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Full Length Article

Exogenously Applied Gibberellic Acid, Indole Acetic Acid and Kinetin as Potential Regulators of Source-Sink Relationship, Physiological and Yield Attributes in Rice (*Oryza sativa*) Genotypes under Water Deficit Conditions

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Abstract

Rice is one of the important cereal crops drastically affected by water scarcity in rice ecosystems. In a pot study, the impact of externally applied gibberellic acid (GA_3), indole acetic acid (IAA) and kinetin was investigated on assimilates mobilization and yield parameters of rice under water stress. Twenty five days seedlings of two rice genotypes KS-133 and Basmati-2000 were transplanted in soil filled pots and sprayed with GA_3, IAA and kinetin each of 10^{-5} M concentration at panicle initiation stage. Water stress was imposed to plants 80 days after transplanting for 10 days. Water stress ($p \leq 0.05$) reduced leaf chlorophyll content and yield of rice but increased the concentration of proline, leaf soluble protein and enhanced mobility of assimilates from leaves to grains. Application of GA_3 and kinetin significantly ($p \leq 0.05$) increased chlorophyll content, growth and yield attributes in both genotypes under water stress conditions. However, IAA application did not improve the grain yield in rice genotypes under water stress. The positive impact of GA_3 and kinetin spray was imitated in the form of enhanced solute accumulation; enhanced growth and greater grain assimilate deposition. This may be concluded that GA_3 and kinetin can be applied exogenously prior to flower initiation in rice to reduce panicle sterility by increasing assimilates mobility under water deficit for optimal economic yield. © 2016 Friends Science Publishers

Keywords: Plant growth regulators; Stem reserve translocation; Proline; Chlorophyll contents

Introduction

Water deficiency is one of major reason for productivity of crops in the world, including rice in rainfall dependent areas (Passioura, 2007). Water deficiency decrease in crop growth may surpass all other factors of yield reduction because both intensity and length of deficiency are critical (Farooq *et al.*, 2008).

Greater than half of world area producing rice is dependent on rainfall, which contributes one fourth of the total rice productivity (Maclean *et al.*, 2002). In order to keep pace with growing requirement for rice till 2030, a substantial rise of about 35% in productivity is required (Bouman *et al.*, 2007). Therefore, there is an urgent need to evolve new rice germplasm with improved yielding ability, water uptake, deficit tolerance and greater water utilization.

Water stress induces various physiological, biochemical and molecular mechanism within the plant through osmotic stress (Witcombe *et al.*, 2008). Re-translocation of photo-assimilates is a vigorous phenomenon involving movement of deposited assimilates from stalks and other parts of plants to caryopses (Zhang *et al.*, 1998). Yield of grains (GY) in cereals depends upon assimilates movement from leaves to grains. The two upper most leaves are the principal basis whereas the panicle's florets are the major deposition place for carbohydrate synthesis process (Hirota *et al.*, 1990; Sicher, 1993). Most of assimilates deposited in rice caryopses are contributed by the two upper most leaves (Gladun and Karpov, 1993). Grain formation in rice is dependent on carbohydrate produced by carbon metabolism, their deposition before anther shedding and transport to the caryopses from asexual plant. Higher proteins and starch deposition in each caryopsis is dependent on the number and size of endospermic cells mainly affected by the pace and length of caryopsis formation. This reduction in assimilates movement during water scarcity affects the final yield and leads to yield reductions (Egli, 1998).

Plant growth regulators play important roles in amelioration of various abiotic stresses in cereals including rice (Gurmani *et al.*, 2013). GA_3 is found to control caryopsis

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formation in crops (Kende and Zeevaart, 1997; Hansen and Grossman, 2000). IAA is known to offset the negative impacts of water deficit in soybean and hence supports their successful growth and development processes (Gadallah, 2000). Cytokinins are known to improve water absorption, cells growth and stimulate tissue formation in plants (Letham and Palni, 1983). Similarly, these help in photosynthetic pigments formation and decrease aging process. Their involvement under hostile ecological conditions is also well documented (Hare *et al.*, 1997). Application of kinetins mitigates the adverse effects of water deficiency by inspiring solute accumulation in chickpea (Yadav *et al.*, 1997). Kinetin's exogenous application has been reported to enhance proline accumulation in the leaves of *Vigna unguiculata* under water stress (Agarwal and Gupta, 1995). As growth regulators play vital role in modifying growth processes in plants, it is likely that they might mitigate the adverse effects of water stress by improving growth.

In present study, we studied water stress tolerance in rice, because it is sensitive to water stress at panicle initiation of reproductive growth stage which may cause significant yield losses. Foliar applied GA_3, IAA and kinetin at panicle initiation stage in rice by investigating sink strength through stem reserve translocation and yield improvement under water stress conditions.

Materials and Methods

Plant Materials and Growth Conditions

Seeds of two rice (*Oryza sativa* L.) genotypes KS-133 and Basmati-2000 were obtained from Rice Research Program, National Agricultural Research center, Islamabad. Pakistan. Seeds were sterilized in 5% Clorox (5.25% Sodium hypochlorite, The Clorox Co, USA) for one hour and then rinsed three times with sterile distilled water. Surface sterilized seeds were germinated on plastic trays. Seedlings were cultured in vitro under 25±3ºC ambient temperature, 60±5% relative humidity (RH) and 60± µmol m^{-2} sec^{-1} photosynthetic photon flux (PPF) provided by fluorescence lamps (TDL 36 W/84 Cool White 3350 1 m, Philips, Pakistan) with photoperiod of 16 h per day (Fig. 1).

Twenty five days old seedlings were transplanted in polyvinyl chloride (PVC) pots containing 10 kg soil. The pots were kept in the green house at National Agricultural Research Centre, Islamabad-Pakistan during 2010. The soil was air dried and sieved through 5 mm sieve and analyzed for physico-chemical properties before filling in pots. Electrical conductivity of soils was 1.00 dsm^{-1} with textural class of silt clay pH; 8.5, extractable phosphorus of 9.9 mg kg^{-1}, exchangeable K 102 mg kg^{-1}, total nitrogen 0.16 % and organic matter was 0.62%. Basal fertilizer was applied to the pots at 125, 95, 65, 5 and 1 kg ha^{-1} N, P_2O_5 and K_2O, Zn and B on soil weight basis in the form of urea, di-ammonium phosphate, sulphate of potash, zinc sulphate and borax. All the P, K, zinc and boron were applied at the time of sowing while N was applied in three equal splits.

Hormonal and Water Stress Treatment

Water stress was imposed at panicle initiation stage 80 days after transplanting in both the rice cultivars for 10 consecutive days till temporary wilting percentage. An aqueous solution of 10^{-5} M each of GA_3, IAA and kinetin was prepared and foliar application each of GA_3, IAA and kinetin @ 50 mL per plant was done at interval of one week. Control plants were untreated and irrigated with tap water keeping 5 cm water from the pot surface till physiological maturity. Plants were re-watered when visual stress symptoms i.e. leaf rolling appeared in the plants.

Morphological Attributes

Agronomic data such as plant height, panicle length, number of seeds per panicle, 100 seed weight and paddy yield were recorded at physiological maturity.

Physiological Attributes

The 2nd leaf was sampled and replicated thrice for measurement of chlorophyll contents by Arnon (1949), leaf proline following Bates *et al.* (1973) and soluble proteins by Bradford (1976).

Stem Reserve Translocation

Photosynthesis stem reserve translocation (PSR) was calculated according to Gallagher *et al.* (1975). Three main stems were taken from each plant as sample one after panicle initiation stage as well as at final harvest for measuring panicle and stem weight. After collection, both panicle and stem were dried at 72^0 C for 48 h and weighed. After drying samples were weighed.

This is based on the net loss in weight of above ground vegetative organs between anthesis and maturity with the differences between yield and net assimilation. It was calculated by the following formula:

$$\text{Photosynthesis Stem Reserve Translocation (\%)} = \frac{S_1 - S_2}{G_1 - G_2}$$

Where S_1= stem dry weight (g) one week after panicle initiation stage, S_2= stem dry weight (g) at maturity, G_1= grain dry weight (g) at one week after panicle initiation stage, G_2 = grain dry weight (g) at maturity.

Statistical Analysis

The collected data were subjected to analysis of variance using statistical package *Statistix 8.1*. The significance of differences among the treatment means was performed using the Tukey's Honest Significant Differences (HSD) test at 5% significance level.

Results

Chlorophyll Contents

Water stress reduced 9.1% and 12.7% leaf chlorophyll contents of KS-133 and Basmati-2000 respectively (Fig. 2). Application of plant growth regulators under water stress cause significant ($p \leq 0.05$) increase in leaf chlorophyll content of both the rice genotypes. Application of GA_3 under water stress caused 19.4% and 11% increase in leaf chlorophyll content in KS-133 and Basmati-2000 respectively. Likely, application of IAA under water stress caused 2.5% and 9.1% significant increase in leaf chlorophyll content of KS-133 and Basmati-2000 respectively.

Proline Contents

Proline content of both the rice genotypes significantly ($p \leq 0.05$) increased under water stress conditions (Fig. 3). Application of GA_3 under water stress caused 23.9% and 29.9% significant increase in leaf proline contents of KS-133 and Basmati-2000, respectively as compared to their respective well watered control. Likely, application of IAA under water stress caused 19.8% significant increase in leaf proline content of KS-133 under induced water stress as compared to its well watered control. Likely, kinetin spray caused 18.7% and 25.4% significant ($p \leq 0.05$) increase in leaf proline content of KS-133 and Basmati-2000 respectively as compared to their respective well watered plants.

Soluble Proteins

Water stress caused significant ($p \leq 0.05$) increase in leaf soluble protein content in both the rice cultivars (Fig. 4). Application of plant growth regulators under water stress caused significant increase in soluble protein contents of both rice cultivars. Application of GA_3 under water stress caused 24.2% and 9.4% significant ($p \leq 0.05$) increase in soluble protein contents of KS-133 and Basmati-2000 as compared to well watered control plants. Likely, Kinetin application under water stress caused 21.9% and 31% significant ($p \leq 0.05$) increase in soluble proteins of KS-133 and Basmati-2000, respectively.

Stem Reserve Translocation

Significant increase ($p \leq 0.05$) in photosynthates stem reserve translocation of both the rice genotypes under simulated water stress was found (Fig. 5). Application of GA_3 under water regimes caused 55.8% and 52.7% significant ($p \leq 0.05$) increase in PSR of KS-133 and Basmati-2000 as compared to well watered control. Likely, IAA application under water stress caused 55.2% and 50.4% significant ($p \leq 0.05$) increase in PSR of KS-133 and Basmati-2000 under water stress as compared to well watered control. Kinetin application under water stress caused 53.3% and 56.9% significant ($p \leq 0.05$) increase in PSR of KS-133 and Basmati-2000 as compared to well watered control.

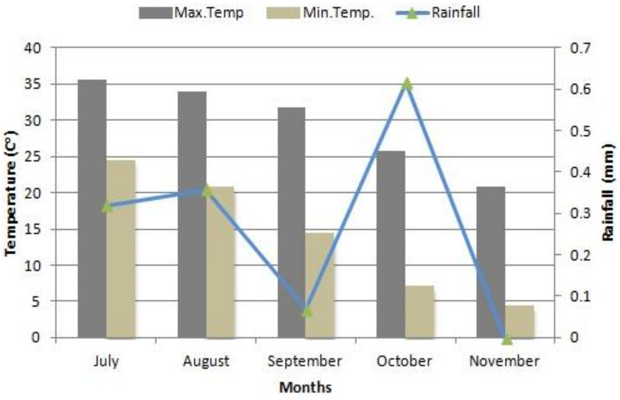

Fig. 1: Average weather conditions during the experimental period

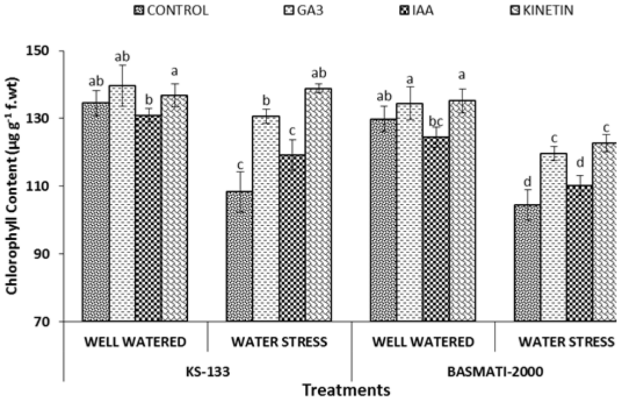

Fig. 2: Impact of foliage applied plant growth regulators on chlorophyll contents (µg g^{-1} f.wt) of KS-133 and Basmati-2000. Bars represent means of 3 replicates ± SE

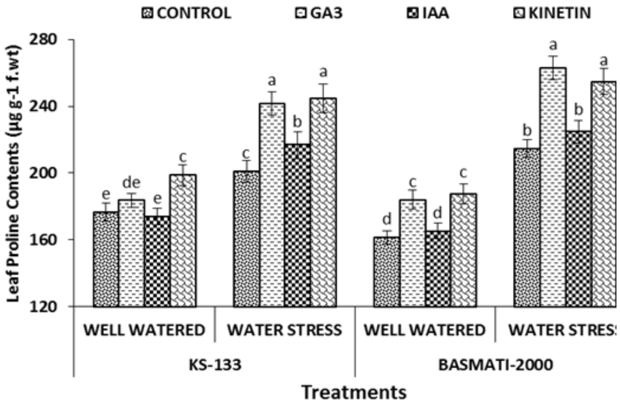

Fig. 3: Impact of foliage applied plant growth regulators on leaf proline contents (µg g^{-1} f.wt) of KS-133 and Basmati-2000 under water stress conditions. Bars represent means of 3 replicates ± SE

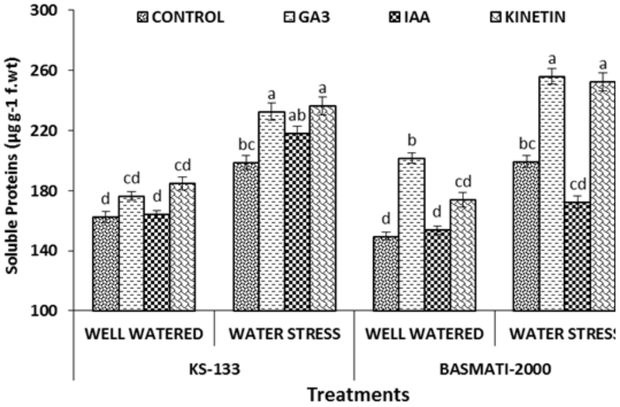

Fig. 4: Impact of foliage applied plant growth regulators on leaf protein contents of KS-133 and Basmati-2000 under water stress conditions. Bars represent means of 3 replicates ± SE

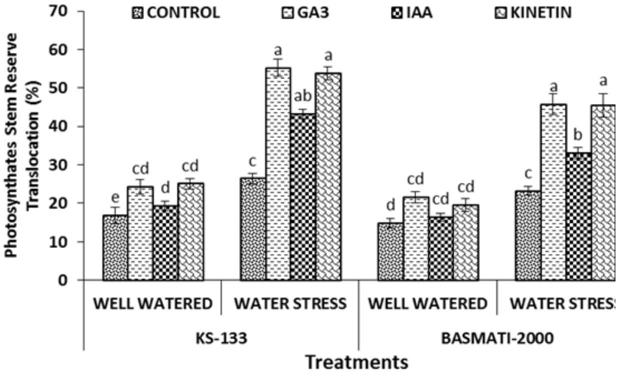

Fig. 5: Impact of foliage applied plant growth regulators on photosynthates stem reserve translocation in KS-133 and Basmati-2000 under water stress conditions. Bars represent means of 3 replicates ± SE

Agronomic Traits

Water stress at panicle initiation had significant effect on plant height, panicle length, number of seeds per panicle, 100 grains weight and paddy yield of KS-133 and Basmati-2000 (Table 1).

Panicle length was significantly ($p\leq 0.05$) reduced under simulated water stress at panicle initiation stage in both the rice cultivars (Table 2). Foliage applied GA_3 on water stresses plants caused 8.6% significant ($p\leq 0.05$) increase in plant height of KS-133 under water stress as compared to well watered control. Likely, kinetin spray on water stressed plants caused 13.8% and 16.6% significant ($p\leq 0.05$) increase in plant height of KS-133 and Basmati-2000 as compared to well watered control, respectively. The overall trend of increase in plant height by plant growth regulators was GA3>Kinetin>IAA.

Application of GA_3 under water stress caused 13.9% and 17.8% significant ($p\leq 0.05$) increase in panicle length of KS-133 and Basmati-2000 as compared to their well-watered control. Similarly, foliage applied IAA under water stress caused 18.7% significant ($p\leq 0.05$) increase in panicle length of KS-133 as compared to its well watered control. Spray application of kinetin caused 8.7% and 21.9% significant ($p\leq 0.05$) increase in panicle length of KS-133 and Basmati-2000 under water stress as compared to well-watered control, respectively.

Significant ($p\leq 0.05$) differences for number of seeds per panicle among different plant growth regulators treatments under induced water stress (Table 3). Water stress caused 45.2% significant ($p\leq 0.05$) reduction in number of seeds per panicle as compared to its well watered control. Application of GA_3 under water stress caused to 43% and 28.6% significant ($p\leq 0.05$) increase in number of seeds per panicle in KS-133 and Basmati-2000 as compared to well-watered control. External application of IAA on water stressed plants resulted to significantly ($p\leq 0.05$) increase the number of seeds per panicle in KS-133 as compared to its well watered control. Kinetin application as spray under water stress caused 37.8% and 27.6% significant ($p\leq 0.05$) increase in number of seeds per panicle of KS-133 and Basmati-2000 as compared to well-watered control.

Significant reduction ($p\leq 0.05$) in 100 grain weight of both the rice cultivars was found under simulated water stress (Table 4). Exogenously applied GA_3 on stressed plants caused 47.5% and 20.6% significant ($p\leq 0.05$) increase in 100 grain weight of KS-133 and Basmati-2000 as compared to well watered (untreated) control. The IAA spray resulted to significantly ($p\leq 0.05$) improve the 100 grain weight of Basmati-2000 as compared to its well watered control. The kinetin spray caused 45.7% and 23.1% significant ($p\leq 0.05$) increase in 100 grain weight of KS-133 and Basmati-2000 respectively as compared to well watered control.

Paddy yield of both the rice cultivars was significantly ($p\leq 0.05$) reduced (43.9% and 44.4%) in KS-133 and Basmati-2000 respectively under water stress conditions (Table 5). The external application of GA_3 to water stressed plants caused 37.2% and 43.5% significant ($p\leq 0.05$) increase in paddy yield of KS-133 and Basmati-2000 as compared to well-watered control. Spray application of IAA and water regimes leads to non-significant increase in paddy yield of both the rice cultivars under water stress. Contrary, Kinetin sprays under water stress caused 38.3% and 41.5% significant ($p\leq 0.05$) increase in paddy yield of KS-133 and Basmati-2000 as compared to well-watered control.

Discussion

Water stress adversely affected growth, yield and biochemical traits of both rice varieties. Foliar application of GA_3, IAA and kinetin at panicle initiation stage was found beneficial for growth, yield and biochemical characters of both the rice genotypes under water stress.

Decrease or no-change in chlorophyll content of plant under water stress has been noticed in different plant species

Table 1: Impact of foliage applied plant growth regulators on plant height (cm) of KS-133 and Basmati-2000 under water stress imposed 80 days after transplanting at panicle initiation stage

Treatments	cv. KS-133		Basmati-2000	
	Control	Water Stress	Control	Water Stress
Control	92.23 a	64.60 f	93.67 cd	75.27 f
GA$_3$	86.60 ab	79.13 cd	117.57 a	84.23 def
IAA	84.66 bc	71.33 ef	105.10 b	77.57 ef
Kinetin	84.43 bc	72.73 de	103.67 bc	86.30 de
LSD	Hormones × Water Regimes:7.4		Hormones × Water Regimes: 10.4	

All such means which share a common letter are statistically similar; otherwise differ significantly at $P \leq 0.05$

Table 2: Impact of foliage applied plant growth regulators on panicle length (cm) of KS-133 and Basmati-2000 under water stress imposed 80 days after transplanting at panicle initiation stage

Treatments	cv. KS-133		Basmati-2000	
	Control	Water Stress	Control	Water Stress
Control	17.73 c	13.90 d	24.90 bc	19.56 d
GA$_3$	24.46 a	21.00 b	20.30 a	24.73 bc
IAA	22.80 ab	18.53 c	28.00 ab	21.86 cd
Kinetin	22.93 ab	20.90 b	30.06 a	23.46 c
LSD	Hormones × Water Regimes: 2.3		Hormones × Water Regimes: 3.6	

All such means which share a common letter are statistically similar; otherwise differ significantly at $P \leq 0.05$

Table 3: Impact of foliage applied plant growth regulators on number of grains per panicle of KS-133 and Basmati-2000 under water stress imposed 80 days after transplanting at panicle initiation stage

Treatments	cv. KS-133		Basmati-2000	
	Control	Water Stress	Control	Water Stress
Control	91.67 c	43.67 f	91.00 bc	53.00 e
GA$_3$	118.67 a	67.67 d	108.33 a	77.33 cd
IAA	107.67 b	54.67 e	97.00 ab	67.00 de
Kinetin	112.0 ab	69.67 d	108.67 a	78.67 cd
LSD	Hormones × Water Regimes: 10.70		Hormones × Water Regimes: 15.13	

All such means which share a common letter are statistically similar; otherwise differ significantly at $P \leq 0.05$

Table 4: Impact of foliage applied plant growth regulators on 100 grains weight (g) of KS-133 and Basmati-2000 under water stress imposed 80 days after transplanting at panicle initiation stage

Treatments	cv. KS-133		Basmati-2000	
	Control	Water Stress	Control	Water Stress
Control	2.18 b	0.72 c	1.53 c	0.49 e
GA$_3$	4.37 a	2.29 b	2.42 a	1.92 b
IAA	3.56 a	1.62 bc	1.85 b	1.25 d
Kinetin	4.37 a	2.37 b	2.42 a	1.86 b
LSD	Hormones × Water Regimes: 0.27		Hormones × Water Regimes: 0.21	

All such means which share a common letter are statistically similar; otherwise differ significantly at $P \leq 0.05$

Table 5: Impact of foliage applied plant growth regulators on paddy yield (g) per plant of KS-133 and Basmati-2000 under water stress imposed 80 days after transplanting at panicle initiation stage

Treatments	cv. KS-133		Basmati-2000	
	Control	Water Stress	Control	Water Stress
Control	30.40 c	15.80 e	25.40 c	13.76 e
GA$_3$	45.00 a	28.26 cd	35.53 a	20.03 d
IAA	39.63 b	18.23 e	30.60 b	16.13 de
Kinetin	38.16 b	23.53 d	32.66 ab	19.10 d
LSD	Hormones × Water Regimes: 5.27		Hormones × Water Regimes: 4.06	

All such means which share a common letter are statistically similar; otherwise differ significantly at $P \leq 0.05$

and its intensity depends on stress rate and duration (Jagtap *et al.,* 1998). Chlorophyll content decreased under water stress in both the rice cultivars. Foliar spray of GA$_3$ ameliorated the water stress induced inhibition in chlorophyll content of both the rice cultivars. It may be attributed to the protective effects of GA$_3$ on photosynthetic machinery of rice plants causing least oxidative stress. Kaya *et al.* (2006) found that foliar spray of GA$_3$ enhanced the water deficit tolerance in maize plants by preserving membrane penetrability, which positively correlated with enhanced chlorophyll content. This may also be due to the GA_3 induced augmentation of ultra organizational morphogenesis of plastids joined with holding of chlorophyll and postponement of senescence induced by the hormone application (Arteca, 1997).

Proline accumulation under water deficit helps the plant to protect cell membrane and leaf proteins from deleterious effects of drought stress (Parida *et al.,* 2007). Under water stress, proline behaves as an osmolite and plays a crucial part in maintaining osmotic adjustment in plants under water stress (Ullah *et al.,* 2012). Proline also works as an electron receptor that protects the photosynthetic machinery during oxidative stress resulting from other abiotic and biotic stresses (Ghorbanli *et al.,* 2013). Proline buildup under water deficiency in plants is a common phenomenon (Zhang *et al.,* 2006), which was also observed in present study. The growth regulators GA_3 and kinetin further augmented the drought induced accumulation of proline and the effect was markedly higher in cv. Basmati-2000. It might be due to involvement of kinetin in osmotic adjustment under water stress. Cha-um *et al.* (2010) found that glycinebetaine and kinetin foliar spray enhanced the level of proline in the leaves of crops under water stress and helped to overcome physiological limitation by proline accumulation.

Foliar spray of GA_3 and kinetin is reported to overcome the physiological constraints under water stress by enhancing the production of proline in many crops (Stevens *et al.,* 2006). Soluble protein contents of rice plants were enhanced under water deficit in both the rice genotypes which further augmented by foliar spray of GA_3 and kinetin. Basmati-2000 was more responsive than KS-133 by accumulating higher protein content by the application of plant growth regulators under water stress conditions. The increase in soluble protein contents in rice plants by exogenous application of plant growth regulators under water stress may be due to their possible involvement in water stress adaptation process (Tuna *et al.,* 2008). The plants produce proteins which are involved in decontamination of free radicals and thus play important role in plants adaptation under stress conditions (Witzel *et al.,* 2009; Bandehagh *et al.,* 2011). It is well documented that the cellular proteins contents of plants pass through variations under stress conditions (Guo and Song, 2009; Mohammadkhani and Heidari, 2008). Ismail (2003) observed increase in proteins contents of sorghum plants by application of gibberellic acid under water stress.

Application of plant growth hormones were found to be effective to rouse physiological reactions of plants under water stress conditions and modify the leaves to grain assimilates transfer through affecting carbohydrate synthesis and grain development. It might be due to the fact that carbohydrates synthesized before anther shedding are stored in the caryopsis through redistribution from assimilate found in the stalks (Iqbal *et al.,* 2011). It was reported that gibberellins play important role in modulating diverse processes throughout plant development. GA_3 improve photosynthesis efficiency of plants by affecting photosynthesis related enzymes, leaf area, light capture and enhance the efficacy of nutrients, consequently improve the source strength and redistribute the assimilates and increase the sink strength (Khan *et al.,* 2007). In present study foliar spray of GA_3, IAA and kinetin were found to enhance the photosynthetic stem reserve translocation 50-57.9% in both the tested rice genotypes under water stress conditions. This might me due to involvement of plant growth regulators in improving the physiological efficiency of both the rice genotypes by changing the equilibrium between carbohydrate metabolism and their breakdown. It was reported that kinetin improves the export of assimilates from source organs and regulate source-sink relations (Roitsch and Ehneß, 2000). Thus GA_3 and kinetin could be regarded as essential regulators in the translocation and partitioning of photo-assimilates for gain filling in rice.

The decline in yield and yield components of rice under water stress have been well documented (Sarvestani *et al.,* 2008) and adverse effects of water stress on paddy yield and yield components were augmented by foliar application of GA_3, IAA and kinetin of present study. Water stress causes inhibition of assimilate movement towards the developing reproductive organs, which might be the reason for the observed decrease in number of grains per panicle, 100 grains weight and paddy yield per plant. Adverse effects of water stress might be ameliorated in rice genotypes mainly by the application of hormones through rejuvenation of the sink potential and enhancement of the duration or rate of dry mass accumulation (Ritenour *et al.,* 1996; Awan *et al.,* 1999; Naeem *et al.,* 2004).

Conclusion

Foliar spray of GA_3 and Kinetin alleviated the deleterious effects of water stress in rice plants by enhancing proline accumulation, increasing soluble proteins, and hence supported to preserve the water requirements of rice plants. These treatments supplementary activated the flow of carbohydrates from leaves to grains and hence produced higher grain yield.

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