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THE ROLE OF GENETIC AND NON-GENETIC FACTORS IN PLANT REGENERATION FROM IMMATURE INFLORESCENCE OF SUGARCANE AND EVALUATION OF REGENERANTS

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Abstract: A total of 35 genotypes of sugarcane were used to study the influence of genetical and environmental factors on plant regeneration from immature-inflorescence culture method used for haploid plant regeneration. In addition to the determination of the responsive sugarcane genotypes, the vegetative progeny of the regenerated plants were also evaluated for some economical characters. The experiment was designated to study the effect of genotypes, the pretreatments, eight types of induction medium and two types of flowering (naturally and artificially induced flowering). The microscopic examination revealed that the microspores were divided within the cultured anthers to produce a multicellular mass which developed into embryoids and finally to plants. However, plants were also regenerated from the somatic tissue of the cultured inflorescence.

The present study revealed that plant regeneration could be obtained from the immature sugarcane inflorescence of both naturally or artificially flowered plants, however the rate of response is controlled by the genotype of the donor plants and non-genetic factors such as cold pretreatment and nutrient medium. It was found that the two media BAT-1 and L were suitable for callus formation from the immature inflorescence. The regeneration rate was independent from each of number of shoots/callus and % of rooting, which suggested that they were under separate mechanisms of genetic control. The vegetative progeny of some regenerated clones exceeded their donor parents in some agronomic characters, especially estimated yield. This suggested the feasability and effectiveness of the immature inflorescence method in improving the agronomic performance of sugarcane.

Introduction

Sugarcane is probably the most genetically complex crop for which genome mapping has been attempted. Sugarcane cultivars are polyploid, aneuploid, interspecific hybrids between the domesticated species *Saccharum officinarum* (2n = ca. 80 chromosomes) and the wild relative *S. spontaneum* (40 to 128 chromosomes, multiples of eight are most frequent, $x = 8$). Cultivars chromosome numbers range from

100 to 130 with ca. 10% contributed by *S. spontaneum* (Price, 1963, Sreenivasan *et al.*, 1987 and Grivet *et al.*, 1996). The selection of desirable combinations of characters at these complex-levels of ploidy is much more laborious and required larger populations that at the diploid level. It is evident that anther culture significantly shortening the breeding cycle and increasing the selection efficiency as shown by the recovery of more recombinants with desirable characteristics (Powell *et al.*, 1990 and Zapata *et al.*, 1991). Through anther culture, haploid and doubled haploid plants could be obtained which permits dominant and recessive traits to be expressed. This enhances selection for favorable traits (Afele and Kannenberg, 1990).

Haploid plants are useful for rapid development of homozygous genotypes. They may arise from microspore, anther, or ovary culture. In sugarcane, a limited success was obtained by isolated microspore culture in which only haploid callus was obtained and not regenerated to plants (Hinchee and Fitch, 1984). Anther culture of sugarcane has proven difficult and very low frequency of haploid plants was obtained only from the wild species *Saccharum spontaneum* L. (Fitch and Moore, 1983 and Hinchee and Fitch, 1984). Haploid sugarcane plants were obtained by culturing part or all of the inflorescence (Fitch and Moore, 1983). The inflorescence method were used in barely (Wilson, 1977 and Thomas and Scott, 1985), tall fescue (Kasperbauer *et al.*, 1980), Gerbera (Preil *et al*, 1977) to regenerate androgenic plants. Plant regeneration from inflorescence cultures has also been reported in sugarcane (Liu, 1993 and Blanco *et al.*, 1997), barley (Thomas and Scott, 1985), corn (Pareddy and Petolino, 1990), rice (Shi *et al.*, 1985), sorghum (Cai and Butler, 1990), triticale (Nakamura and Keller, 1982) and wheat (Maddock *et al.*, 1983).

Furthermore, the regeneration of plants from anthers or immature inflorescence is influenced by both genetical as well as environmental factors (Powell, 1989). Factors known to influence sugarcane anther culture response include the developmental stage of the microspores, pre-treatment of anthers, culture media composition and the genotype of the donor plant. The present investigation aimed to study the influence of genetical and environmental factors on plant regeneration from immature-inflorescence-culture method used for haploid plant regeneration. In addition to the determination of the responsive sugarcane genotypes, the vegetative progeny of the regenerated plants were also evaluated in the field for some economical characters. This method offers the opportunity of obtaining

haploid and/or doubled haploid sugarcane plants which could be used for cane improvement.

Materials and Methods

Plant materials: Thirty-five sugarcane hybrid genotypes (*Saccharum officinarum* x *S. spontaneum*) were kindly obtained, cultivated and flowered in El-Hawamdya research station of Sugar and Integrated Industries Company. The selected genotypes were either flowered naturally in the field or artificially induced to flowering in the photoperiod houses when needed (Fig. 1a).

In order to study the effect of natural and artificially induced flowering in the response of inflorescence cultures, four genotypes were used for both naturally and artificially flowering.

Preparation of explants: Unemerged inflorescences (35 – 80 cm in length) were clipped off from field/greenhouse grown sugarcane plants during the period of end of October to early January in the two seasons 1997/1998 and 1998/1999. Cane-panicles were either (1) used directly for explant preparation and culture, or (2) pretreated with a cold shock (10° C) for 5 days. Because the tissue age is different between the upper and lower portions of a single panicle, the stages of microspore development in the inflorescence segments were determined by acetocarmine method before inoculation, as described by Liu *et al.*, (1980). Panicles-cylinders (5 cm in length) are corresponding to tetrad and early to late uninucleate stages were selected. Then, their leaf-sheets were carefully stripped off except for the last one. The 5 cm panicle-cylinder was opened aseptically and the tissues at both ends were cut off. Thirty to forty inflorescence segments in lengths of 1.2 – 1.5 cm were picked up from the remaining tissue cylinder and explanted on callus-induction medium. Because the inflorescence tissues are wrapped in leaf sheath, they are sterile and thus do not need to be disinfected.

Experiments: Two experiments were designated in the present investigation. In the first experiment, the influence of genetical and environmental factors on the response of immature inflorescence culture was studied. The experiment was designated on the basis of the following factors: 1) four genotypes (87-26-5, 87-27-2, 84-N-5 and 84-E-1), 2) two types of flowering (naturally and artificially induced flowering), 3) the pretreatments (cold pretreatment and no pretreatment), and 4) eight types of callus induction media (BAT-1, BAT-2, N6-1, N6-2, H-1, H-2, M-1 and L, Table 1). The experiment was replicated three times. The cultures were incubated at 16°C in the dark and after 6 weeks of incubation, the

Table (1):The composition of different media used for regeneration of sugarcane plants from immature inflorescence culture.

Code name	Medium composition (mg/l)
N6-1	NN69 + 20,000 sucrose + 100 myo-inositol + 2.0 2,4-D + 2.0 Kinetin + 500 casein hydrolyzate + 300 glutamine + 8,000 Agar
N6-2	NN69 + 200,000 sucrose + 100 myo-inositol + 2.0 2,4-D + 2.0 Kinetin + 1,000 casein hydrolyzate + 600 glutamine + 8,000 Agar.
BAT-1	Mod. MS (MS macro and microelements + 165 NH4 NO3 + 1,900 KNO_3 + MS vitamins + 0.02 biotin + 10.0 Na-pyruvate + 10.0 citric acid) + 500 myo-inositol + 1,000 casein hydrolyzate + 500 glutamine + 30,000g sucrose + 3.0 2,4-D + 1.0 BAP + 8,000 Agar.
BAT-2	Mod. MS (MS macro and microelements + 165 NH4 NO3 + 1,900 KNO_3 + MS vitamins + 0.02 biotin + 10.0 Na-pyruvate + 10.0 citric acid) + 100 myo-inositol + 20,000 sucrose + 3.0 2,4-D + 1.0 BAP + 8,000 Agar.
H-1	NN69 + 20,000 sucrose + 100 myo-inositol + 2.0 2,4-D + 1.0 BAP + 300 glutamine + 8,000 Agar.
H-2	NN69 + 2,000 sucrose + 5,000 myo-inositol + 2.0 2,4-D + 1.0 BAP + 600 glutamine + 8,000 Agar.
M-1	Mod. MS (1/2 macro + microelements + vitamins) + 15,000 sucrose + 500 glutamine + 500 myo-inositol + 1.0 2,4-D + 1.0 BAP + 8,000 Agar.
L	Mod. MS (1/2 MS macro + micro + NN69 Vitamins) + 4.5 2,4-D + 2.5 Kinetin + 500 inositol + 600 glutamine + 1,000 casein hydrolyzate + 30,000 sucrose + 8,000 Agar.
SC	MS salts (MS macro + micro) + NN69 Vitamins + 0.5 folic acid + 0.05 biotin + 10.0 Na-pyruvate + 10.0 citric acid + 100 myo-inositol + 1.0 2,4-D + 1.0 BAP + 500 inositol + 600 glutamine + 1,000 casein hydrolyzate + 30,000 sucrose + 8,000 Agar.
MS-R1	MS + 0.5 folic acid + 0.05 biotin + 10.0 Na-pyruvate + 10.0 citric acid + 100 myo-inositol + 500 casein hydrolyzate + 30,000 sucrose + 10,000 activated charcoal + 2.0 mg/l IAA + 8,000 Agar.
MS-R2	MS + 0.5 folic acid + 0.05 biotin + 10.0 Na-pyruvate + 10.0 citric acid + 100 myo-inositol + 500 casein hydrolyzate + 30,000 sucrose + 10,000 activated charcoal + 4.0 IAA + 8,000 Agar.
MS-R3	MS + 0.5 folic acid + 0.05 biotin + 10.0 Na-pyruvate + 10.0 citric acid + 100 myo-inositol + 500 casein hydrolyzate + 30,000 sucrose + 10,000 activated charcoal + 1.0 NAA + 8,000 Agar.
MS-R4	MS + 0.5 folic acid + 0.05 biotin + 10.0 Na-pyruvate + 10.0 citric acid + 100 myo-inositol + 500 casein hydrolyzate + 30,000 sucrose + 10,000 activated charcoal + 5.0 NAA + 8,000 Agar.
MS-R5	MS + 0.5 folic acid + 0.05 biotin + 10.0 Na-pyruvate + 10.0 citric acid + 100 myo-inositol + 500 casein hydrolyzate + 30,000 sucrose + 10,000 activated charcoal + 0.2 IBA + 2.0 IAA + 8,000 Agar.

Where: NN69 : Nitsch and Nitsch (1969) medium.
M&S : Murashige and Skoog (1962) medium
IAA : 3-Indol acetic acid NAA : naphthalene acetic acid
IBA : Indol butyric acid BAP : Benzyl amino purine

percentage of explants produced callus was calculated.

The second experiment was conducted to evaluate the response of more sugarcane genotypes to immature inflorescence culture. A total of 31 sugarcane genotypes were cultured on the two suitable media (BAT and L) for callus formation from the immature inflorescence explants. The experiment was replicated three times.

Shoot regeneration: The formed calli were sub-cultured two times on the same medium and incubated in 16/8h light/dark cycle at 25±2°C. After four weeks of subculture, callus pieces (0.5-0.6 cm width x 0.9-1.0 cm length x 0.2-0.3 cm diameter) from each responsive genotype were cultured on the regeneration medium. The regeneration medium (SC-medium, Table 1) consisted of MS (Murashige and Skoog, 1962) salts (MS macro- + micro-salts) + NN69 Vitamins (Nitsch and Nitsch, 1969) + 0.5mg/l folic acid + 0.05mg/l biotin + 10.0mg/l Na-pyruvate + 10.0mg/l citric acid + 100mg/l myo-inositol + 600mg/l glutamine + 1000mg/l casein hydrolyzate + 30g/l sucrose + 1.0mg/l 2,4-D + 1.0mg/l BAP + 8g/l Agar. After 6 weeks of incubation in 16/8h-light/dark cycle at 25±2°C, the regeneration rate (% of calli produced shoots) and number of shoots per callus were determined.

Root formation and plant development: When shoots were 3-5 cm in length, they transferred to the rooting medium. Five types of rooting medium (MS-R1, MS-R2, MS-R3, MS-R4 and MS-R5) were used (Table 1). The rooted shoots were counted after 4 weeks and transferred to hormone free medium for further development. The well-rooted plantlets were potted in pots contained a 1:1:1 mixture of peat, sand, and soil. Pots were covered with a transparent plastic cover for two weeks to keep the humidity and transferred to the greenhouse for adaptation and further growth. Healthy plants (Ro) were then transferred to the field (experimental farm of Genetics Department, Faculty of Agriculture, Assiut university).

Evaluation of the regenerated plants: At the second season (2000/2001), the vegetative progeny of the regenerated plants (R1) and their donor parents were cultivated in the field in a randomized complete block design with three replicates. Stalk number per plot (N), stalk length (L), and stalk diameter (D) were measured at harvest. The percentage of total soluble solids (T.S.S.%), which reflect the density of sugar contents, was measured by using a hand refractometer according to Blackburn (1984). Stalk volume (V) was calculated as $V = \frac{1}{4} \pi D^2 LN$ and yield was estimated from "stalk

volume x T.S.S. ratio" (Miller and James, 1974 and Nagai et al., 1991).

Results and Discussion

The first experiment in the present study was designated to investigate the role of the genotypes, the pre-treatment, flowering type (naturally or artificially induced), and culture medium in mean percentages of callus formation from immature inflorescence of four sugarcane genotypes. The mean percentages of callus formation and the analysis of variance are found in Tables (2&3).

The inflorescence segments taken either from cold pretreated or not pretreated cane-panicles were capable for producing callus. However, the cold pretreated panicles produced a significantly higher percentage (17.98% average over treatments) than the non-pretreated panicles (7.48%). In a wild species of sugarcane, *Saccharum spontaneum*, prolonged pre-culture of panicle segments at 10°C promoted microspore development (Fitch and Moore, 1983). Liu (1993) found that Cold (13°C) storage of immature inflorescences for 2 or 3 days enhanced callus frequency significantly higher than natural night temperature-treatment.

The cold treatment reduce the brownish substances that may be due to the inactivation of the polyphenol oxidase (Monaco *et al*, 1977), and induce the nongametogenic microspore divisions which lead to embryogenesis by triggering physiological changes which result in embryogenic development (Wenzel *et al*., 1977; Dunwell, 1978; Rashid & Reinert, 1980, Hinchee *et al*, 1984 and Liu, 1993 and El-Aref *et al*, 1998). Powell and Uhrig (1987) reported that response to temperature pretreatment is genetically controlled. The availability of responsive genotypes that differ in their response to temperature pretreatment will allow this phenomenon to be genetically investigated in more detail. After approximately 3 weeks of culture the microspores within the anthers responded by dividing to form a multicellular mass (Fig.1b).

In addition to cold pretreatment, other manipulations such as dark incubation, frequent subculture and the addition of high level of glutamine in the medium were used in the present study to minimize the negative effect of browning. The different stages of plant regeneration from immature inflorescence containing anthers at tetrad to uninucleate microspores are illustrated in Figs. (1b-g and 2a-h).

In order to determine the appropriate medium for callus formations from the immature inflorescence, eight types of callus-induction medium were tested (Tables 1 and 2). Overall genotypes,

Table (2): Percentage of callus formation from immature inflorescence of four sugarcane genotypes flowered naturally (N) or artificially induced flowering (I), either cold pretreated (CPT) or not pretreated (NPT), and cultured on eight types of induction medium for six weeks.

Genotypes	Flowering	BAT-1		BAT-2		L		H-1		H-2		M-1		N6-1		N6-2	
		CPT	NPT	CPT	NPT	CPT	NPT	CPT	NPT	CPT	NPT	CPT	NPT	CPT	NPT	CPT	NPT
87-26-5	I	40.0	19.3	5.0	1.7	13.7	8.0	2.3	1.0	4.7	1.7	2.3	1.3	4.7	2.0	1.3	1.0
	N	20.7	8.3	2.3	1.3	18.3	8.7	4.3	1.3	6.7	2.3	1.0	1.0	3.7	1.3	2.3	1.0
87-27-2	I	79.3	25.0	24.0	12.3	19.3	8.7	61.0	25.0	39.3	19.7	39.3	16.0	40.3	22.3	3.3	1.3
	N	79.7	18.3	76.0	31.7	55.7	24.0	40.0	18.3	11.7	2.7	6.0	2.7	2.7	1.3	8.3	2.3
84 N 5	I	79.3	21.0	6.3	3.0	23.3	11.0	1.7	1.0	2.0	1.0	4.7	2.3	3.3	1.7	3.7	1.3
	N	29.7	14.7	4.0	1.0	65.7	30.0	2.7	1.3	4.3	2.0	2.3	1.0	7.3	3.7	40.3	21.3
84 E 1	I	8.7	2.7	4.0	2.3	20.0	9.7	6.3	2.0	3.3	1.3	5.7	2.7	1.0	1.0	4.0	1.7
	N	22.0	10.7	4.3	1.7	50.7	23.7	4.7	1.7	2.0	1.0	4.7	2.0	2.7	1.0	6.7	3.3
Average		44.9	15.0	15.7	6.9	33.3	15.5	15.4	6.4	9.2	4.0	8.2	3.6	8.2	4.3	8.7	4.2
MEAN		29.95		11.30		24.40		10.90		6.60		5.90		6.25		6.45	

L.S.D. 0.05 = 5.539 L.S.D.0.01 = 7.263

Table (3): Percentage of callus formation from immature inflorescences of 31 sugarcane genotypes flowered naturally and cultured on two types of induction medium for six weeks.

Genotypes	Media		Genotypes	Media		Genotypes	Media	
	BAT-1	L		BAT-1	L		BAT-1	L
54 B 469	80.0	20.3	86-2-8	80.0	15.3	87-28-6	80.3	10.0
65-11-19	8.0	20.0	87-3-9	42.7	20.0	87-28-9	66.7	79.7
82-G-98	40.0	4.7	87-19-13	7.7	78.7	87-28-13	75.7	79.7
82-14-1	2.3	1.3	87-12-23	3.3	15.7	87-28-16	82.7	53.3
83 B 51	19.3	38.0	87-14-4	62.0	14.7	89-97-33	40.0	2.3
83 C 51	54.0	60.0	87-15-3	58.0	16.0	89 F 43	14.3	36.3
83 C 59	31.7	47.0	87-23-30	80.0	4.3	89-60-14	38.7	33.7
84 T 5	80.0	12.3	87-25-12	73.3	61.3	CP-57-614	83.3	8.3
85-14-1	8.0	13.0	87-25-35	84.0	79.3	BOT 7	7.3	1.3
85-14-4	66.0	3.0	87-27-22	70.0	50.7			
86 L 37	73.3	66.7	87-28-2	7.7	4.0			

$LSD_{0.05}$ = 11.262, $LSD_{0.01}$ = 14.768

pretreatment and flowering type, the results in Table 2 showed that the medium BAT-1 followed by L exhibited the highest percentage of callus formation (29.95% and 24.40%, respectively). The other media exhibited lower percentages of callus formation in ranged from 5.9% to 11.3%. These differences between the tested mediums were highly significant (Table 4). These results suggested that the two media BAT-1 and L were suitable for callus formation from the immature inflorescence. Blanco *et al.*, (1997) found that embryogenic callus culture was initiated from immature inflorescence segments of sugarcane var. CP 5243 after 30 days of culture on MS medium supplemented with 13.5μM 2,4-D.

High significant differences were found between the tested genotypes in the percentage of callus formation. However, the genotypes performed differently from one medium to another and revealed high significant interaction.

Both inflorescence segments taken from cane plants flowered naturally or artificially were capable for producing callus and exhibited non-significant differences. Meanwhile, the interaction between the genotypes and their flowering type was significant. This interaction indicated that the performance of the genotypes depended upon their flowering type in the same medium. For example on BAT-1 medium, the two types of flowering revealed a similar % of callus formation in the genotype 87-27-2, however the genotype 84N5 exhibited 79.3% callus from induced flowering and 29.7% callus from naturally flowering, while the naturally flowered plants of 84E1 exhibited a higher % of callus (22.0%) than those induced flowering plants (Table 2). These results indicated that callus formation could be obtained from the inflorescences of both naturally or artificially flowered sugarcane plants, however the % of callus formation depending upon the type of medium as well as the genotypes.

The analysis of variance (Table 3) provide the importance of genotype, cold pretreatment, media and their interactions on the mean percentages of callus formation from immature sugarcane inflorescence. The non-significant triple interaction (genotype x flowering x pretreatment) was turned to be significant when interacts with media, which reflect the magnification role of media composition. The significance between replicates may be due to the field conditions, since the experiment was repeated three times during the period of end of October to early January, in the season 1997/1998.

The above results revealed that the two media BAT-1 and L were feasible for callus formation from

sugarcane immature inflorescence. The two media were used to evaluate 31 genotypes as shown in Table (3) and statistically analyzed in Table (4). The results also confirmed the dependence of callus formation on the genotype as well as the type of induction medium. The genotype 87-25-35 (84%) followed by CP-57-614, 87-28-16, 54B469, 84T5, 86-2-8, 87-23-30 and 87-28-6 (83.3-80%) were the highest responsive genotypes on the BAT-1 medium. However, the highest responsive genotypes on the L-medium were 87-28-9, 87-28-13, 87-25-35 and 87-9-23 (79.7-78.7%). The other genotypes exhibited high (66%) to very low (1.3%) response for callus induction (Table 3). The differences between the genotypes, media and their interaction were high significant (Table 4).

For callus proliferation, calluses were sub-cultured two times on the same medium and then transferred to the regeneration medium (SC-medium, Table 1). The results revealed that shoot regeneration was only obtained from the embryogenic callus i.e. via embryogenesis (Figs. 2a-e). Similar observation was also found by Blanco *et al.*, (1997). However, Liu (1993) reported that the regeneration of plants from immature inflorescence of most tested genotypes was by the way of embryogenesis, whereas the pattern of plant regeneration was through a typical embryogenesis—organogenesis in few genotypes.

The regeneration rate was ranged from 67.0% in the genotype 87-15-3 to 1.7% in 87-97-33 (Table 5). However, the number of regenerated shoots/callus ranged from 54.3 shoots in the genotype 87-27-2 to one shoot in 87-28-16 (Table 5). These differences between genotypes were high significant (Table 6). Although calli of the genotype 87-15-3 exhibited the highest rate of regeneration, a low number of shoots (8.7 shoots) were regenerated from each callus (Table 5). Meanwhile, the lower regeneration-rate genotypes (3.0 – 13.7%), such as 82-G-98, 87-14-4, 87-23-30, 87-28-6 and 87-28-9, exhibited higher numbers of shoots/callus (13.7 – 26.0 shoots). These results revealed that the regeneration rate was independent from the numbers of shoots/callus (r = 0.208), which suggested that regeneration rate and numbers of shoots/callus were under separate mechanisms of genetic control. M'Ribu and Veilleux (1990) concluded that there was no direct relationship between the regeneration frequency and number of shoots per explant.

The developed shoots were transferred to five types of rooting medium (Table 1). Roots were formed only on the two media MS-R2 (modified MS + 4.0mg/l IAA) and MS-R4 (modified MS + 5.0mg/l

Table (4): Analysis of variance for the percentage of callus formation from: (A) 4 sugarcane genotypes cultured on eight types of medium, and (B) 31 genotypes cultured on two types of induction medium.

Source	A		B	
	DF	MS	DF	MS
Reps.	2	335.36**	2	126.715
Genotypes (G)	3	7786.16**	30	3035.405**
Flowering (F)	1	60.17		
G x F	3	362.72**		
Pretreatment (P)	1	10584.00**		
G x P	3	1603.02**		
F x P	1	10.67		
G x F x P	3	13.77		
Media (M)	7	4136.18**	1	15665.855**
G X M	21	821.10**	30	1872.840**
F x M	7	1284.93**		
G x F x M	21	583.52**		
P x M	7	988.34**		
G x P x M	21	191.98**		
F x P x M	7	217.64**		
G x F x P x M	21	128.15**		
Error	254	35.93	122	49.529

Table (5): Regeneration rate and number of shoots/callus of immature inflorescence derived calli cultured on the regeneration medium.

NO	Genotypes	Reg.* Rate	No. of Shoots/ Callus	NO	Genotypes	Reg. rate	No. of Shoots/ Callus	NO	Genotypes	Reg. rate	No. of Shoots/ Callus
1	54 B 469	3.0	6.3	13	86 L 37	4.0	2.7	25	87-27-22	11.3	6.7
2	65-11-19	18.0	12.0	14	86-2-8	3.7	15.7	26	87-28-2	3.3	13.7
3	82-G-98	12.0	16.3	15	87-3-9	6.3	11.7	27	87-28-6	13.7	21.7
4	82-14-1	18.3	21.3	16	87-19-13	5.0	11.0	28	87-28-9	7.7	26.0
5	83 B 51	4.0	10.0	17	87-12-23	6.0	3.3	29	87-28-13	50.7	3.0
6	83 C 51	40.3	2.3	18	87-14-4	3.0	13.7	30	87-28-16	5.3	1.0
7	83 C 59	3.7	3.3	19	87-15-3	67.0	8.7	31	89-97-33	1.7	4.3
8	84 T 5	3.7	9.7	20	87-23-30	5.3	17.0	32	89 F 43	15.7	14.7
9	84 E 1	59.7	6.7	21	87-25-12	14.0	5.3	33	89-60-14	36.3	33.7
10	84 N 5	65.3	14.7	22	87-25-35	62.0	26.0	34	CP-57-614	3.3	8.7
11	85-14-1	3.3	2.7	23	87-26-5	60.3	19.7	35	BOT 7	20.0	2.3
12	85-14-4	33.7	4.3	24	87-27-2	31.7	54.3				
$LSD_{0.05}$										9.14	4.98
$LSD_{0.01}$										11.99	6.53

* Reg. Rate = Regeneration rate was calculated as the percentage of calli produced shoots.

Table (6): Analysis of variance for the regeneration rate and number of shoots per callus.

Source	DF	Regeneration rate	No. of Shoot/ Callus
		MS	MS
Replicates	2	28.638	23.210
Genotypes	34	1381.884**	399.511**
Error	68	32.648	9.670

Table (7): The percentage of rooted shoots, number of plants transferred to the greenhouse, and number of plants established in the field.

Genotypes	% of rooted shoots	No. of plants transferred to the greenhouse	No. of field established plants	Genotypes	% of rooted shoots	No. of plants transferred to the greenhouse	No. of field established plants
85-14-1	3.53	6	1	87-14-4	9.72	7	0
84 T 5	20.0	10	1	87-27-2	8.99	40	11
84 N 5	20.0	52	4	87-28-6	20.0	22	0
86-2-8	31.25	50	5	87-28-13	7.14	10	0
87-3-9	3.33	4	0	89 F 43	59.51	147	12
87-19-13	20.0	21	2	89-60-14	59.22	302	7

NAA). However, Gallo-Meagher et al., (2000) reported that the shoots regenerated from the sugarcane hybrid cv. CP84-1198 were rooted efficiently on MS medium supplemented with 19.7 µM IBA (indole-3-butyric acid). Not all genotypes formed roots that indicated the dependence of root formation on the genotypes as well as the growth regulators. High rooting was observed in the genotypes 89-E-43 (59.51%), 89-60-14 (59.22%), and 86-2-8 (31.25%). Non-significant negative correlation ($r = -0.139$) was observed between the regeneration rate and % of rooting, which suggested that they were under separate mechanisms of genetic control (Tables 5 and 7).

The regenerated plants maintained in the greenhouse and finally transferred to the field for further development (Table 7). The vegetative progeny of the regenerated plants (R1) and their donor parents were cultivated in the next season and evaluated for stalk number/plot, stalk length, stalk diameter, the degree of total soluble

solids (T.S.S.%), stalk volume and estimated yield (Table 8). The analysis of variance revealed the presence of high significant differences between the regenerated clones as well as among the regenerated clones and their donor parents in most studied characters (Table 9).

The results revealed that some regenerated clones exceeded their donor parents in different characters, especially estimated yield. In this respect, the two clones C11 and C12 exceeded their donor parent 89-60-14 in all studied characters and exceeded their parent in estimated yield by 3 – 4 folds. Similarly, the clones C9 and C10 were superior in all studied characters, except stalk number/plot and showed at least two fold increase in their yield, as compared with their parent 89-F-43. The three clones C4, C8 and C14 also showed significant development in different agronomic characters including estimated yield (Table 8). Selected sugarcane somaclones with thick stalk, increased stalk number and high sugar contents were also reported by Chen, (1986) and Jimenez *et al.*, (1991). Among hundreds of sugarcane plants regenerated from anther culture only five plants were transplanted to the field, Chen et al., (1983) found only one plant was superior to its parent in many agronomic traits.

Table (8) also revealed that the five sugarcane clones C1, C2, C3, C6 and C7 exhibited significant decrease in stalk diameter, stalk volume and estimated yield, as compared with their donor parents. However, the two clones C5 and C13 revealed mean performance similar to their parents in most studied characters including estimated yield. In addition, all tested clones revealed mean values of stalk number and sugar contents (T.S.S.%) similar or higher to that of their donor parents. Miller (1985) found no significant differences between the means of the sugarcane donor clones and their tissue culture-derived subclones in stem diameter and stem number.

The results showed that the vegetative progeny of some regenerated clones (e.g. C4, C8, C9, C10, C11, C12) exceeded their donor parents in some agronomic characters, especially estimated yield. This suggested that through immature inflorescence culture, sugarcane clones that are superior in agronomic performance could be obtained.

The present study revealed that plant regeneration could be obtained from the immature sugarcane inflorescence of both naturally or artificially flowered plants, however the rate of response is controlled by the genotype of the donor plants and

Table (8): The mean values of stalk diameter (mm), length (cm), number and volume (cm^3); sugar contents (T.S.S.%) and estimated yield (cm^3 %) for the vegetative progeny of the regenerated sugarcane clones and their donor parents.

Genotypes	Stalk Diameter	Stalk Length	Stalk No.	Stalk Volume	T.S.S %	Estimated Yield
85-14-1	23.0	210	9.2	8023.0	17.0	136389.0
C1	15.4	212	11.8	4657.0	16.9	78986.0
LSD 0.5	3.199	10.539	1.865	929.576	2.130	212.987
0.1	5.2946	17.439	3.086	1538.148	3.524	352.424
84 N 5	20.0	190	29	17301.0	17.4	301043.8
C2	15.6	244	33.8	15755.0	17.2	271620.0
C3	14.6	222	31.2	11515.0	18.2	209577.0
LSD 0.5	1.168	13.557	1.651	350.0922	0.979	199.981
0.1	1.698	19.720	2.402	509.225	1.425	290.882
87-9-23	22.0	254.8	16.0	15569.0	18.2	282216.00
C4	24.8	288	14.8	20293.0	18.4	372707.00
C5	23.0	249.6	15.4	15962.0	17.3	276144.00
LSD 0.5	1.004	9.743	1.420	245.961	1.0125	196.136
0.1	1.460	14.171	2.065	346.124	1.473	285.289
87-27-2	25	219	14.4	15331.0	13.02	199610.0
C6	21.8	203	13.2	9996.0	17.04	170342.0
C7	19.6	208	14.2	8907.0	16.96	151063.0
C8	22.0	176	22.0	14711.0	17.28	254210.0
LSD 0.5	1.772	13.424	1.357	254.164	0.395	141.706
0.1	2.487	18.842	1.905	356.762	0.555	198.909
89 F 43	18	162	17.2	7086.0	15.8	113213.0
C9	23	217	18.4	16580.0	17.9	297789.0
C10	20.8	212	18.0	12959.0	17.8	231168.0
LSD 0.5	0.927	14.146	1.410	215.183	0.65073	1772.67
0.1	1.349	20.576	2.050	312.994	0.9465	2578.43
89-60-14	21	210	11	7996.0	13.6	108757.0
C11	26.2	246	21	27837.0	17.8	495503.0
C12	23.6	248	18.8	20384.0	17.4	355528.0
LSD 0.5	1.7893	14.861	1.624	131.708	0.4671	472.691
0.1	2.603	21.616	2.363	191.576	0.6794	687.550
84 T 5	19	235	9.2	6126.0	18.02	110404.0
C13	18.6	227	10.0	6164.0	17.76	109487.0
C14	17.4	251	11.4	6618.0	17.72	117283.0
LSD 0.5	1.615	14.936	1.300	144.066	0.4239	314.16
0.1	2.349	21.725	1.891	209.551	0.6166	456.96

Table (9): The analyses of variance for stalk diameter, length, number and volume; T.S.S.ratio and estimated yield for the vegetative progeny of the regenerated sugarcane clones and their donor parents.

Geno types	S.V.	DF	Stalk Diameter	Stalk Length	Stalk No.	Stalk Volume	T.S.S %	Estimated Yield
			MS	MS	MS	MS	MS	MS
85-14-1	Repslicates	4	1.150	28.75	2.00	280121.75	1.474	13339.25
	85-14-1 vs. C1	1	144.40**	10.00	16.90**	28324890.0**	0.004	8237761022.5**
	Error	4	2.65	28.75	0.90	223619.75	1.174	11739.25
84 N 5	Repslicates	4	1.067	130.83	9.833*	19624.33	0.309	1530.067
	Beteen all Genotypes	2	41.267**	3686.67**	28.867**	44871260.0**	1.323	10901057798.067**
	C2 vs. C3	1	3.8	1210.0**	17.9**	44944000.0**	2.304	9623334622.5**
	Error	8	0.767	103.33	1.533	68906.833	0.539	22485.817
87-9-23	Repslicates	4	4.067**	89.267	1.233	46124.167	0.572	25827.833
	Between all Genotypes	2	10.067**	2169.87**	1.800	34356821.667**	1.658	14624919361.667**
	C4 vs. C5	1	4.592*	1686.40**	0.900	4689390.250**	1.572	13311032422.500**
	Error	8	0.567	53.367	1.133	31835.167	0.576	21627.833
87-27-2	Repslicates	4	1.575	370.0	2.050	16480.125	0.245	6502.800
	Between all Genotypes	3	24.60**	1668.33**	82.717**	55120651.25**	20.832**	10099640980.050**
	Between C6, C7, C8	2	22.42*	1481.67**	68.667**	47586301.67**	0.331	1503719082.807**
	Error	12	2.642	151.667	1.550	54371.792	0.132	16901.467
89 F 43	Repslicates	4	1.233	135.00	3.267	1032.667	0.420	1403997.433
	Between all Genotypes	2	31.400**	4625.00**	1.867	114783171.67**	7.368**	43683400424.600**
	C9 vs. C10	1	12.100**	187.53	0.400	32779102.50**	0.360	11095760860.900**
	Error	8	0.483	112.5	1.117	26032.667	0.238	1766667.433
89-60-14	Repslicates	4	0.900	514.167*	0.233	24352.667	0.583*	13229.5
	Between all Genotypes	2	33.800**	2286.67**	138.067**	502229195.00**	27.096**	1526197.12**
	C11 vs. C12)	1	32.70**	34.92	12.1*	138868022.5**	0.324	48982501562.5**
	Error	8	1.800	124.167	1.483	9752.667	0.123	125618.00
84 T 5	Repslicates	4	4.567	319.167	0.600	8460.667	0.055	26255.333
	Between all Genotypes	2	4.467	681.667*	6.200*	374686.667**	0.133	90782621.667**
	C13 vs. C14	1	4.90	1322.5*	7.161*	515290.00**	0.004	151944040.00**
	Error	8	1.467	125.417	0.950	11668.667	0.101	56109.333

non-genetic factors such as cold pretreatment and nutrient medium. Whilst studies on the non-genetic components have produced some dramatic increases in the regeneration response, some of these factors are more difficult to fix than the genetic component (Powell, 1989 and Afele and Kannenberg, 1990). The identification of responsive genotypes and their regenerated clones will extend the pool of sugarcane genotypes amenable to immature inflorescence and anther culture technique.

A few publications dealt with haploid plant regeneration were

found in sugarcane. These publications demonstrated that the regeneration of haploid plants from isolated microspores was not succeeded, and the regeneration from anther culture has proven difficult and very low frequency of haploid plants was obtained only from the wild species *Saccharum spontaeum* (Fitch and Moore, 1983 and Hinchee and Fitch, 1984). Therefore, immature inflorescence culture of sugarcane becomes an important consideration for the regeneration of haploid sugarcane plants (Fitch and Moore, 1983, Liu, 1993 and Blanco *et al.*, 1997). The present investigation revealed that the microspores were divided within the anthers to produce a multicellular mass which developed into embryoids and finally to plants (Figs. 1 and 2). In addition, plants were also regenerated from the somatic tissue of the cultured inflorescence (Fig. 1g). These observations suggested that some of the regenerated plants were arisen from the development of microspores. The ploidy level of these regenerants must be recognized. Because sugarcane plants are mixoploid, that is not all somatic cells have the same number of chromosomes, meiotic chromosome count becomes an excellent method to determine the ploidy level (Price, 1963; Chaleff, 1983, Blackburn, 1984 and Al-Janabi *et al*, 1994). The ploidy level of the regenerated sugarcane plants recovered during the present study will be determined at the meiotic stage in the future.

Since some of the regenerated plants were arisen from the development of microspores and other plants were also regenerated from the somatic tissue of the cultured inflorescence, in this study it is not known whether mutations (gametoclonal/somaclonal variations), recombinations of genes or both are the causes of the exhibited variability in the tested sugarcane clones. While, the superiority of the vegetative progeny of some regenerated clones reflects the effectiveness of immature inflorescence culture in improving cane yield.

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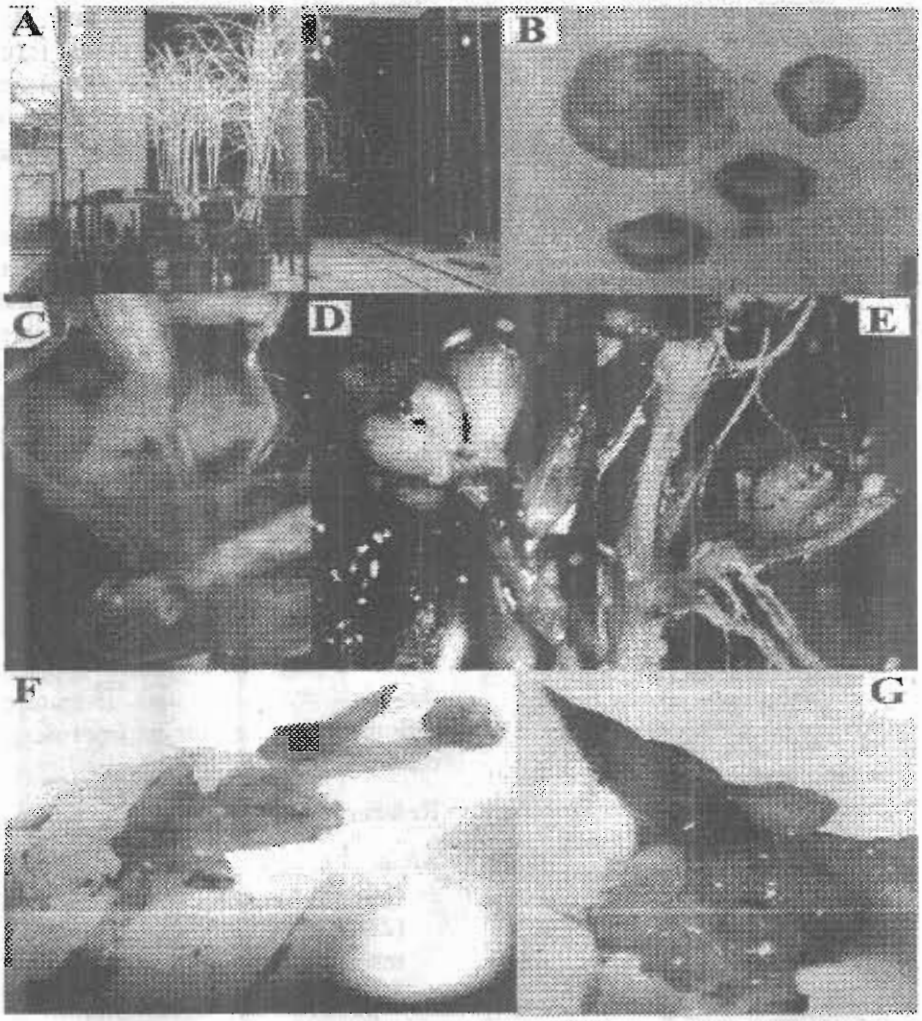

Fig. (1 A-G): Immature inflorescence culture in sugarcane. A: The donor parents during artificial flowering. B: Multicellular microspore after 3 weeks of culture. C: Micro-calli breaking out the floweret. D-E: Responded sugarcane flowerts exhibited calli proliferated from the anthers. F: Responded branch of immature inflorescence exhibited calluses proliferated from the anthers. G: In some cases calli initiated from the articulation of a rachis and the base of a pedicellate spikelet.

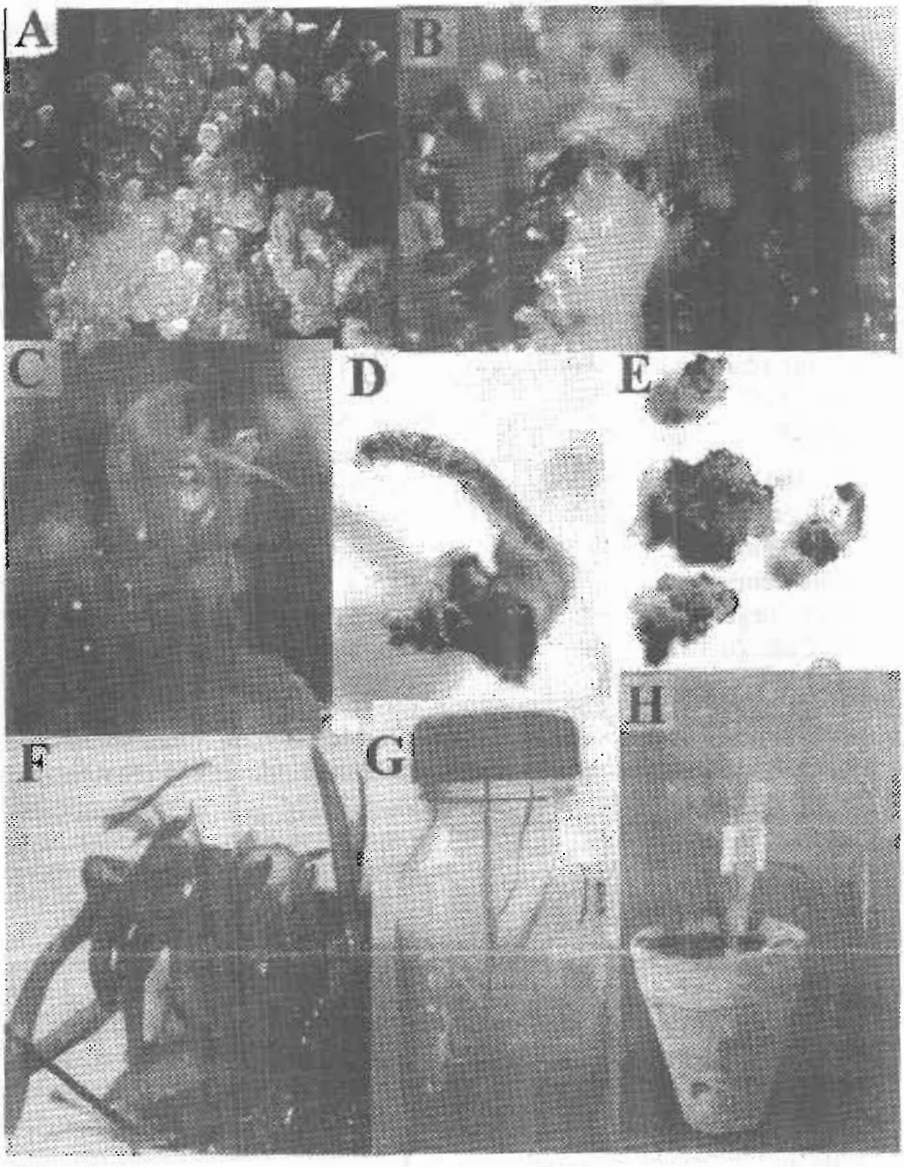

Fig (2 A-H): The development of sugarcane plants from immature inflorescence. A: Embryogenic calli derived from anthers. B and C: The germination of embryo like structures. D and E: sugarcane embryoids exhibiting shoot and root primordia. F and G: Regenerated sugarcane shoots and plantlets having shoot and roots. H: Immature-inflorescence drived sugarcane plant grown in pots after 6 months of culture.

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دور العوامل الوراثية وغير الوراثية في تكشف النباتات من النورات الزهرية غير الناضجة في قصب السكر وتقييم النباتات المتكشفة

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تم في هذا البحث استخدام ٣٥ تركيب وراثي من أصناف قصب السكر لدراسة دور العوامل الوراثية وغير الوراثية في تكشف النباتات بواسطة طريقة زراعة النورات الزهرية غير الناضجة والمستخدمة في تكشف النباتات الأحادية. وقد استهدف البحث أيضا تحديد الأصناف ذات الاستجابة العالية لهذه الطريقة وكذلك تقييم النسل الخضري للنباتات المتكشفة بالنسبة لبعض الصفات الاقتصادية الهامة. تم تصميم التجربة لدراسة تأثير التراكيب الوراثية ، المعاملة التمهيدية بالبرودة ، ٨ طرز من البيئات الغذائية وكذلك نوعين من الإزهار (طبيعي أو مستحدث) علي طريقة زراعة النورات الزهرية غير الناضجة. أوضح الفحص المجهري أن خلايا الميكروسبور تنقسم داخل المتوك مكونة كتل متعددة الخلايا تنمو مكونة مشابهات الأجنة والتي تتكشف في النهاية إلى نباتات. وبالإضافة إلي ذلك فان هناك بعض النباتات تكشفت من الأنسجة الخضرية (الجسمية) للنورات الزهرية المنزرعة.

أوضحت الدراسة أن تكشف النباتات يمكن الحصول علية من زراعة النورات الزهرية غير الناضجة والناتجة من الإزهار الطبيعي أو الصناعي علي السواء ، إلا أن معدل الاستجابة يتوقف علي التركيب الوراثي للنباتات المستخدمة والعوامل غير الوراثية مثل المعاملة التمهيدية بالبرودة والبيئة الغذائية المستخدمة. وجد أن البيئتين BAT ، L كانتا اكثر مناسبة لتكوين الكالوس الناتج من زراعة النورات الزهرية غير الناضجة. وجد أن معدل التكشف كان مستقلا عن كل من عدد الأفرع بكل كالوس و نسبة تكوين الجذور مما يدل علي انهما تحت ميكانيكيات منفصلة من التحكم الوراثي.

أوضحت الدراسة أن النسل الخضري لبعض الكلونات المتكشفة قد تفوق علي آبائها في عدد من الصفات المحصولية وخاصة المحصول التقديري. ويستنتج من ذلك انه باستخدام طريقة زراعة النورات الزهرية غير الناضجة يمكن الحصول علي كلونات من قصب السكر متفوقة علي آبائها في بعض الصفات المحصولية الهامة.