Pak. J. Bot., 38(5): 1511-1518, 2006.

EFFECT OF ABSCISIC ACID AND CHLOROCHOLINE CHLORIDE ON NODULATION AND BIOCHEMICAL CONTENT OF *VIGNA RADIATA* L. UNDER WATER STRESS

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Abstract

The present study was carried out to evaluate the effect of abscisic acid (ABA) and chlorocholine chloride (CCC) on growth, nodulation and changes in endogenous level of plant hormones indole acetic acid (IAA) and Gibberellic acid (GA) of mung bean grown under normal or water stress conditions. Two varieties (cv.NM 98 and cv.HCM 209) of mung bean were soaked in 10^{-6} M ABA or in chlorocholine chloride and grown for five weeks under non-stress conditions after which water stress was imposed at 50% flowering (37 days after sowing). Drought stress reduced fresh and dry weight of root and shoot,decreased the diameter of pink bacteroid tissue and number of root nodules. However, ABA and CCC pre-soaking treatments partially alleviated the inhibitory effect of drought. Pre-soaking with ABA or CCC in non-stressed or stressed plants caused increase in proline, protein, sugar and chlorophyll content and peroxidase activity of leaves. However, ABA treatment caused a maximal increase in proline accumulation while maximal increase in peroxidase activity was observed in plants raised from seeds treated with CCC. Likewise, endogenous levels of phytohormones GA and IAA were also increased following ABA application.whereas, CCC increased IAA. Maintenance of water budget by increase in relative water content of leaves,geater increase in proline production,higer activity of antioxidant enzyme, peroxidae and better survival of *Rhizobium* in soil following ABA and CCC application appears to be the mechanism for providing mung bean tolerance to water stress. The effects of CCC was similar to ABA under water stress and may be implicated to combat water stress on farmers level because of better cost benefit ratio as compared to ABA.

Keyword: nodulation, mungbean, water stress, abscisic acid, chlorocholine

Introduction

Drought is one of major abiotic stresses that causes heavy crop production losses world wide. Furthermore, the climatic-change models predict that in many regions of world, crop losses due to increasing aridity will further increase in future (Athar & Ashraf, 2005). Grain legumes respond to drought differently and express various drought tolerance strategies (Subbarao *et al*., 1995). Mungbean like many other crops is sensitive to water availability though it is sensitive to water stress at all growth stages, it is more sensitive to drought at flowering and grain development stage (Zubair *et al*., 2002; Thaloot *et al*., 2006). Of various plant responses to water shortage, enhanced accumulation of ABA is one of key mechanism of adaptation to water stress (Esther *et al*., 2000). Water stress also induced the accumulation of other compatible solutes such as glycerol, sugar, betaines and proline. The accumulation of free proline in plants may be part of a general adaptation to water stress (Ashraf & Foolad, 2007). Free proline has been suggested as a metabolic measure of drought, and is suggested to play an important role as an organic osmolyte. Various studies have focused on the ability of proline as a

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compatible osmolyte involved in osmotolerance; however, its specific role is still unclear (Ashraf & Foolad, 2007). In view of various strategies adapted by plants to acclimatize abiotic stresses, Ashraf & Foolad (2005; 2007) proposed that exogenous application of plant growth regulators, compatible solutes, antioxidants or inorganic salt can induce stress tolerance by modulating various biochemical and physiological processes. For example, Raza *et al*. (2006; 2007) found that foliar application of glycinebetaine improved the growth of wheat by improving photosynthesis, K^+/Na^+ ratio, and antioxidant capacity. Similarly, root applied salicylic acid improved growth of wheat under salt stress conditions (Arfan *et al*., 2007) as well as under water stress conditions (Waseem *et al*., 2006). Thus, in view of role of chlorocholine chloride (CCC) in thickening of culms that enhances plant stability improvement in water stress tolerance by its exogenous application is expected. Similarly, ABA has a role in number of physiological functions such as photosynthesis, ion and water homeostasis and signal transduction. Although comprehensive effects of ABA on the water use efficiency is known, scanty information is available on the water budget of leguminous plants exposed to moisture stress. Furthermore, their role in nodulation in mung bean, on the survival efficiency of *Rhizobium* in soil and the symbiotic efficiency under water stress were also assessed at flowering stage and relationship was drawn between endogenous level of growth promoting hormones, IAA and GA and various biochemical attributes.

Materials and Methods

Seeds of two varieties of mungbean cv NM 98 and NCM 209 were obtained from pulses program, National Agricultural Research Center (NARC), Islamabad. Prior to sowing seeds were soaked for 7h in aqueous solution of ABA and CCC each of (10^{-6}M) and distilled water (in case of control).Seeds of both cultivars were sown in pots under natural condition during the monsoon season (July-mid – September, 2005. Water stress was induced at 50% flowering (37 DAS) by withholding the supply of water. Water stress was imposed for 12 days till 50% decrease in soil moisture. Thereafter plants were harvested. A group of plants were re-watered for 12 days and plants were harvested. Before harvesting various physiological and biochemical attributes were measured. Relative water content of leaves (leaf no. 4 from top) was determined following the method given by Gupta *et al*. (1996). Protein and proline content of leaves were determined following the method of Lowry *et al*. (1951), and Bates *et al*. (1973). While leaf chlorophyll contents were measured and calculated following Arnon (1949) and Kirch (1968). Total chlorophyll was determined for the equation:

$$\text{Total chlorophyll (mg/l)} = (20.2 \times A645) + (8.02 \times B663)$$

Total soluble sugar estimation of leaves was done by the method of Dubo *et al*. (1956). Peroxidase activity was determined by the method of Vetter *et al*. (1958) as modified by Gorin & Heidema (1976). Extraction and purification of hormones (IAA and GA) was made following the method of Kettner & Doerffling (1995). The detection was made with HPLC equipped with variable UV detector the wavelength used for detection of IAA was 280 nm (Sarwar *et al*., 1992) whereas for GA analysis it was adjusted at 254 nm (Li *et al*., 1994). The data recorded were analyzed statistically by Analysis of Variance (ANOVA) and treatment means were compared by “Duncan’s Multiple Range Test” (DMRT) using MSTAT-C version 1.4.2.

Results and Discussion

Imposition of drought stress decreased relative water content of leaves of both cultivars of mung bean (Table-1). Furthermore, cultivars did not differ significantly under water stress or non-stress conditions. However, seed soaking with ABA or CCC enhanced RWC of both cultivars under non-stress or water stress conditions. The magnitude of increase was greater under stressed conditions. Laila *et al.* (2002) demonstrated decline in leaf relative water content in Safflower plant under water stress and the ameliorating effect was more pronounced with ABA. Pospisilova & Batkova (2004) also observed ABA- induced increase in RWC in French bean under mild stress. Imbamba (1993) observed that stomatal opening was suppressed by CCC but the number of stomata per unit leaf area was increased resulting in increased relative water content.

Pre sowing seed treatment with ABA partially counteracted the decrease in shoot fresh and dry weight as compared to drought stressed plants (Table 2). In contrast, CCC soaking treatment further augmented the inhibitory effect of water stress on shoot growth but was stimulatory to root growth and the magnitude of stimulation was greater than that of ABA. Previous reports indicates the inhibitory effect of water stress on decreased plant biomass which may be attibuted to inhibition of cell division and cell enlargement (Thaloot *et al*., 2006). ABA was reported to increase the photosynthetic rate and chlorophyll content under water stress (Dong *et al*., 1995). The anti gibberellin CCC was found to induce reduction in aerial plant parts by directing the assimilates more to roots (Peltonen-sainio *et al*., 2003; Thaloot *et al*., 2006).

The proline content was significantly increased under water stress in both the varieties and there was no marked effect of rewatering on proline content. Seed soaking treatments with growth regulators enhanced proline accumulation under both water stress and unstressed conditions as compared to control (Table-3), but the magnitude of stimulation was more under drought stress and ABA being more effective than CCC. The cv. NCM-209 was less responsive to ABA and CCC. Maiti *et al.* (2000) reported that increased proline accumulation is a mechanism for plant adaptation to abiotic stress as it protect cells from damage resulted by stress (Demir, 1999). Yang *et al*. (1995) found ABA induced proline accumulation and postulated that drought resistant plants reduce their water stress by increasing proline content when ABA was exogenously applied. CCC is also involved in osmoregulation as reported by Rademacher (2000).

Protein content was decreased under water stress but was increased to the level of control by re-watering (Table 3). The ABA and CCC overcame the adverse effects of water stress on protein content as compared to control and under unstressed condition they were more stimulatory to protein production. The accumulation of proteins in leaves under water stress is an adaptation mechanism as it bounds to membranes and regulate membrane water permeability in cells and may influence water movement among tissues and organ (Ashraf *et al*., 2003) Exogenous application of ABA results in synthesis of proteins in different species under water stress (Frederique *et al*., 1998). Ibrahim *et al.* (2001) found that the protein and amino acid contents were higher in seedlings of CCC treated seeds than those of untreated ones.

The antioxidant content of a plant is closely related to its stress tolerance (Alscher and Hess, 1993; Smirnoff, 1995). The water stress significantly increased the peroxidase activity in leaves of mung bean plants (Table-3). Re-watering decreased it to the level of control. Peroxidase activity was stimulated by ABA and CCC seed soaking treatments under both water stress and un-stressed conditions. The stimulation was more with CCC

Table 1. Effect of ABA and CCC on leaf relative water content (%) of *Vigna radiata* under water stress.

Treatments	leaf relative water content	
	Varieties	
	NM 98	NCM 209
Control	57.40a	60.15a
Water stress	37.31c	42.70c
Rewatering	56.08a	58.14a
Water stress + ABA	51.69b	55.84b
Water stress + CCC	46.93b	51.89b
ABA	60.09a	63.19a
CCC	56.93a	57.88ab
L.S.D	4.760	3.951

Table 2. Effect of ABA and CCC on shoot and root fresh and dry weight (g) of *Vigna radiata* under water stress.

Treatments	Shoot fresh		Shoot dry weight		Root fresh weight		Root dry weight	
	Varieties		Varieties		Varieties		Varieties	
	NM 98	NCM 209	NM 98	NCM 209	NM 98	NCM 209	NM 98	NCM 20
Control	38.50a	35.443a	10.37a	10.827a	9.893b	10.110b	5.147b	6.101
Water stress	14.92d	21.448c	3.263e	2.631d	4.797d	5.037e	2.930e	2.013
Rewatering	28.11b	28.737b	7.137c	6.637c	7.650c	8.967dc	4.100cd	4.963
Waterstress+ABA	26.37b	26.44b		6.285c	6.050c	7.623d	3.980d	3.847
Waterstress+CCC	11.32d	12.64d	2.627e	2.757d	9.760b	10.290b	4.840c	5.20
ABA	19.41c	21.27c	8.420b	8.647b	8.773bc	9.280c	3.810d	3.780
CCC	15.54d	15.35e	4.280d	5.393c	13.370a	14.080a	6.170a	7.78
L.S.D	4.827	5.040	1.537	1.542	2.625	1.709	2.99	6.07

Table 3. Effect of ABA and CCC on leaf proline (µg/g) protein content (mg/g) and peroxidase activity (mg / min x g fresh weight) of *Vigna radiata* under water stress.

Treatments	Proline content		Protein content		Peroxidase activity	
	Varieties		Varieties		Varieties	
	NM 98	NCM 209	NM 98	NCM 209	NM 98	NCM 209
Control	167.533e	200.00b	10.200c	12.300c	0.103b	0.343b
Water stress	515.767c	491.700c	7.744d	6.024d	0.151d	0.737d
Rewatering	404.900c	400.133c	10.867c	12.400c	0.113b	0.252b
Water stress+ABA	673.800a	587.270	10.20b	17.150b	0.184a	0.891a
Water stress + CC	595.667b	300.00b	14.90b	16.613b	0.221a	0.980a
ABA	330.500d	402.10c	19.23a	21.567a	0.32c	0.415d
CCC	261.300d	313.767b	18.00a	20.400a	0.146b	0.580c
L.S.D	64.02	50.27	3.008	9.356	0.071	0.639

Table 4. Effect of ABA and CCC on leaf sugar content (mg/l) and Chlorophyll a + b (mg/l) of *Vigna radiata* under water stress.

Treatments	sugar content		Chlorophyll a + b	
	Varieties		Varieties	
	NM 98	NCM 209	NM 98	NCM 209
Control	214.67c	278.67c	34.133a	36.303a
Water stress	357.83b	388.267b	22.680e	19.943d
Rewatering	300.00bc	319.40b	29.517c	30.607b
Water stress + ABA	458.200a	496.67a	28.693c	28.053c
Water stress + CCC	434.67a	473.267a	26.993d	26.723c
ABA	299.267ab	386.360b	29.160c	33.527ab
CCC	293.933b	374.700b	32.00b	32.967b
L.S.D	88.94	19.98	4.45	4.028

Table 5. Effect of ABA and CCC on IAA and GA concentration (μg/g) in leaves of *Vigna radiata* under water stress.

Treatments	IAA concentration		GA concentration	
	Varieties		Varieties	
	NM 98	NCM 209	NM 98	NCM 209
Control	0.768b	0.819b	641.8a	779.8a
Water stress	0.325e	0.472e	433.7c	468.3c
Rewatering	0.655c	0.777c	514.6b	593.7b
Water stress + ABA	0.508d	0.688d	543.7b	548.9b
Water stress + CCC	0.483d	0.591d	119.3d	124.0d
ABA	0.563d	0.586d	579.6b	556.7b
CCC	0.997a	1.456a	224.5d	306.7d
L.S.D	0.365	0.698	64.96	47.90

Table 6. Effect of water stress on diameter of pink bacteriod tissues (mm^{-3}), number of nodules/plant of and the Cfu for *Rhizobium* per g of soil.

Treatments	diameter of pink bacteriod tissues		number of nodules		Cfu for *Rhizobium* After Harvest	
	Varieties		Varieties		Varieties	
	NM 98	NCM 209	NM 98	NCM 209	NM 98	NCM 209
Control	0.309a	0.348a	16.6a	18.3a	22×10^4	23×10^4
Water stress	0.157bc	0.123d	10.6d	9.6d	15×10^4	12×10^4
Rewatering	0.247a	0.162c	11.6b	10.3b	17×10^4	14×10^4
Water stress + ABA	0.210b	0.207bc	14.0b	15.0b	20×10^4	19×10^4
Water stress + CCC	0.172b	0.160cd	9.3d	10.0d	22×10^4	19×10^4
ABA	0.235ab	0.253b	12.0c	13.6c	26×10^4	25×10^4
CCC	0.210b	0.288ab	14.0bc	14.6bc	25×10^4	24×10^4
L.S.D	0.0818	0.0818	2.40	3.30	22×10^4	23×10^4

soaking treatments under water stress conditions. Zhang (1998) also observed that the concentration of antioxidants is positively correlated to the growth of Kentucky bluegrass under low or high soil moisture condition. Closure of stomata and the reduction in photosynthesis interfere with gas exchange, as a result excess excitation energy may be diverted to activate molecular oxygen and excess reactive oxygen species are produced .A high status of antioxidant enzymes may be considered adaptive mechanism for plants under drought stress.

Water stress induced the accumulation of sugar in leaves of mung bean plants (Table-4), which was non-significantly affected by re-watering but was further increased with ABA and CCC. The magnitude of increase was greater under unstressed condition.. Bajji *et al.* (2001) reported sugar as osmotica, which accumulates in leaves under water stress (Kameli & Loesel, 1996; Ndung *et al*.,1997). El-komy (1998) also reported that under water stress important role of exogenous ABA is to accumulate soluble sugar through activation of amylase for osmotic adjustment and improvement of plant water balance.

Water stress significantly decreased the chlorophyll a+b in the leaves of mung bean plants (Table-4). Re-watering was unable to restore the chlorophyll contents to the level of control though there was increase in sugar content as compared to the water stress. Decrease in chlorophyll content in the leaves of plant may be attributed to high rate of degradation of chlorophyll more than its biosynthesis under water stress (Yang *et al*., 2001). Furthermore Schtz & Fangmeier (2001) and Bano & Aziz (2003) added that water stress accelerate chlorophylls break down. Thaloot *et al*., (2006) also observed that drought stress at any growth stage decreased the chlorophyll a and b and carotenoid in leaves of mung bean plants. Pre- sowing soaking treatment with ABA and CCC partially ameliorated the adverse effect of water stress on chlorophyll content. Both the treatments increased the chlorophyll content under water stress. Thaloot *et al*. (2006) also observed drought induced decrease in Chlorophyll a and b and carotenoids in mung bean. Gadallah (1995) reported that high chlorophyll content with ABA treatment is due to stability of chlorophyll with ABA.

IAA and GA content in the leaves were decreased under water stress (Table 5). Pre-sowing seed treatment with ABA and CCC partially overcame the decrease in IAA content observed under drought stress; CCC soaking treatment was more stimulatory under water stress. ABA resulted in increase in IAA and GA content of leaves under stress. (Maiti *et al*., 2001). Mian *et al*., (1994) found that when CCC is applied exogenously it has improved the root growth this may be due to a slight increase in IAA content (Shakiba *et al*., 1996). Decrease in GA concentration on CCC application may be due to the fact that CCC interfere with the early stages of gibberellin biosynthesis primarily by blocking the activity of *ent*-kaurene synthesis (Rademacher, 2000) whereas, ABA was stimulatory to GA production both under stress and unstressed condition. Under water stress soaking with either ABA or CCC stimulated the number of rhizobial colony as compared to control. Interesting to note that water stress markedly reduced the cfu of *Rhizobium* and rewatering has not restored the number of *Rhizobium* closer to the control though the value was significantly higher than stressed ones. The ABA and CCC under unstressed condition was equally effective to maintain the colony forming unit (cfu) of *Rhizobium* g^{-1} of soil over that of water stressed treatment. The favourable effect of ABA and CCC was more pronounced in NM 98 as compared to NCM-209 under drought stress.

Drought stress decreased the number of nodules and pink bacteroid tissue of nodules in the mung bean plants (Table 7). The inhibitory effect of drought was decreased by ABA seed soaking treatment, CCC being less effective (Albrecht *et al*., 1994; Suzuki *et al*., 2004). Zahran (1999) reported the adverse effects of water stress on *Rhizobium*. The observed increase in the cfu of *Rhizobium* following ABA and CCC treatment may possibly be the growth regulators modulated root exudation of host plant which serve as source of nutrient and energy for the *Rhizobium* consequently increasing their survival in soil.

The inhibitory effects of water stress on plant growth was partially ameliorated by both ABA and CCC treatments, the underlying mechanism of their effect were increase in RWC of leaves, to maintain the turgidity of plants, the maintenance of osmotic potential by increasing the production of proline and sugar as osmoregulants and by affecting the membrane permeability through increased protein accumulation. The root-to-shoot ratio was altered via increased production of growth promoting hormones IAA and GA in ABA and CCC treatments.

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(Received for publication 18 September, 2006)