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Effect of Foliar Spray of Kinetin and Brassinosteroid during Drought Period on Biochemical Parameters of Groundnut (*Arachis hypogaea* L.)

Punnia Viswan, K L Narasimha Rao, Y Ashoka Rani and M Lal Ahamed
Department of Crop Physiology, Agricultural College, Bapatla 522 101, A P

ABSTRACT

A field experiment was conducted in Agricultural College Farm, Bapatla, during *rabi* 2012-13 to study the effect of foliar sprays of kinetin and brassinosteroid during drought period on biochemical parameters *viz*., chlorophyll stability index, leaf proline content and nitrate reductase activity of groundnut. The treatments comprised of foliar sprays of kinetin @ 5 ppm and 10 ppm and homobrassinolide (HBL) @ 0.5 ppm, 1 ppm and 2 ppm at 32 DAS and at 32 and 45 DAS, water stress and irrigation without foliar spray as control in RBD with three replications. The treatment plots were exposed to water stress by withholding irrigation at 30 DAS, continuing for 20 days and relieving at 50 DAS. Foliar spray of homobrassinolide @ 1 ppm at 32 and 45 DAS during water stress gave higher values for all the biochemical parameters of groundnut in the study.

Key words : Biochemical parameters, Drought period, Groundnut, Spray of kinetin.

The most important districts of groundnut production in Andhra Pradesh are Prakasam, Kurnool, Guntur and Krishna. Groundnut is mainly grown as a *rabi* crop in coastal sandy loam soils of Andhra Pradesh in irrigated rice fallows, unirrigated rice fallows and unirrigated upland areas. During *rabi* groundnut cultivation is limited by drought stress which ultimately leads to lower yields than the potential yield.

Brassinosteroids and kinetin are two important plant hormones in the field of Crop Physiology which provide a wide scope in plant hormone research. They are proved to be effective in improving crop health to increase productivity. Brassinosteroids and kinetin increase crop yields by changing plant metabolism and protecting plants from environmental stresses. Fariduddin *et al.* (2004) identified that foliar application of kinetin and 28-homobrassinolide increased the activities of nitrate reductase and carbonic anhydrase, chlorophyll and total protein contents, net photosynthetic rate in leaves and number of pods and seed yield at harvest in *Vigna radiata.* Prakash *et al.* (2006) reported that foliar application of BRs increased photosynthesis, dry matter production and protein synthesis. Optimization of antioxidant defense mechanisms and physiological processes by kinetin and a significant role of exogenous phytohormones in conferring salt tolerance were reported by Shah (2011).

The present study has been undertaken with a view to study the effect of kinetin and brassinosteroid on drought tolerance ability of groundnut under water stress condition.

MATERIAL AND METHODS

The field experiment was conducted using TAG-24 variety of groundnut during *rabi* season (2012-13) in Agricultural College Farm, Bapatla. The experimental plot was laid out in randomized block design with three replications and 12 treatments. The soil type was sandy with a pH of 7.6 which was low in organic carbon (0.4%) and available nitrogen (164.5 Kg ha^{-1}), medium in available phosphorus (25 Kg ha^{-1}) and available potassium (346.5 Kg ha^{-1}). The treatments included, water stress (T_1), water stress + kinetin @ 5 ppm at 32 DAS (T_2), water stress + kinetin @ 10 ppm at 32 DAS (T_3), water stress + kinetin @ 5 ppm at 32 and 45 DAS (T_4), water stress + kinetin @ 10 ppm at 32 and 45 DAS (T_5), water stress + HBL @ 0.5 ppm at 32 DAS (T_6), water stress + HBL @ 1 ppm at 32 DAS (T_7), water stress + HBL @ 2 ppm at 32 DAS (T_8), water stress + HBL @ 0.5 ppm at 32 and 45 DAS (T_9), water stress + HBL @ 1 ppm at 32 and 45 DAS (T_{10}),

water stress + HBL @ 2 ppm at 32 and 45 DAS (T_{11}) and control without water stress and without hormone spray (T_{12}). Data were recorded on biochemical parameters *viz*., leaf proline content, chlorophyll stability index and nitrate reductase activity of groundnut

1.Leaf proline content (µg g^{-1} fresh weight)

Proline content in leaves was estimated by following the method given by Bates *et al*. (1973). Fresh leaf material of 500 mg was weighed and homogenized in 10 ml of 3 per cent suplphosalicylic acid. The homogenized mixture was filtered and the volume of the filtrate was made up to 25 ml. From the extract, 2 ml was taken in a test tube and 2 ml of glacial acetic acid was added to it and thoroughly mixed. To that solution 2 ml of acid ninhydrin was added (acid ninhydrin was prepared by dissolving 2.5 g of ninhydrin powder in 60 ml of glacial acetic acid and 40 ml of orthophosphoric acid) and kept in a boiling water bath maintained at 100^0c for 60 minutes. The test tube was brought to room temperature and 4 ml of toluene is added to it. After thorough shaking, the toluene fraction was separated and the optical density was determined at 520 nm using spectrophotometer.

$$\text{Proline (µg g}^{-1}\text{ fresh weight)} = \frac{\text{O.D. x 36.231 x V}}{\text{Y x W}}$$

Where,

O.D. — Optical density at 520 nm
V — Final volume of the extract
Y — Volume of the aliquot taken
W — Weight of the plant material

2. Chlorophyll stability index (%)

Chlorophyll stability index (CSI) was estimated by Spectrophotometric method suggested by Rajagopal *et al.* (1990).

The leaf samples were collected, surface cleaned and made into small pieces. Leaf sample of 250 mg is immersed in 50 ml of distilled water in two test tubes. One tube was subjected to heat in a water bath at 56°C for 30 minutes. The other tube was kept as control. After the completion of the reaction time the leaf samples were macerated with 80% acetone. The O.D. of the chlorophyll extract is then measured with spectrophotometer. From the O.D. values, total chlorophyll content of the treated and untreated samples are calculated by using the following formula.

$$\text{Total chlorophyll content (mg g}^{-1}) = \frac{\text{O.D. at 652 nm}}{34.5} \times \frac{V}{W}$$

The CSI is the ratio of the chlorophyll content of treated and untreated samples which is expressed as percentage.

$$\text{CSI (\%)} = \frac{\text{Total chlorophyll content of treated leaf}}{\text{Total chlorophyll content of untreated leaf}} \times 100$$

3.Nitrate reductase activity (µM NO_2^- g^{-1} hr^{-1})

To estimate nitrate reductase activity in the leaf, the method suggested by Hageman and Flesher (1960) was followed. The nitrite formed was estimated by the method described by Nicholas *et al*. (1976) by measuring the absorbance of the pink colour at 540 nm using spectrophotometer.

200 mg weight of leaf material was taken as pieces into culture tubes. To that 2.5 ml of 0.1 M phosphate buffer of 7.5 pH was added followed by 2.5 ml substrate solution and 0.1 ml n-propanol. The contents of the culture tubes were allowed for infiltration for about 20 minutes. Afterwards the culture tubes with its contents were incubated at 30^0C for 30 minutes and taken out. Following the incubation the enzyme action was stopped by vigorous shaking of the culture tubes. The incubation was carried out in complete darkness.

The extract of 1 ml from culture tube was transferred into a test tube and to that 1 ml of 1% sulphanilamide solution was added followed by 1 ml of 0.02% NEDD and 3 ml of distilled water and the final volume was made to 6 ml. The readings of the pink colour intensity were recorded at 540 nm. The concentration of nitrite formed was noted with the help of the standard curve and expressed as micro moles of NO_2^- formed g^{-1} fresh weight hr^{-1}.

Preparation of standard curve

Different dilutions of 1 ml of standard nitrite solution *viz*., 0, 10, 20,…, 200 micro moles were taken in serially numbered test tubes. To these test tubes 1ml of 1% sulphanilamide followed by 1 ml of 0.02% NEDD were added. Then volume of the standard solution in test tubes was made up to 6 ml with distilled water. The readings of the pink colour developed were recorded at 540 nm in terms of

Table 1. Effect of foliar spray of kinetin and brassinosteroid during drought period on biochemical parameters of groundnut.

Treatments	Proline content (µg g^{-1} fresh weight)	Chlorophyll stability index (%)	Nitrate reductase activity (ìM NO_2^- g^{-1} hr^{-1})
T_1:Water stress	89.6	63.6	11.28
T_2:Water stress+kinetin @ 5ppm at 32 DAS	132.7	76.4	14.72
T_3:Water stress+kinetin @ 10ppm at 32 DAS	112.8	72.2	12.85
T_4:Water stress+kinetin @ 5ppm at 32 and 45 DAS	147.9	81.1	16.58
T_5:Water stress+kinetin @ 10ppm at 32 and 45 DAS	140.5	79.6	15.72
T_6:Water stress+HBL @ 0.5ppm at 32 DAS	124.3	74.0	13.54
T_7:Water stress+HBL @ 1ppm at 32 DAS	178.4	87.8	19.33
T_8: Water stress+HBL @ 2ppm at 32 DAS	164.4	86.4	18.51
T_9:Water stress+HBL @ 0.5ppm at 32 and 45 DAS	149.4	83.7	17.65
T_{10}:Water stress+HBL @ 1ppm at 32 and 45 DAS	202.6	93.5	20.15
T_{11}:Water stress+HBL @ 2ppm at 32 and 45 DAS	197.8	89.6	19.72
T_{12}:Control (without water stress and without foliar spray)	72.9	68.7	11.80
SEm ±	2.32	1.68	0.35
CD	6.80	4.94	1.02
CV(%)	2.81	3.66	3.70

O.D. The standard curve of nitrite was thus obtained by plotting different concentrations of nitrite taken versus corresponding O.D. values obtained.

The data were analyzed statistically following analysis of variance (ANOVA) technique suggested by Panse and Sukhathme (1978) for randomized block design. The statistical hypothesis of equalities of treatment means was tested by F-test in ANOVA at 5 per cent level of significance. Critical difference was correlated at 5 per cent level of significance to compare different treatment means.

RESULTS AND DISCUSSION

Higher values for leaf proline content (202.6 µg g^{-1} fresh weight), chlorophyll stability index (93.5%) and nitrate reductase activity (20.15 µM NO_2^- g^{-1} hr^{-1}) were recorded in water stressed plants treated with 1 ppm HBL at 32 and 45 DAS (T_{10}). Water stressed plants (T_1) recorded lower values in all the three parameters followed by irrigated plants without hormone spray (T_{12}).

An increase in proline content was observed in groundnut leaves both by water stress and water stress + hormone sprays. The water stressed plants subjected to HBL spray @ 1 ppm at 32 and 45 DAS (T_{10}) recorded higher values compared to other treatments in the range of 2.4 to 79.6 per cent over water stress (T_1) by 2.3 folds and over control (T_{12}) by 2.8 folds. Water stress (T_1) increased proline content by 22.9 per cent over irrigated plants (T_{12}). Proline is the common osmolyte, whose accumulation provides an osmoprotection to the plants. From this experiment it is evident that both water stress and hormone sprays increased proline content significantly over irrigated plants without hormone spray. Application of HBL and kinetin to stressed plants enhanced proline content in leaves and also enabled them to maintain higher levels of proline even after relieving stress. This might be due to the mitigating effects of kinetin and BRs under stress condition in plants. These findings of BR-increased proline content were in tune with the reports of Vardhini and Rao (2001 and 2005) in sorghum and Anuradha and Seeta Rama Rao (2002). Kinetin-induced osmotic adjustment was found in callus cultures of cowpea in the experiment by Agarwal and Gupta (2005).

Water stress + HBL @ 1 ppm at 32 and 45 DAS (T_{10}) showed 1½ folds increase in CSI over water stressed plants (T_1) and by 36.1 per cent over irrigated plants without hormone spray (T_{12}). This treatment (T_{10}) increased CSI over other treatments in the range between 4.4 and 29.5 per cent. Irrigation (T_{12}) increased CSI over water stress (T_1) by 8.0 per cent. The better performance of brassinosteroid (BR) in maintaining CSI might be due to the positive effect of BR treatment in increasing chlorophyll content and reducing leaf senescence in water stress conditions. These findings were in tune with those of Rao *et al*. (2002) in which they reported an inhibitory effect of BRs on stress resulting in high membrane stability, chlorophyll stability and production of stress related proteins against stress.

The HBL application @ 1 ppm at 32 and 45 DAS (T_{10}) resulted in an increase in nitrate reductase activity (NRA) over all other treatments at all stages of the crop. This treatment (T_{10}) increased NRA by 1.8 folds and 1.7 folds over water stressed plants and irrigated plants without foliar spray, respectively. NRA could be increased by about 4.6 per cent in irrigated plants (T_{12}) when compared to water stressed plants (T_1). The increase in NRA by kinetin might be due to the positive effect of cytokinin in nodulation (Gonzalez-Rizzo *et al.*, 2006, Murray *et al*., 2007 and Tirichine *et al*., 2007).

CONCLUSIONS

From the present investigation, it is concluded that the double spray of brassinosteroids @ 1ppm at 32 and 45 DAS significantly increased biochemical parameters like leaf proline content, chlorophyll stability index and nitrate reductase activity and ultimately helped to mitigate water stress in groundnut.

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