# Selection of High Productive Cell Lines through Someclonal Variation of *Sorghum bicolor* L. Monch. (Var. Tracy).

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## ABSTRACT

Seventy seven plants regenerated from embryogenic calli of leaf culture of *Sorghum bicolor* L.Mnch (var. Tracy) were morphologically evaluated at dough ripening stage. The first generation selfed lines drived from 37 selected plants were compared with the original genotype to select superior plants to control for performing 2 nd generation which their progenies (nine clones) were grown in two different locations (Sabhia and Nubaria) for two different successive seasons. Morphological and technological characters were measured in comparison with their control (donor explant). Peroxidase zymorgram of selected superior clones with their control, cytological examination for them and statistical analysis were performed. The results have shown observable genetic variants due to a wide range of recorded values for each measured character through the two cycles of selection in two seasons. The final selected clones (nine clones and their control) revealed that some studied characters have no significant differences in both locations (sucrose % and stem diameter), while the following characters: plant height, juice weight, number of nods , spikes length , fresh and dry weights of leave manifested superiority in the field of Nubaria. On the contrary, the following characters showed significant superiority in the locations of Sabhia; T.S.S %, leaf area, chlorophyll, total weight and stalk weight. Due to improved yield and genetic changes obtained by zymogram patterns (number of bands, their distribution, and light density) in clones and their control, hence, tissue culture technique can be used to creat superior varieties for agriculture use. However, the effect of culture media on chromosome number was not observable.

## INTRODUCTION

Genetic variability due to the tissue culture process are greater than that of created by convential mutagensis (Edallo *et al;* 1981). This variability has been shown to be genetically distinct from the original source of the plant. In some cases, this variability has been heritable over one or more generations of sexual reproduction (Larkin, 1987).

The production of a genetically improved variety of a plant species with superior disease resistance and / or agronomic yield is a difficult and need long time consuming process. However, tissue culture technique has provided a new tool for plant breading programs. (Linacero and Vazquez, 1993; Seliskar and Gallagher, 2000 and Tregear *et al.*, 2002). Ouf *et al.* (1996) performed an efficient regeneration system for *Sorghum bicolor* L.Monch. (Var *Tracy)* in order to produce a somaclonal variation required for selection. The present work sought to assess the highest productive cell lines among regenerated populations of *Sorghum bicolor* L. Manch for several generations required for such breeding programs at two different locations (Sabahia and Nubaria) in two seasons 2001 and 2002.

## MATERIALS AND METHODS

### 1. Establishment of somaclones:

*Sorghum bicolor* L., Monch (Var. *Tracy)* was used as an explant donnor for the *in vitro* culture. Production of embryogenic calli, plant regeneration and transplanting into soil were designed as early described by Ouf *et al.* (1996). These clones were grown in the field of sabaheia research station.

### 2. Measurement of morphological characters on somaclones (R0):

The selection was based on the high productivity of the given clones, which is affected the following characters: plant height, (m), number of nods, stem diameter (cm) and leaf area ($m^2$) per plant.

### 3. Progenies of selected clones:

Each selected clones (R1) were sown in determined lines in the same filed as mentioned above to be evaluated at the dough ripening stage. Seeds of selected R1 were finally considered as a new population (R2) which were morphologically and technologically assessed at two locetions in different areas (the first at Sabahia station and the other one at Nubaria station) for two successive generations where their physical and chemical proprties at 2001 and 2002 seasons were analyzed according to Piper, 1950 (Table 1). The following characters were measured on such a new population ($R_2$) for two successive seasons (2001 and 2002) at Sabahia and Nubaria areas. a.) Morphlogical caharaters: plant weight (kg); number of nods; plant height (m) with and without spike; stalk weight (kg); stem diameter (cm), Leaf area ($m^2$) was calculated by Montogomery, 1911, spike hight (m) and chlorophyll (mg/gm dry weight), which was directly measured by using a hand chlorophyll meter model Spad- 502. b.) Technological characters: Juice yield (Kg), Total soluble solids (T.S.S%) and sucrose percentge.

### 4. Acrylamid gel electrophoresis for peroxidase:

In order to detect genetic variation in some clones, the progeny of selected clones were examined in comparison with their mother explant donor (control) according to the method of Laemmli (1970) as modified by Studier (1973).

A gram of new initiated leaves was homogenized in peroxidase extraction buffer (tris 1.21 g, boric acid 0.62 g, Triton x 100 2 ml, mercaptoethanol 1 ml at PH 7). Samples were diluted in tris- HCl buffer 6.8,15%sucrose mercaptoethanol and bromophenol. Samples were placed on the vertical electrophoresis wells in 12 % acrylamid separating gel. In order to determine enzymatic activity, the approeriate-stain mixture of $H_2O_2$ and benzidine 0.1 % solution were used.

Table (1a and b). Physical and chemical properties of the soil surface layer (0-30 cm) for Sabahia and Nubaria areas during 2001 and 2002 seasons.

Table 1 a. At Sabahia station.

| Characters | Field 1: Sabahia station | |
|---|---|---|
| | 2001 | 2002 |
| $Ca^{2+}$ meq/l | 17.25 | 16.31 |
| $Mg^{2+}$ meq/l | 4.3 | 10.2 |
| $Na^{+}$ meq/l | 14.6 | 13.9 |
| $K^{+}$ meq/l | 1.25 | 0.96 |
| $CO_3^{-2}$ meq/l | 0.00 | .01 |
| $Hco^{-3}$ meq/l | 2.5 | 2.62 |
| $Cl^{-1}$ meq/l | 27.12 | 25.0 |
| $SO_4^{-2}$ meq/l | 13.5 | 14.5 |
| EC $dSm^{-1}$ | 4.75 | 4.62 |
| pH: soil:water (1:2.5) | 7.78 | 8.02 |
| Total N % | .10 | .13 |
| Organic matter | 1.38 | 1.25 |
| Clay % | 42.3 | 43.1 |
| Silt % | 43.1 | 42.8 |
| Sand % | 14.6 | 14.1 |
| Text .class | Clay loam | Clay loam |
| $CaCO_3$ % | 5.8 | 6.9 |

Table 1 b. At Nubaria station.

| Characters | Field 2: Nubaria station | |
|---|---|---|
| | 2001 | 2002 |
| Soil PH (1:2,soil:water) | 7.8 | 7.9 |
| E.C.,ds/m(soil paste extract) | 2.00 | 1.68 |
| CaCo3 % | 33.00 | 31.0 |
| Organic matter % | 0.52 | 0.56 |
| DEC,mg/100g soil | 14.18 | 13.57 |
| Na Hco3 - ext. µg/g | 2.4 | 2.5 |
| DTPA-ext., µ/g soil | 2.6 | 2.6 |
| DTPA-ext. Mn, µ/g soil | 2.2 | 2.4 |
| DTPA-ext. Zn, µ/g soil | 1.00 | 1.3 |
| DTPA –ext. Cu, µ/g soil | 0.3 | 0.3 |
| Sand % | 52.00 | 49.00 |
| Clay % | 10.00 | 12.00 |
| Silt % | 38.00 | 39.00 |
| Texture | Sandy clay loam | |

### 5.Cytological examination:

Seeds were thoroughly washed with running tap water for 3 hours and soaked in water for 24 hours. Germenating seeds were kept in petri dishes until roots had reached 1cm length. Root-tips were removed and fixed in 3.1 (ethanal: glacial acetic acid) solution for 3 hours and stored in 70 % thanal.

**Staining:** Root-tips were stained in vial samples containing aceto-orcein for 15 min., washed, transferred to 45% acetic acid and 1000 cells at both prophase and metaphase stages were examined.

### 6. Statistical estimations of somaclonal variation:

The mean, range and number of clones superior to control for each characters were analyzed by using least significant differences (L.S.D.) according to Steel and Torrie (1981).

## RESULTS AND DISCUSSION

### 1-Morphological variability induced by tissue culture:

The growth of plantlets transferred into soil (into clay pots) have shown a varied variability before transferring them into the field (Fig. 1). The recorded values estimated on somaclones at the dough ripening stage in the field indicated that the *Sorghum bicolor* L., Monch can reported to be excellent material for induction of somaclones (Table, 2 and Figs. 2 a & b and 3 a & b). The results obtained from this part of investigation indictated that there was a wide range of variations in all characters under study. These results are in accordance with those obtained in maize (Edallo *et al.*,1981) and in sugar cane ( Liu & Chen; 1978, 1980 and 1982; Ramos leal *et al.*, 1996 and Sharaf *et al.*, 2000). The variability could be explained as a result of cell exposuriall to cuture media containing phytohormones, especially cytokinins (Jones, 1979, Smith and Nightingsle, 1979).

In adduition, the influence of culture age on variation has been recognized (Karp, 1989).

Lee and Phillips (1987) demonstrated that the inherited variation tend to the increment with culture age.

Table 2. Range of variability in $R_0$ and number of clones superior to control mean for their important characters of 77 regenerants ($R_0$).

| Character | Range | The mean of control | No of clones superior to control | % Lines sup. To cont. |
|---|---|---|---|---|
| Plant hight (cm) | 1.40-4.33 | 1.9 | 20 | 26 |
| Stem diameter (cm) | 1.0-3.7 | 2.25 | 24 | 31 |
| Leaf area ($cm^2$) | 0.28-0.33 | 0.37 | 22 | 28.5 |

**2-Selection in $R_1$ progenies:**

Each clone in R0 manifested superiority to control in one or more characters were involves in the next selection cycle ($R_1$) and the others were eleminated seeds of 2625 resulting from 37 selected R0 were sown in lines (there lines for progeny of each somaclone). The evaluations of these plants are reported in (Table 3).

The wide range of variability observed in Table (3) is due to the segregation occurred during meiotic cycle (Sharaf *et al.* 2000).

Table 3. Evaluation of somaclonal progenies (R1).

| Characters | Range | Mean of control | N° of cell lines superior to cont. | % of cell lines sup.to cont. |
|---|---|---|---|---|
| Plant height(cm) | 1.05-4.33 | 2.30 | 29 | 78 |
| Stem diameter(cm) | 1.3-3.0 | 2.2 | 15 | 40.5 |
| Leaf area ($cm^2$) | 0.27-0.25 | 0.17 | 9 | 24.3 |

**3-Comparative assessment of selected clones in two different areas:**

The nine clones were selected for their superiority in R2 (Table, 3), their progenies were sown in two different areas (Sabahia and Nubaria Res. Station) for two successive seasons. Finally, the analysis of morphological and technological characters were measured as reported in (Table, 4).

Table 4. Evaluation of selection R2.

| Characters | Range | Control | No superior clones to control | % superior clones |
|---|---|---|---|---|
| Plant height (m) | 1.36-2.80 | 2.40 | 5 | 55 |
| Stem diameter (cm) | 1.3-3.1 | 2.29 | 6 | 66 |
| Leaf area ($m^2$) | 0.45-0.70 | 0.23 | 1 | 11 |

These results could explain the stability of new genetic mutations through several generations and the effect of location could be reacted with genes of some characters as shown in (Table, 4). Also, differences between progeny of each clone may due to segregation and new recombinants occurred during meiotic division (Ramos leal *et al.*, 1996 and Sharaf *et al.*, 2000).

Some clones possessed one or more superior characters to control the evaluation of selected clons at the two different areas in two successive seasons were reported in Tables (5 to 9).

Table 5. Evaluation of selected clones for the characters of chlorophyll , plant weight and stalk weight.

| Clones No. | Chlorophyll (mg/gm dry wt.) | | | | Plant weight ( Kg) | | | | Stalk weight (Kg) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sabahia | | Nubaria | | Sabahia | | Nubaria | | Sabahia | | Nubaria | |
| | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year |
| 1 | 37.2 | 42.6 | 33.5 | 29.9 | 4.4 | 5.3 | 3.6 | 3.4 | 2.6 | 3.9 | 2.6 | 2.1 |
| 2 | 31.2 | 56.3 | 30.3 | 35.9 | 3.2 | 3.6 | 2.9 | 3.1 | 2.2 | 2.7 | 1.8 | 1.5 |
| 3 | 40.1 | 42.3 | 40.3 | 33 | 5.1 | 4.9 | 3.2 | 3.5 | 2.2 | 3.7 | 2.6 | 2 |
| 4 | 31.7 | 48.6 | 29.2 | 36.4 | 3.2 | 4.9 | 3.6 | 3.5 | 2.3 | 3 | 2.3 | 2.4 |
| 5 | 53.2 | 41.5 | 37.2 | 38.4 | 3.7 | 4.9 | 3.6 | 3.4 | 2.2 | 2.3 | 2.6 | 2.4 |
| 6 | 39.4 | 51.1 | 37 | 36 | 2.6 | 4.6 | 4.2 | 4.6 | 1.2 | 2.5 | 2.9 | 3 |
| 7 | 46.5 | 50.3 | 38.1 | 31 | 3.3 | 5.6 | 3.5 | 4 | 2.6 | 3.7 | 2.2 | 2.6 |
| 8 | 47.5 | 44.2 | 34.2 | 36 | 5.4 | 5.6 | 3.6 | 4.1 | 3.5 | 2.9 | 2.4 | 2.9 |
| 9 | 45.1 | 53.9 | 37.5 | 37 | 4.03 | 4.03 | 3.5 | 3.4 | 3.8 | 3.6 | 2.5 | 2.6 |
| 10 | 47.8 | 44.2 | 31.7 | 35.3 | 3.1 | 4.6 | 3.5 | 4 | 2.2 | 2.1 | 2.4 | 2.7 |
| P $LSD_{0.05}$ | | | 0.69 | | | 0.06 | | | | 0.15 | | |
| Y $LSD_{0.05}$ | | | 0.90 | | | 0.07 | | | | 0.17 | | |

P = place Y = year

Table 6. Evaluation of some selected clones for the characters of total plant height , plant height without spike and spike length.

| Clones No. | Total plant height (m) | | | | Plant height with out spike (m) | | | | Spike length (Cm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sabahia | | Nubaria | | Sabahia | | Nubaria | | Sabahia | | Nubaria | |
| | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year |
| 1 | 3.4 | 2.9 | 3.6 | 3.2 | 3.1 | 2.1 | 3.3 | 2.9 | 27 | 25.3 | 30 | 30 |
| 2 | 1.7 | 2.6 | 3.1 | 3.1 | 1.6 | 2.3 | 2.8 | 2.7 | 18 | 30 | 30 | 40 |
| 3 | 3.6 | 2.4 | 3.7 | 3.7 | 3.4 | 2.1 | 3.4 | 3.5 | 23 | 29.3 | 30 | 20 |
| 4 | 3.2 | 2.7 | 4.03 | 4.3 | 3 | 2.4 | 3.6 | 3.7 | 16.5 | 30 | 40 | 60 |
| 5 | 3.8 | 2.7 | 3.2 | 3.2 | 3.4 | 2.3 | 2.9 | 2.8 | 25.6 | 41.3 | 30 | 40 |
| 6 | 3.6 | 2.2 | 2.6 | 2.7 | 3.4 | 2 | 2.3 | 2.4 | 22.6 | 19.6 | 30 | 30 |
| 7 | 3.4 | 2.6 | 3.6 | 3.6 | 3.1 | 2.3 | 3.2 | 3.2 | 24.6 | 34.3 | 40 | 40 |
| 8 | 3.2 | 2.5 | 1.8 | 2.1 | 3 | 2.1 | 1.7 | 1.8 | 17 | 30 | 10 | 30 |
| 9 | 2.5 | 3.4 | 2.9 | 2.8 | 2.3 | 2.6 | 2.6 | 2.5 | 19.6 | 36.6 | 30 | 30 |
| 10 | 2.7 | 2.1 | 3.14 | 3.2 | 2.5 | 1.7 | 2.8 | 2.9 | 20.3 | 35 | 30 | 30 |
| P $LSD_{0.05}$ | | | 0.12 | | | 0.15 | | | | 0.04 | | |
| Y $LSD_{0.05}$ | | | 0.17 | | | 0.19 | | | | N.S | | |

P = place Y = year

Table (5 and 6) represent the evaluation of selected colnes for the characters of chlorophyll, plant weight, stalk weight, total plant height, plant height without spik and spik length. The obtained data revealed that, there were a manifested superiority in Nubaria location for plant height, spik length, while a significant superiority in clorophyll, total weight and stalk weight was observed at Sabahia location.

Table 7. Evaluation of some selected clones for the characters of fresh and dry weight of leaves and leaf area.

| Clones No. | Fresh weight of leaves (gm) | | | | Dry weight of leaves (gm) | | | | Leaf area ($m^2$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sabahia | | Nubaria | | Sabahia | | Nubaria | | Sabahia | | Nubaria | |
| | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year |
| 1 | 82.9 | 79.6 | 66.9 | 66.5 | 39.0 | 37.9 | 30.3 | 30.1 | 0.39 | 0.45 | 0.39 | 0.44 |
| 2 | 69.6 | 67.6 | 68.3 | 68.1 | 35.0 | 33.1 | 29.5 | 32.8 | 0.57 | 0.43 | 0.57 | 0.42 |
| 3 | 74.8 | 75.0 | 82.6 | 81.9 | 47.0 | 43.4 | 40.3 | 39.2 | 0.65 | 0.63 | 0.65 | 0.64 |
| 4 | 100.2 | 90.7 | 106.1 | 106.3 | 54.3 | 99.6 | 49.4 | 51.5 | 0.49 | 0.45 | 0.48 | 0.39 |
| 5 | 79.4 | 74.4 | 92.4 | 91.8 | 36.0 | 34.9 | 42.1 | 43.1 | 0.47 | 0.50 | 0.47 | 0.43 |
| 6 | 53.0 | 60.7 | 117.8 | 117.2 | 20.7 | 22.3 | 52.3 | 54.2 | 0.94 | 0.90 | 0.49 | 0.54 |
| 7 | 88.5 | 82.0 | 94.06 | 94.6 | 42.0 | 41.2 | 43.4 | 42.5 | 0.51 | 0.54 | 0.51 | 0.46 |
| 8 | 107.9 | 103.5 | 86.5 | 86.8 | 56.0 | 53.5 | 42.4 | 47.6 | 0.55 | 0.46 | 0.55 | 0.50 |
| 9 | 87.9 | 90.3 | 66.8 | 67.2 | 43.2 | 44.7 | 30.8 | 33.1 | 0.46 | 0.42 | 0.46 | 0.42 |
| 10 | 82.5 | 83.1 | 100.4 | 90.4 | 40.3 | 39.1 | 51.8 | 52.5 | 0.59 | 0.50 | 0.59 | 0.51 |
| P $LSD_{0.05}$ | | | 4.84 | | | 2.86 | | | | 0.40 | | |
| Y $LSD_{0.05}$ | | | 7.67 | | | 0.82 | | | | 0.19 | | |

P = place Y = year

It is well known that the leaf is the organ in which sugar processed. The breeders always select plants of families with beg blade and long petiole to get more photosynthesis. Also, large number of yields more photosynthesis (Spiller, 1980; Smith & Hadley, 1989 ;Patel *et al.* 1992 and Attalah, 1997). In the preent study Sabahia area showed significant differences in fresh and dry weight of leaves and leaf area (Table, 7)

The data obtained from Tables (8 and 9) revealed that some studied characters have no significanr difference in the two areas of culture (sucrose percentage and stem diameter), while the following characters showed superiority in the area of Nubaria; juice weight and number of nods. In contrast, T.S.S% showed a significant superiority in Sabahia location.

Concerning the following characters; plant weight, stalk weight, fresh and dry weight of leaves, chlorophyll, number of nods and total plant height, there were significant differences between the two year of culture in both locations.

Table 8. Evaluation of some selected clones for the characters of number of nods and stem diameter.

| Clones No. | No. of nods | | | | Stem diameter (cm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Sabahia | | Nubaria | | Sabahia | | Nubaria | |
| | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year |
| 1 | 9.6 | 19 | 22.6 | 25.3 | 2.3 | 2.6 | 2.4 | 2.3 |
| 2 | 14 | 16.6 | 23.6 | 21.6 | 2.9 | 2 | 2.1 | 2.8 |
| 3 | 13 | 16 | 22.6 | 20.6 | 1.9 | 2.2 | 2.6 | 2.5 |
| 4 | 13 | 19 | 25.6 | 22 | 1.7 | 2.3 | 2.5 | 2.4 |
| 5 | 12 | 17 | 23 | 21 | 2.8 | 2.4 | 2.5 | 2.5 |
| 6 | 12 | 17 | 14 | 12.3 | 1.9 | 2.3 | 2.3 | 2.4 |
| 7 | 12 | 17 | 17 | 24.3 | 4.2 | 2 | 2.2 | 2.2 |
| 8 | 13 | 17 | 17.3 | 15 | 2.6 | 2.2 | 2.5 | 2.1 |
| 9 | 13 | 16 | 19.3 | 15.3 | 2.3 | 2.8 | 2.2 | 2.2 |
| 10 | 14 | 18 | 13 | 13.6 | 2.03 | 2 | 2.5 | 2.6 |
| P $LSD_{0.05}$ | | 0.47 | | | | 0.143 | | |
| Y $LSD_{0.05}$ | | 0.82 | | | | 0.061 | | |

P = place Y = year

Table 9. Evaluation of some selected clones for the characters of sucrose % , total soluble solids (T.S.S%) and juice yield.

| Clones No. | Sucrose % | | | | T.S.S % | | | | Juice yield (kg) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sabahia | | Nubaria | | Sabahia | | Nubaria | | Sabahia | | Nubaria | |
| | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd year | 1st Year | 2nd Year | 1st Year | 2nd year |
| 1 | 8.2 | 5.6 | 3.4 | 3.6 | 17 | 16 | 12.3 | 16 | 0.5 | 0.45 | 1.5 | 1.2 |
| 2 | 10.03 | 8.4 | 5.4 | 5.1 | 17 | 16 | 12 | 14 | 0.3 | 0.2 | 2.3 | 2.1 |
| 3 | 5.3 | 6.3 | 4.4 | 4.6 | 15 | 16 | 12 | 13 | 0.5 | 0.45 | 2.1 | 1.9 |
| 4 | 5.5 | 5.5 | 6.2 | 5.3 | 14 | 16 | 14 | 12 | 0.35 | 0.45 | 1.2 | 1.2 |
| 5 | 6.6 | 6 | 6.1 | 5.4 | 14 | 14 | 12.3 | 11 | 0.4 | 0.166 | 3.2 | 2.9 |
| 6 | 8.3 | 7.4 | 6.9 | 6.3 | 16 | 14 | 13 | 13 | 0.3 | 0.183 | 1.8 | 1.6 |
| 7 | 10 | 8.7 | 6.7 | 5.8 | 16 | 14 | 14 | 12 | 0.5 | 0.7 | 2.6 | 2.2 |
| 8 | 9 | 7 | 5.4 | 4.6 | 18 | 18 | 12 | 13 | 0.5 | 0.33 | 1.2 | 1.9 |
| 9 | 6.2 | 8.9 | 6.3 | 6.1 | 16 | 18 | 12 | 14 | 0.6 | 0.48 | 1.2 | 1.7 |
| 10 | 7.5 | 8.5 | 5.4 | 5 | 17 | 18 | 15 | 12 | 0.6 | 0.3 | 1.2 | 1.2 |
| P $LSD_{0.05}$ | | | 4.84 | | | 2.86 | | | | 0.40 | | |
| Y $LSD_{0.05}$ | | | 7.67 | | | 0.82 | | | | 0.19 | | |

P = place Y = year

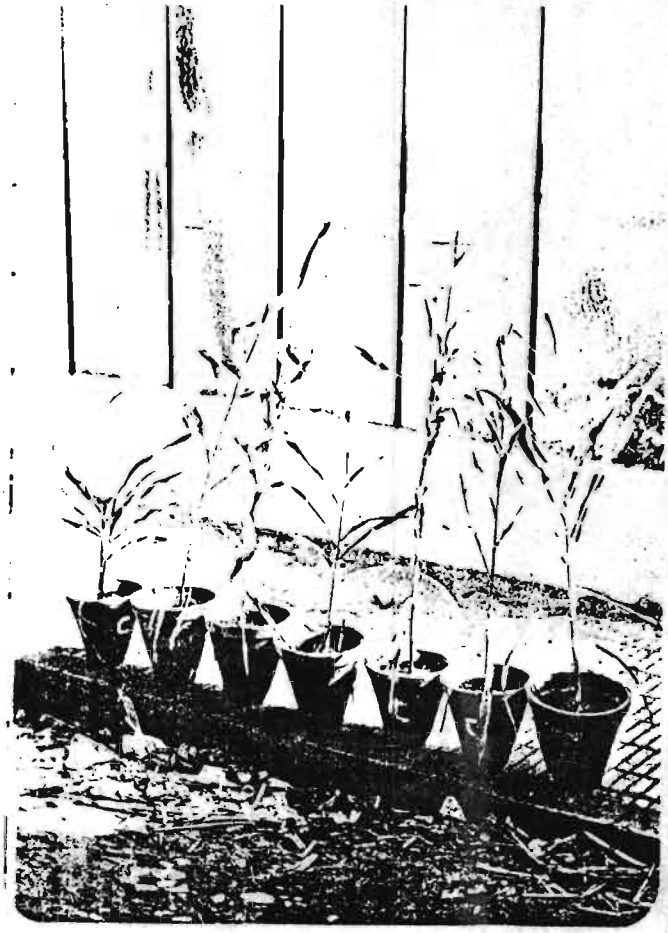


**Figure (1)**: Variants plants due to somaclones grown in clay pots under condition of greenhouse

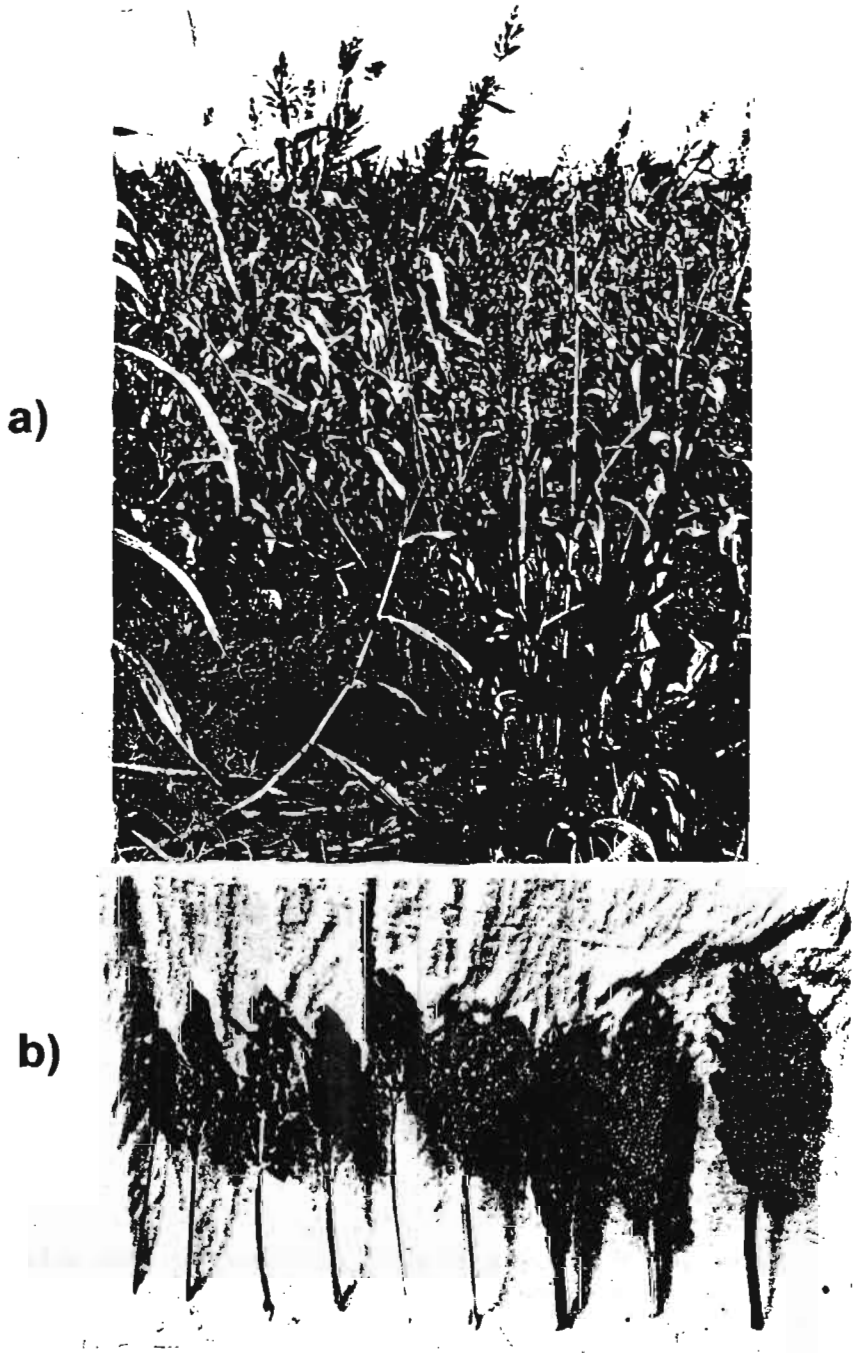
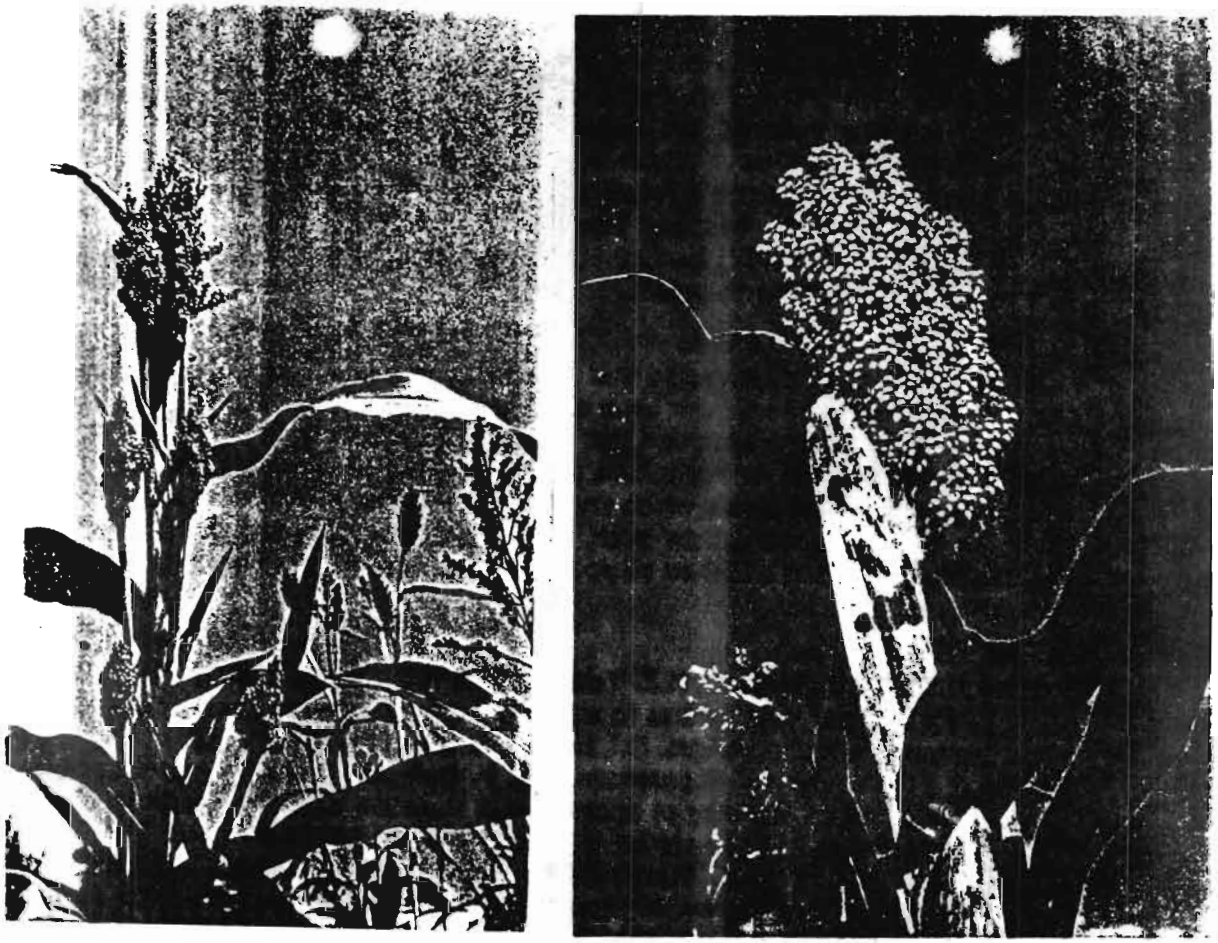



**Figure (2):** Morphological variation in some somaclones
a) General view in the field.
b) Spike size.



a b

**Figure (3):** Superiority of selected clones in:
a) plant height and stem diameter.
b) spike size and leaf area.

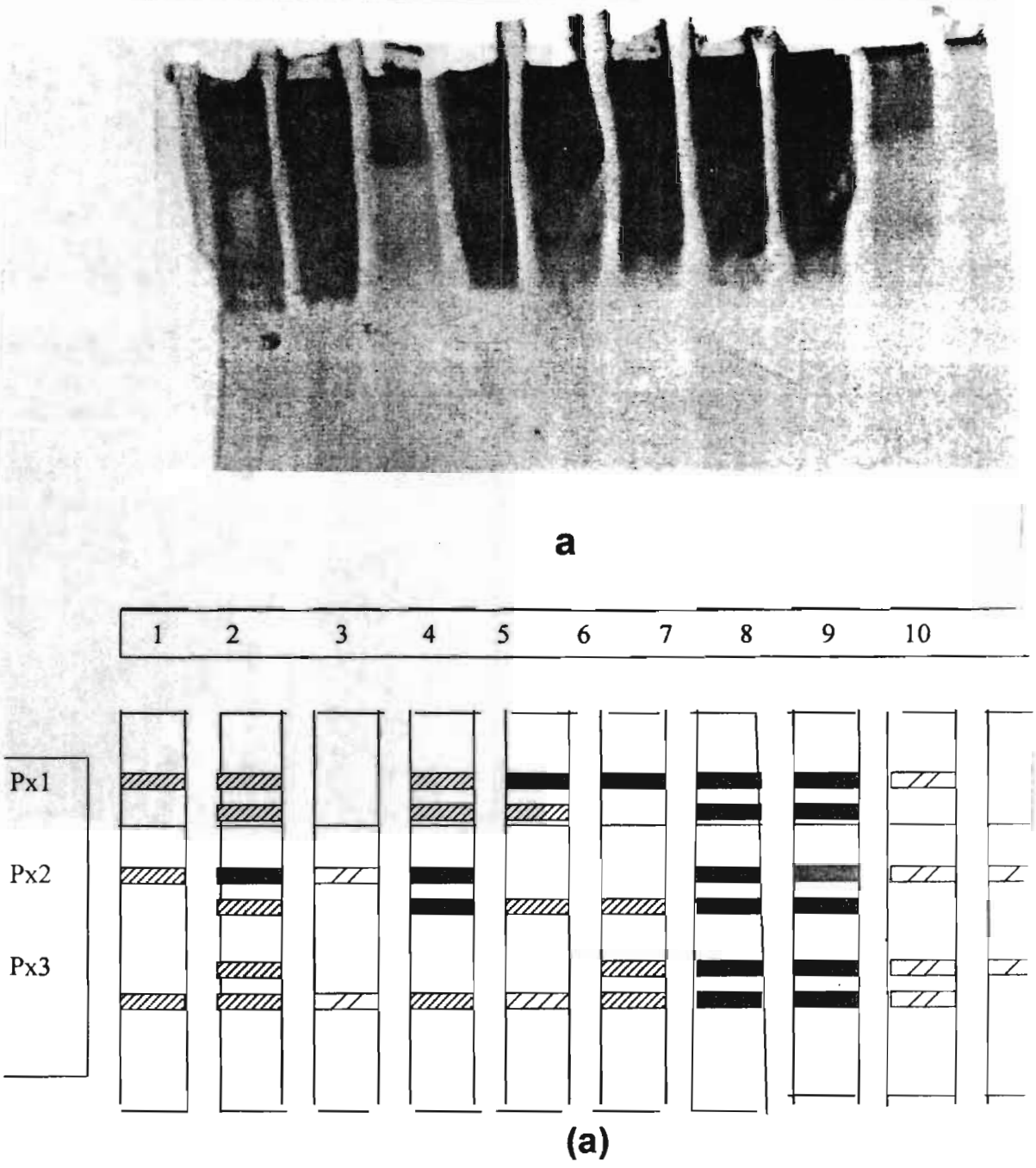



**Figure (4): (a)** Peroxidase isozyme and **(b)** Zymogram banding patterns of selected clones (1-9) and their explant donner (control 10).

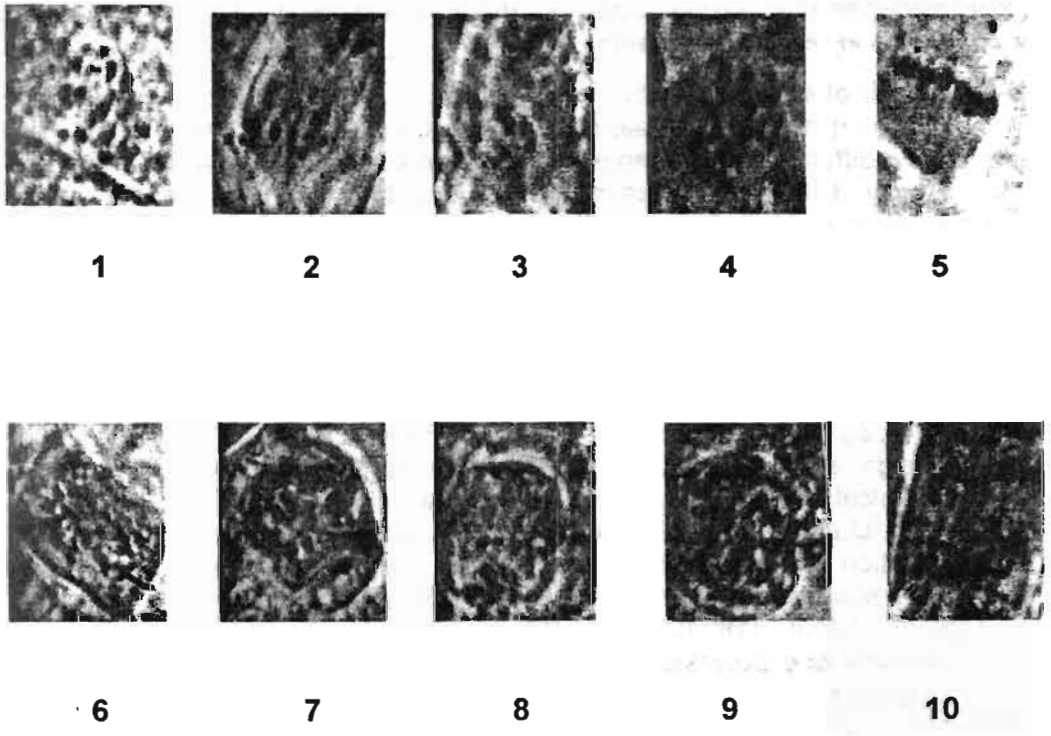



**Figure (5):** Photomicrographs showing normal metaphases in selected clones (1-9) and their explanted donner (Tracy).

### 4- Electrophoresis:

Peroxidase isozyme zymogram patterns showed three loci migrated to the cathode which designated Px1, Px2 and Px3 (Fig. 4 a & b). In general, it can be observed that the patterns are highly variable, therefore, all differences in enzymatic activities between clones and their control are modified because of tissue culture process. Such a result is in harmony with those obtained by Abd El-Hamid (1997) in sugar beet and Sharaf *et al.*, (2000) in sugar cane.

Kaeppler *et al.*, (2000) found that the modifications in DNA are less *in vitro* culture than in seed grown plants.

### 5-Cytological examination:

This part of study revealed that all clones possessed 2n=20 (fig. 5) and showed no differences between each other concerning chromosome number.

Finally, it can be concluded that tissue culture technique can be used to creat superior varieties for agriculture use and the obtained results can help the sourghum producer to chose which one of clones suitable for him. In addition to that, the breader could utilize such a result in select genotype with some parameters have good effect on sucrose percentage and / or juice yield.

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## الملخص العربي

# انتخاب سلالات فائقة الإنتاجية من خلال الاختلافات الناتجة عن زراعة الأنسجة للذرة السكرية صنف (Tracy)

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معمل البيوتكنولوجي ** قسم الفسيولوجي

مركز البحوث الزراعية – معهد بحوث المحاصيل السكرية

تم دراسة ٧٧ سلالة ناتجة عن زراعة الأنسجة للذرة السكرية (صنف Tracy ) من حيث الصفات المورفولوجية لدورتين انتخاب متتاليتين انتج عنها ٩ سلالات تم زراعتها بالبذرة موسمين متتاليين في حقلي الصبحية والنوبارية وتم تقييم السلالات المنتجة مورفولوجيا وتكنولوجيا لمعرفة التأثير الناتج من زراعة الأنسجة في إحداث التغيرات الوراثية اللازمة لعملية الانتخاب ثم دراسة ما إذا كانت هذه الصفات ناشئة عن تغيرات وراثية باستخدام تكنيكيات الفرد الكهربي لمشابه الأنزيم بيروكسيديز لتلك السلالات المتفوقة مع النبات الأصلي. وقد أظهرت النتائج التباين الكبير في قياسات الصفات المسجلة وتم التأكد من تفوق التسع سلالات المنتخبة في حقلي الزراعة المختلفين إلا أن بعض الصفات تفوقت في المنطقة الأولي كنسبة المواد الصلبة الذائبة الكلية ومساحة الورقة ونسبة الكلوروفيل والوزن الكلى للنبات (قبل وبعد التقشير) بينما في المنطقة الثانية تفوقت النباتات في صفات الطول الكلى و وزن العصير و عدد العقل و طول النورة و وزن الأوراق (الأخضر و الجاف) إلا أن صفتي نسبة السكروز وسمك العود لم تتأثر باختلاف الحقلين،كذلك تم التحقق من أن هذه الاختلافات ناشئة عن تغير في التركيب الوراثي كما أوضحت نتائج الفرد الكهربي لمشابه الأنزيم بيروكسيديز في اختلاف عدد وتوزيع الحزم الناتجة وكثافتها الضوئية مقارنة بالنبات الأم. من هذا يتضح أهمية عمليات زراعة الأنسجة في إنتاج أصناف جديدة من الذرة السكرية متفوقة في المحصول وكذلك محصول السكر. أظهرت النتائج أن عمليات زراعة الأنسجة لم تؤثر في هذه الحالة على عدد الكروموسومات.