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Proline Accumulation and Growth of Soybean Callus Under Salt and Water Stress

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ABSTRACT

Calli of soybean [*Glycine max* cv. Pershing] were grown on "MS" media supplemented with different levels of KCl and NaH_2PO_4 for 45 days. The effects of salt stress were evaluated on the production and accumulation of proline and growth content of calli. Proline content increased as growth occurred. The maximum amount of proline and the highest content of growth were observed in calli grown on media supplemented with 60 mM KCl along with 1 mM NaH_2PO_4 (the highest salts levels) and 45 mM KCl along with 1 mM NaH_2PO_4, respectively. Proline accumulation was correlated with absorption of water from environment by calli that were grown on media containing 60 mM KCl and 1 mM NaH_2PO_4. The results suggest a positive role for proline accumulation in adaptation of cells to salt and water stress.

Key Words: Callus; Proline; Salt stress; Soybean

INTRODUCTION

Plants exposed to stresses undergo changes in their metabolism in order to adapt with changes in their environment. Agricultural productivity worldwide is subjected to increasing environmental constraints, particularly to drought and salinity due to their high magnitude of impact and wide distribution. Salt stress changes the morphological, physiological and biochemical responses of plants. On the molecular level these responses are manifested as changes in the pattern of gene expression (Fabre & Planchon, 2000; Maggio, 2002). Mostly callus cultures are used as an *in vitro* technique for biochemical and physiological studies in response to salt and water stress at the cellular level (Liu & Van Staden, 2001; Basu *et al.*, 2002; Jantaro *et al.*, 2003; Liu *et al.*, 2006). Production and accumulation of Free Amino Acids (F.A.A.), especially proline by plant tissue during drought, salt and water stress is an adaptive response. Proline has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm (McCue & Hanson, 1990; Porgali & Yurekli, 2005; Arshi *et al.*, 2005; Caballero *et al.*, 2005; Bartels & Sunkar, 2006). Thus proline can be used as a metabolic marker in relation to stress (Burton, 1991). Proline produces immediately after encounter of cells with salt stress and protects the plasma membrane and proteins against stress (Santoro *et al.*, 1992). Understanding of plant ability in fight to stresses open a way for crops manipulations for their ability in tolerance, adaptation or resistant to stresses (Hare *et al.*, 1999).

The aim of this work was to study the effects of various concentrations of salts (KCl & NaH_2PO_4) in the culture media on calli of soybean during 45 days of growth and to evaluate the proline level and growth of callus in order to recognition of the resistant of soybean to the salinity.

MATERIALS AND METHODS

Seeds of soybean [*Glycine max* (L.) Merr. cv. Pershing] were used. Seeds were disinfected for two min in ethanol 70% (v/v), rinsed, then sterilized by 2% sodium hypochlorite (w/v) for 15 min. Seeds were cultured on water – agar media (0.7%) for production of the seedling. After a week, hypocotyls of seedling were divided into sections of 8 to 10 mm and cultured on "MS" solid media (Murashige & Skoog, 1962) along with KCl (0, 15, 30, 45 & 60 mM) and NaH_2PO_4 (0, 0.01, 0.1 & 1 mM) salts (20 treatments).

For estimation of fresh weight, dry weight and proline content, calli with 45 days old were used. For estimation of fresh weight, calli were weighed immediately after isolation of culture media. Calli were dried at 60°C for 18 h, and their dry weights were measured. For measurement of proline content, 20 mg dried callus was homogenized in 5 mL 3% aqueous sulfosalicylic acid and filtered by whatman filter paper (No. 1). To 1 mL extract, 1 mL acid ninhydrin and 1 mL of glacial acetic acid were added and the reaction mixture incubated at 100°C for 1 h. The reaction mixture was placed on ice and extracted with 2 mL toluene. Proline content in the extract was subjected to the spectrophotometric assay of Wren and Wiggal (1965).

The experiments were set up in a "Factorial" with completely randomized design, which was done with unequal repetition. Analysis of variance was performed on the data and means were compared at 5% and 1% levels of probability.

RESULTS AND DISCUSSION

This study revealed that synthesis of proline and fresh and dry weights increased in calli of soybean grown on media supplemented with high levels of KCl and NaH_2PO_4. Accumulation of proline is caused for survival of calli. This result had also been reported in other studies (Liu & Van Staden, 2001; Basu *et al.*, 2002).

The highest content of fresh weight (1965 mg) and dry weight (120 mg) were obtained in calli grown on media contain 45 mM KCl and 1 mM NaH_2PO_4. Also the highest content of proline accumulation (71.01 $mol.g^{-1}$ dry wt) was in calli grown on media include the highest levels of KCl and NaH_2PO_4 (60 mM & 1 mM) respectively. The least content of fresh weight (416 mg) dry weight (40 mg) and proline accumulation (36.42 $mol.g^{-1}$ dry wt) were observed in calli of control (without KCl & NaH_2PO_4) (Table I). The role of proline in adaptation and survival of plants had been observed by several researchers (Basu *et al.*, 2002; Porgali & Yurekli, 2005; Arshi *et al.*, 2005; Djanaguiraman *et al.*, 2006). The study of Harinast *et al.* (2000) on mulbery have shown that when the concentration of NaCl increased to 200 mM the growth was completely inhibited and the necrosis of leaves tissue observed. The studies on rice, maize, ice plant and arabidopsis grown on salinity environment have revealed that salt shock, initially decrease the growth, fresh and dry weight, but after a time, content of the compatible solute, particularly proline increased and growth occurred (Rodriguez *et al.*, 1997; Yang & Yen, 2002; Djanaguiraman *et al.*, 2006). In several types of plant, the accumulation of proline and increasing of water has been observed in response to salt stress (Hohl & Peter, 1991; Agarwal *et al.*, 1994; Pandy & Agarwal, 1998). Some workers have reported the similar results (Delauney & Verma, 1993; Samaras *et al.*, 1995; Taylor, 1996). Handa *et al.* (1986) have shown that transfer of cultured tomato cells to a low water potential environment resulted in an increased dry weight to fresh weight ratio accompanied by a rapid accumulation of proline. According to study on senna, proline content in leaves treated with 10 mM CaCl2 and 160 mM NaCl was 8-fold more than to that in leaves without CaCl2 and NaCl treatment (Arshi *et al.*, 2005). These observation have led to the suggestion that proline could be used as a metabolic marker (Burton, 1991).

In this study, levels of 45 mM KCl along with 0.1 and 1 mM NaH_2PO_4, also 60 mM KCl along with all of the levels of NaH_2PO_4 (0, 0.01, 0.1 & 1 mM) were stressor.

Fresh weight, dry weight and proline content were significantly increased in calli treated with levels of salts mentioned above compared to that in calli of control (Table I). The most content of proline in calli treated with 60 mM KCl and 1 mM NaH_2PO_4 have established that the highest levels of salts caused the highest stress and the highest stress caused the most content of synthesis of proline. The "ANOVA" results showed significant differences were found between different levels of KCl and NaH_2PO_4 related to synthesis and accumulation of proline. Differences between concentrations of KCl, NaH_2PO_4 and KCl along with NaH_2PO_4 were significant (Table II). The water content of calli treated with the highest level of salts showed that proline plays a role in water aquilibrium. In addition to proline, salt stress alter the synthesis of other amino acids also polyamines such as putresine and spermine (Jantaro *et al.*, 2003; Pandy *et al.*, 2004; Mutlu & Bozcuk, 2005; Liu *et al.*, 2006). Abscisic acid (ABA) plays an important role in content of proline synthesis. Content of ABA and proline synthesis depends on tolerance or sensitivity to salt stress (Mills *et al.*, 2001; Woodward & Bennett, 2005). Some studies, particularly work on callus of rice and aquatic macrophytes have revealed presence or retention of K^+ in cells is a key factor for salt tolerance. The results indicated

Table I. Fresh and dry weight and proline content of calli derived from media supplemented with different concentrations of KCl and NaH_2PO_4

KCl (mM)	NaH_2PO_4 (mM)	Fresh weight (mg)	Dry weight (mg)	Proline content (µmol g^{-1} dry wt)
0	0	416	40	36.42
0	0.01	807	64	47.68
0	0.1	684	56	48.01
0	1	938	75	48.82
15	0	960	65	53.45
15	0.01	812	60	54.88
15	0.1	968	64	55.89
15	1	970	77	56.81
30	0	898	78	47.61
30	0.01	724	55	50.94
30	0.1	846	59	53.12
30	1	957	67	57.66
45	0	737	52	47.79
45	0.01	728	61	49.49
45	0.1	1903	111	61.84
45	1	1965	120	64.14
60	0	1697	107	60.98
60	0.01	1588	108	62.16
60	0.1	1498	112	63.77
60	1	1573	115	71.01

Table II. Analysis of variance (ANOVA) the effect of different concentrations of KCl and NaH_2PO_4 on proline content of calli

Source	Degrees of freedom	Sum of squares	Mean squares	F value
Replication	2	8.939	4.469	1.4568
Factor A	4	2314	578.5	188.5545**
Factor B	3	932.567	310.856	101.3194**
A x B	12	407.974	33.998	11.0811**
Error	38	116.587	3.068	
Total	59	3780.067		

Coefficient of variation: 3.20%
**: Significant at 1% levels of probability.

that K^+ is the first candidate to counteract the negative water potential of outside milieu, while proline is probably the last metabolic device that rice calli opted for when exposed to salt stress.

In summary, plants possess mechanisms of adaptation in response to the change in salt concentration in their environments. This research has demonstrated that when the concentration of salt in the environment increased, proline content in calli of soybean also increased.

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