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EFFECTS OF DIFFERENT CONCENTRATIONS OF POLYETHYLENE GLYCOL ON CELLS OF THREE SOYBEAN CULTIVARS

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ABSTRACT

The responses of three cultivars of Soybean, *Glycine max* (L) Merr to water stress was studied using different concentrations of Polyethylene glycol (PEG) namely: 0%, 10% and 15% for 7 and 14 days. Results showed that after a 7 day exposure of the cells of the cultivars to the stress agent, the fresh weight of cultivars Tracy, Hill and Forest were the same in the control groups and the experimental groups with an average of 1.5810g for the control , 1.5310g and 1.5002g for the 10% and 15% PEG treatments respectively. This indicates that growth rate in the experimental groups for the three cultivars was not affected by the PEG treatments after 7 days of growth. After 14 days of exposure, the fresh weight results for cultivar Hill showed that from an initial fresh weight of 0.2771g, fresh weight of the control increased to 1.6375g, while that of 10% and 15% PEG treatments increased to 0.9650g and 0.8700g respectively. This low growth rate in the 10% and 15% PEG treatments was the same in all the cultivars after 14 days exposure to the stress agent. Total protein measurements of cultivar Tracy after 14 days of growth increased from 681.3ug/gcell in control to 932.60ug/gcell and 946.72ug/gcell for the 10% and 15% PEG treatments. This pattern indicated that there was an increase in protein synthesis when the cells were exposed to longer periods of stress. Cultivar Forest responded differently from the other two cultivars by producing a high amount of protein after 7 days of growth in the PEG treatments indicating that the increased protein, 1058.86ug/g cell in the cells growing in 15% PEG in contrast to 769ug/gcell of the control, allowed the cells to adapt to the stress and this adaptation was evidenced by the fact that there was no decrease in growth rate.

KEYWORDS: Soybean cultivars, Polyethylene glycol (PEG), drought stress, growth rate, total protein.

INTRODUCTION

Drought conditions have been blamed as the major cause of famine in most countries (TVAB 1989). and this is largely because water is a limiting factor in crop production. When drought occurs, crop yield is adversely affected, especially when it occurs during critical periods in plant growth (Blum and Ebercon, 1976). Although the initial cost of this lowered yield is borne by the farmers, the consumers are ultimately affected as food items become scarce in the market and prices are increased.

Most of the researches on drought stress have been concentrated on the responses of whole plants at the field level, but with increasing interest in biotechnology, more focus is being directed towards the application of tissue culture techniques to the selection of drought tolerant plant cultivars (Boyers, 1973). The use of tissue culture allows careful measurements of growth and other parameters in response to various changes in osmotic environment simulated by stress agents like sodium chloride (NaCl) and Polyethylene glycol (PEG). Polyethylene glycol is widely used as a non-penetrating stress agent to lower the water potential of the medium in which cells are grown. Thus, it is possible to select cells that are resistant to water stress from populations by using PEG as the stress agent (Bressan *et al.*, 1981).

When plants undergo periods of limited water availability, a variety of modifications occur. Such modifications include the release of substances like proteins, organic salts, sugars, proline or other amino acids. These substances appear to convey adaptive value to the stressed cells (Handa *et al*, 1982). Since protein synthesis in plants responds dramatically to changes in environmental conditions, environmental stresses are known to lead to quantitative and qualitative changes in the pattern of protein synthesis in plants (Ramagopul, 1988). There is, therefore, the need to study the process of osmoregulation in plants towards a better understanding of the genotypic diversity of responses to water stress and with the ultimate goal of developing plant cultivars with some degree of tolerance to the stress.

In many instances, there are syntheses of new polypeptides which are not found in the absence of the stress conditions. These "stress proteins" seem to be related to the adaptation process of the plants to the stress and also the genetic constitution of selected tolerant varieties (Dell'Aquila and Spada 1993).

The objectives of this study were to

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determine the water stress response of three soybean cultivars; to determine which of the three cultivars studied show adaptation to simulated drought stress and to determine whether or not "stress proteins" were synthesized which would enable the adapted varieties to adapt to the stress conditions.

MATERIALS AND METHODS

Soybean seeds cultivars: Tracy, Hill and Forest were obtained from the Department of Agriculture, Tennessee State University, Nashville Tennessee, USA, where the research was carried out . The seeds, 25 seeds per cultivar, were surface sterilized with 50% chlorox for 8 minutes. Excess chlorox was removed by rinsing 3 - 5 times with deionized distilled water. The surface sterilized seeds were placed aseptically on petri dishes (5 seeds per petri dish) containing solid Murashige and Skoog (MS) Medium which had been autoclaved for 20 minutes at 121°C. There were 5 petri dishes per cultivar. Hypocotyl sections were aseptically excised from 2 weeks old seedlings and placed on 5 plates per cultivar each containing solid MS medium with 2mg/1 of 2, 4-dichlorophenoxyacetic acid (2,4-D) added as a supplement to induce callus formation. The plates were incubated in the cell culture room at 25°C for about 4 weeks in diffused light. The callus pieces formed after 4 weeks were removed and placed in 125ml Erlenmeyer flasks containing 20 ml of MS media to initiate suspension cultures. The flasks were incubated on a gyratory shaker (100- 125 rev/min.) to allow callus pieces to break up into smaller clumps of cells. As new cells were formed, they were dispersed throughout the medium. Stock cultures of untreated cells were maintained in 125ml flasks of MS media supplemented with 2,4-D.

Water stress was simulated by adding PEG of molecular weight 8000 in MS media supplemented with 2 mg/l of 2, 4-D. 20 ml of the media containing PEG concentrations of 0%, 10% and 15% was poured into 125ml flasks and autoclaved for 20 minutes at 121°C before using for suspension cultures. 4 flasks of each of the 0%, 10% and 15% PEG treatments were maintained as replications.

The cells grown in 0, 10 and 15% PEG were harvested at the end of 7-day periods of exposure for a total exposure of 14 days. A 2.5 ml aliquot was aseptically removed and collected on Whatman #8 filter paper dried in a Buchner funnel by aspiration and weighed to obtain fresh weight. These cells were then dried in an oven at 70°C overnight and later weighed to obtain the dry weight. Packed cell volume (PCV) which was also used in determination of growth rate was obtained by centrifuging the total volume of cells at 1300g for 5 minutes and the volume of cells was recorded. The remaining cells were subcultured in a 1: 4 dilution in fresh media with the same make-up as the original media and allowed to grow for another 7-days.

TOTAL PROTEIN MEASUREMENTS:

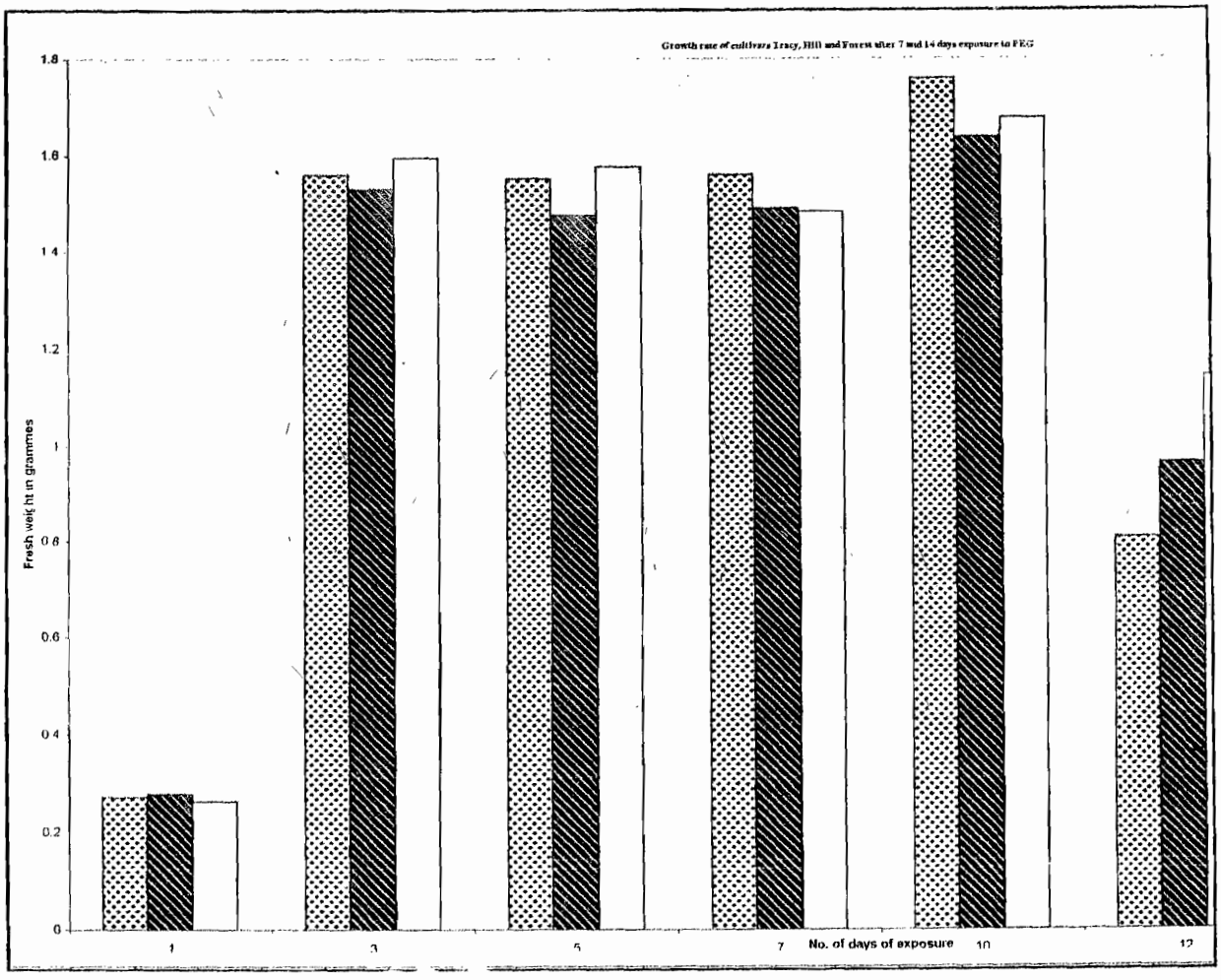
Protein extraction was done using an extraction buffer of tris (hydroxymethyl) aminomethane. The cells were homogenized and then centrifuged at 17000 rpm in a refrigerated superspeed centrifuge. After centrifugation, the supernatant was collected. Sodium deoxycholate and trichloroacetic acid were added and the mixture was centrifuged at room temperature in a table top centrifuge for 30 minutes to precipitate the protein. The actual assay was done using the Lowry Method (Lowry, 1981) and the Bradford Method (Bradford, 1976) with bovine serum albumin as the standard. The reagents used for this method were reagent A and reagent B. To the precipitated protein 1 ml of reagent A and 1 ml of deionized distilled water was added. The mixture was vortexed and allowed to stand at room temperature for 15 minutes. 3 mls of reagent B was then added and allowed to stand at room temperature for 35 minutes. Total protein was determined by reading at 625 nm on a Shimadzu QV-50 spectrophotometer.

A students T-test was used in statistical analyses of data obtained.

RESULTS:

The results of the exposure of soybean cells of cultivars Tracy, Hill and Forest to different concentrations of PEG are summarized in Figures 1 and 2 and in Table 1. Figure 1 shows the effects on fresh weights of Tracy, Hill and Forest cultivars after 7 and 14 day exposure to 0%, 10% and 15% PEG concentrations. The initial fresh weight of the cells of cultivar Tracy was 0.2718gm for all the treatments. After a 7 day exposure, the average weight of 4 replications was 1.5597gm, 1.5526gm and 1.5594gm for 0, 10 and 15% PEG treatments respectively. The fresh weight results for cultivar Hill is also seen in Figure 1. The initial fresh weight for all the treatments was 0.2771g but at the end of a 7 day period it increased to 1.5298g for the control group 1.4758g and 1.4900g for the 10% and 15% PEG treatments respectively. After 14 days of exposure the data showed an increase in fresh weight of the control from the initial weight of 0.2771g to 1.6375g. At 10%, fresh weight increased to 0.9650g while at 15% it increased to 0.8700g.

The initial fresh weight of all the treatments of cultivar Forest was 0.2628g. After



a 7 day exposure to 0%, 10% and 15% PEG treatments, the fresh weight increased to 1.5947g, 1.5766g and 1.4843g respectively. After 14 days of exposure to 0%, 10% and 15% PEG fresh weight increased from 0.2628g to 1.6754g for the control, 1.1423 g for the 10% treatment and 0.8943 g for the 15% treatment.

The results for total protein are summarized in Figure 2. After 7 days of exposure, cultivar Tracy had a total protein measurement of 688.05 ug/gcell while at 10% and 15% PEG concentrations total protein was 681.3 ug/g cell and 688.0 ug/g cell respectively. After 14 days exposure the total protein measurements were 684.2ug/g cell, 932.60ug/g cell and 946.72 ug/g cell respectively at 0%, 10% and 15% PEG concentrations respectively.

After 7 days of growth in 0%, 10% and 15% PEG concentrations the total protein measurements for cells of cultivar Hill were 692.5ug/g cell, 683ug/g cell and 694.1ug/g cell respectively. After 14 days of growth the amount of total protein increased to 687.655ug/g cell, 898.19ug/g cell and 946.72ug/g cell for the 0%, 10% and 15% PEG treatments respectively.

Results for the total protein measurements of cultivar Forest showed that after 7 days of growth in 0%, 10% and 15% PEG the total protein of the cells was 769.0 ug/g cell, 883.5ug/g cell and 1058.86ug/g cell respectively. After 14 days exposure to the same PEG concentrations, the total protein for the cells were 685.78ug/g cell, 1039.23ug/g cell and 1229.5ug/g cell for the cells in 0%, 10% and 15% PEG respectively.

DISCUSSION:

The results for cultivar Tracy showed a 5.7- fold increase in fresh weight over a 7 day period of growth in different concentrations of PEG. The increase was the same in the control group (0% PEG) and the experimental groups (10% and 15%PEG). The 5.5- fold and 5.9- fold increases observed in cultivar Hill and in cultivar Forest respectively after the first 7 days of growth was also the same in the control groups and the experimental groups. These results indicate that PEG concentrations of 10% and

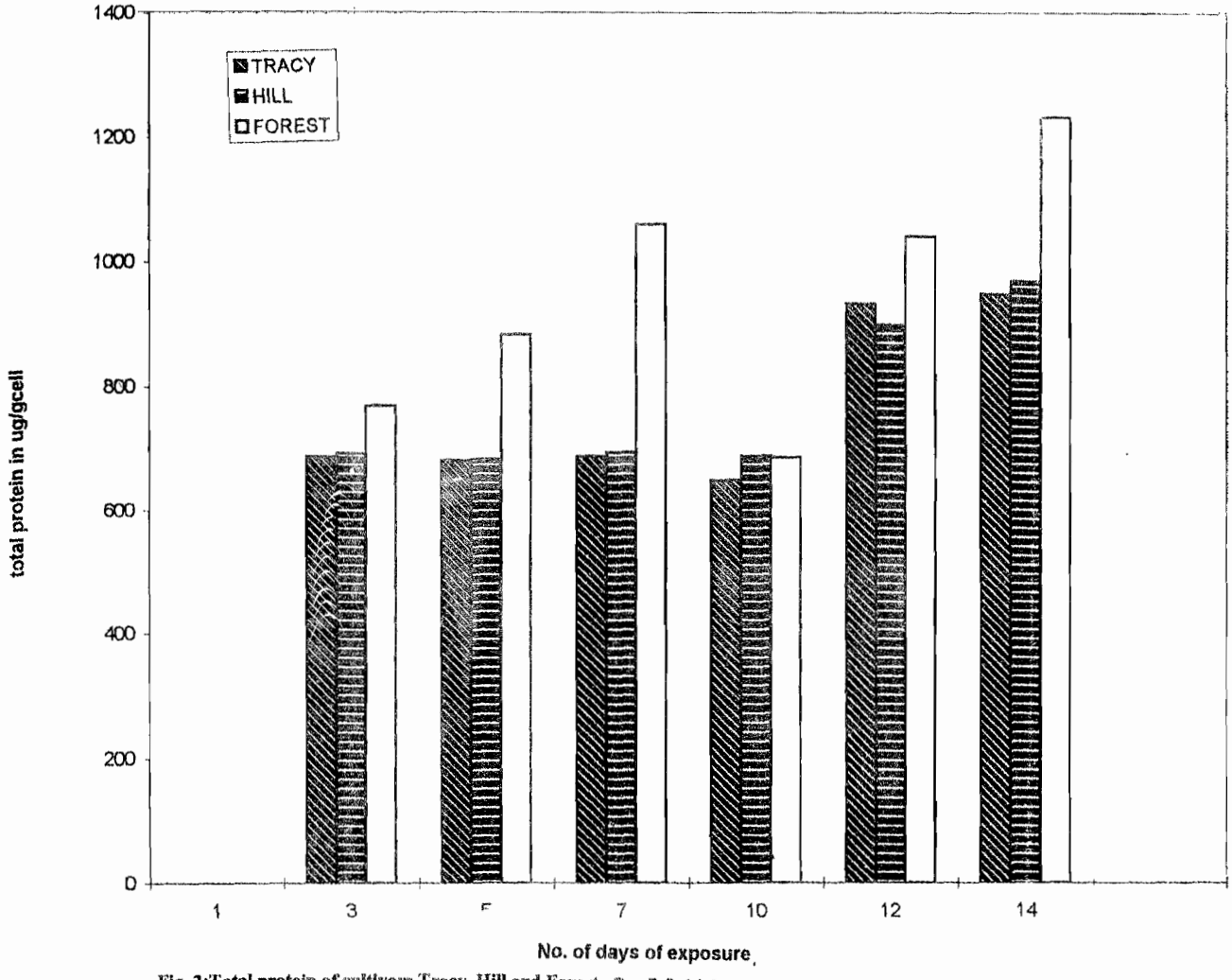


Fig. 2:Total protein of cultivars Tracy, Hill and Forest after 7 & 14 days exposure to PEG.

15% were not sufficient levels of stress to result in significant changes in fresh weight of the three cultivars after 7days of exposure. The results confirm previous work in which soybean cultivars exposed to PEG concentrations as high as 20% showed no differences in fresh weight or dry weight when exposed for 7 days (McCuller, 1985).

The results after a 14 day exposure to 0%, 10% and 15% PEG indicate that all three cultivars showed reduced growth rate. The PEG concentrations of 10% and 15% for 14 days was sufficient to induce water stress in the plant cells and the cells responded to this stress by a reduction in growth rate. The consistency of these results among the three cultivars would seem to indicate that there was no difference among the genotypes represented by the three cultivars as far as growth rate response was concerned.

The average total protein of three replications of cultivars Tracy and Hill and Forest after a 7 day exposure to 0%, 10% and 15% PEG would seem to indicate that protein metabolism is not affected in any significant way in cultivars Tracy and Hill. This confirms the earlier assumption that a 7 day exposure to various levels of PEG was not sufficient to

TABLE 1: Percentages of Packed cell volume (PCV) in Tracy, Hill and Forest cultivars after 7 and 14 day exposure to 0, 10 and 15% PEG conc

AFTER 7 DAYS				AFTER 14 DAYS			
PEG conc.	TRACY	HILL	FOREST	PEG conc.	TRACY	HILL	FOREST
0% 98.8	100.0	100.0	100.0	0%	100.0	100.0	
10% 85.8	99.7	99.3	100.0	10%	86.0	86.0	
15% 78.0	98.0	98.0	98.7	15%	75.0	78.0	

induce stress conditions in the cells. The increase in total protein of the experimental groups of cultivar Forest as compared to the control group would seem to indicate that even though growth rate of this cultivar was not affected after a 7-day exposure to PEG concentrations of 10% and 15%, the stress levels were sufficient to induce production of higher amounts of total protein in response to the stress and this increased amount of protein was sufficient to adapt the cells to water stress and therefore no reduction in growth was observed (Ramagopul, 1988.)

Total protein measurements of the three cultivars after 14 day exposure to simulated drought stress indicate that there was a significant increase between total protein of the 0% treatment and that of the other experimental groups at 5% level of probability using a tudent's *t*-test. There is also a significant increase in total protein measurements of the 14 day exposure over total protein measurements of the first 7 day stress period in the experimental groups. It has already been shown that the cells of cultivar Tracy and Hill responded to stress levels of 10% and 15% PEG for a 14 day period by a decrease in fresh and dry weight. The total protein measurements further show that they respond to the same treatment by an increase in total protein. The increased protein synthesis in response to the stress was not sufficient to alleviate total stress as there was a decrease in growth rate of stressed cells. This increase in total protein of the experimental group over the control group after a 14 day exposure is also seen in the response of cultivar Forest. Total protein increased as PEG concentration increased. Accumulation of protein in response to environmental stress has been reported (Erickson and Alfinito1984; Kanabas et al., 1984; Ramagopul, 1988 and Reinhold, 1973). It has been postulated that the accumulation of protein in the cells exposed to sufficient levels of PEG is involved in the cells' ability to withstand osmotic stress. (Handa et al., 1983). Identification and characterization of these proteins would aid significantly in the understanding of the mechanism of stress tolerance in plants. The use of electrophoresis to determine whether the accumulation of protein is as a result of synthesis of new "stress proteins" or an increased synthesis of already existing proteins would also be helpful in providing insight into osmoregulation in plants. Such a study is intended as a follow up to this research.

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