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Effect of Light and Salinity on Growth of Soybean (*Glycine max.*, L.) Callus Established from Adapted and Unadapted Stem Cell Culture

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Abstract. Calli of Soybean (*Glycine max.*, L. Maple Arrow) from cell cultures unadapted (control) and adapted to 85 mM NaCl cells cultures were established under 0 - 160 mM NaCl stress treatments. Light induced 45% increase in fresh weight of preadapted but not in unadapted calli. NaCl decreased growth (as fresh weight) with more or less equal rates in preadapted and unadapted calli incubated in dark or light conditions. Callus cultures of soybean were established from cell suspension of stem tissues on MS medium containing 1 mgL-1 BAP and 4 mgL-1 IBA from cell suspension exposed (adapted) and unexposed (unadapted) to 85 mM NaCl. Callus tissues of adapted soybean exhibited green pigmentation and grew better, while callus tissues of unadapted soybean were pale-yellow and grew less. Callus cultures of adapted and unadapted soybean were sensitive to the presence of 80 mM NaCl in the culture medium. The growth of the unadapted callus cultures was more sensitive than the adapted callus. The significance of the soybean callus responses in relation to the mechanisms of salt tolerance is discussed

Key words. Soybean (*Glycine max*, L. Maple Arrow) cells, NaCl salinity, Callus Culture and Growth.

Abbreviations. BAP: 6-benzylaminopurine; 2, 4-D: 2, 4-dichlorophenoxyacetic acid; IAA: Indole-3-acetic acid, IBA: Indole-butyric acid; MS: Murashige and Skoog; GB5: Gamborg B5.

Introduction

Soybean (*Glycine max,* L.) is an economically important crop of tropical and suptropical regions, which is sensitive to salinity. It is an important world crop since it is a source of protein. Therefore, it has been a subject of plant tissue culture research for many years. Until recently, however, attempts to regenerate intact soybean plants from tissue culture have been unsuccessful [1-3].

During the last two decades, tissue culture has been recognized as a powerful tool for physiological research including studies on the mechanisms of stress tolerance. Cells and tissues cultured *in vitro* have been used to study salinity tolerance in halophytes such as *Salicornia herbacea* [4], *Salicornia maritima* [5] and *Atriplex undulate* [6]. Comparative studies have also been carried out on salt tolerance in plants and callus of *Hordeum vulgare* and *Hordeum jubatum* [7; 8].

The objective of the present work was to study the response of soybean callus fresh weight (F.W.) which was produced from adapted and unadapted cells cultures to salinity and light for future purpose developing salt tolerant strains of soybean.

Materials and Methods

Preparation of cell suspension and callus culture growth

A cell suspension of soybean (*Glycine max*, L. Maple Arrow) was established from callus culture of the stem tissues and grown on 3.78 gL-1 Gamborg B5 salt medium [9], 30 gL-1 sucrose, 0.75 mg L-1 IAA, 1 mg L-1 kinetin, (5-50 uM) 2,4-D, and different concentrations of NaCl (0, 85, 170, 340, 510 and 680 mM NaCl). Cells were left in continuous subculture in the range of salinity concentrations at 25 °C under fluorescent light with a 14 h photoperiod (3,000 lux), [10]. The cells were then inoculated on MS media [11] supplemented with 1 mg L-1 IBA, 20 gL-1 sucrose, and 10 gL-1 agar with 4.708 gL-1 MS media. The calli were grown in petri dishes on solid MS medium at 25 °C under fluorescent light with a 14 h photoperiod (3,000 lux). Soybean callus cultures in the active growth phase (15 d after subculture) were used to test salt tolerance. Portions of NaCl adapted and unadapted callus (1 g F. W.) were inoculated on the medium used for callus establishment supplemented with 0 - 160 mM NaCl. All treatments were incubated at 25 ± 2 °C for 15 days in either continuous light (daylight fluorescent lamps; 100 u Em-2 S-1) or in the dark. Each treatment had five replicates and all experiments were carried out at least twice.

Callus Growth

The callus cultures were weighed every week from the first week after inoculation (beginning of stationary phase) and the effect of salinity treatments analysed by the evaluation of growth (F.W.) and photosynthetic pigmentation. The cells were collected from control (unadapted) and 85 mM NaCl (adapted) cultures and callus initiated on MS solid medium.

Results and Discussion

Calli of soybean from the cell suspension cultivated on MS media containing different concentrations of NaCl showed different responses. Adapted calli were green (chlorophyllaceous) while the callus tissues of unadapted pale-yellow in color (non-chlorophyllaceous). At 0 - 20 mM NaCl adapted soybean calli grown in the light gave higher fresh weights than those incubated in darkness (Fig. 1A). Generally, the calli maintained in the dark acquired a green-yellowish pigmentation. Callus tissues from unadapted cells grown in light however, were not significantly different in fresh weight from those cultured in darkness (Fig. 1B). It is possible, therefore, that the increase in growth observed in adapted calli incubated in the light was due to the production of cell material via the photosynthetic processes.

Calli of adapted soybean (*Glycine max,* Maple Arrow) cultured in the light on media containing increasing NaCl concentrations, produced decreasing F.W. (Fig. 1A), but callus cultures incubated in darkness exhibited less reduction in growth (Fig. 1A). Callus tissue of unadapted soybean cultures also showed a decline in growth as the NaCl concentration was increased. In contrast to adapted soybean cultures, there were no differences between calli maintained in either light or in darkness (Fig. 1B). Acording to Gale [12, pp. 168-185] in general, photosynthetic activity declines in proportion to salt concentration. However, an exception can be seen in halophytes, where low concentrations of salt may even enhance photosynthesis [13]. In the case of callus cultures of adapted soybean incubated in the light, it is possible that the inhibition of growth by NaCl may be mediated mainly through interference with photosynthesis. In the non-green callus of unadapted soybean culture, processes other than photosynthesis could be inhibited in the presence of salt.

Ahmed, *et al.* [14], and Helal and Mengel [15] concluded that salinity appeared to affect the rate of photosynthesis and this might be associated with changes in pigment composition. The minimum NaCl concentration capable of affecting the growth of adapted and unadapted callus cultures was 80 mM. Both callus cultures from adapted and unadapted cells showed a similar degree of inhibition at 85 mM and higher concentrations. According to the growth response curves of halophytes and glycophytes described by [16] it was suggested that callus tissues of these species could be considered salt-sensitive. Furthermore, it seems that the mechanisms of salt tolerance in callus cultures of adapted soybean are not operating at the cellular lèvèl but depended on the physiological and anatomical integrity of the whole plant. A lack of correlation has also been found between the response of calli originating from the halophytic species Salicornia herbacea [4], Atriples undulata and Suaeda australis [6] and the corresponding whole plants. Positive correlations have been observed however, in *Suaeda maritima* [5] and in *beta vulgaris* [6]. It is possible that the increase in growth observed in NaCl-adapted and unadapted soybean calli incubated in the light was due to the production of cell material via the photosynthetic process.

Soybean calli produced from salinity adapted cell suspension cultures attained about 45% higher fresh weight in light than in dark, while those from unadapted cultures attained slightly higher fresh weight in dark than in the light (Figs. 1A and 1B).

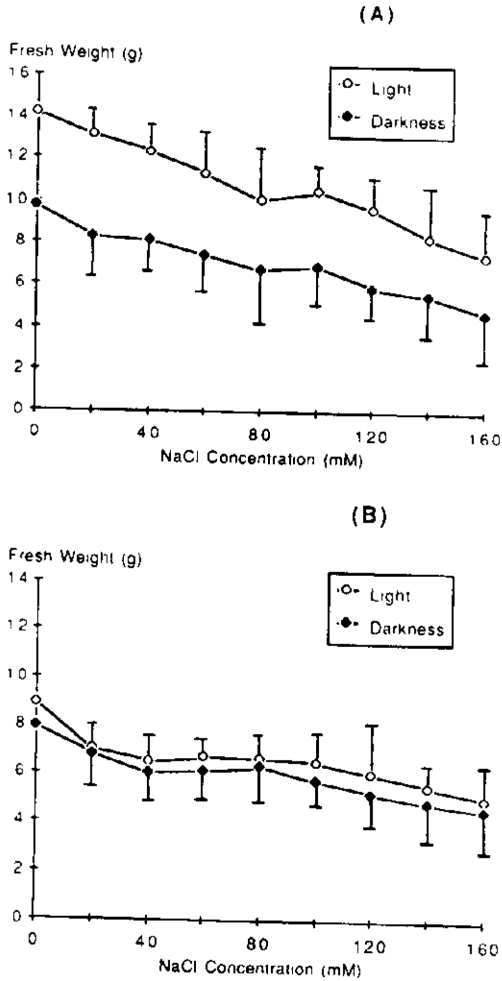

Fig.1. Influence of light and NaCl concentration on the growth in 15 d of callus cultures ofsoybean (Glycine max, L. maple arrow) from cells adapted (A) and unadapted (B) to NaCl salinity. Vertical bars, Standard deviation of fivereplicates.

Generally, adapted cell cultures produced calli which could survive and aquire higher fresh weight under the osmotic stress treatments irrespective of light or dark incubation. This may indicate higher cell division activity in calli which slightly survive osmotic stress (85 mM NaCl). There is evidence of photosynthetic activity increase by low concentration of salt in intact plants [13] and this may apply to preadapted callus. The growth rates decreased, however, with increasing NaCl concentration in adapted and unadapted calli and with more or less equal rates in light and dark incubated calli. This reduction in fresh weight may be due to expenditure of metabolites for osmoregulation in response to salt stress.

The calli from NaCl adapted cells were green in comparison with those from unadapted cells which were yellow. The incubation of chlorophyllous calli in light and dark did not affect the rate of decrease in growth by salt stress which indicates that growth inhibition was not through light induced processes. On the other hand, chlorophyll in calli of adapted cells could be the reason for their greater fresh weight especially in light incubated calli which attained the greatest fresh weights under all osmotic stress treatments. Further studies at different levels of cell tisssue organization are necessary to define the mechanisms of salt tolerance in soybean and results presenting these aspects are presented elsewhere [10].

It may be concluded that the responses of soybean callus cultures adapted and unadapted to NaCl treatments are as follows: (1) At all treatment levels, growth of adapted soybean was greater than unadapted soybean particularly in light at low salinity level. The decline in growth in response to concentration was greater in adapted than unadapted soybean, especially when exposed to light. (2) Adapted callus tissues exhibited green pigmentation and grew better up to 80 mM NaCl while the unadapted callus of soybean changed to pale-yellow coloration and grow less well. (3) The reduction in growth (F.W.) under saline stress by NaCl (adapted and unadapted cells) may be due to expenditure of energy on the synthesis of organic and inorganic solutes for osmotic adjustment rather than for growth [10].

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تأثير الضوء والملوحة على تحقيق نمو الكالس المستخلص من خلايا ساق نبات فول الصويا المتأقلمة وغير المتأقلمة للملوحة

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ملخص البحث. لقد تحقق نمو الكالس الخاص لفول الصويا من خلايا الساق لنباتات غير متأقلمة بدون ملح ومتأقلمة (٨٥ مليمولار كلوريد الصوديوم) تحت ظروف الإجهاد الملحي من صفر وحتى ١٦٠ مليمولار.

الكالس المتأقلم كان لونه أخضر، ولوحظ زيادة في الوزن الطازج، وقد وجد أن تأثير الضوء حث نحو ٤٥٪ إلى زيادة في الوزن الطازج للكالس المتأقلم ولكن العكس مع الكالس غير المتأقلم. انقص كلوريد الصوديوم النمو (الوزن الطازج) مع زيادة أو نقصان بمستوى متساوٍ من الكالس المتأقلم وغير المتأقلم والمؤثرة عليها بالضوء أو الظلام.

تحقق نسيج الكالس لنبات فول الصويا من نسيج الساق على وسط غذائي MS يحتوي على ١ مجم/لتر BAP و ٤ مجم/لتر IBA من مستخلص الخلايا المعرضة (المتأقلمة) وغير المعرضة (غير متأقلمة) للتركيز ٨٥ مليمولار كلوريد الصوديوم. أنقص زراعة الكالس لفول الصويا المتأقلمة الصبغة الخضراء وأعطي نمو أفضل بينما كان لون نسيج الكالس لفول الصويا غير المتأقلمة أصفر شاحب والنمو أقل قدرة. زراعة الكالس لفول الصويا المتأقلمة وغير المتأقلمة كان حساسًا جدًا في وجود ٨٠ مليمولار كلوريد الصوديوم في وسط الزراعة ونمو زراعة الكالس غير المتأقلم يظهر أكثر حساسية عن الكالس المتأقلم. نوقشت الاستجابة المعنوية لكالس فول الصويا وعلاقته بميكانيكيّة التحمل الملحي.