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Full Length Research Paper

Effect of genotype on callus induction and plant regeneration from leaf explants of sugarcane (*Saccharum* sp.)

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Nine sugarcane genotypes (CP59-73, CP63-588, CP80-314, SP71-1081, F160, L62-96, CP70-321, CP57-614 and Clone III) were evaluated for their callus induction capacity, embryogenic callus production and plant regeneration ability. Leaf cylinders were used as explants using Murashige and Skoog (MS) based medium supplemented with 3 mg l^{-1} 2,-4 dichlorophenoxyacetic acid. Plant regeneration was accomplished on hormone free modified MS medium supplemented with casein hydrolyzate. The genotypes tested showed high callus induction percentage (69 to 95%) and high embryogenic callus percentage (60 to 100%). These genotypes also showed excellent regeneration capacities, with regeneration percentages ranged between 88 and 100%. Significant differences were observed between genotypes for callus induction capacity, embryogenic response and plant regeneration ability indicating that these criteria are genotype dependent. Plant regeneration ability is highly correlated with embryogenic callus production. The *in vitro* regenerated plants were successfully rooted and well acclimatised in growth cabinet conditions.

Key words: Tissue culture, embryogenic callus, genotypic variation, plant regeneration, sugarcane, leaf explants.

INTRODUCTION

Sugarcane (*Saccharum sp.*) is the source of 65% of sugar production in the world (Alam et al., 1995). Unfortunately, the production of this crop is restrained by several diseases and abiotic stresses such as salinity, drought and freezing. The improvement of sugarcane plant resistance to these stresses is of great importance. *In vitro* selection of favorable somaclonal variant strains from callus culture is a supplementary tool to traditional breeding for production of stress-resistant plants (Larkin and Scowcroft, 1981; Dix, 1993; Ashraf, 1994). The introduction of a given genotype in *in vitro* selection program depends on its aptitude to *in vitro* culture, essentially to embryogenic callus induction and plant regeneration.

The success of *in vitro* culture depends mainly on the growth conditions of the source material (Caswell et al., 2000; Delporte et al., 2001), medium composition and culture conditions (Saharan et al., 2004) and on the genotypes of donor plants. Among those factors, the genotype appears to be important factor influencing the efficiency of *in vitro* culture. In *Triticum,* for the explants with same age and the same growth regulator combination, callus production and plant regeneration capacity depend essentially on genotype (Arzani and Mirodjagh, 1999; Zale et al., 2004). The same results were reported in *Oryza sativa* (Hoque and Mansfield, 2004) and *Primula* ssp. (Schween and Schwenkel, 2003).

In sugarcane, little is known about the importance of genotype of *in vitro* culture ability. In this plant, except for the work of Burner (1992) who studied the response of three sugarcane cultivars to callus production and plant regeneration *in vitro* using mature caryopses as explants

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and revealed that genotype affected callusing response, data concerning the importance of genotype on *in vitro* response of sugarcane are scarce. In our previous study using three sugarcane cultivars, we have reported that callus production ability in sugarcane is genotype dependent (Gandonou et al., 2005).

The purpose of this study was to test the ability of nine cultivars of sugarcane to callus induction, embryogenic callus production and plant regeneration using leaf explants in order to determine genotypic influences within *in vitro* response in sugarcane and to identify the cultivars with high regeneration capacities for *in vitro* selection programs.

MATERIALS AND METHODS

PLANT MATERIALS

The nine sugarcane (*Saccharum sp*.) commercial cultivars were obtained from the Technical Center of Sugars Cultures (C.T.C.S.), Morocco. These varieties are: CP59-73, CP63-588, CP80-314, SP71-1081, F160, L62-96, CP70-321, CP57-614 and Clone III. Stalk segments were surface disinfected with 70% ethanol and sown in pots containing soil in greenhouse. Pots were irrigated every two days with tap water. After germination, sugarcane plants were grown in the same conditions until approximately 6 months.

CALLUS INDUCTION

The explants used for callus induction are leaf cylinders provided from the sheath of the three youngest sheets. The basal part of the stem (constituted by the sheath of leaves) was surface sterilized for 10 min in 0.03% mercuric chloride supplemented with Tween 80, followed by three rinses with sterile distilled water (10 min each). After drying on sterile filter paper, leaf cylinders were aseptically placed on based MS (Murashige and Skoog, 1962) medium supplemented with 3 mg l^{-1} 2,4 Dichlorophenoxyacetic acid and 30 g l^{-1} sucrose. The pH was adjusted to 5.8 with 0.1 N NaOH and all media were solidified with 8 g l^{-1} agar before autoclaving during 20 min at 120°C. Five explants per petri dish were cultivated and cultures were kept in dark at 25±1°C. Callus induction percentage was determined after 4 weeks.

EMBRYOGENIC CALLUS EVALUATION

Distinction between embryogenic and non-embryogenic callus was performed on the basis of callus external aspect (Nabors et al., 1983; Pellegrineschi et al., 2004). Embryogenic calli are of glossed aspect, compact, characterized by their white to cream colour and their nodular structure, while non-embryogenic callus are of wet aspect, translucent or of brownish colour. After 4 weeks of culture, the number of embryogenic calli was recorded for each cultivar. The data were expressed as a percentage of embryogenic calli per total number of calli obtained.

Plant regeneration

After two subcultures (4 weeks each), calli were transferred in bottle containing the medium of regeneration (MS modified) with 60 g l^{-1} of sucrose and 500 mg l^{-1} of casein hydrolyzate. Cultures were incubated in growth cabinet at 25±1°C under 16-h photoperiod.

Calli that regenerate plant were recorded after 5 weeks and the data were expressed as a percentage of regenerated calli per total number of calli transferred for regeneration. Considering the very high number of plant obtained per callus, plant density per callus was estimated visually.

Acclimatation of regenerated plants

The plantlets obtained were aseptically transferred to the same regeneration medium for 5 other weeks. Plantlets with at least five well-developed roots were transferred to a pot containing soil under high humidity (> 90%) by covering the plants with plastic envelops after cutting their leaves (Paulet et Glaszmann, 1994). Pots were placed in growth cabinet at 25±1°C under 16-h photoperiod.

Statistical analysis

Number of explants that induced callus, the number of embryogenic callus and the number of regenerable callus were analysed as binomial-distribution variates with a number of explants ranging between 20 and 86. Correlation coefficients between the different parameters were calculated using SAS program (SAS institute, 1992)

RESULTS AND DISCUSSION

Callus induction rate varied from 69.23 to 95.87% (Figure 1). These high callus induction percentages observed revealed the high capacity of the sugarcane cultivars tested to induce callus from leaf explants. Significant differences ($p < 0.05$) were observed between cultivars with distinct groups: Clone III and L62-96 have the highest callus induction percentage (94.87 and 93.02%, respectively) while CP80-314 has the weakest (69.23%). CP63-588, CP59-73, CP57-614, CP70-321, F160 and SP71-1081 showed an intermediary behaviour (92.85, 90, 89.53, 88.68, 83.87 and 82.5%, respectively).

These results indicated that callus induction ability are greatly influenced by the genotype and are in agreement with those reported in *Oryza sativa* (Abe and Futsuhara, 1986; Mikami and Kinoshita, 1988; Hoque and Mansfield, 2004), in *Primula* ssp. (Schween and Schwenkel, 2003) and in *Triticum* (Zale et al., 2004).

Embryogenic characteristic of callus is a very important parameter. It reveals the capacity of the callus to regenerate plant from one cell or few numbers of cells. Distinction between embryogenic and non-embryogenic callus was performed on the basis of callus external aspect as reported by Gandonou et al. (2005). In addition to these two previous types, we observed an intermediar type with a non-embryogenic tissue covered by an embryogenic tissue. This type of callus had been already observed for sugarcane (Guiderdoni, 1986) and sorghum (Mackinnon et al., 1986). For embryogenic calli percentage determination, we classified the intermediar type as embryogenic because, in further subcultures, the embryogenic tissue grows faster than non-embryogenic tissue.

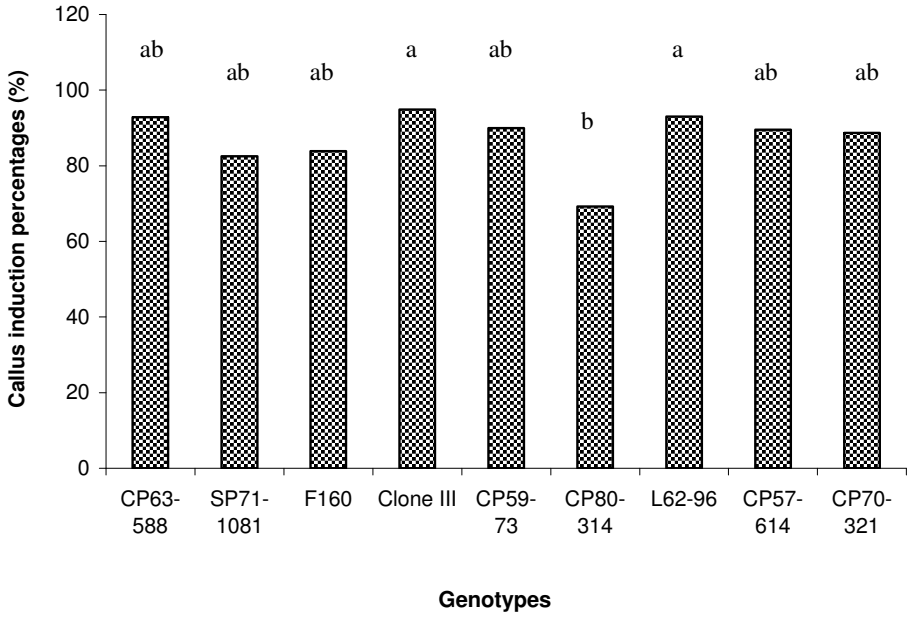

Figure 1. Callus induction percentage of nine sugarcane genotypes after 4 weeks of culture. Values with same letter are not different at $p < 0.05$.

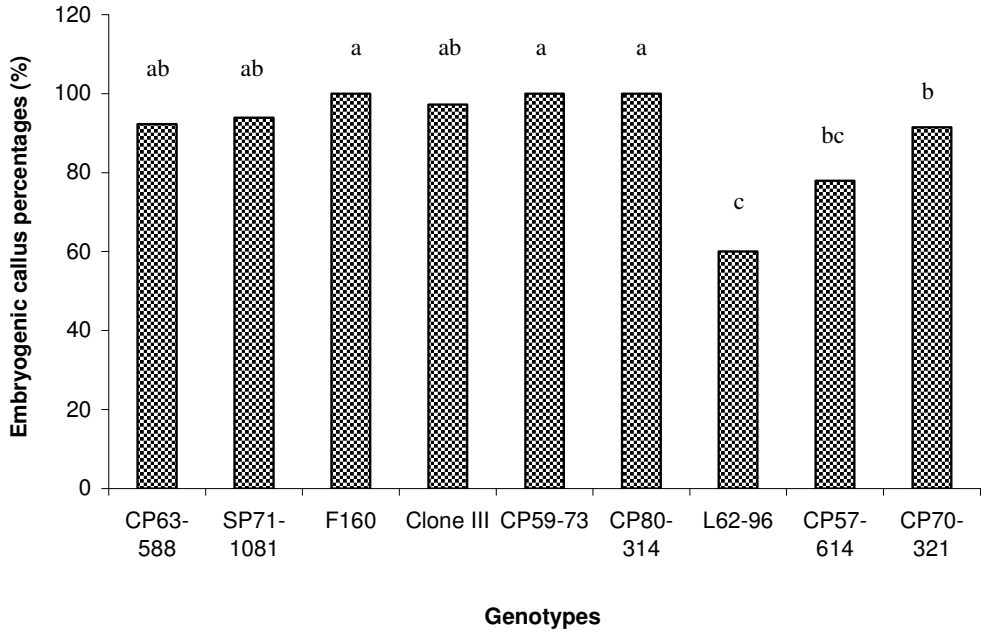

Figure 2. Embryogenic Callus percentage of nine sugarcane genotypes after 4 weeks of culture. Values with same letter are not different at $p < 0.05$.

The nine cultivars reported herein showed high embryogenic callus percentages (> 60%) which reached 100% for some cultivars (Figure 2). There were significant differences between cultivars ($p < 0.05$). F160, CP59-73 and CP80-314 had the highest embryogenic callus percentage (100%) while L62-96 had the weakest (60%). Clone III, SP71-1081, CP63-588, CP70-321 and CP57-614 were intermediary (97.29, 93.93, 92.3, 91.49

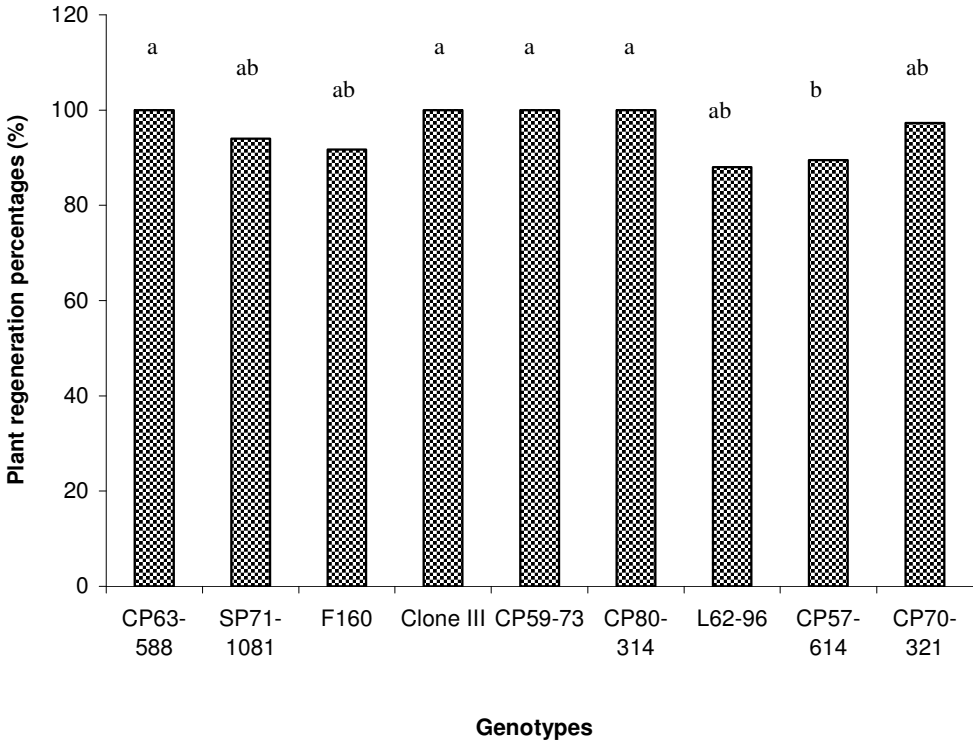

Figure 3. Percentage of plant regeneration from callus cultivated during 8 weeks (2 x 4 weeks on callus induction medium and 5 weeks on regeneration medium of nine sugarcane genotypes. Values with same letter are not different at $p < 0.05$.

Figure 4. Regenerated plants obtained from leaf explants callus of sugarcane after 8 weeks (2 x 4 weeks) on callus induction medium and 10 weeks (2 x 5 weeks) on regeneration medium (variety Clone III).

and 77.92%, respectively).

The capacity to produce embryogenic callus depends on genotype. The genotype effect on embryogenic callus ability was also reported previously in rice (Van Sint Jan et al., 1990) and in coffee (Molina et al., 2002). These studies have shown that embryogenic capacity is a stable trait since the first generations and that it would be possible to predict the embryogenic capacity of a given line by evaluating the embryogenic rate of its ancestors.

With *in vitro* breeding program, selection must be followed by plant regeneration. The choice of the potential genotypes that could be improved depends mainly on their capacity to regenerate plant. Figure 3 shows that plant regeneration percentages ranged between 87% and 100%. These results revealed the efficacy of the regeneration medium used and confirmed the results previously reported in sugarcane by Aftab et al. (1996), who also successfully used the casein hydrolyzate as a natural complex nutrient source for plant regeneration from sugarcane callus. The number of plant regenerated per callus was also very high (Figure 4).

As for callus induction and embryogenic callus production, significant differences were observed between cultivars. Thus, CP63-588, Clone III, CP59-73 and CP80-314 presented the high percentage of plant regeneration (100%). Such genotypes which have high regenerable callus frequencies are advantageous in

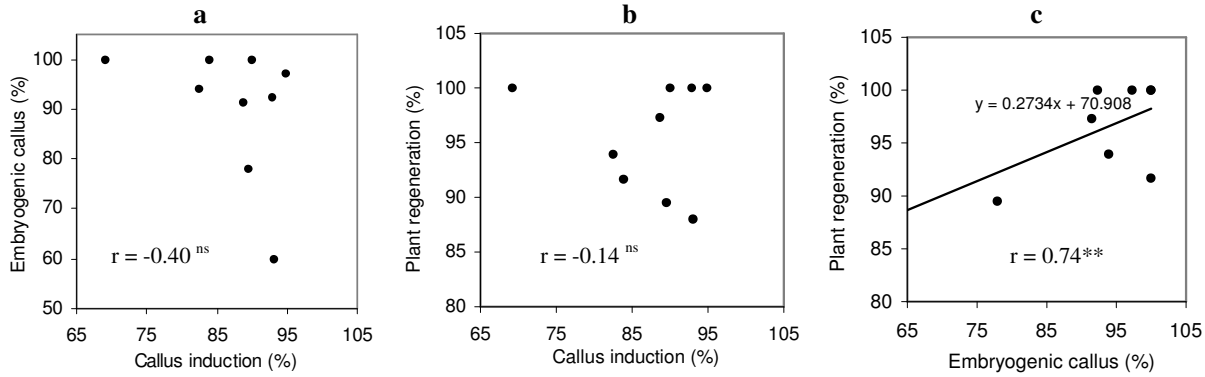

Figure 6. Relation between callus induction capacity and embryogenic callus production (a), between callus induction capacity and plant regeneration ability (b) and between embryogenic callus production and plant regeneration ability (c) in nine sugarcane genotypes. **Significant at P < 0.01; ns: non significant.

Figure 5. Acclimatized plants obtained from leaf explants callus of sugarcane after 8 weeks (2 x 4 weeks) on callus induction medium, 10 weeks (2 x 5 weeks) on regeneration medium and 10 weeks in acclimatation pots (variety SP71-1081).

tissue culture programs. L62-96 showed the weakest regeneration rate (88%). CP70-321, SP71-1081, F160 and CP57-614 presented intermediary behaviour (97.3, 93.93, 91.66 and 89.5%, respectively). These results indicated that plant regeneration ability is a genotypic character and suggested the presence of gene or block of genes involved in embryogenic callus initiation. These data confirm the results reported in *Primula* ssp. (Schween and Schwenkel, 2003), *Oryza sativa* (Hoque and Mansfield, 2004), and *Triticosecale* (Birsin et al., 2004).

The *in vitro* regenerated plants were successfully rooted on the regenerated medium used (Figure 4). Futhermore, the regenerated plants responded well to acclimatation (Figure 5).

No correlation was observed between callus induction percentage and embryogenic callus percentage (r = -0.40, value non significant at p<0.05 at degree of freedom = 7, Figure 6a) and between callus induction percentage and plant regeneration percentage (r = -0.14, value non significant at p<0.05 at degree of freedom = 7, Figure 6b). The absence of correlation between callus induction frequency and regeneration capacity of callus clearly indicated callus induction and regeneration capacity may be controlled by different mechanisms. However, high positive correlation was observed between embryogenic callus percentage and plant regeneration percentage (r = 0.74, value significant at p<0.05 at degree of freedom = 7, Figure 6c).

The high correlation observed between the ability of cultivars to produce embryogenic callus and their capacity for plant regeneration indicate that embryogenic callus percentage constitute a good index for callus ability to regenerate later on plantlets. The cultivars that presented high embryogenic callus percentages at the first weeks of culture have high chance to regenerate plants after several weeks of culture.

Our findings showed that callus induction ability, embryogenic response and plant regeneration capacity in sugarcane are significantly affected by genotype. Furthermore, the nine cultivars reported in this study responded well to *in vitro* culture especially to embryogenic callus production and plant regeneration. These results showed the importance of these varieties in

in vitro selection programs for tolerance to different stresses in sugracane.

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