Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 2, No. 12, p. 8-15, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

The role of exogenous salicylic acid (SA) on phytohormonal changes and drought tolerance in common bean (*Phaseolus vulgaris* L.)

O. Sadeghipour*, P. Aghaei

Department of Agronomy, Shahre-Rey Branch, Islamic Azad University, Tehran, Iran

Received: 18 November 2012
Revised: 06 December 2012
Accepted: 07 December 2012

Keywords: Auxin, gibberellic acid, cytokinin, abscisic acid, salicylic acid, water stress.

Abbreviations: ABA (Abscisic acid); GA_3 (Gibberellic acid); CK (Cytokinin); IAA (Auxin or Indole-3-acetic acid); SA (Salicylic acid).

Abstract

Salicylic acid (SA) is an endogenous plant growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants and also plays an important role in provoking plant resistance to various abiotic stresses. An experiment was therefore, conducted to evaluation the effect of exogenous application of SA on the changes of phytohormones and biomass of common bean under water stress conditions during 2011 in Iran. Seeds were soaked at various concentrations of SA (0, 0.25, 0.5, 0.75 and 1 mM) for 6 h. Then plants were subjected to normal and drought conditions. Drought decreased significantly endogenous auxin (IAA), gibberellic acid (GA_3) and cytokinin (CK) levels while increased abscisic acid (ABA) content. Water stress also reduced biomass production. SA-pretreatment (especially 0.5 mM) enhanced IAA, GA_3 and CK levels whereas decreased ABA content under both conditions and so improved biomass production. Results suggest that SA can alleviate the adverse effects of drought in common bean via changes in levels of endogenous plant hormones.

***Corresponding Author:** O. Sadeghipour ✉ osadeghipour@yahoo.com

Introduction

In the face of a global scarcity of water resources, water stress has already become a primary factor in limiting crop production worldwide. It has become urgent to elucidate the responses and adaptation of crops to water stress and take action to improve the drought tolerance of crops. Plants respond to water deficit and adapt to drought stress through various physiological and biochemical changes including changes of the endogenous phytohormone levels, especially that of ABA (Wang *et al.,* 2008). Generally, ABA increases markedly under drought (Xie *et al.,* 2003; Pospisilova *et al.,* 2005; Wang *et al.,* 2008). However, the variation of IAA content under water stress is very contradictory. It was reported that drought resulted in a decrease of IAA content in the leaves of wheat (Xie *et al.,* 2003). Wang *et al.,* (2008) reported that there was a transient increase in the IAA content during the initial stage of adaptation to water stress in maize leaves, but it dropped sharply thereafter in response to water stress. However, other evidence has shown that the adaptation to drought was accompanied with an increase in the IAA content (Sakurai *et al.,* 1985; Pustovoitova *et al.,* 2004). Under osmotic stress, GA_3 and CK are able to increase germination percentage and seedling growth in chickpea (Kaur *et al.,* 1998). Yet, knowledge about the variation of CK and GA_3 contents in plants under water stress is scarce (Pospisilova *et al.,* 2005; Yang *et al.,* 2001; Xie *et al.,* 2003). Hormonal regulation of plant growth and metabolism is complex and interactions among phytohormones are widespread. The interactions include both positive and negative reciprocal effects on the phytohormone synthesis and different relations between signaling pathways. Water stress affects many metabolic pathways, mineral uptake, membrane structure, etc. Therefore it is not surprising that hormone contents can be also changed by water stress. This is very important because plant hormones are considered as main signals in root-to-shoot communication and vice versa (Pospisilova *et al.,* 2005).

Understanding of methods to induce stress tolerance in plants against adverse environmental conditions such as drought is vital and necessary. A possible approach to minimize drought damages that induces crop losses is the exogenous application of chemicals. SA is a common plant-produced signal molecule of phenolic nature which participates in the regulation of numerous physiological processes (Shakirova *et al.,* 2003). The exogenous application of SA was reported to have an effect on a wide range of physiological processes including increasing plants tolerance to different abiotic stresses such as drought, salinity, cold, heat, heavy metals and UV (Reviewed by Hayat *et al.,* 2010).

Common bean (*Phaseolus vlugaris* L.) generally, is known to be drought-sensitive crop (Beebe *et al.,* 2008). In spite this fact, most dry bean production in the world takes place under rainfed conditions and drought due to insufficient or unpredictable rainfall limits yield. Nearly 60% of bean production occurs in agricultural land prone to water deficit, where the costs of irrigation or the lack of precipitation are major difficulties for producers (Graham and Ranalli, 1997). That is why the mechanisms involved in the formation of drought tolerance are of great importance with regard further improvement of common bean agronomic performances and obtaining of more resistant cultivars (Subbaro *et al.,* 1995).

There are little evidences about impact of exogenous SA application on changes of plant hormone levels in common bean under water stress conditions; therefore the present study was performed to evaluation of the effect of SA application on phytohormonal changes and common bean tolerance to water stress.

Materials and methods

The experiment was conducted during summer 2011 in research farm of the Shahre-Rey Branch, Islamic Azad University, Tehran, Iran. The experimental area lies between longitude 51° 28´ E, latitude 35° 35´ N and 1000 m altitude. The mean annual rainfall and temperature are 201.7 mm and 20.4° C respectively. Soil texture was sandy clay loam containing nitrogen 0.091%, phosphorous 9.1 ppm, potassium 350 ppm, EC 2.8 dS m^{-1} and pH 7.8. The experiment was laid out in a split plot on the basis of randomized complete block design with four replications. Each replication had two main plots as irrigation levels viz, I0: Irrigation after 50 mm evaporation from class A pan and I1: Irrigation after 100 mm evaporation from class A pan, as control and water stress conditions, respectively. Each main plot consisted of five sub plots as common bean (cv. Derakhshan) seeds were soaked for 6 h in SA solutions (0, 0.25, 0.5, 0.75 and 1 mM). Seeds before treatment were sterilized with sodium hypochlorite solution (1%) for 5 min and then washed thoroughly with distilled water. Seeds were treated with Bavistin and then were sown by hand on 12 June 2011 in 4 cm depth of soil. At the same time plots were fertilized with 100 kg ha^{-1} ammonium phosphate. Each sub plot had 4 planting rows with length of 5 m thus; size of each plot was 10 m^2. Distances on and between rows were 10 and 50 cm respectively. All plots were irrigated immediately after sowing, but subsequent irrigations were carried out according to the treatments. Crop management practices such as hand weeding and thinning were done as required. At the flowering stage, plant hormone levels were measured. For estimation of growth regulators, 3-5 g fresh samples of the youngest fully expanded leaf were frozen in liquid nitrogen and stored at -20° C until analysis. The method of extraction in ethanol and the fraction of the ethanol extract were carried out according to the method described by Shindy and Smith (1975). The acidic fraction contain the acidic hormones (IAA, GA_3 & ABA) while the aqueous fraction comprised the CK. The growth promoters (IAA, GA_3 & CK) and the growth inhibitors (ABA) were estimated using high performance liquid chromatography (HPLC). Finally at physiological maturity, plants in 2 m^2 of each plot were harvested and above ground dry matter was recorded after drying in oven at 75° C for 48 h. Collected data were analyzed by MSTAT-C statistical software and the means were compared by Duncan's Multiple Range Test (DMRT) at the 5% probability level (Steel and Torrie, 1980).

Results and discussion

Our study showed that water stress significantly decreased growth promoting hormones (IAA, GA_3 and CK) level in common bean plants about 11%, 8% and 14% respectively; as compared with control, while reversibly increased ABA content about 7% (Fig. 1-4). These results were similar to those of Abass and Mohamed (2011); Abdalla (2011); Abdalla and El-Khoshiban (2007); Zhang *et al.,* (2011) and Shi *et al.*, (1994) who found that drought decreased growth promoting hormone levels but increased ABA content. Drought stress appeared to inhibit the biosynthesis of IAA and GA_3 and/or increase their degradation (Poljakoff-Mayber and Lerner, 1994). Bano and Yasmeen (2010) also reported that drought significantly decreased IAA and GA_3 concentration in wheat leaves than that of control. It may be due to decrease IAA and GA_3 synthesis (Xie *et al.*, 2004) or increase in the destruction of these by increasing the activity of oxidase (Davenport *et al.*, 1980). It can be supposed that the decrease of growth promoting hormones under drought is related to reduce growth performance. Decline of IAA content under water stress is a well known phenomenon (Wang *et al.*, 2008; Yang *et al.,* 2001), this being in accordance with our results.

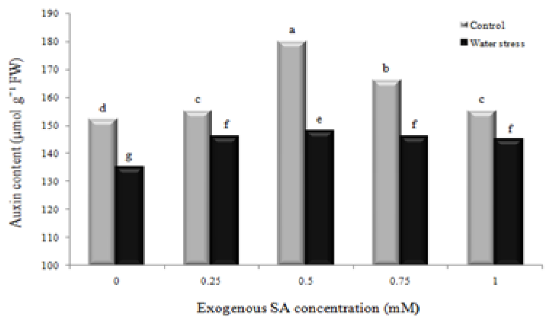

Fig. 1. Effect of exogenous salicylic acid application on endogenous auxin content under control and water stress conditions.

Under drought conditions an increase in the ABA content seems to be related to rapid defense responses. It is usually believed that such responses include stabilization of membranes, closing of stomata, which decreases water loss by leaves, and an increased water uptake by roots (Pustovoitova *et al.,* 2004).

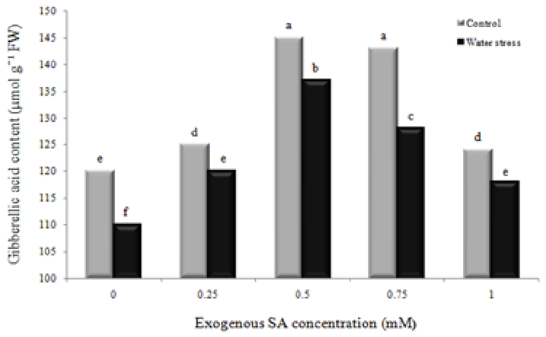

Fig. 2. Effect of exogenous salicylic acid application on endogenous gibberellic acid content under control and water stress conditions.

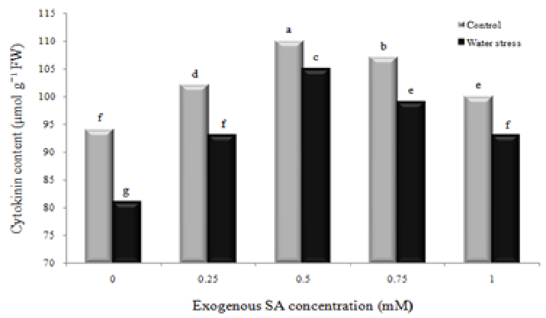

Fig. 3. Effect of exogenous salicylic acid application on endogenous cytokinin content under control and water stress conditions.

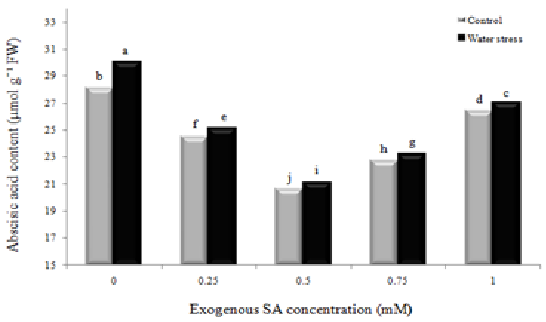

Fig. 4. Effect of exogenous salicylic acid application on endogenous abscisic acid content under control and water stress conditions.

The biosynthesis of ABA have previously been thought to occur only in the roots, but more recent studies show that ABA is also synthesized in mesophyll cells, vascular tissue and stomata. As stated above increased levels of ABA in leaves induces and regulates stomatal closure, while the increased levels of ABA in roots increase the hydraulic conductivity increasing the water uptake and transportation (Parent *et al.,* 2009). It has been proposed that ABA and CK have opposite roles in drought stress. Increase in ABA and decline in CK levels favor stomatal closure and limit water loss through transpiration under water stress (Morgan, 1990). Above results show that drought is able to changes the hormonal balance in plants towards increasing the level of growth inhibitor (ABA) while decreasing the growth promoting hormone (IAA, GA_3 and CK) levels which resulting to change their mechanism to overcome drought by stomata closure so as to reduce water loss, which in turn, reduces gas exchange thus causing low CO_2 content, low photosynthetic and growth rate and finally low dry matter production. In our study, water stress increased ABA content while reduced growth promoting hormones (IAA, GA_3 and CK) content and finally decreased biomass production (Fig. 5).

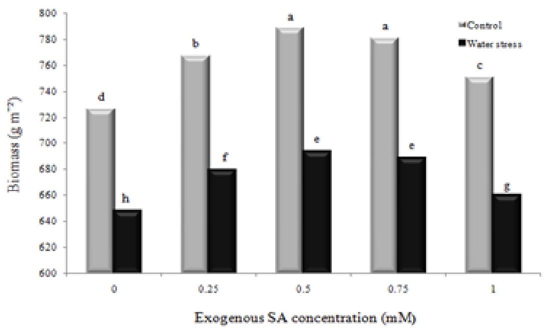

Fig. 5. Effect of exogenous salicylic acid application on biomass production under control and water stress conditions.

Our research also revealed that the exogenous application of SA (especially 0.5 mM) under both normal and water stress conditions caused significant increase in IAA, GA_3 and CK contents; in contrast, decreased ABA level. These changes in plant hormone levels caused to improved biomass production (Fig. 5). These results are in line with those of Saruhan *et al.,* (2011); Farooq *et al.,* (2009) and Haji *et al.,* (2009) who observed that water stress decreased biomass production but SA application improved this trait. We observed that seeds soaking in 0.5 mM SA as compared to untreated seeds; increased IAA content by 10% and 18% under water stress and control conditions, respectively (Fig. 1). Exogenous application of 0.5 mM SA than that of no application, raised GA_3 level by 25% and 21% under drought and normal conditions, respectively (Fig. 2). Pretreatment of seeds with 0.5 mM SA as compared to untreated, increased CK content by 30% and 17% under water stress and no stress conditions, respectively (Fig. 3). Seeds soaking in 0.5 mM SA than that of untreated seeds; decreased ABA level by 29% and 27% under drought and control conditions, respectively (Fig. 4). Similar to our results the positive effect of exogenous SA on growth promoting hormone levels has been reported by other researchers. For example; Gharib and Hegazi (2010) found that the content of IAA and GA_3 was increased in the different bean varieties, in response to seed soaking in 10^{-4} M SA at 15°C. Shakirova *et al.,* (2003) also observed that SA-treatment diminished changes in phytohormone levels in wheat seedlings under salinity. It prevented any decrease in IAA and CK contents and thus reduced stress-induced inhibition of plant growth. Sakhabutdinova *et al.,* (2003) reported that the SA treatment caused accumulation of IAA in wheat seedlings. However, did not influence CK content. SA is a phenolic compound naturally occurring in plants in very low amounts. Phenolics participate in some way on IAA metabolism by regulating IAA degradation or by controlling the formation of IAA conjugate (El-Mergawi and Abdel-Wahed, 2007). The sustained level of SA may be a prerequisite for the synthesis of IAA and/or CK (Metwally *et al.*, 2003). There is evidence that GA regulates dry matter partitioning between source and sink. Noushina *et al.,* (2011) recently reported that GA signaling is involved in adjustment of plants under limiting environmental conditions and maintained source-sink relation. This adjustment could be mediated through the GA and SA interaction.

Many studies about the effect of SA application on endogenous ABA and stomatal movements produced contradictory findings. However there are many reports that show exogenous SA application increase ABA content and thus induce the reduction of stomatal aperture and conductance (Hao *et al.,* 2011; Bandurska and Stroinski, 2005; Shakirova *et al.,* 2003) on the other hand in conformity with our findings; some researchers demonstrated that exogenous application of SA has negative effect on endogenous ABA level and so induce the increasing stomatal conductance. For example; Rai *et al.,* (1986) observed that SA application can reverse the stomatal closure induced by ABA. Waseem *et al.,* (2006) also reported that exogenous SA application caused an increase in stomatal conductance in wheat cultivars under drought stress as compared to control. Khan *et al.,* (2003); Idrees *et al.,* (2011) and Saruhan *et al.,* (2011) also obtained alike results. With respect to conflicting impacts of exogenous SA on endogenous ABA level and stomatal conductance it seems that depending on the exogenous SA concentration, kind of stress, stress intensity and duration, plant species and application method it can induces stoma closing or opening. The results of present study showed that pretreatment with SA reduced ABA content and thus raised stomatal conductance. This supports the conclusion that the effect of exogenous SA on diminish drought injury not only was not connected with increasing of ABA but also was related to increasing IAA, GA_3 and CK contents. SA also participates in the induction of different anti-stress programs. There are data supporting that SA increase the activity of antioxidant enzymes, synthesis of substances such as proline, maintenance chlorophyll content, stomatal conductance, net photosynthetic rate and relative water content under water stress conditions (Sadeghipour and Aghaei, 2012 a, b).

Conclusion

Drought decreased IAA, GA_3 and CK levels while increased ABA content in common bean. Nonetheless; SA application increased IAA, GA_3 and CK against decreased ABA levels and therefore raised biomass production. The exogenous application of

0.5 mM SA improved growth in common bean under water stress conditions. The role of SA in alleviating drought damages may be attributed to its ability to changes in plant hormone levels. This study showed an interesting effect of SA in drought stress response that should be important not only for a basic understanding of the role of the hormone but also for potential use of the chemical in agriculture.

Acknowledgements
The authors would like to thank the Shahre-Rey Branch, Islamic Azad University, Tehran, Iran for funding of this project.

References

Abass SM, Mohamed HI. 2011. Alleviation of adverse effects of drought stress on common bean (*Phaseolus vulgaris* L.) by exogenous application of hydrogen peroxide. Bangladesh Journal of Botany **41 (1),** 75-83.

Abdalla MM. 2011. Beneficial effects of diatomite on the growth, the biochemical contents and polymorphic DNA in *Lupinus albus* plants grown under water stress. Agriculture and Biology Journal of North America **2 (2),** 207-220.

Abdalla MM, El-Khoshiban NH. 2007. The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two *Triticium aestivum* cultivars. Journal of Applied Sciences Research **3 (12),** 2062-2074.

Bandurska H, Stroinski A. 2005. The effect of salicylic acid on barley response to water deficit. Acta Physiologiae Plantarum **27 (3B),** 379-386.

Bano A, Yasmeen S. 2010. Role of phytohormones under induced drought stress in wheat. Pakistan Journal of Botany **42 (4),** 2579-2587.

Beebe S, Rao IM, Cajiao C, Grajales M. 2008. Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. Crop Science **48,** 582-592.

Davenport TL, Morgan PW, Jordan WR. 1980. Reduction of auxin transport capacity with age and internal water deficit in cotton petioles. Plant Physiology **65,** 1023-1025.

El-Mergawi R, Abdel-Wahed M. 2007. Diversity in salicylic acid effects on growth criteria and different indole acetic acid forms among faba bean and maize. International Plant Growth Substances Association.19 Annual meeting, Puerto Vallarta, Mexico, July 21-25, 2007.

Farooq M, Basra SMA, Wahid A, Ahmad N, Saleem BA. 2009. Improving the drought tolerance in rice (*Oryza sativa* L.) by exogenous application of salicylic acid. Journal of Agronomy and Crop Science **195 (4),** 237-246.

Gharib FA, Hegazi AZ. 2010. Salicylic acid ameliorates germination, seedling growth, phytohormone and enzymes activity in bean (*Phaseolus vulgaris* L.) under cold stress. Journal of American Science **6 (10),** 675-683.

Graham HP, Ranalli P. 1997. Common bean (*Phaseolus vulgaris* L.). Field Crops Research **53,** 131-146.

Haji MAA, Bukhsh A, Malik AU, Ishaque M, Sadiq SH. 2009. Performance of sunflower in response to exogenously applied salicylic acid under varying irrigation regimes. The Journal of Animal & Plant Sciences **19 (3),** 130-134.

Hao JH, Wang XL, Dong CJ, Zhang ZG, Shang QM. 2011. Salicylic acid Induces stomatal closure by modulating endogenous hormone levels in cucumber cotyledons. Russian Journal of Plant Physiology **58 (5),** 906-913.

Hayat Q, Hayat S, Irfan M, Ahmad A. 2010. Effect of exogenous salicylic acid under changing environment: A review. Environmental and Experimental Botany **68,** 14-25.

Idrees M, Naeem M, Aftab T, Masroor M, Khan A, Moinuddin. 2011. Salicylic acid mitigates salinity stress by improving antioxidant defense system and enhances vincristine and vinblastine alkaloids production in periwinkle [*Catharanthus roseus* (L.) G. Don]. Acta Physiologiae Plantarum **33 (3),** 987-999.

Kaur S, Gupta AK, Kaur N. 1998. Gibberelic acid and kinetin partially reverse the effect of water stress on germination and seedling growth. Plant Growth Regulation **25,** 29-33.

Khan W, Prithviraj B, Smith DL. 2003. Photosynthetic responses of corn and soybean to foliar application of salicylates. Journal of Plant Physiology **160,** 485-492.

Metwally A, Finkemeier I, Georgi M, Dietz K. 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiology **132,** 272-281.

Morgan PW. 1990. Effects of abiotic stresses on plant hormone systems. In: Stress Responses in Plants: Adaptation and Acclimation Mechanisms. Alscher, R. G. and J. R. Cumming (Eds). Wiley-Liss, New York, pp. 113-146.

Noushina I, Rahat Nazar M, Iqbal R, Khan AM, Nafees AK. 2011. Role of gibberellins in regulation of source-sink relations under optimal and limiting environmental conditions. Current Science **100,** 998-1006.

Parent B, Hachez C, Redondo E, Simonneau T, Chaumont F, Tardieu F. 2009. Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: A trans-scale approach. Plant Physiology **149,** 2000-2012.

Poljakoff-Mayber A, Lerner HR. 1994. Plants in saline environments. In: Handbook of Plant and Crop Stresses. Pessarkali, M. (Ed.). Marcel Dekker Inc., New York, pp. 65-96.

Pospisilova J, Vagner M, Malbeck J, Travnickova A, Batkova P. 2005. Interactions between abscisic acid and cytokinins during water stress and subsequent rehydration. Biologia Plantarum **49 (4),** 533-540.

Pustovoitova TN, Zhdanova NE, Zholkevich VN. 2004. Changes in the levels of IAA and ABA in cucumber leaves under progressive soil drought. Russian Journal of Plant Physiology **51,** 513-517.

Rai VK, Sharma SS, Sharma S. 1986. Reversal of ABA-induced stomatal closure by phenolic compounds. Journal of Experimental Botany **37,** 129-134.

Sadeghipour O, Aghaei P. 2012a. Biochemical changes of common bean (*Phaseolus vulgaris* L.) to pretreatment with salicylic acid (SA) under water stress conditions. International Journal of Biosciences **2 (8),** 14-22.

Sadeghipour O, Aghaei P. 2012b. Response of common bean (*Phaseolus vulgaris* L.) to exogenous application of salicylic acid (SA) under water stress conditions. Advances in Environmental Biology **6 (3),** 1160-1168.

Sakhabutdinova AR, Fatkhutdinova DR, Bezrukova MV, Shakirova FM. 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. Bulgarian Journal of Plant Physiology, special issue 314-319.

Sakurai N, Akiyama M, Kuraishi S. 1985. Role of abscisic acid and indoleacetic acid in the stunted growth of water-stressed, etiolated squash hypocotyls. Plant and Cell Physiology **26,** 15-24.

Saruhan N, Saglam A, Kadioglu A. 2011. Salicylic acid pretreatment induces drought tolerance and delays leaf rolling by inducing antioxidant systems in maize genotypes. Acta Physiologiae Plantarum **34 (1),** 97-106.

Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fatkhutdinova RA, Fatkhutdinova DR. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Science **164 (3),** 317-322.

Shi JP, Li GM, Chi SM. 1994. The effect of calcium on endogenous hormones of maize seedlings under water stress. Journal of Hebei Agricultural University **17,** 48-51.

Shindy WW, Smith OE. 1975. Identification of plant hormones from cotton ovules. Plant Physiology **55,** 550-554.

Steel RGD, Torrie JH. 1980. Principles and procedures of statistics. A biometrical approach. 2nd Ed. McGraw-Hill Book Co. Inc., New York, 633 p.

Subbaro GV, Johansen C, Slinkard AE, Nageswara Rao RC, Saxena NP, Channah YS. 1995. Strategies for improving drought resistance in grain legumes. Critical Reviews in Plant Sciences **14 (6),** 469-523.

Wang C, Yang A, Yin H, Zhang J. 2008. Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. Journal of Integrative Plant Biology **50 (4),** 427-434.

Waseem M, Athar UH, Ashraf M. 2006. Effect of salicylic acid applied through rooting medium on drought tolerance of wheat. Pakistan Journal of Botany **38 (4),** 1127-1136.

Xie Z, Jiang D, Cao W, Dai T, Jing Q. 2003. Relationships of endogenous plant hormones to accumulation of grain protein and starch in winter wheat under different post-anthesis soil water statuses. Plant Growth Regulation **41,** 117-127.

Xie Z, Jiang D, Dai T, Jing Q, Cao W. 2004. Effect of exogenous ABA and cytokinin on leaf photosynthesis and grain protein accumulation in wheat ears cultured In vitro. Plant Growth Regulation **44,** 25-32.

Yang JC, Zhang JH, Wang ZQ, Zhu QS, Wang W. 2001. Hormonal changes in the grains of rice subjected to water stress during grain filling. Plant Physiology **127,** 315-323.

Zhang J, Smith DL, Liu W, Chen X, Yang W. 2011. Effects of shade and drought stress on soybean hormones and yield of main-stem and branch. African Journal of Biotechnology **10 (65),** 14392-14398.