Phyton (Horn, Austria)	**Vol. 32**	Fasc. 2	263–275	29. 12. 1992

Response of Adapted and Unadapted Soybean Cell Suspension Cultures to Water Stress.

By

Hameda ELSAYED*) and Ralph C. KIRKWOOD**)

With 5 Figures

Received October 22, 1991

Accepted March 18, 1992

Key words: Water stress, cell culture, soybean, *Glycine max* L., maple arrow, osmotic adaption, solute accumulation.

Summary

EL SAYED H. & KIRKWOOD R. C. 1992. Responses of adapted and unadapted soybean cell suspension cultures to water stress. – Phyton (Horn, Austria) 32 (2): 263–275, 5 figures. – English with German summary.

The responses of soybean cells to polyethylene glycol (PEG) induced water stress were studied after transfer to culture medium containing PEG at concentrations between 0% and 25%. Changes in growth characteristics, cellular osmotic potential and organic solute concentration were followed in unadapted cells and in cell lines adapted to growth in various PEG concentrations. A decline in fresh and dry weight increase occurred in unadapted cells with increasing water potential, while dry weight gain was unaffected in adapted lines. Substantial osmotic adjustment was observed in adapted lines, due mainly to increased glucose, fructose and sucrose. Proline concentration increased up to 40-fold in adapted and 12-fold in unadapted cells and other amino acids including alanine, histidine and arginine showed similar, though smaller responses. Polyamines and glycine betaine did not increase significantly in either adapted or unadapted cells. Changes leading to long-term adaptation to water stress are discussed in relation to short-term stress-shock responses.

*) Dr. Hameda EL SAYED, Department of Biology, Faculty of Education, University of Tanta, Kafr El Sheikh, Egypt.

**) Dr. Ralph C. KIRKWOOD, Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow G4ONR, Scotland, U.K.

Zusammenfassung

EL SAYED H. & KIRWOOD R. C. 1992. Reaktionen angepaßter und unangepaßter Zell-Suspensions-Kulturen von Sojabohnen auf Wasserstreß. – Phyton (Horn, Austria) 32 (2): 263–275, 5 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die Reaktionen von Zellen von Sojabohnen auf Polyethylenglucol (PEG) induzierten Wasserstreß wurde untersucht, in dem sie in ein Kulturmedium überführt wurden, welches PEG-Konzentrationen zwischen 0 und 25% enthielt. Veränderungen im Wachstumsverhalten, im osmotischen Potential der Zellen und in der Konzentration gelöster organischer Substanzen wurden in Zellen verfolgt, welche an das Wachstum in verschiedenen PEG-Konzentrationen angepaßt und unangepaßt waren. Das Frisch- und Trockengewicht, verbunden mit ansteigendem Wasserpotential, nimmt in nicht angepaßten Zellen ab, während das Trockengewicht in angepaßten Zellen unbeeinflußt bleibt. Eine grundlegende osmotische Anpassung kann in adaptierten Zellen beobachtet werden, was hauptsächlich auf dem Anstieg von Glucose, Fructose und Saccharose beruht. Die Prolinkonzentration ist bis auf das 40fache in den angepaßten Zellen und das 12fache in unangepaßten Zellen erhöht, andere Aminosäuren wie Albumin, Histidin und Arginin zeigen ein ähnliches, jedoch weniger ausgeprägtes Verhalten. Polyamine und Glycin-Betain steigen nicht signifikant in adaptierten und nicht adaptierten Zellen an. Änderungen, welche zu einer langfristigen Anpassung an den Wasserstreß führen, werden in Relation zu kurzfristigen Schockreaktionen auf Streß diskutiert.

Introduction

The physiological responses of plants to water-stress have long been of interest, mainly because of the need to understand better the effects on economically important crop plants when water is a limiting factor. KRAMER (1980) has estimated that losses in production due to lack of water exceed those of all other factors combined. One mechanism of resistance to water-stress is drought tolerance (LEVITT 1980), which involves the reduction of cell water potential through intracellular solute accumulation, allowing the cell to retain turgor and enhancing survival in an environment of fluctuating osmotic potential (TURNER & JONES 1980).

Research into drought stress has until now been mainly directed towards whole plants, with particular emphasis on stomatal behaviour. Suspension-cultured plant cells offer however, a relatively homogeneous and experimentally controlable alternative for the study of cellular responses to water stress. HEYSER & NABORS (1979) first reported the selection of cultured cell lines resistant to the stress induced by polyethylene glycol (PEG). Adaptation procedures and growth responses have been examined (BRESSAN & al. 1981) and physiological changes associated with water-stress adapted cells investigated in detail (HANDA & al. 1983).

HARMS & OERTLI (1985) employed mannitol-adapted carrot cell suspension cultures to study the interaction of osmotic and ionic stresses. EL SAYED & KIRKWOOD (1981) found that the proline concentration was positively correlated with cell osmotic potential. While, the average

concentrations of soluble sugars and total free amino acids increased as a function of the level of adaptation, the levels of these solutes did not approach those observed for Na^+ and Cl^-. Further they indicated that although Na^+ and Cl^- are the principal components of osmotic adjustment, organic solutes also make significant contributions.

Two components of the response of plant cells to decreased water potential may be identified, long-term changes in cells adapted to continuous growth at low water potential, here referred to as stress-adaptation, and stress-shock responses occurring in the short-term in cells transferred abruptly to lower water potential environments. In this study, both responses were examined in soybean suspension cells transferred to fresh medium of the same or lower water potential for a period of 10 h. For stress-shock changes, cells of a line adapted to growth in a standard tissue culture medium (control cells) were transferred directly to media containing various concentrations up to 25% PEG. For stress-adaptation, cell lines previously adapted to similar PEG concentrations were transferred to fresh medium with appropriate PEG levels. Changes in growth rate, water potential and solute content for both control and adapted cells were determined.

Materials and Methods

Suspension cultures of soybean (*Glycine max* L., Maple Arrow) were maintained on Murashige & Skoog (1962) medium and fresh and dry weights determined as previously described by El Sayed & Kirkwood 1991. Five cell-lines were used; unadapted cells were grown in standard culture medium without PEG, while four cell-lines were adapted to growth in medium containing 10, 15, 20 and 25% (w/v) PEG (BDH-MW 4,000, purified by ion exchange with BDH-Duolite MB 5113 mixed resin) by repeated subculture until consistent final fresh weights were obtained in consecutive passages; the adaptation period was for a minimum of eight sub-cultures. For the experimental treatments, adapted cells were transferred to fresh medium of corresponding PEG concentration. For each cell-lines, cells from stationary phase cultures were collected by nylon mesh filtration, allowed to drain, and resuspended in the same volume of fresh medium. Water potential and solute determinations were made 10 h after transfer.

The osmotic potential of cell contents of media was determined by the use of a vapour pressure osmometer (Wescor 5100 C). Samples of fresh and spent media were sampled directly, while for the determination of cellular solute potential, filtered and spin-dried tissue was stored frozen at -20° C, and allowed to reach room temperature prior to determination.

Fresh weight was determined by weighing the cells left on Whatman Glass Microfibre (GF/C, 2.5 cm) filters after filtering under vacuum; the cells had been washed three times in distilled water (El Sayed & Kirkwood 1991).

Free amines were determined by HPLC, as described by Fallon & Philips (1988). Proline was determined by the ninhydrin technique of Troll & Lindsley (1955), and glycinebetaine by the colorimetric method of Stumpf (1984). Free amino

acids were determined by HPLC following extraction with sulphosalicylic acid and OPA/mercaptoethanol derivatization (JARRETT & al. 1986). Sugars were determined by the method of SWEELEY & al. (1963) with modifications recommended by HOLLIGAN (1971) on a Shimadzu GC Mini 3/CR 3A gas chromatograph with an SE 30 packed column.

Results

On inoculation into fresh medium both adapted and unadapted cells showed a characteristic sigmoidal growth pattern over an 18 d period, at all concentrations of PEG (Fig. 1). In unadapted cells (Fig. 1 b), increasing PEG concentration progressively increased the length of the lag period, and decreased the rate of fresh weight increase. The lag time was extended from 2–3 d in control medium to 10 d with 25% PEG, and the rate of fresh weight increase reduced from 0.75 g/d to 0.25 g/d. The final fresh weight attained was reduced at all PEG concentrations, from a 15% reduction in 10% PEG to a 63% reduction in 25% PEG.

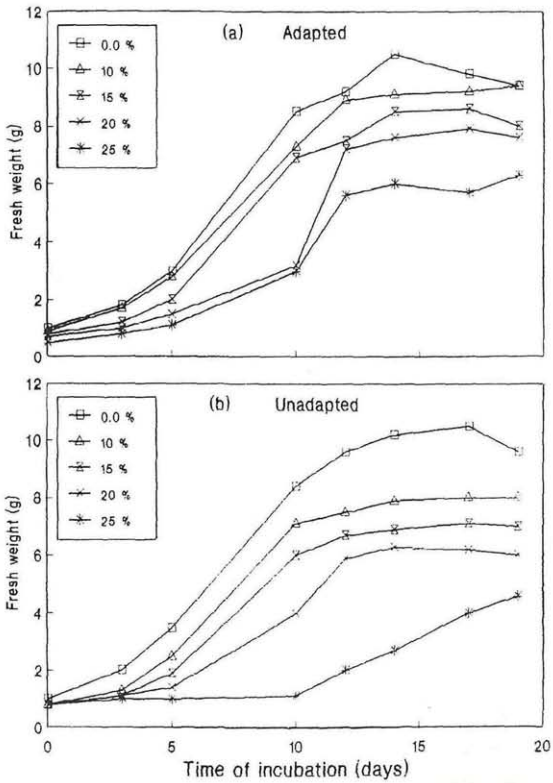

Fig. 1. Changes in fresh weights of adapted (a) and unadapted (b) cells over the culture period at a range of PEG concentrations; means of three replicates.

In adapted cells the effects of PEG on growth are less severe (Fig. 1 a). There is an effect on lag phase only at the two highest concentrations, extending it from 3 d in the control to 5 d with 25% PEG, and little changes in the rate of fresh weight accumulation were observed. However, final fresh weight was affected by increasing PEG concentration, though to a lesser degree than in unadapted cells.

The distinct responses of adapted and unadapted cells to water stress are illustrated by comparison of final fresh and dry weights after 18 d of growth in media of varying PEG content (Fig. 2). Fresh weight yields declined with decreasing osmotic potential, the decline being greater in unadapted than adapted cells. Dry weight yields, on the other hand, were reduced only in unadapted cells, with a decrease of approximately 40% in 25% PEG. Clearly adapted cells have an enhanced capacity for both water retention and dry weight accumulation compared to unadapted cells. A reduction in water content relative to control cells of up to 36% for adapted and 54% for unadapted cells was observed (Fig. 2).

The solute potentials of media containing 0, 10, 15, 20 & 25% PEG were found to be –0.34, –0.55, –0.83, –1.13 and –1.67 MPa respectively. The osmotic potential of adapted cells was maintained at a substantially lower value than that of the corresponding medium, the deficit actually increasing at higher PEG concentrations, such that in the line adapted to 25% PEG the solute potential decreased 4-fold relative to control cells (Fig. 3). Unadapted cells were in equilibrium with the medium (and were usually plasmolysed) at 15% PEG and above.

Putrescine (put) and spermidine (spd) were the major polyamines (PA_s) present, with spermine (spm) as a minor component (Table 1). The total PA

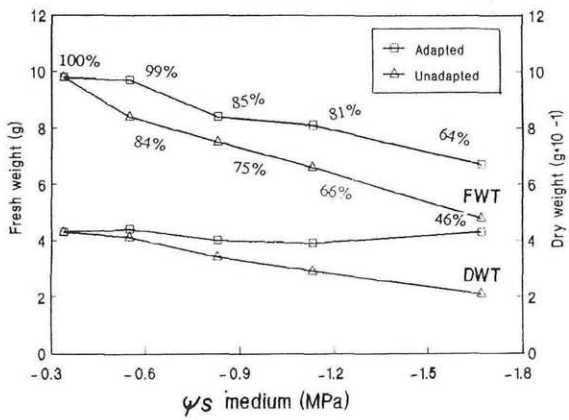

Fig. 2. Changes in fresh weight, dry weight and water content in adapted (A) and unadapted (U) cells after 19 days incubation at a range of PEG concentrations; means of three replicates. Water content is expressed as a percentage of that of control cells.

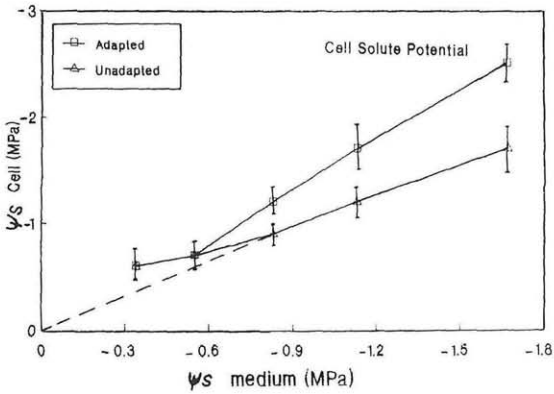

Fig. 3. Solute potentials of adapted (A) and unadapted (U) cells after 10 hours incubation at a range of PEG concentrations; means of three replicates. The broken line indicates the equivalence of cell and medium potentials.

concentration increased with PEG concentration in an approximately linear fashion; any differences between the adapted and unadapted lines were small and showed no clear trends. Increased total concentrations were primarily due to increases in putrescine and the rather small increases in concentration (approximately 2-fold) can be accounted for by concentration due to water loss (Fig. 2).

The cellular concentration of proline rose significantly in both normal and adapted cells grown in media of decreasing solute potential (Fig. 4 b). Proline concentration increased 12-fold in unadapted cells, and >40-fold in adapted cells compared with the controls. Proline levels continued to

Table 1

Free amine concentrations in unadapted (U) and adapted (A) cell lines after treatment with PEG in various concentrations, related to the respective control = 100%. Means of 3 replicate determinations.

Free Amine (mol m^{-3})		concentrations, related to control							
		unadapted (U)				adapted (A)			
PEG (%)	Control (0.0%)	10	15	20	25	10	15	20	25
Putrescine	1.36 = 100	114	174	293	286	185	218	235	268
Spermidine	1.34 = 100	137	140	151	101	135	131	128	126
Spermine	0.09 = 100	189	267	256	422	89	122	356	544
Total	2.79 = 100	127	161	224	201	158	172	187	206

increase in adapted cells up to the highest concentration tested, while in unadapted cells no increase occured beyond 20% PEG.

The combined cellular concentration of the major free amino acids shows a strong correlation with medium solute potential in adapted cells (Fig. 4 a). The response was approximately linear at lower solute potentials and was not saturated at the highest PEG concentration employed. In unadapted cells, however, the response was far less pronounced, with a doubling in concentration over the control, as opposed to an 8-fold increase in adapted cells. While the response of individual non-proline amino acid varies considerably (Table 2), higher concentrations are generally found in adapted rather than unadapted cells. Alanine concentration increased up to 18-fold over control values (adapted cells), but the highest concentrations observed were those of histidine, arginine, and valine, which were relatively abundant in control cells. With the exception of serine, all amino acids were present at higher concentration in adapted than unadapted cells at the lowest solute potential. However, proline was the most abundant amino acid by a factor of 2- and constituted 30% of the total amino acid concentration. Betaine concentration changed little in either cell line response to increasing water stress (Table 2).

The cellular sucrose concentration increased approximately 3-fold up to 120 mol m^{-3} in adapted cells under water stress, and doubled in non-adapted cells (Fig. 5 a). Concentrations in adapted cells were significantly higher than in unadapted cells at all solute potentials.

Glucose concentrations rose 4.5-fold in adapted cells at the lowest solute potential reaching a cellular concentration of nearly 350 mol m^{-3} (Fig. 5 c). Levels also rose 3.5-fold in unadapted cells to a concentration exceeding 240 mol m^{-3}. Concentrations of fructose increased 5-fold in

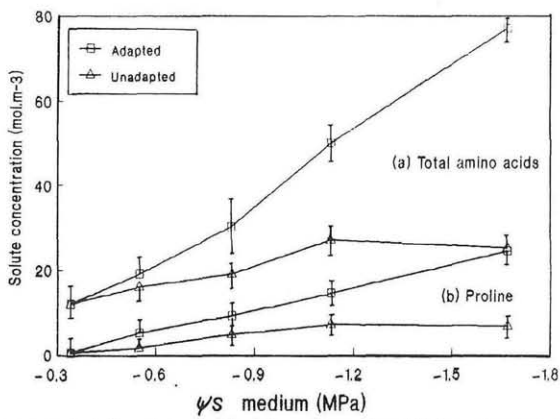

Fig. 4. Changes in total amino acids (a) and proline (b) in adapted (A) and unadapted (U) cells after 10 hours incubation at a range of PEG concentrations; means of three replicates.

Table 2

Free amino acid and glycinebetaine concentrations in unadapted (U) and adapted (A) cell lines after treatment with PEG in various concentrations, related to the respective control = 100%. Means of 3 replicate determinations.

Amino Acids & Betaine ($mol\ m^{-3}$)	Control	concentrations, related to control							
		unadapted (U)				adapted (A)			
PEG (%)	(0.0%)	10	15	20	25	10	15	20	25
Asp	0.57 = 100	87.7	63.2	114.0	112.3	152.6	149.1	186.0	275.4
Gln	0.71 = 100	101.4	111.3	104.2	100.0	139.4	156.3	157.8	156.3
Ser	1.67 = 100	109.0	126.4	144.3	146.7	71.3	112.0	251.5	116.8
His	2.16 = 100	99.1	119.0	115.7	126.9	144.9	172.7	305.6	587.5
Gly	0.33 = 100	84.9	118.2	136.4	175.8	109.1	357.6	251.5	339.4
Thr	0.89 = 100	95.5	111.2	118.0	122.5	85.4	171.9	200.0	257.3
Arg	1.32 = 100	115.2	143.2	147.7	147.0	137.1	203.0	267.4	681.1
Ala	0.29 = 100	117.2	286.2	382.8	375.9	82.8	137.9	1834.5	1889.7
Tyr	0.17 = 100	100.0	105.9	123.5	141.2	164.7	200.0	211.8	341.2
Met	0.25 = 100	136.0	140.0	148.0	148.0	128.0	212.0	232.0	268.0
Val	0.83 = 100	149.4	173.5	224.1	228.9	224.1	312.1	557.8	851.8
Phe	0.86 = 100	132.6	139.5	167.4	218.6	75.6	119.8	167.4	201.2
Ile	0.41 = 100	119.5	156.1	195.1	258.5	141.5	202.4	273.2	702.4
Leu	0.22 = 100	131.8	168.2	218.2	268.2	181.8	245.5	281.8	809.1
Betaine	0.73 = 100	106.9	138.4	116.4	127.4	90.4	109.6	113.7	105.5

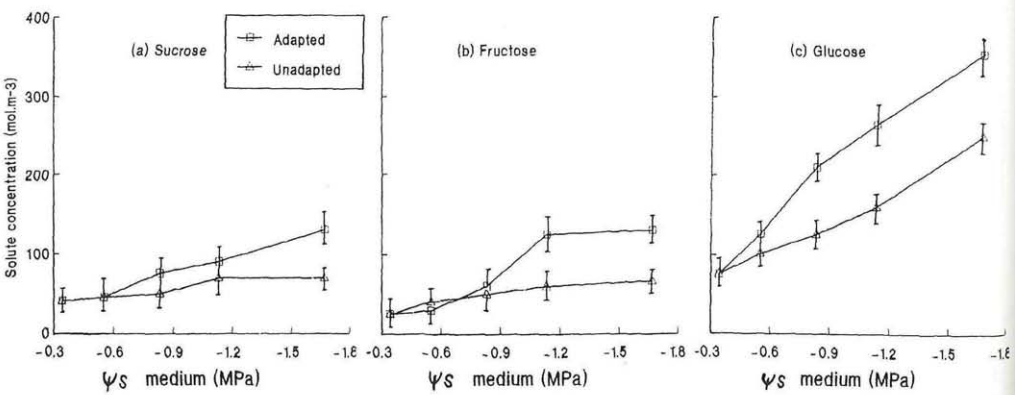

Fig. 5. Changes in sucrose (a), fructose (b) and glucose (c) concentrations in adapted (A) and unadapted (U) cells after 10 hours incubation at a range of PEG concentrations; means of three replicates.

adapted and 3-fold in unadapted cells; reaching a peak of more than 130 mol m^{-3} in adapted cells (Fig. 5 b). Glucose, fructose and sucrose are major contributors to solute concentration, contributing over 60% of the osmolarity of stressed cells (Table 3).

To illustrate the contribution of each solute investigated to the overall cell water potentials, values for medium and cell water potentials and solute concentrations have been calculated in milliosmoles (mOsm), and the percentage contribution of each expressed in Table 3. Glucose, fructose and sucrose were the principal osmotic agents, contributing 61.5% of the cell water potential of adapted cells in 25% PEG. Under the same conditions, proline contributed 2.4% and other amino acids 5.1%. Polyamines and betaine represented an osmotic contribution of only 0.5%. The organic solutes investigated contribute 69.5% of the total potassium and other ionic species have not been determined.

Discussion

The adaptation of cultured plant cells to both water and NaCl stress has been reported (BRESSAN & al. 1981, BEN-HAYYIM 1986, HARMS & OERTLI

Table 3

Medium and cell osmolarity and percentage of each solute in unadepted (U) and adapted (A) cell lines after treatment with PEG in various concentrations, related to the respective control = 100%. Means of 3 replicate determinations.

Concentration of Solutes to cell (%)		concentrations, related to control							
		unadapted (U)				adapted (A)			
PEG (%)	Control (0.0 %)	10	15	20	25	10	15	20	25
med (mos)	140.0 = 100	160.7	242.9	332.1	489.3	160.7	242.9	332.1	489.3
cells (mos)	278.0 = 100	103.6	122.7	164.0	247.1	105.4	168.0	242.5	353.6
Glucose	25.6 = 100	130.5	127.0	137.9	138.3	169.9	168.8	154.3	138.7
Fructose	6.8 = 100	204.4	227.9	219.1	164.7	186.8	192.7	276.5	200.0
Sucrose	14.0 = 100	104.3	96.4	119.3	80.0	107.9	103.6	94.3	88.6
A. A.	3.9 = 100	107.7	105.1	89.7	64.1	112.8	105.1	125.6	130.8
Proline	0.2 = 100	300.0	700.0	800.0	550.0	750.0	900.0	1050.0	1200.0
Polyamine	1.0 = 100	120.0	130.0	140.0	80.0	150.0	100.0	80.0	60.0
Betaine	0.2 = 100	150.0	150.0	100.0	50.0	100.0	100.0	50.0	50.0
Total	51.7 = 100	131.9	132.7	142.4	120.5	152.6	150.7	153.6	134.8

All solute concentrations were recalculated in terms of mOsm and expressed as a percentage of corresponding cell osmotic potential. A. A. = Total amino acid excluding proline.

1985, EL SAYED & KIRKWOOD 1991). Adapted cells characteristically show a shorter lag phase and increased growth rate compared to non-adapted cells exposed to similar stress conditions. Comparable responses were obtained with soybean suspension cultures in this study. This adaptation involves a decrease in cell solute potential (Fig. 3) which is substantially greater in adapted than in unadapted cells and directly related to a reduction in cell water content (Fig. 2), and an accumulation of intracellular solutes compared to controls. This is similar to the response of sorghum (BHASKARAN & al. 1985), tomato (HANDA & al. 1983) and soybean (EL SAYED & KIRKWOOD 1991) cells to a low water potential environment.

When considering changes in solute concentration, it should be noted that the water contents of cells of either line transfered to medium or low water potential are sharply reduced as compared with control cells (Fig. 2). This is due to water loss to the medium, and will result in increased solute concentrations even in the absence of any absolute rise in solute content. For example, the water content of unadapted cells transferred to 25% PEG, falls by 54% (Fig. 2); concentrations of all solutes will thus double without any net accumulation. This point has been considered by some investigators, (TURNER & STEWART 1988) and should be noted, particularly when concentration changes of the order of 1–2 × are considered. The accumulation of solutes has been widely reported under stress conditions (HANSON & HITZ 1982, STEWART & HANSON 1980) with polyamine accumulation in particular receiving attention in the last decade (SMITH 1984). Large increases in polyamines (50- to 60-fold) have been reported in detached oat leaves and protoplasts after 6 h exposure to osmotic stress (FLORES & GALSTON 1982). In similar experiments with barley leaf sections, however, TURNER & STEWART (1988) found only a 3- to 4-fold increase in putrescine levels over intact leaf controls, and suggested that the massive increase reported by FLORES & GALSTON (1982, 1984) was artefactual and due in plant to low control values.

In this study, no increase in total polyamines and only a small increase in putrescine over that dictated by concentration due to water loss, was detected. This may be due to the high levels of putrescine found in control cultures, reported previously (FALLON & PHILLIPS 1988). It may be that putrescine accumulation is saturated at the control values of approximately 1.3 mol m^{-3}, but in any case no significant increase in putrescine was detected either in adapted or stress-shocked cells. The minor component spermine increased 4- to 5-fold in concentration, suggesting rather more than a doubling in net content. Spermine has not been previously identified as a stress-indicator.

Large increases in proline concentration were observed, relative to control values. In cells exposed to 25% PEG, proline increased 12-fold under stress-shock conditions and 40-fold in adapted cells, to a maximum

of 23 mol m^{-3}. HANDA & al. (1983) reported a 100-fold increase in proline in tomato cells adapted to 25% PEG up to a similar maximum concentration; comparable increases in proline content have been found in drought-stressed barley (HANSON & NELSEN 1978) and sorghum (STEWART & HANSON 1980, BLUM & EBERCON 1976), and thus the response of soybean cells is in agreement with that widely reported for both cell and whole plant systems subjected to water stress (EL SAYED & KIRKWOOD 1991). A significant difference in the degree of response to adaptation and stress-shock was observed; proline accumulation apparently continues to increase in adapted cells to substantially higher levels than are produced by a 10 h stress-shock period. In salt-stressed barley leaves, proline accumulation increased over a 16 h period (VOETBERG & STEWART 1984) but did not increase further with osmotic adjustment. HANDA & al. (1983) have discussed the possibility that proline is derived from glutamate under stress conditions. These observations are similar in that glutamate levels decline somewhat in low water potential medium when water loss is taken into account. The observed values in control cells however, are not suffeciently high to account for the increase in proline.

The varied response of the individual non-proline amino acids in adapted cells supports speculation (HANDA & al. 1983) that water stress adaptation involves changes in the rates of assimilation, synthesis, utilization and interconversion of amino acids; certainly no common mechanism can be evoked. While proline is clearly unique in the extent of its response, it may be noted that the increase in osmotic contribution due to non-proline amino acids is nearly twice that of proline itself. In stress-shocked cells, on the other hand, the concentration of non-proline amino acids only increases by 70%. This means that the net control falls, when concentration effects are taken into account. Adaptation and stress shock processes do not, therefore, seem closely related for these amino acids.

Reducing sugars, particularly glucose, together with sucrose, provide the major contribution to solute accumulation in adapted cell lines. This is in agreement with findings in stressed tomato cells by HANDA & al. (1983), who suggest that such increases might be due to a reduction in cell wall synthesis mediated by reduced expansion. It should be noted, however, that the culture medium initially contains 78 mol m^{-3} sucrose, and that high levels of sugars may well be due to uptake and catabolism from this source; in sorghum and sunflower leaves levels of sucrose, and reducing sugars were approximately an order of magnitude lower than in the present study (JONES & al. 1980). It would be of interest to investigate sucrose uptake rates to clarify this point.

This study reveals substantial differences in cellular responses to stress shock as compared to long term adaptation to water stress, when concentration effects are discounted stress shocked cells show major

increases in proline and, to a lesser extent, reducing sugars. Long term adaptation is accompanied by enhanced levels of these solutes as well as sucrose and other amino acids. It is uncertain whether this is solely a function of the duration of exposure to water stress or qualitatively different mechanisms are involved.

Adaptation involves a substantial reduction in cellular water potential, allowing turgor to be maintained. The organic solutes investigated here account for about 70% of the osmotic potential change observed between control and adapted cells. The nature of the remaining 30% must be speculative, although inorganic solutes (mostly potassium ions) represented 20% of the osmotic potential in adapted tomato cells (HANDA & al. 1983). Part of the short-fall might be due to underestimation of cell osmotic potential by mixing of extracellular water with cellular content during osmometer measurements. In a major study using adapted tomato cells, HANDA & al. (1983) were able to account for only 65% of the observed osmotic potential, including organic solutes; other workers (JONES & al. 1980, MEYER & BOYER 1981) accounted for almost 100% of the observed osmotic potential in whole plant organs, the major contribution being from sugars and amino acids.

References

BEN-HAYYIM G. 1986. The relationship between salt tolerance and resistance to polyethylene glycol induced water stress in cultured citrus cells. – Plant Physiol. 85: 430–433.

BLUM A. & EBERCON A. 1976. Genotypic responses in sorghum to drought stress. III-Free proline accumulation and drought resistance. – Crop Sci. 16: 428–431.

BRESSAN R. A., HASEGAWA P. M. & HANDA A. K. 1981. Resistance of cultured higher plant cells to polyethylene glycol induced water stress. – Plant Sci. Lett. 21: 23–30.

BHASKARAN S., SMITH R. H. & NEWTON R. J. 1985. Physiological changes in cultured sorghum cells in response to induced water stress. – Plant Physiol. 79: 266–269.

EL SAYED H., KIRKWOOD R. C. 1991. Solute accumulation in soybean (*Glycine max* L.) cells adapted to NaCl salinity. – Phyton (Horn, Austria) 31: 233–249.

FALLON K. M. & PHILLIPS R. 1988. Polyamines in relation to growth in carrot cell cultures. – Plant Physiol. 88: 224–227.

FLORES H. E. & GALSTON A. W. 1982. Polyamines and plant stress: Activation of putrescine biosynthesis by osmotic shock. – Science 217: 1259–1261.

— & — 1984. Osmotic stress induced polyamine accumulation in cereal leaves. I-Physiological parameters of the response. – Plant Physiol. 75: 102–109.

HANDA S., BRESSAN R. A., HANDA A. K., CARPITA N. C. & HASEGAWA P. M. 1983. Solute contributing to osmotic adjustment in cultured plant cells adapted to water stress. – Plant Physiol. 73: 834–843.

HANSON A. D. & HITZ W. D. 1982. Metabolism responses of mesophytes to plant water deficits. – Annu. Rev. Plant Physiol. 33: 163–203.

— & NELSEN C. E. 1978. Betaine accumulation and formate metabolism in water stressed barley leaves. – Plant Physiol. 62: 305–312.

HARMS C. T. & OERTLI J. J. 1985. The use of osmotically adapted cell structures to study salt tolerance in vitro. – Plant Physiol. 120: 29–38.

HEYSER J. W. & NABORS M. W. 1979. Osmotic adjustment of tobacco cells and plants to penetrating and non penetrating solutes. – Plant Physiol. Suppl. 63: 63–77.

HOLLIGAN P. M. 1971. Routine analysis by gas liquid chromatography of soluble carbohydrates in extracts of plant tissues. – New Phytol. 70: 239–269.

JARRETT H. W., COOKSY K. D., ELLIS B. & ANDERSON J. M. 1986. The separation of O-phthalaldehyde derivatives of amino acids by reversed-phase chromatography on octylsilica columns. – Ann. Biochem. 153: 189–198.

JONES M. M., OSMOND C. B. & TURNER N. C. 1980. Accumulation of solutes in leaves of sorghum and sunflower in response to water deficits. – Aust. J. Plant Physiol. 7: 193–205.

KRAMER P. J. 1980: Drought stress and the origin of adaptation. – In: TURNER N. C. & KRAMER D. J. (Eds.), Adaptation of plants to water and high temperature stress, pp. 7–20. – J. Wiley & Sons, New York

LEVITT J. 1980. Responses of plants to environmental stress. 2 nd edition. – Academic Press, New York.

MEYER R. F. & BOYER J. S. 1981. Osmoregulation, solute distribution and growth in soybean seedlings having low water potentials. – Planta 151: 482–489.

MURASHIGE T. & SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. – Physiol. Plant. 15: 473–479.

SMITH T. A. 1984. Putrescine and inorganic ions. – In: TIMMERMANN B. N., STEELINK C. & LOEWUS F. A. (Eds.), Phytochemical adaptations to stress 8: 6-54. – Plenum Press, New York.

STEWART C. R. & HANSON A. D. 1980. Proline accumulation as a metabolic response to water stress. – In. TURNER N. C. & KRAMER P. J. (Eds.), Adaptation of plants to water and high temperature stress, pp. 155-172 – J. Wiley & Sons, New York.

STUMPF D. K. 1984. Quantitation and purification of quaternary ammonium compounds from halophyte tissue. – Plant Physiol. 75: 273–274.

SWEELEY C. C., BENTLEY R., MAKITA & WELLS W. W. 1963. Gas liquid chromatography of trimethylsilyl derivatives of sugars and related substances. – J. Amer. Chem. Soc. 85: 2497.

TROLL W. & LINDSLEY J. 1955. A photometric method for the determination of proline. – J. Biolog. Chem. 215: 655–660.

TURNER L. B. & STEWART G. R. 1988. Factors affecting polyamine accumulation in barley (*Hordeum vulgare* L.) leaf sections during osmotic stress. – J. Exp. Bot. 39: 311-316.

— & JONES M. M. 1980. Turgor maintainance by osmotic adjustment: A review and evaluation. – In: TURNER N. C. & KRAMER P. J. (Eds), Adaptation of plants to water and high temperature stress, pp. 87–104. – J. Wiley & Sons, New York.

VOETBERG G. & STEWART C. R. 1984. Steady-state proline levels in salt-shocked barley leaves. – Plant Physiol. 76: 567–576.