y. Ashraf, A. Ali and H.N. Asghar. 2012a. Glycinebetaine applied under drought improved the physiological efficiency of wheat (*Triticum aestivum*L.) plant. Soil. Environ. 31:67-71.

Raza, M.A.S., M.S. Zaheer, M.F. Saleem, I.H. Khan, F. Khalid, M.U. Bashir, M. Awais, R. Iqbal, S. Ahmad, M.U. Aslam and I. Haider. 2017b. Investigating drought tolerance potential of different Wheat (*Triticum aestivum* L.) varieties under reduced irrigation level. Int. J. Biosci. 11:257-265.

Rivero, R.M., V. Shulaev and E. Blumwald. 2009. Cytokinin-dependent photorespiration and the protection of photosynthesis during water deficit. Plant. Physiol. 150:1530-1540.

Saleem, M.F., M.A.S. Raza, S. Ahmad, I.H. Khan and A.M. Shahid. 2016. Understanding and mitigating the impacts of drought stress in cotton- a review. Pak. J. Agri. Sci. 53:609-623.

SalehiGharaviran, L., E. Nabizadeh and S. Yezdanseta. 2014. Study of impacts of plant growth regulators foliar spray on yield and yield components of wheat cv. zarrin at different growth stages. Adv. Env. Biology. 8:134-138.

Sofo, A., A. Scopa, M. Nuzzaci and A. Vitti. 2015. Ascorbate Peroxidase and Catalase Activities and Their Genetic Regulation in Plants Subjected to Drought and Salinity Stresses. Int. J. Mol. Sci. 16: 13561-13578.

Steel, R.G.D., J.H. Torrie and D. Dickey.1997. Principles and Procedure of Statistics. A Biometrical Approach 3rd Ed. McGraw Hill Book Co. Inc., New York.: 352-358.

Thomas, H. and C.J. Howarth. 2000. Five ways to stay green. J. Exp. Bot.51:329-337.

Vanacker, H., T.L.W. Carver and C.H. Foyer. 2000. Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during the hyper sensitive response in the barley-powdery mildew interaction. Plant Physiol. 123:1289-1300.

Wang, W.Q., Q.Q. Hao, F.X. Tian, Q.X. Li and W. Wang. 2016. The stay-green phenotype of wheat mutant tasg1 is associated with altered cytokinin metabolism. Plant Cell Rep. 35:585-599.

Werner T., V. Motyka, M. Strnad and T. Schmülling. 2001. Regulation of plant growth by cytokinin. Proc. Natl. Acad. Sci. USA, 98:10487-10492.

Yang, D., Y. Li, Y. Shi, Z. Cui, Y. Luo, M. Zheng, J. Chen, Y. Li,Y. Yin and Z. Wang. 2016. Exogenous cytokinins increase grain yield of winter wheat cultivars by improving stay-green characteristics under heat stress. PLoS One 11:e0155437.

Zhang M, L. Duan, Z. Zuho, J. Li, X. Yian, B. Wang, Z. He and Z. Li. 2004. Effects of plant growth regulators on water deficit induced yield loss in soybean. *In*: Proceedings of 4th International Crop Science Congress. pp. 571-575.

Zaheer, M.S., M.A.S. Raza, M.F. Saleem, K.O. Erinle, R. Iqbal and S. Ahmad. 2019. Effect of rhizobacteria and cytokinins application on wheat growth and yield under normal vs drought conditions. Commun. Soil. Sci. Plant Anal.50:2521-2533.

[Received 03 Jan 2019: Accepted 07 March 2020: Published (online) 08 June 2020]