WATER STRESS EFFECTS ON GROWTH RELATED MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERS IN MUNGBEAN (*Vigna radiata* (L.) wilczek) AT DIFFERENT GROWTH STAGES

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ABSTRACT

Water stress effect on some growth related morphological, physiological and biochemical characters was studied in ten mungbean genotypes (*Vigna radiata* (L.) wilczek) subject to phasic drought at vegetative, flowering and pod development stages. The first recorded physiological parameters in vegetative stage for both the non-stress and stress conditions increased further in the successive flowering and pod development stages. Water stress induced significant reduction in some characters and increase in others. Significant reduction in the measurements was observed in leaf area, dry matter production, relative water content, leaf water potential, transpiration rate, chlorophyll content, soluble protein and nitrate reductase activity. Leaf diffusive resistance, proline content, catalase activity and peroxidase activity had increased measurements in the water stress condition. Maximum reduction was observed for leaf area, dry matter production, relative water content, chlorophyll content and soluble protein in the vegetative stage, for nitrate reductase in the flowering stage and leaf water potential and transpiration rate in the pod development stage. Both proline content and per peroxidase activity, catalase activity and leaf diffusive resistance had the maximum expression in the vegetative, flowering and pod development stage respectively. The physiological processes that were possibly involved in suppression or promotion of the physiological activities of the characters under study on account of water stress are discussed in the light of information available in related studies.
Key words: water stress, Green gram, RWC, Leaf Water Potential and physiological & biochemical parameters.

INTRODUCTION

Mungbean, popularly known as greengram (*Vigna radiata* (L.) wilczek) is an important legume crop of the arid and semi arid regions. It is mostly grown as a rainfed crop and is often exposed to drought at different stages of growth. Depending upon the intensity and duration of drought, a yield loss upto 60% has been recorded in mungbean. Moisture stress effect on germination, seedling characters, yield and yield related attributes have been studied by few workers. Moisture stress, in general, has reduced growth and yield in many crops including mungbean. Water stress at vegetative stage in mungbean has been found to have irreversibly reduced plant height, root growth, leaf area, number of clusters, number of pods and dry matter accumulation (Sadasivam *et al.*, 1988). Based on germination stress index, genotypes with variable levels of drought resistance have been identified in mungbean. Studies relating to physiological aspects of growth, especially the water stress effects on morpho-physiological and biochemical characters in different stages of crop growth are limited. The present investigation was taken up to find out the effects of water stress on physiological processes in different crop growth stages.

MATERIALS AND METHODS

A pot culture experiment with ten genotypes of greengram subject to water non-stress and stress conditions in each of vegetative, flowering and pod development stages of growth was conducted during summer 1997 in the glass house of the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. The experimental design adopted was a factorial experiment, completely randomized with three replications. Seed materials were

obtained from the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore. Each pot (30 x 30 cm) was filled with 12 kg of homogenized mixture of red soil, sand and FYM in 2:1:1 ratio and N:P fertilizers at 25 and 50 kg h^{-1}. Five seeds were space planted in each pot and after germination and establishment, thinning was done leaving one good plant in each pot. The soil properties of the experimental plot were: Bulk density: 1.42 gcc^{-1}, field capacity: 14.80%; permanent wilting point: 7.5%; pH: 8.5; EC: 0.2 m hec cm^{-1} and available N:P:K-196:16:380 kg ha^{-1}. Soil moisture content was recorded gravimetrically. Stress plots were maintained at 40% available soil moisture (ASM) at the vegetative, flowering and pod development stages for a period of 15 days. Control plots were fully irrigated and maintained at 60% ASM. Observations on two morphological characters *viz*., leaf area (LA) and total dry matter production (DMP), four physiological characters *viz*., relative water content (RWC), leaf water potential (LWP), leaf diffusive resistance (LDR) and transpiration rate (TR) and five biochemical characters *viz*., chlorophyll content (CC), soluble protein (SP), proline content (PC), nitrate reductase (NR) and two AOS scavenging enzymes *viz*., catalase activity (CA) and peroxides activity (PA) were recorded following appropriate experimental procedures, just prior to termination of water stress in the vegetative, flowering and pod development stages of growth.

RESULTS AND DISCUSSION

The mean measurements for all the morphological physiological and biochemical characters are presented in Table 1. For easy reference, the percentage increase or decrease due to water stress has been calculated for each character, stage wise and given in Table 1 itself.

The measured leaf area was 206.1, 442.3 and 440.1 cm^2 in the non-stress and 117.6, 288.0 and 386.7 cm^2 in the stress conditions in the vegetative, flowering and pod development stage respectively. There was progressive increase in the leaf area in the successive stages of crop growth. Water stress significantly reduced the leaf area to an extent of 42.9%, 34.9% and 12.1% in the vegetative, flowering and pod development stage respectively. Maximum reduction in the vegetative stage pointed out to the sensitive nature of the grand growth period to water stress. Negative influence on the cell enlargement and cell division as pointed out by Ludlow and Muchow (1990) might have resulted in reduced leaf area. As in the case of leaf area, dry matter production steadily increased in the successive crop growth stage in both the non-stress and stress conditions. Water stress also caused a significant reduction in dry matter production in each crop growth stage, the maximum (33.4%) being in the vegetative stage. Such a reduction in leaf area and dry matter production on account of moisture stress has already been observed in mungbean and cotton (Pannu and Singh, 1988; Sadasivam *et al*., 1988, Singh and Bhardwaj, 1983 and Nandini, 1994).

The relative water content remained at 85.9%, 83.4% and 81.1% in the non-stress and at 74.8%, 73.6% and 74.7% in the stress conditions in the respective vegetative, flowering and pod development stage. The RWC at the vegetative stage was more or less maintained at the same level in the subsequent flowering and pod development stages in both the non-stress and stress conditions. Water stress reduced the RWC to 12.9%, 11.8% and 7.9% in the vegetative, flowering and pod development stage respectively. Reduction in RWC might be due to the hardship in maintaining the internal water balance because of continuous evaporational loss even in moisture stress situations. Water stress induced reduction in RWC is common in crop plants (Begg and Turner, 1976). Reduction in RWC due to moisture stress has been observed in blackgram (Seetharani, 1990) and french-bean (Upreti *et al*., 1998).

The leaf water potential at –0.33 MPa, -0.36 MPa and –0.41 MPa in the non-stress and at –0.80 MPa, -0.88 MPa and 1.02 MPa and in the stress conditions in the respective vegetative, flowering and pod development stage indicated the level of LWP to be nearly the same in all three stages of growth under non-stress and stress conditions also noticed in case of RWC. Water stress significantly reduced the LWP in each crop growth stage, the differences between the stages, however, being insignificant. Reduction in LWP worked out to 142.4% in vegetative stage 144.4% in flowering stage and 148.8% in pod development stage. Decreased stomatal conductance, leaf area and transpiration in response to moisture stress would be causes of action to reduce LWP in the present study. Reduced LWP under drought has been

noticed in mungbean (Pannu and Singh, 1988), chickpea (Singh *et al.*, 1987) and blackgram (Seetharani, 1990).

The mean value of leaf diffusive resistance was 3.53, 3.51 and 4.44 in the non-stress and 8.76, 9.28, 13.82 in the stress conditions in the respective vegetative, flowering and pod development stage. The LDR was at a lower level in the non-stress and at a higher level in the stress conditions in all the stages of crop growth. Water stress promoted LDR and it worked out to 148.2%, 164.4% and 211.3% increase in the vegetative, flowering and pod development stage respectively. Reduced turgidity in plant cell and accelerated stomatal closure could have led to an increase in LDR, as also observed by Kramer (1983), Sahay (1989) and Nandini (1994) in drought affected cotton crop.

The mean value of transpiration rate was 7.50, 7.80 and 6.72 in the non-stress and 2.75, 3.02 and 1.56 in the stress conditions in the respective vegetative, flowering and pod development stage. The level of TR was higher in the non-stress and lower in the stress. Water stress significantly reduced TR in each crop growth stage, the extent of reduction working out to 63.3%, 61.2% and 76.8% in the vegetative, flowering and pod development stage respectively. Water stress could have created a situation of lowly available moisture and enhanced stomatal resistance inducing stomatal closer culminating in reduced TR as observed in drought affected soybean (Chen *et al.*, 1993), blackgram (Seetharani, 1990) and sugarcane (Srivastava *et al.*, 1996).

The soluble protein estimated to be 22.28, 28.09 and 16.69 mg g^{-1} in the non-stress and 11.0, 14.1 and 13.2 mg g^{-1} in stress condition in vegetative, flowering and pod development stage respectively showed that water stress caused a significant reduction amounting to 50.7, 49.7 and 20.8% in the respective vegetative, flowering and pod development stage. The decrease in soluble protein under water stress might be either due to increased proteolysis or decreased synthesis or both (Hsio, 1973; Gang *et al.*, 1981; Kumar, 1983). Similar was the case with nitrate reductase activity. The mean value corresponding to the non-stress and stress conditions at 1.80:0.78, 1.54:0.96 and 1.04:0.80 in the vegetative flowering and pod development stage respectively indicated the phenomenon of reduction taking place on account of water stress. The reduction worked out to 29.1%, 37.9% and 16.5% in the respective vegetative, flowering and pod development stage. The reduction in nitrate reductase activity might have been brought about by the reduction in enzyme level or inactivation of enzymes as suggested by Bardzik *et al.* (1971) and Nicholas *et al.* (1976). Yadav (1997) reported that reduced nitrate reductase activity was due to decrease in nitrate content caused by reduced nutrient uptake under stress condition in chickpea.

The chlorophyll content at 1.00, 1.43 and 0.63 mg g^{-1} in the non-stress and at 0.73, 1.15 and 0.51 mg g^{-1} in the stress condition in the respective vegetative, flowering and pod development stage showed that the water stress had significantly reduced the chlorophyll content by 26.8%, 19.6% and 20.1% in vegetative, flowering and pod development stage respectively. Water stress has been found to have caused damage to chlorophyll in the form of chlorophyll loss from mesophyll cells (Alberte *et al.*, 1977), or lack of metabolites resulting in retarded chloroplast membrane synthesis (Henningsen, 1970), or inhibition in the synthesis of chlorophyll precursor (Makhmudo, 1983) or disintegration of chloroplast membrane (Viera de Silva *et al.*, 1974). The decline in chlorophyll content under water deficits has been observed in blackgram (Gopal Singh *et al.*, 1985; Chandra Babu *et al.*, 1988 and Sadasivam, 1996). When water stress was found to have decreased chlorophyll content, soluble protein, nitrate reductase etc. it was found to have increased to level of proline content to more than eight times in both the vegetative and flowering stages and six times in pod development stage. The proline accumulation was maximum in the flowering stage in both the non-stress and stress conditions as it could be seen from the observed absolute level of proline content in the non-stress and stress conditions at 1.87:17.89, 2.7:198.52 and 1.87:14.48 in the respective vegetative, flowering and pod development stage. Proline is known to accumulate in plants under stress condition (Hsiao, 1973) and taken as an index of drought resistance (Singh *et al.*, 1972). The accumulation of proline takes place due to simulation of its synthesis from glutamate by loss of feedback inhibition, decline in proline oxidation and decreased incorporation into protein (Kramer, 1983). Proline is synthesized to depress the internal osmotic potential to maintain a positive gradient for water uptake under water stress

condition (Handa *et al.*, 1986). Reduction in leaf water potential or its related measure such as relative water content increased the proline synthesis in crop plants (Blum and Ebercon, 1976).

The catalase activity at 2.77, 3.61 and 3.28 in the non-stress and at 4.40, 6.84 and 5.30 in the stress condition in the respective vegetative, flowering and pod development stage indicated the progressive increase over the crop growth stages in both the non-stress and stress conditions. Water stress induced increased expression to the tune of 58.8% in vegetative, 89.5% in flowering and 61.6% in pod development stage, the expression being maximum in the flowering stage. The peroxidase activity at 55.88, 74.00 and 105.54 in the non-stress and at 62.56, 79.14 and 105.54 in the stress in the vegetative, flowering and pod development stage respectively also pointed out to progressive increase over the crop growth stages. Water stress resulted in a marginal increase in peroxidase activity amounting to 11.9% in vegetative, 6.9% in the flowering and 1.0% in pod development, the maximum increase being in vegetative stage. It was obvious that there was no water stress effect on the peroxidase activity in the pod development stage. Free radicals (OH^+) and single oxygen and superoxide oxygen (O_2) were destroyed by SOD, catalase and peroxidase so as to reduce the damage to lipid peroxidation during period of stress (Kellog and Fridovich, 1975). Chempakam *et al.* (1993) observed a direct relationship between drought tolerance and lipid peroxidation related enzyme activities in coconut. These reports will lend support to the findings in the present study.

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Table 1. Effect of water stress on growth, physiological and biochemical characters in mungbean at vegetative, flowering and pod development stage

Characters	Water non-stress (C) / Stress (S) at								
	Vegetative stage			Flowering stage			Pod development stage		
	Non-stress control (C)	Stress (S)	% Reduction (-) Increase (+) due to stress	Non-stress control (C)	Stress (S)	% Reduction (-) Increase (+) due to stress	Non-stress control (C)	Stress (S)	% Reduction (-) Increase (+) due to stress
I. Morphological characters									
1. Leaf area index cm^2 $plant^{-1}$	206.14	117.61	-42.9	442.30	288.03	-34.9	440.10	386.71	-12.1
2. Total dry matter production g $plant^{-1}$	1.44	0.96	-33.4	6.44	4.63	-28.2	12.06	9.99	-17.2
II. Physiological characters									
3. Relative water content (%)	85.90	74.81	-12.92	83.41	73.60	-11.81	81.12	74.71	-7.9
4. Leaf water potential (-MPa)	0.33	0.80	-142.4	0.36	0.88	-144.4	0.41	1.02	-148.8
5. Leaf diffusive resistance (s cm^{-1})	3.53	8.76	+148.2	3.51	9.28	+164.4	4.44	13.82	+211.3
6. Transpiration rate (μg H_2O cm^2 s^{-1})	7.50	2.75	-63.3	7.80	3.02	-61.2	6.72	1.56	-76.8
III. Bio chemical characters									
7. Chlorophyll content (mg g^{-1})	1.00	0.73	-26.8	1.43	1.15	-19.6	0.63	0.51	-20.1
8. Soluble protein (mg g^{-1})	22.28	10.98	-50.7	28.09	14.12	-49.7	16.69	13.22	-20.8
9. Proline content (μ moles g^{-1})	1.87	17.89	+856.7	2.07	19.52	+843.0	1.87	14.48	+674.3
10. Nitrate reductase (μ moles No_2-h^{-1} g^{-1})	1.10	0.78	-29.1	-1.54	0.96	-37.9	1.04	0.80	-16.5
11. Catalase activity (x 10^4 enzyme units g^{-1} min^{-1})	2.77	4.40	+58.8	3.61	6.84	+89.5	3.28	5.30	+61.6
12. Peroxidase activity (enzyme units mg^{-1} protein)	55.88	62.56	+11.9	74.00	79.14	+6.9	104.47	105.54	+1.0