Genetic Diversity in Sugarcane (Var Gt54- C9) During Tissue Culture Stages

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ABSTRACT

The main purpose of this investigation was to detect the variation among sequential tissue culture stages of sugarcane varity GT54- C9. In order to achieve such a purpose, peroxidase isozymes and total protein patterns were studied and used as a tool to detect these differences during the different stages of tissue culture. The obtained results indicated that, significant differences among different tissue culture stages for sugarcane varity GT54- c9 were observed and detected as a result of presence or absence of some bands among the different tissue culture stages or the presence the same bands as a homo or heterozygous band then turned to be in another form. Based on these observations, different bands were observed for peroxidase isozymes patterns among the different stages of tissue culture technique. also, different protein patterns for sequential stages of tissue culture technique which applied on sugarcane varity gt54- c9 were confirmed. 90, 73, 65, 20, 19.7, 19.5, 18.8,11 and 8 kda could be considered a differential protein bands among the different stages of tissue culture

INTRODUCTION

Somaclonal variation was reported in sugarcane. In order to produce better somaclonal via rearmament system Sharaf *et al.*, (2000). it is necessary to take advantage of exciting diversity due to mutations at the DNA levels. IT is very important to determine the degree of genetic diversity present in different stages acceding to different culture media used. These changes result from genetic differences pre-existing in somatic cells and genetic changes occurring during the tissue culture process (D, amato, 1995). These changes include numerical and structural chromosomes, point mutations, transposition of DNA sequences and modification in mitochondria and chloroplast genome (Karp, 1991). The differentiation phase (callus) from tissue culture cycle known to show variation for many species (Edallo *et al.*, 1981, Liu and Chen, 1980, Chopra et al., 1988). The high concentration of cytokinin, required for shoot regeneration, might result high degree of genetic variation (Jones, 1979, Smith and Nightingsle, 1979).

The purpose of this work is to determine the degree of genetic diversity in each stage of tissue culture cycle by using protein patterns and isozymes patterns to quality the validity used culture media to produce a wide range of variability.

MATERIALS AND METHODS

Plant material

Changes in protein content during sequenced stages of tissue culture technique, sugarcane (*Saccharum officinarum L.*) var GT54- C9 was used as explant donor to produce embryogenic calli on MS medium containing 2.4-D 3 mg/L, shoot (produced by exposure the calli on MS medium containing IAA 3 mg/L and kinitine 4 mg /L) and root (on MS medium containing 1 mg/L 2.4-D) system according to Sharf and Ouf 1995.

Protein patterns

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was employed via discontinuous buffer system described by Laemmli (1970). Also, the protein concentration was recorded through Total lab program (Version 1.11, 2000). Nevertheless, based on this analysis, similarity value among the same varity which produced from different procedures was estimated using past program (2.1 Version).

Peroxidase isozymes patterns:

To detect the differences among different tissue culture technique stages of sugarcane Varity, peroxidase isozymes patterns was performed for each control and embriogenic and nonembryogenic calli, shooted and rooted plantlets through P.V.P Agar Starch technique according to Sabrah *et al.*, 1990.

RESULTS AND DISCUSSION

Isozymes patterns

Significant differences among different tissue culture stages for sugarcane Varity GT54- C9 was observed and detected (Figure). The first cathodal band was expressed as a heterozygous band in control plants by contrary of embryo and non embryogenic calli which turned to be homozygous band. Interestingly, shooted and rooted plantlets do not express this band. Moreover, the second cathodal band was expressed succefully in all stages of tissue culture except shooted sample. The third cathodal band was shown as a heterozygous band in all stages sample except control sample which turned to be homozygous band. In final, control and embryogenic calli do not produce the fourth cathodal band by

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contrary the nonembryogenic calli, shooted and rooted samples.

The first anodal band was found as a heterozygous band in all samples except shoot and root stage which were a homozygous band. Control and calli samples showed the second anodal band. The third anodal band was only excised in shoot and root stage of tissue culture technique. Different isozymes systems were employed to follow and detect the variation during calli development process (Sanders et al., 2000). Also, variation in all of number and activity of peroxidase and infertase isozymes bands for different sugar crops was indicated (Anderson and Sherman 1988, Simamoto et al., 2002).

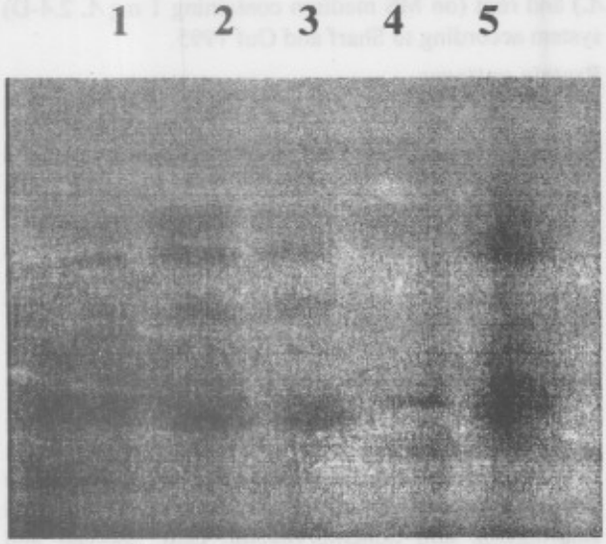

Figure 1a. Peroxidase isozymes patterns for sequential stages of tissue culture technique for sugarcane varity GT54- C9.

Where:

1. control
2. Embryogenic calli
3. Non Embryogenic calli
4. Shooted plantlets
5. Rooted plantlets

Protein patterns:

Figures (2a, b) and show different protein patterns for sequential stages of tissue culture technique which applied on sugarcane Varity GT54- C9. The obtaining result might be indicated that protein band with 90 KDa was expressed in all samples except in embryogenic calli and protein band with 73 KDa were only presence in control, embryogenic and non embryogenic calli samples. Furthermore, embryogenic calli and shooted plantlet were suppressed bans with 65 KDa. Unexpectedly, protein band with 36 KDa was presence in all tissue culture stages except shooted plantlets. By contrary, all stages expressed 20 KDa protein band

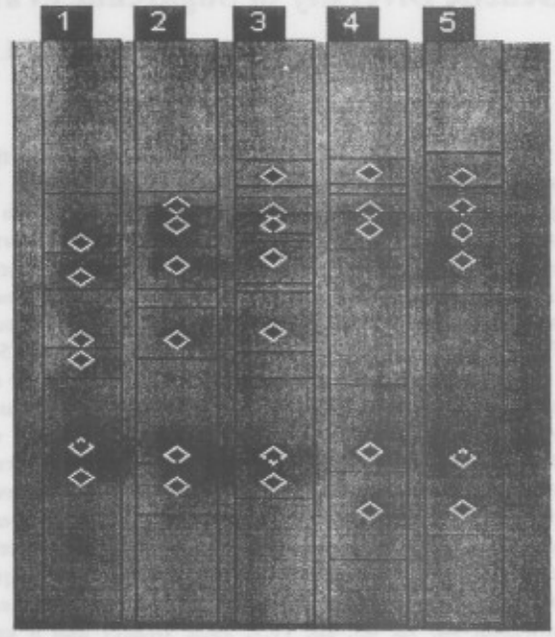

Figure 1b. Computerized shape of peroxidase isozymes patterns for sequential stages of tissue culture technique for sugarcane varity GT54- C9.

Where:

1. control
2. Embryogenic calli
3. Non Embryogenic calli
4. Shooted plantlets
5. Rooted plantlets

with 36 KDa was presence in all tissue culture stages except shooted plantlets. By contrary, all stages expressed 20 KDa protein band except control sample. Also, protein band with 19.7 KDa was suppressed in nonembryogenic calli by contrary the other stages samples. Nevertheless, embryogenic calli was the only tissue culture stage which expressed protein band of molecular weight 19.5 KDa. Control and rooted plantlets were the only samples which shown protein band with 18.8 KDa. Although, shoot stage was the only sample which showed protein band with 11KDa. But, it don not contained protein band with 8 KDa. The obtaining results were in agreement with the result mentioned by Mo *et al.*, 1993. They follow the protein content of different stage of tissue culture technique which applied on sugarcane. Significant variation in protein bands was recorded and mentioned which could characterize every stage. By the way of conclusion, used the culture media in this tissue culture technique are capable to induce a wide range of variability so called somaclonal variation.

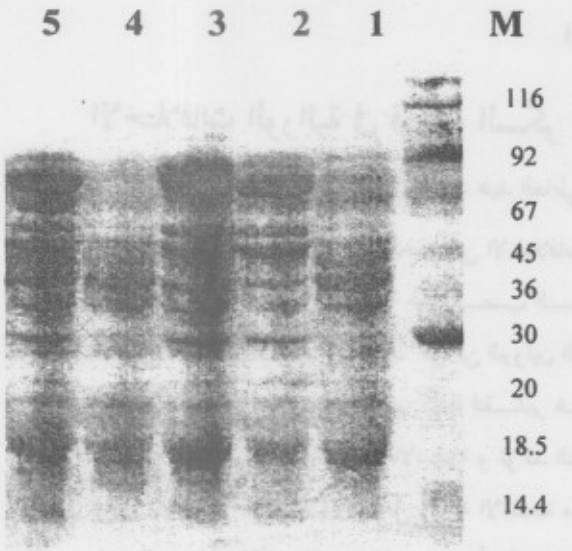

Figure 2-a. Protein patterns of sequential stages of tissue culture technique for sugarcane varity GT54- C9.

Where:

1. control
2. Embryogenic calli
3. Non Embryogenic calli
4. Shooted plantlets
5. Rooted plantlets

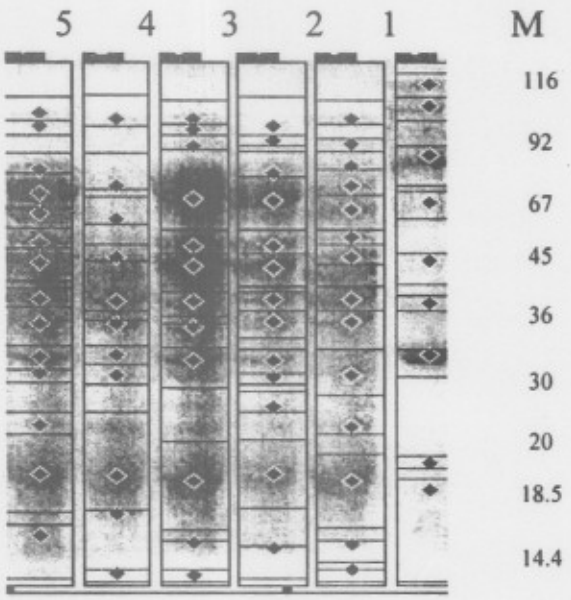

Figure 2-b. Computerized shape of protein patterns of sequential stages of tissue culture technique for sugarcane varity GT54- C9.

Where:

1- control
2- Embryogenic calli
3- Non Embryogenic calli
4- Shooted plantlets
5- Rooted plantlets

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الملخص العربي

الاختلافات الوراثية فى قصب السكر الصنف GT54- C9 خلال مراحل زراعة الانسجة

محمد عبد العاطى شرف و عاطف احمد عوف

ان الهدف الأساسي لهذا البحث هو التحقق من الاختلافات ما بين المراحل المختلفة لزراعة الانسجة المطبقة على قصب السكر الصنف GT54- C9 لتحقيق هذا الهدف فان كل من البروتين الكلى ونشاط انزيم البيروكسيديز قد تم استخدامهم كاداة لتقدير هذة الاختلافات ما بين المراحل المختلفة لزراعة الانسجة و تؤكد النتائج المتحصل عليها وجود الاختلافات فى مراحل زراعة الانسجة من خلال رصد و تقدير الاختلافات المعنوية فةى وجود أو غياب بعض حزم مشابهات الأنزيمات فى احد المراحل غيابها فة المراحل الاخرى او وجودها فى حالة خليطة أو متجانسة فى اليلاتها و تحولها للحالة الأخرى فى المراحل المختلفة. و ايضا فان الاختلافات الواضحة فى المحتوى البروتينى للمراحل المختلفة تم التاكد منها و على هذا فان يمكن اعتبار كل من الحزم البروتينية ذات الأوزان 90, 73, 65, 20, 19.7, 19.5, 18.8,11 و١٨,٨ و١١ كيلو دالتون من الحزم التفريقية التى يمكن استخدامها للتفرقة بين المراحل المختلفة لزراعة الانسجة.