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GENETIC STUDIES ON RICE (*Oryza sativa* L.)

b. Isolation and biochemical characterization of albino somaclonal variants

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ABSTRACT

Egyptian cultivated rice varieties Giza 172, 175 and 181 were manipulated for tissue culturing. During the course of regeneration induction, we selected albino rice plantlets as somaclonal variants. The variants were stable and hold promise for rice improvement by protoplast fusion. As albino plantlets offered the best abnormal plant phenotypes, that did not severely affect plant maturation or viability, to be able to recover hybrids from the fusion of two complementing albino mutants. Variants characterization were done on the molecular bases by testing peroxidase, esterase, and catalase isozymes comparing to the orginated parental plantlets. It appeared that, the albino morphology exhibited by the variants were reflected on the biochemical expression concerning peroxidase, catalase and esterase isozymes. In general the three tested rice varieties variants showed absence of isozyme bands and reduced quantity (activity).

Key words *: Somaclonal variation, Isozymes, Albinism rice.*

INTRODUCTION

It is well established fact that plant cells in culture exhibit abnormalities and modified karyotype (Jordan and larter 1984).

A number of factors can be identified that could affect the origin and causes of these variations. These include genotype and source of explant, and aspects of the procedure (Bebeli *et al.*, 1988). Larkin and Scrowcroft (1981) proposed the occurance of culture

variation to drastic genetic rearrangments rather than simple base changes or deletions caused by some chemical mutagen components of the medium. Later on Landsman and Uhrig (1985) have been domonstrated the cause of somaclonal variations to alterations at the DNA level. This genetic instability in culture, giving rise to somaclonal variation, would be possible use for plant improvement by generation of mutant plant (Carlson and Polacco, 1975 and Green, 1977). The significance of somaclonal variation for realizing this goal have increased dramatically to include selection for many desirable traits, herbicide resistance, stress tolerance and disease resistance (Heath - Pagliuso *et al.* (1987). Concerning rice, Somaclonal variation have been detected by Nishi *et al.* (1968). and Henke *et al.* (1978). As variation hold promise as an adjacent to protoplast fusion. It appeared that plants obtained by protoplast fusion was much more variable than that by sexual hybridization for Nicotiana interspecific hybrids (Evans *et al.* (1984).

The efficiency of crop improvement programs might be enhanced if molecular markers such as allozymes, could be used to manipulate quantitative trait loci (Guse *et al.*, 1988). Tanksley *et al.* (1982). suggested that significant associations between isozyme genotype and quantitative characters in interspecific crosses could be advantgeous in an introgression programme.

Moreover, electrophoretic zymograms are stable varietal characteristics and could be used in varietal identification (Gorman and Kiang 1977). In our research we isolated number of albino rice plantlets as somaclonal variants from three Egyptian cultivated rice varieties, Giza 171, 175 and Giza 181. we intended to characterize

them biochemically to use them as an applicable tool for rice improvement by protoplast fusion.

MATERIALS AND METHODS

1. Material preparation :

Fresh albino plantlets (0.200 gm) were homogenated in cool mortar and diluted with 0.2 ml d.H_2O. The homogenates were centrifuged for 10 min at 12000 rpm and 4°C. 10 µl of supernatent pipetted onto gel (27 x 20 x 0.3 cm) for electrophoresis.

2. Gel and Buffer preparation :

i. Peroxidase :

1.5% bacteriological agar gel was used for peroxidase isozyome. 0.03 M. Borate buffer pH 7.9 was used as gel buffer, while 0.3 M borate buffer pH 7.9 was used as electrode buffer.

ii. Catalase:

1.0% bacteriological agar with 0.5% commercial maize starch were used together as gel for catalase isozymes. Gel and electrode buffers used for catalase were the same bufferes used for peroxidase.

iii. Esterase:

1.2% bactoriological agar in 0.03 M borate buffer pH 7,9 used as gel buffer for esterase. 0.3 M Borate buffer pH 7.9 used as electrode buffer.

3. Electrophoresis conditions :

peroxidase, catalase and Esterase isozymes were separated at 5°C and 16 m A/tray for 3 hrs.

4. staining Solutions and procedures:

i. peroxidase:

50 mg benzidine disolved in 1 ml acetone, up to 98 ml with d.H_2O then, 2 ml of H_2O_2(20 vol.) were added immediatly. The gel flooded with staining solution for 10 min at 30°C, wash gel with d.H_2O after developing bands colour (Tanksley and Rick , 1980).

ii. Catalase:

Flood the gel with 0.1 M potassuim iodide (KI) for 1 min. at room temprature. Wash gel with tap water, then add 100 ml of sodium thiosulfate (0.1 M) with 1% H_2O_2 (20 vol.) to the gel for 5 min at 30°C. Wash gel tray again with d. H_2O and flood the gel with 0.1 M KI until gel became blue and catalase bands remain colourless. (Hoelzel, A.R. ed. 1992) with modification in staining steps and gel).

iii. 100 mg of α - Naphthyleactate and 100 mg of β-Naphthyleacetate were disolved in 20 ml acetone (sol. a), 100 mg of fast Blue RR salt and 100 mg of fast Blue BB salt were disolved in 80 ml d. H_2O (sol. b), then mix sol a and b and filtrate. Filtrated solution used for esterase staining for 2 hrs at least. (Hoelzel, A.R. (ed) (1992) with modifications).

RESULTS AND DISSCUSION

Successful regeneration of rice plantlets from tissue culture were achieved from three Egyptian rice varieties, Giza 172, 175, and Giza 181, thereby confirming the possible applicability of tissue culturing system in Egyptian varieties (Abou-Ghalia, *et al.*, 1995).

We were able to isolate an albino rice variant out of the three used varieties. Fig. (1 a) illustrate the regeneration steps albino somaclonal variant of Giza 172. A relatively high percentage of calluses produced albino shoots (15%) (data not shown) sometimes all the shoots arising from the same callus were albino. White leaves with areas of brown pigmentation were also seen (Fig. 1 b). This occurance of albino plants supported what Fukui (1983) suggested that albino rice plants is controlled by a single recessive gene and the callus itself had a heterozygous constitution of this gene.

In fact somaclonal variations in rice is not new, it has been described in both quantitative and qualitative genetic traits by Sun *et al.* (1983). Meanwhile, Sun *et al.* (1977). considered that the albinism was due to lack of ribosomes. Later they reported that the albino plants lacked one of the major soluble protein bands and also 23 S and 16 S. r.RNA (Sun *et al.*, 1977).

In an attempt to characterize and identify the isolated albino variants on the molecular level, we detected three isozymes peroxidase, esterase and catalase in comparison with green plantlets of the parental varieties. This was conducted due to the fact that proteins comprise the majority of the stable functional genetic products, each variety with a unique genotype should differ from

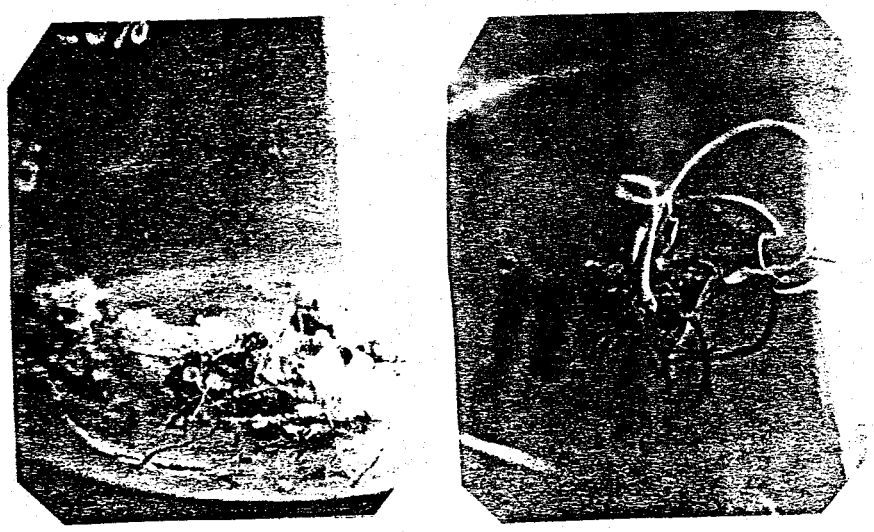


Fig. (1. a) : Regeneration steps of albino plantlets in Rice var. Giza 172

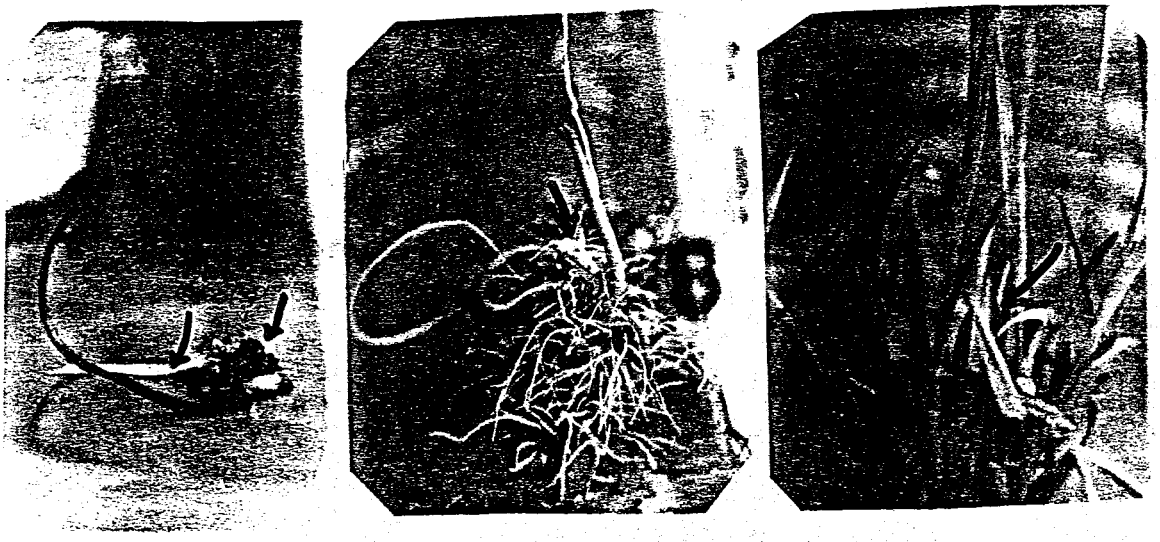


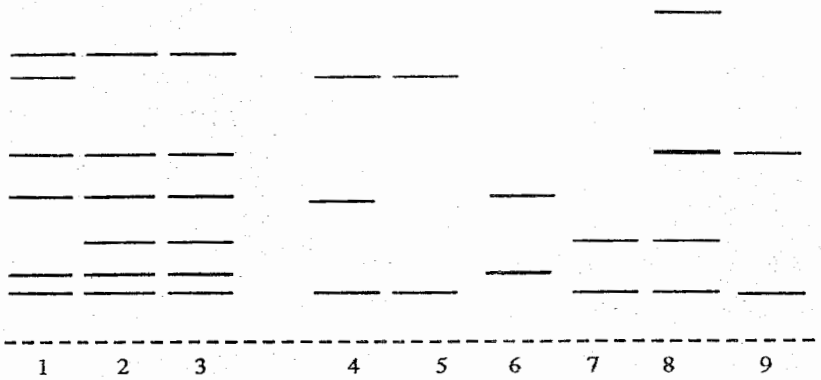
Fig. (1. b) : Albino plantlets in Rice var. Giza 175 and Giza 181

other varieties by one or more proteins (Gorman and Kiang 1977).

Preparation of peroxidase isozymes out of the three tested varieties showed a wide varietal differences especially between Giza 172 in one side and Giza 175 and Giza 181 in the other side. Rice variety Giza 172 showed six bands, two of them are small, whereas Giza 175 and Giza 181 have a higher molecular weight band (Fig. 2). Two isolates of Giza 172 albino variant plantlets were used to prepare peroxidase isozymes and presented in lanes (4 and 5) Inspite of both of them appeared as albino, they showed different peroxidase pattern. Both of them differed greatly from the parental peroxidase pattern lane (1). Some results have been obtained in albino isolates of variety Giza 175 lanes (6 and 7), both having two peroxidase bands but are different in migration level on gel. Albino variants of variety Giza 181 exhibited less bands 4 and 3 in lanes 8 and 9 respectively. Albino variants originated from var. Giza 175 (lane. 6) and variant originated from var. Giza 181 (lane. 9) both of them showed two peroxidase bands, though they are different in size. As extra band in isozymes could be due to increased ploidy levels, which possibly occured during cell culture at very high rates (Evans and Sharp, 1983), missing or loss of band (s) could be due to loss of chromosomes or segments and / or loss of activity giving rise to phenotypic alterations (albino plantlets).

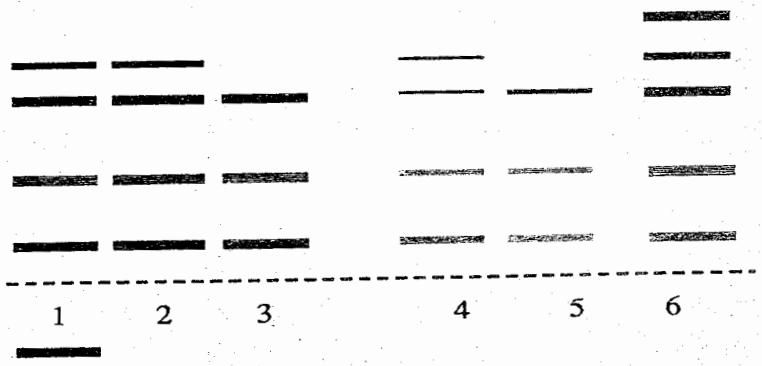
Concerning catalase isozymes in rice, we tested its activity in albino variant plantlets compared with normal green plantlets and data were presented in (Fig. 3). Variety Giza 172 variant showed loss of an anodic band and less enzyme activity pronounced in thin bands (lane 4). Whereas, Giza 175 showed loss of one cathodic band.

Fig. 2. Peroxidase isozymes in cultivated Egyptian Rice varieties, Giza 172, 175, 181 and albino plantlets variants.



Lane :
1. Green plantlet of Giza 172.
2. Green plantlet of Giza 175.
3. Green plantlet of Giza 181.
4. and 5 Albino variants of Giza 172.
6. and 7 Albino variants of Giza 175.
8. and 9 Albino variants of Giza 181.

Fig. 3 : Catalase isozymes variation in cultivated Egyptian Rice varieties, Giza 172, 175 and 181 and albino variants.

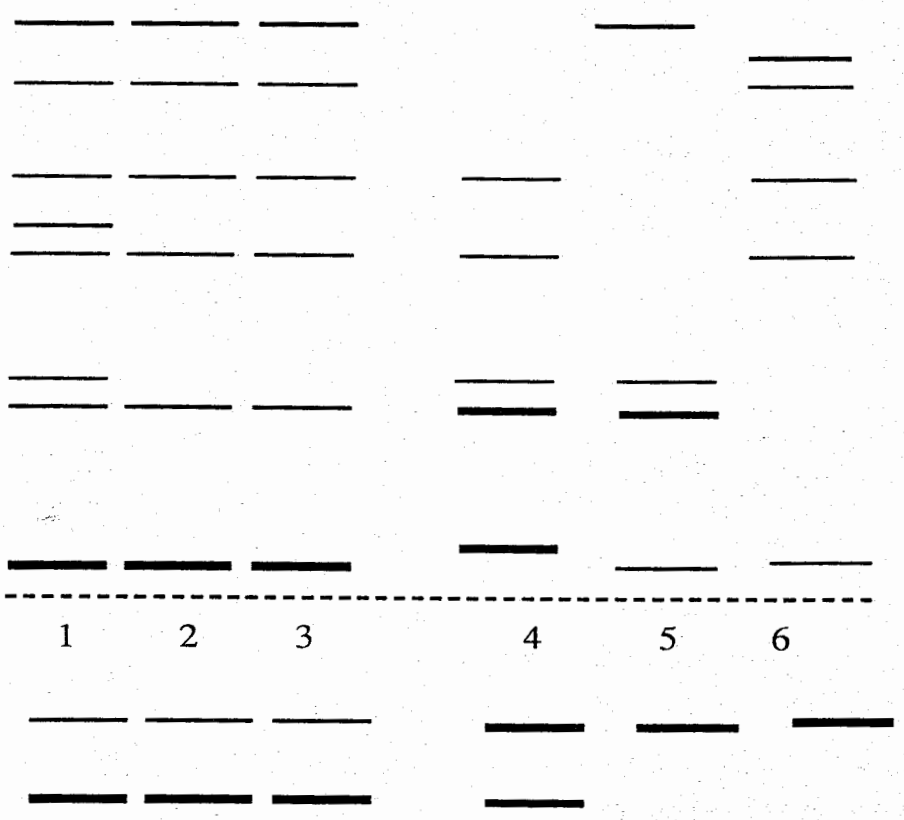


Lane :
1. Green plantlet of Giza 172.
2. Green plantlet of Giza 175.
3. Green plantlet of Giza 181.
4. Albino variant of Giza 172.
5. Albino variant of Giza 175.
6. Albino variant of Giza 181.

Albino variant of rice variety Giza 181 showed two extra bands (lane 6) accompanied with high activity (thick bands) This could be explained by occurance of ploidy which apparently happen at high rates in tissue cultures. While the exact cause of this high catalase yield remains to be fully characterized, it neverstheless appears that we were able to isolate a useful rice genotype with catalase marker. Varietal differences are pronounced among the three tested varieties regarding catalase isozymes (Fig. 3 lanes 1, 2 and 3). Same figure displayed that, rice variety Giza 172 have only one anodic catalase band, wherease Giza 175 and 181 did not have it. This anodal band disappeared in albino variant (Fig. 3 lane 4). This observation could indicate that this band did not involve dirctly in albino phenotye production as it is missing in normal green plantlets of the other tested varieties.

Detection of esterase isozymes in rice varieties and their somaclonal variants showed vareital differenes and supported what published before that esterase isozymes in rice are polymorphic (Romero *et al.*, 1993). Rice variety Giza 172 showed heterozygousity in two loci (Fig. 4 - lane 1). Meanwhile varieties Giza 175 and 181 showed homozygousity in all loci (lanes 2 and 3). In fact, rice var, Giza 172 possessed two co-dominant esterase alleles (5A and 7A in lane 1) and this is supported what Nakagahara (1986) cited. Spontonus mutations that cause albinism in rice was reflected in esterase zymograms (Fig. 4 lenes 4, 5 and 6). Rice var. 172 variants suffered an absence of three esterase isozymes. Meanwhile Giza 175 showed loss of four isozymes, one anodic and three cathodic bands (Fig. 4 lane 4). Somalclonal variant of Giza 181 displayed an alteration of

Fig. 4 : Esterase isozymes in Cultivated Rice varieties, Giza 172, 175, 181 and albino variants.



Lane :
1. Green plantlet of Giza 172.
2. Green plantlet of Giza 175.
3. Green plantlet of Giza 181.
4. Albino variant of Giza 172.
5. Albino variant of Giza 175.
6. Albino variant of Giza 181.

cathodic band and loss of two bands, one cathodic and one anodic compared to the parental zymogrames (Fig. 4) lanes 6 and 3 respectively.

It might be helpful to mention that, resemblance of Giza 175 and 181 esterase zymogromes(Fig. 4 lanes 2 and 3) was not maintained in their albino somaclonal variants (Fig. 4 lanes 5 and 6), which indicate difference (s) in the site of alteration. This might be due to genotype response toward surrounding environmental conditions (Bebeli *et al.*, 1988).

In fact, with this obtained observations in tested Egyptian rice varieties, we could possibly use those albino variants in protoplast fusion to construct desired rice variety with wanted characters adjacent with biochemical identification as an early test to ensure the presence and occurance of the fusion. Then, we would fulfill our aim of this prelimenary study. We hope to be able to perform this study and represent it later.

Biochemical variations appeared in all tested varieties indicated that more than one mutational event could have occured in those variants and might have some form of gene amplifications.

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الملخص العربى

دراسات وراثية على الأرز

ب – عزل وتحديد الخصائص الكيموحيويه لطفره الألبينو فى الأرز

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استخدمت أصناف الارز المصرية جيزه ١٧٢ ، ١٧٥ ، ١٨١ للزراعه النسيجيه ، وأثناء عمليه التكشف تم أنتخاب طفرات الألبينو المكتشفه كتباينات جسميه؛ وهذه التباينات كانت ثابته ويمكن استخدامها فى تحسين محصول الازر بإستخدام الدمج الخلوى وتعتبر كأفضل التباينات المظهريه التى لاتؤثر بشده على نضج أو حيويه هذه النبايات والتى يلغى تأثيرها الضار بالدمج الخلوى .

وقد تم تحديد الاختلافات على المستوى الجزئيى بأختبار مشابهات انزيمات البيروكسيديز والكاتاليز والإستيريز ، وقد أمكن ربط هذه الاختلافات المظهريه للطفرات الألبينو بالإ ختلافات فى التعبير الجينى للجينات المسئوله عن إنزيمات البيروكسيديز والإستيريز والكاتاليز ؛ وبصفه عامه وجد أن الأختلافات بين أصناف الازر الثلاثه المختبره غير موجودهسواء فى المشابهات الأنزيميه أو فى نشاطها.