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ACCUMULATION OF PROLINE AND PROTEIN CONTENT IN RELATOIN TO ENVIRONMENTAL STRESS IN REGENERATED SUGRACANE VARIETY GT54-9

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ABSTRACT

This present investigation was carried out to monitor and follow the protein content and concentration tolerant plants against salinity and drought in comparing to explant donor (GT54-9) and its non tolerant clones. SDS-PAGE technique and proline determination were performed to study the differences among sugarcane variety GT54-9, and its somaclones which produced through tissue culture technique and were tolerant to drought and salinity. The obtained results could be summarized that differentiation in proline concentrations were observed for GT54-9, control, somaclones of GT54-9, GT54-9 salinity and drought tolerant mutants which were 17, 37, 55 and 115 mmol respect. Protein band with 67 KDa which presence in all samples was absence in salinity tolerant plant. Also, protein bands with 50 KDa which expressed in control plant and drought tolerant plant were none expressed in salinity tolerant plant and somaclones. Furthermore, protein band with 32 and 21 KDa was recorded in drought tolerant plant and somaclones plant. Then, disappeared in control and salinity tolerant plant. In final, 20 KDa protein band was only presence in control plant. Base on Total lab program, many of protein bands which have the same molecular weight were varied in protein concentration for different plants under study.

INTRODUCTION

Sugarcane (*Saccharium officinarum L*) provides 90 % of sugar supply in Egypt. Thus, production of salt and drought tolerant plants are a strategically goal. But, the classical methods of breeding are very slow so we have to use the mutations and cell selection to produce salt and drought tolerant sugarcane plant. The possible contributions to agriculture was the spontaneous or induced mutations which could be selected thought tissue culture methods reviewed by Nabors, 1976, Hanning and Narobs 1988; Handa *et al.*, 1983; Kishnamurthi, 1974; Larkin and Scowcroft, 1981; Liu, 1981 and Liu and Chen, 1982). Monitoring of protein content and concentration for salt and drought tolerant mutants plants which produced through tissue culture technique was considered as an important procedure which help to understand the process and mechanism of salinity and drought stresses tolerance of mutant somaclones.

The present work is directed to follow the proline and protein content and their concentration in salinity and drought tolerant plants with comparing to explant donor (GT54- 9) and its non tolerant clones.

MATERIALS AND METHODS

Plant materials.

Sugarcane (*Saccharum officinarum L*.) var GT54-9 and its somaclones which produced through tissue culture technique and those of drought and salinity tolerant somaclones were used as plant material in this

work and produced according to Sharaf and Ouf 1995a,b and Sharaf and Ouf 1998.

Proline determinations.

Proline content for in vivo and in vitro plants were determined spectrophotometrically via ninhydrin method according to by Bates *et al.,* (1973) as followed, approximately 300 mg of dry tissue was homogenized in 10 mL of 3% (w/v) aqueous sulphosalicylic acid and filtered. Then, 2 mL of acid ninhydrin was added followed by the addition of 2 mL of glacial acetic acid and boiling for 60 min. Mixture was extracted with toluene, and the free proline was quantified spectrophotometrically at 520 nm from the organic phase using a Shimadzu spectrophotometer (Duisburg, Germany)

Protein patterns:-

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using discontinuous buffer system described by Laemmli (1970) through SDS-PAGE unit (Pharmacia, Sweden). Also, the protein concentration was recorded through Total lab program (version 1.1, 2002). Nevertheless, based on this analysis, similarity value among the same varity which produced from different procedures was estimated using past program (2.1 Version) which could design a phylogeny tree for samples based on protein patterns results after turned to digital form (0 and 1).

RESULTS AND DISSCUSION

Proline determinations.

Different proline concentrations were recorded for sugarcane variety GT54-9 which produced through different procedure as follow: 17, 55, 115 and 37 mmol for GT54-9 control, somaclones of GT54- C9, GT54-9 salinity and drought tolerant mutants. The obtaining results seems to add more support to the results which presented by Hussain, 2003 Kingston *et al.,* 2003 and Khan *et al.,* 2004,. They observed and recorded the differences in proline accumulation for salinity and drought tolerant sugarcane mutants which produced through tissue culture technique comparing with *in vivo* plants.

Protein patterns results :-

As shown in Figure (1), differentiation in protein patterns was detected through two main procedures. The first capable us to confirm of presence and absence of protein bands for different characterized plants under study. According to this procedure, it was found that, protein band with 67 KDa which presence in all samples was absence in salinity tolerant plant (pattern number 2). Also, protein bands with 50 KDa which expressed in control plant and drought tolerant plant were none expressed in salinity tolerant plant and somaclones which produced via tissue culture technique (pattern number 2 and 3). Furthermore, protein band with 32 and 21 KDa was recorded in drought tolerant plant and somaclones plant. Then, disappeared in control

and salinity tolerant plant. Only plant which produced from tissue culture technique do not produced 24 KDa protein bands which characterized different protein patterns. In final, 20 KDa protein band was only presence in control plant. The second procedure (base on Total lab program) showed variation in protein concentration even in bands which expressed in all samples. Although, protein band with 67 KDa was presence in all samples (except in salinity tolerant plant). But, different protein concentration with 5, 17 and 9% of total protein content in these patterns were recorded for control plant and plant which produced from tissue culture technique and drought tolerant plant. Moreover, clear decrease was found in protein band with 50 KDa which presence in control plant (with 14 % of total protein content in this pattern) which turned to be only 9%. Interestingly, differentiation in protein band with 38 KDa could not detect as a result of its presence in all samples. Interestingly, differentiation could be performed based on variation in protein concentration in this band. Consequently, 27, 17, 18 and 8 % of total protein content were accumulated in this band. The presented results were in accordance with Schmidit *et al.,* (2001) who recorded three new protein bands with 88, 65 and 50 KDa which were suppressed in non treated sample of sugarcane. Then turned to be expressed after the treatment of different concentrations for NaCl. As similar, Wang *et al.,* (2002) found two protein bands with 110 and 85 KDa which only expressed after the irrigation with different concentration of saline water in Sorghum bicolor genotypes.

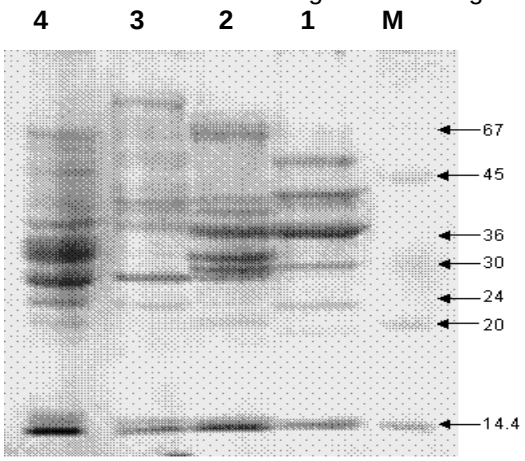

Figure (1): Shows protein patterns for sugarcane variety GT54-9 which obtained from different procedure. Where:

1- GT54- C9, control
2- Somaclones of GT54- C9
3- GT54-9 salinity tolerant
4- GT54-9 drought tolerant

The obtained results which indicated the differentiation between the same varieties as a result of applying tissue culture technique were in agreement with the morphological observation of Hanning and Narobs (1988). They observed sudden decrease in calli number after the first NaCl

concentration and Handa *et al.*, (1983) who observed 34-86 % drop in calli number depending on NaCl concentrations which could be due to osmotic readjustment. Furthermore, genetic variability occurred thought tissue culture process was exploited for plant improvement in several works (Krishnamurthi, 1974; Larkin and Scowcroft, 1981; Liu, 1981; Liu and Chen, 1982). These results indicated that the resistances may controlled by more than one gene with certain effects to tolerate high degree of salinity in the medium and calli cells must possess more than dominant gene.

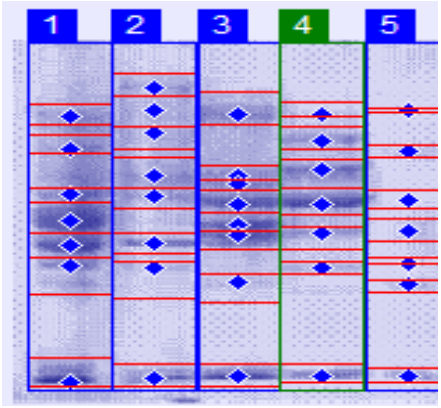

Figure (2): Zymogram of sugarcane variety GT54-9 which obtained from different procedure (identified by computer analysis program). Where,

1- GT54- C9, control
2- Somaclones of GT54- C9
3- GT54-9 salinity tolerant
4- GT54-9 drought tolerant

REFERENCES

Bates, L.S; R.P. Waldren and I.D. Tear (1973). Rapid determination of free proline for water stress studies. Plant Soil 39:205-207.

Handa, S.; R.A. Bressan; A. K. Handa and C.C. Nicolos (1983). Solutes contribution to osmotic adjustment in cultured plants cell adapted to water stresses. P. Physiol. 73: 834-843.

Hanning, G and Narobs M.W (1988). In vitro tissue culture selection for sodium chloride (NaCl) tolerance of the regeneration under saline condition. In Review of Advances in Plant Biotechnology, 1985- 1988, 2nd International Symposium on Genetic Manipulation in Crops, Tech. eds. Mujeeb A. and Sitch L.A., 239- 248.

Hussain, .A.(2003). Isolation, purification and characterization of invertases from sugarcane grow under slain condition. Director library. Higher education commission.(H-9).Islamabad.

Khan, S. J.; M. A. Khan ; H.K. Ahmed; R.D. Khan and Y. Zafar.(2004). Somaclonal variation in sugarcane through tissue culture and subsequent screening for salt tolerances . Assian fournal of plant sciences,3(3) :330-334,ISSN 1682-3974.

Kingston.G and C.M Anink.(2003),Assessing the impact of salinity on yielded and quality of sugarcane . ISSCT Agronomy workshop, Msiri, reduit, Mauritus. Agronomy & Agricultural Engineering. (21-21 Julay 2003).

Larkin, P and D.A. Scowcroft. (1981): Eyespot disease of toxin induction and its interaction with leaf cells. P. Physiol 67:408- 414.

Liu M.C and W. H Chen. (1982): Application of tissue and cell culture technique for sugarcane improvement. Annual Report Research development Council, 14-15 Taiwan sugar Crop., Taiwan (In Chinease).

Liu, M.C. (1981): *In vitro* methods applied to sugarcane improvement in plant tissue culture: methods and applications in Agriculture (T. A. Thrope, ed.) 299- 323.Academic press. USA.

Schmidit, D.S, L.E. Llewellyn; C.A. Motti and D.M. Tapiolas. (2001) Purification and biochemical characterization of two sugarcane varity. Process Biochemistry, Volume 40, Issue 5,1823-1828. Sharaf, M. A and A. A. Ouf. (1995 a): High efficient regeneration system of sugar cane (GT54-9) required for gene transfer. J. Agric. Sic. Mansoura Univ. 20(1): 421-432.

Sharaf, M. A and A. A. Ouf. (1995b): Embryogenic calli induction and their regeneration in three varieties of sugar beet. J. Agric. Sic Mansoura Univ. 20 (5): 2274-2286.

Sharaf, M. A and A. A. Ouf. (1998): Selection of salt- tolerance mutants from sugarcane calli (Var. GT 54- GT54-9). Proceedings of the 26 th Annals meeting of Genetics Alex. 29-30 Sep Vol pp 139-147.

Sharaf, M. A; A. A. Ouf and Manal. M. abdel Rahman .(1999): In vitro selection of drought tolerant sugarcane (variety GT54-9). J. Agric. Sci. Mansoura Univ. 20 (5): 2269-2277.

تراكم البرولين و المحتوى البروتينى و علاقتة بالضغوط البيئية على سلالات قصب السكر الصنف GT54-C9 الناتجة من زراعة الانسجة

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****قسم فسيولوجى النبات**

تهدف هدة الدراسة للتحقق من الاختلافات ما بين صنفين من قصب السكر و حيث تمت المقارنة ما بين الصنف الناتج حقليا و الصنف التاتج من تطبيق برنامج زراعة الانسجة و و كل من النباتات المقاومة للملوحة و الجفاف. و للمقارنة ما بين النباتات تحت الدراسة ، فقد تمت دراسة كل من البروتين الكلى و تركيز البرولين للنباتات تحت الدراسة. و بدراسة تركيز الحمض الامينى البرولين فقد تاكد وجود اختلافات واضحة فى تركيزات البرولين ما بين اوراق النباتات تحت الدراسة حيث بلغ اقصى تركيز لة فى النباتات المقاومة للملوحة(115 m mol) و هدا على العكس من النباتات المقاومة للجفاف حيث بلغ اقل قيمة لة (17 m mol). و بعمل الفرد الكهربى للبروتين الكلى لكل من النباتات تحت الدراسة تبين وجود اختلافات واضحة على مستويين. المستوى الاول و يتمثل فى وجود بعض الحزم دات الاوزان الجزيئية المحددة و التى تختفى فى بعض المعاملات بينما ظهرت فى البعض الاخر (للحزم البروتينية دات الاوزان التالية KDa 67 و 50 و 32 و 21 و20كيلو دالتون) . و يتضح المستوى الثانى من الاختلافات للمحتوى البروتينى فى التباين الواضح فى تركيز البروتين للحزم البروتينية و التى لها نفس الوزن الجزيئى. و من هدة الدراسة يتضح وجود الاختلافات على مستوى البروتين الكلى و تركيزالبرولين بين النباتات تحت الدراسة مما يمكن ارجاعة لدور زراعة الانسجة فى انتاج الاختلافات الوراثية ما بين الخلايا الجسمية.