BIOLOGIA PLANTARUM 40 (3): 351-356, 1997/98

Effect of water and salt stresses on growth, chlorophyll, mineral ions and organic solutes contents, and enzymes activity in mung bean seedlings

M.A. ZAYED and I.M. ZEID

Botany Department, Faculty of Science, El-Menoufiya University, Shibin El-Kom, Egypt

Abstract

The experiment was made by using different concentrations of polyethylene glycol (PEG) or salt solutions to decrease the osmotic potential of the growth medium to reveal the response of mung bean (*Vigna radiata*) to water and salt stresses. No germination (emergence of the seedling) occurred at medium osmotic potential lower than -1.0 MPa in all treatments. It was found that the activity of α-amylase and protease, and contents of proline, saccharides and the soluble proteins decreased in the germinating seeds during 3-d stress. However, after 10-d stress, the contents of organic solutes and the activity of the hydrolytic enzymes increased. Growth, chlorophyll content and mineral uptake were also significantly reduced under stress. The seedlings under water stress induced by PEG were affected much more than under salinity. This may be due to the maintenance of a higher succulence under salt stress than under PEG-induced water stress.

Additional key words: α-amylase, proline, protease, proteins, saccharides, *Vigna radiata*.

Introdution

Water stress reduces growth and manifests several morphological, anatomical and biochemical alternations in plants, including modification in gene expression ultimately leading to a massive loss in yield. Water stress reduces nutrient uptake by roots and transport from roots to shoots because of restricted transpiration rates and impaired active transport and membrane permeability.

Plants may increase its drought tolerance by decreasing osmotic potential by accumulation of solutes, which allows cell enlargement, plant growth and keeps open stomata and CO_2 assimilation under water stress. Many solutes may be used in

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Received 11 February 1997, *accepted* 2 July 1997.

Abbreviations: Chl - chlorophyll; d.m. - dry mass; f.m. - fresh mass; PEG - polyethylene glycol; ψ_s - substrate water potential.

Fax: (+20) 2777620

osmotic adjustment including inorganic ions, such as Na^+, K^+ and Cl^- (*e.g.*, Ford and Wilson 1981, Wyn Jones and Gorhan 1983), and saccharides and amino acids, especially proline (Morgan 1984, Drossopoulos *et al.* 1985, Navari-Izzo *et al.* 1990).

The present study aims to know the extent to which the mung bean plants (which is recently introduced to Egypt), can tolerate the water and salt stresses. This paper includes the effect of water stress induced by polyethylene glycol (PEG) and salinity-induced stress on growth, chlorophyll contents, mineral composition, activity of the hydrolytic enzymes, α-amylase and protease, and the accumulation of proline, saccarides and soluble proteins in germinating seeds and seedlings of mung bean.

Materials and methods

Seeds of mung bean (*Vigna radiata* L.) were sown in sand and irrigated with Hoagland nutrient solutions, which adjusted to a definite osmotic values. Polyethylene glycol (PEG) was added at concentrations to give medium osmotic potential -0.05, -0.2, -0.5 and -1.0 MPa, according to Lawlor (1970). Salinity stress was induced by adding NaCl and $CaCl_2$ at concentrations to give the same osmotic potentials. 3 d or 10 d after the sowing the samples of shoots and roots were taken for analyses.

Nitrogen analyses were carried out using a C/N analyser *Model Na-1500* (*Carlo Erba,* Göttingen, Germany) Samples are combusted with added oxygen at 1020 °C. NO_x is then reduced to N_2 and analysed by gas chromatography. 2.3 mg of the re-dried samples were weighed into zinc containers. Atropin samples were used as calibration standards. Empty zinc containers were run as blanks. The analysis was carried out twice for each sample.

The activity of the hydrolytic enzymes, α-amylase and protease was determined according to Bergmeyer (1974), the content of proline according to Bates *et al.* (1973), and the contents of saccharides and soluble proteins according to Gallop *et al.* (1957), Umbreit *et al.* (1959), and Lowry *et al.* (1951), respectively. Chloroplast pigments were measured spectrophotometrically in 85 % acetone extracts of leaves according to Metzner *et al.* (1965).

Results and discussion

Effect of water and salt stresses on growth and water content: Generally, there is a reversible relationship between the osmotic potential of the growth medium, and the water content and growth parameters (Table 1). The reduction of water content under PEG treament was about 2, 8 and 42 % at the osmotic potential -0.05, -0.2 and -0.5 MPa, respectively. Under salinity induced stress, the reduction was only about 0.5, 4 and 10 %, respectively.

Growth was also greatly affected, since the shoot length and leaf area decreased paralelly with the water content.

Table 1. Effect of PEG-induced water stress and salt stress of mung bean seedling growth and water content (mean ± S.E.).

Treatment	ψ_s [MPa]	Shoot length [cm]	Leaf area [cm^2]	Water content [%]
Control	0.0	25.5 ± 1.8	3.04 ± 0.09	88.10
PEG	-0.05	24.0 ± 1.6	2.32 ± 0.07	86.58
	-0.20	16.5 ± 1.3	1.15 ± 0.05	81.09
	-0.50	8.0 ± 0.9	0.18 ± 0.03	51.40
Salinity	-0.05	25.5 ± 1.8	3.03 ± 0.08	87.70
	-0.20	18.5 ± 1.4	1.94 ± 0.07	84.62
	-0.50	9.5 ± 1.1	0.49 ± 0.03	79.00

Contents of minerals in roots and shoots: Under salinity, the mung bean accumulated Na in roots while the Na content in plant shoots was reduced. According to Boursler and Läuchli (1990) regulatory mechanism located within the roots prevents translocation of excessive cations such as Na, from roots to aerial parts, resulting in Na retention. This may be make the mung bean plants relatively tolerant to salinity. Potassium content, on the other hand, has been reduced markedly, similarly as was found previously (*e.g.*, Levitt 1980, Behboudian *et al.* 1986, Burgos *et al.* 1993). The internal Ca content in mung bean roots did not significantly changed with increasing salinity. On the other hand, Ca was accumulated in the plant shoots. Chloride content increased slightly in the plant shoots as a result of salt treatments, but its accumulation in the root cells was high. Mg decreased in plant shoots with increasing salt stress conditions. However, the reduction of Mg content in the root cells was

Table 2. Effect of PEG-induced water stress and salt stress on element contents [%(d.m.)] in mung bean seedling shoots and roots.

Organ	Treatment	ψs [MPa]	N	P	S	Ca	Mg	Na	K	Cl
Shoots	control	0.0	7.44	1.2	5.6	0.43	0.33	0.69	1.34	3.6
	salts	-0.05	8.17	0.9	8.1	0.77	0.24	0.63	0.25	3.6
		-0.20	8.12	1.0	4.1	1.51	0.17	0.34	0.06	4.2
		-0.50	4.94	1.1	1.9	1.71	0.18	0.17	0.03	4.0
	PEG	-0.05	9.18	1.3	6.0	0.51	0.28	0.62	0.51	5.0
		-0.20	7.39	1.1	5.5	0.33	0.24	0.38	0.33	2.9
		-0.50	5.15	1.5	4.1	0.46	0.44	0.45	0.21	1.5
Roots	control	0.0	3.78	1.0	3.0	0.49	0.14	0.33	0.06	1.8
	salts	-0.05	4.55	1.0	4.6	0.36	0.17	0.29	0.07	2.9
		-0.20	4.92	0.9	1.9	0.47	0.21	0.42	0.06	4.6
		-0.50	2.37	0.9	0.0	0.48	0.11	0.40	0.04	3.4
	PEG	-0.05	5.19	1.1	2.5	0.42	0.18	0.32	0.04	3.7
		-0.20	5.29	1.3	3.2	0.12	0.10	0.14	0.04	3.3
		-0.50	3.38	0.8	2.7	0.50	0.19	0.26	0.07	1.1

more obvious at severe stress (-0.5 MPa). Nitrogen uptake was slightly higher at low and moderate concentrations of salts (-0.05 or -0.2 MPa). However, at severe salt stress (-0.5 MPa), the N accumulation in the plants and shoots was reduced greatly (Table 2). The possible decrease in N uptake by increasing salinity has been partly attributed to a probable substitution of Cl^- for NO_3^- (Rendig and Papadopoulos 1983). Sulphur content decreased markedly with increasing the salts concentration in the growth medium. Phosphorus content, on the other hand, generally did not reduced significantly by salt treatments.

Under PEG-induced water stress the contents of Na K, N, Cl and S in shoots decreased. However, the K and S content in the roots was more or less as in the control plants. Ca, Mg and P did not show a clear tendency (Table 2).

The results reveal that under both water and salt stresses, the N, S and K content, generally decreased, whilst the P content was not affected significantly.

The reduction of total ions content under severe water and salt stresses, suggests also that mung bean plant depends on the accumulation of organic solutes, not on the mineral ions in its cytoplasmic osmoregulation.

Organic solutes accumulation and acting of hydrolytic enzymes: Reduction of water potential in the growth medium, resulted in a reduced water content in the cells of the germinating seeds and so, the activity of the hydrolytic enzymes such as α-amylase and protease decreased and the content of soluble saccharides, soluble proteins and amino acids, also decreased (Table 3).

However, in the 10-d-old seedlings, the activity of α-amylase and protease in the plant leaves was significantly stimulated under stress conditions, particularly under

Table 3. Effect of PEG-induced water stress and salt stress on contents of soluble sugars, soluble proteins and proline [mg g^{-1}(d.m.)] and activity of α-amylase and protease [U g^{-1}(f.m.) h^{-1}] in mung bean germinating seeds and seedlings.

Age	Treatment	ψs [MPa]	Saccharides	Proteins	Proline	α-Amylase	Protease
3 d	control	0.0	57.07±0.87	25.41±1.22	1.71±0.03	180.49± 6.91	14.13±0.97
	PEG	-0.05	53.26±2.64	17.08±1.10	1.50±0.04	210.06±10.46	14.75±0.77
		-0.20	30.04±1.47	16.71±1.12	1.31±0.10	194.01± 8.78	15.00±1.01
		-0.50	37.91±1.70	17.23±0.89	0.88±0.02	161.88± 7.91	11.26±0.78
	salts	-0.05	55.12±4.84	26.48±1.94	1.26±0.03	171.37±15.02	13.69±1.00
		-0.20	35.86±1.05	15.97±0.93	1.28±0.02	172.11± 5.07	12.77±0.84
		-0.50	14.12±0.10	12.72±0.81	1.01±0.01	83.95± 2.11	12.59±0.80
10 d	control	0.0	58.48±0.78	76.25±0.62	3.03±0.03	50.75± 6.12	23.07±2.26
	PEG	-0.05	56.83±0.38	81.93±0.62	3.65±0.03	60.02± 0.39	25.13±2.76
		-0.20	71.21±1.46	85.08±0.38	8.47±0.04	154.62± 4.12	55.54±1.46
		-0.50	139.97±1.50	85.47±0.96	32.09±0.20	198.16± 4.12	72.13±2.11
	salts	-0.05	64.07±1.77	89.32±0.67	2.08±0.07	67.52± 4.00	23.26±0.28
		-0.20	62.47±1.17	78.50±1.50	3.72±0.04	87.68± 2.98	28.56±3.89
		-0.50	91.99±0.57	83.62±1.23	4.63±0.14	134.82± 2.81	43.95±3.98

the PEG-induced water stress. Also the contents of saccharides, soluble proteins and proline increased. Concentration of the soluble saccharides and proline was much higher in plants under PEG-induced water stress than in salinity-stressed plants (Table 3). The accumulation of such organic solutes may improve the cytoplasmic osmoregulation and thus, increase plant tolerance.

Chloroplast pigments: Chlorophyll (Chl) *a* and *b*, and carotenoid contents were greatly reduced with decreasing water potential of the growth medium by addition of PEG. Plants under salt-induced stress, also showed a reduced content of these pigments under severe stress (-0.5 MPa), but the content was higher than that of plants under PEG-induced stress (Table 4).

Table 4. Effect of PEG-induced water stress and salt stress on contents of chlorophylls (Chl) *a* and *b*, and carotenoids (Car) [mg g^{-1}(d.m.)], and Chl *a*/*b* and Chl/Car ratios in 10-d-old mung bean seedlings.

Treatment	ψs [MPa]	Chl *a*	Chl *b*	Car	Chl *a*/*b*	Chl/Car
Control	0.0	9.93±0.02	5.86±0.03	7.08±0.02	1.69	2.23
PEG	-0.05	8.46±0.02	5.09±0.03	4.44±0.02	1.66	3.05
	-0.20	3.07±0.03	1.72±0.04	0.51±0.01	1.78	9.39
	-0.50	1.65±0.02	1.10±0.04	0.18±0.01	1.50	15.27
Salts	-0.05	9.45±0.01	5.57±0.02	5.39±0.02	1.69	2.53
	-0.20	9.17±0.02	5.22±0.03	5.69±0.01	1.75	2.52
	-0.50	4.11±0.03	2.33±0.01	0.82±0.01	1.76	7.85

The ratio Chl/Car increased under stress conditions. The increament was much higher in plants under PEG-induced water stress than that under salinity-induced stress.

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