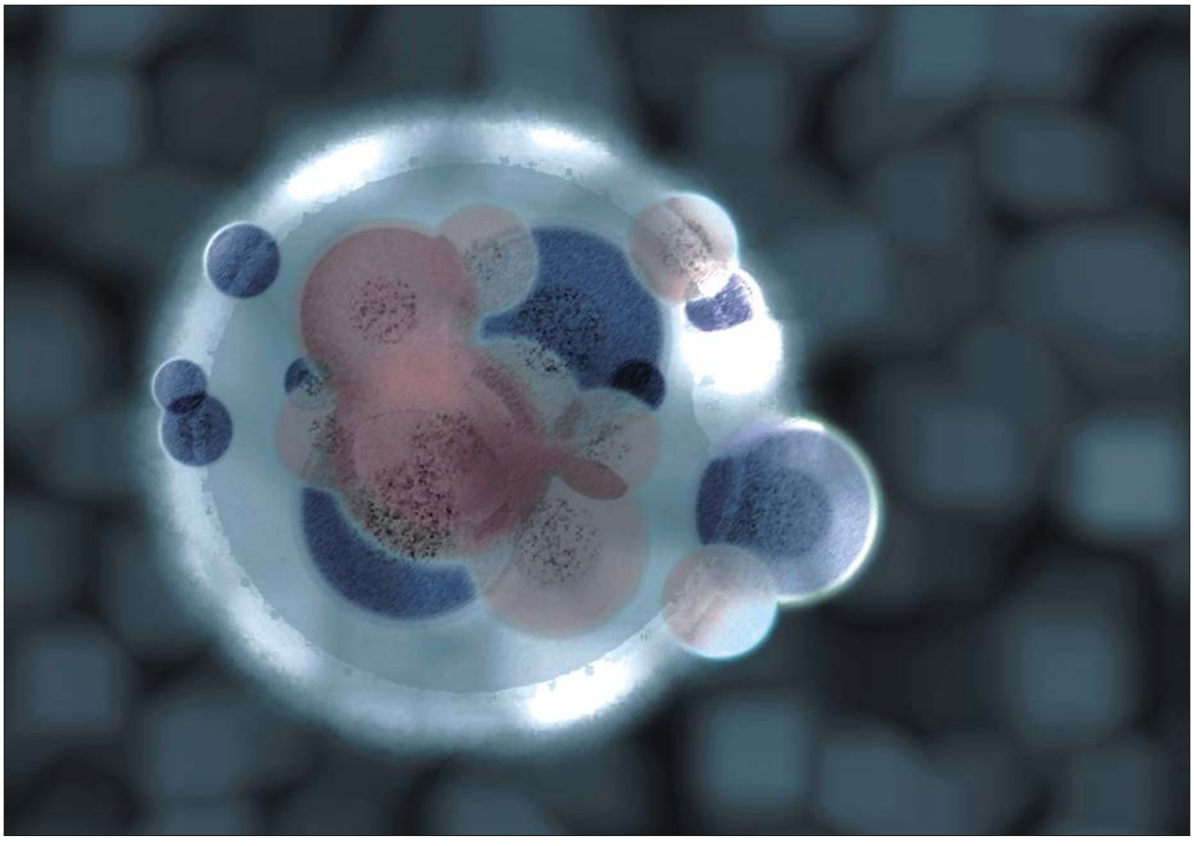

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Sulphate Supply Promotes Somatic Embryogenesis in *Theobroma cacao* L.

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Abstract: The present study examines the possibility that exogenous sulphate improve induction of cacao (*T. cacao*) somatic embryos. The investigation was conducted on five genotypes IMC67, P7, SCA6, UPA409 and IFC5. Sulphate was supplied as K_2SO_4 during the induction phase. Six induction media were defined: PCG_0 (0.000 g L^{-1}), PCG_1 (1.559 g L^{-1}), PCG_2 (3.118 g L^{-1}), PCG_3 (6.236 g L^{-1}), PCG_4 (9.354 g L^{-1}) and PCG_5 (10.913 g L^{-1}). Staminodes and petals from immature flowers buds were used as explants. In the absence (PCG_0) of K_2SO_4, there was no embryo expression from any of the five genotypes. The percentage of embryo induction increased with the sulphate content from PCG_0 (negative control) to PCG_3 (4 folds sulphate content compared to the positive control PCG_1) dependently to genotype and the explant type. In a given Petri plate, the highest percentage of somatic embryogenesis induction was obtained with PCG_2 (2 folds sulphate content compared to the positive control PCG_1) with: petals from IMC67 (61.41%) and staminodes from UPA409 (87.26%), IFC (57.35%) while Sca6 (56.85%) and P7 (30.84%) were most responsive in PCG_3. The probability of the occurrence of somatic embryos was highest in PCG_2 with staminodes from IMC67 (0.55), P7 (0.57), UPA409 (0.75) and IFC5 (0.56). Sca6 genotype displayed a probability of somatic embryos induction of 0.38 when cultured on PCG_3 medium. The embryos obtained were converted to plantlets. These results indicate that sulphate supply improves cacao somatic embryogenesis. The importance of sulphate metabolism in embryos differentiation is discussed.

Key words: Cacao, somatic embryogenesis, genotype, sulphate

INTRODUCTION

Theobroma cacao L. is a small perennial tropical tree belonging to the Malvaceae family (Whitlock *et al*., 2001). Its culture provides both economic and ecological benefits to farmers and producing countries (Despréaux, 2001). The world trade from dried cacao beans has an annual estimated value of 2.9 millions US dollars per year (Gray, 2000). Due to increasing pressures (diseases and low yielding) cacao farmers are in urgent need of high yielding and pest and pathogen resistant varieties. Selection of genotypes with desirable yields and qualities becomes an integral component of the overall strategy for yield improvement. But cacao is naturally and mainly propagated by seeds. As a consequence of this and the high heterozygosis of the crops and its allogamous character, wide genetic variation is observed among the seed-derived plants, resulting in a large proportion of low-yielding trees in a single plantation (Irizarry and Rivera, 1999). Development and practical application of fast and highly-efficient systems for vegetative propagation of superior new cacao varieties selected in breeding programs and newly identified wild germoplasm is therefore needed. For vegetative (asexual) multiplication of cacao, classical methods such as, grafting and rooted cuttings have been applied to multiply cacao tree but with limited success (Lopez-Baez *et al*., 1993). An alternative method for clonal production is somatic embryogenesis (technique of *in vitro* tissue culture) as it could enable multiplication and distribution of elite genotypes. Somatic embryos from maternal tissues were obtained. The process was later improved using staminodes and petals from immature flower buds (Lopez-Baez *et al*., 1993; Alemanno *et al*., 1996; Li *et al*., 1998). Recent advances in

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cacao somatic embryogenesis have enabled the production of large number of aseptically-grown cacao plants from flower staminodes and petal cultures (Li *et al*., 1998; Maximova *et al*., 2002). Despite the high multiplication rate, its application is limited by the long period of time from culture initiation to embryo production (6-8 months) and the relatively high cost per plant produced. Additionally, protocols used, lack reliability or have limited regeneration efficiency. Furthermore, the response of explants to somatic embryogenesis is highly genotypic and medium dependant. For these reasons, there is a need to improve protocols in order to increase the yields of micropropagated plantlets. Among the possible ways of improving somatic embryogenesis, modifications of the culture media are undoubtedly the method most commonly used. Given the large number of factors to be modified, several authors chose to consider the plant as a guide in their search for the optimal media. In that way, El Badaoui *et al*. (1996) designated a culture medium based upon mineral analysis of the plant itself to obtain better growth and multiplication rates for Solanum paludosum microcuttings as well as an increase in Solamargine production. Carman *et al*. (1988) characterised the environment during zygotic embryogenesis of wheat as a guide for designing appropriate media for somatic embryogenesis. Sossou Dangou *et al*. (2002) reported on biochemical characterizations (minerals) of cacao endosperm, that sulfate which is the primary source of sulfur for plants was in high concentration at different stages of zygotic embryo development, (for subsequent possible use for improving cacao somatic embryogenesis). In a previous study, we reported an implication of cysteine, glutathione and cysteine synthase in cacao zygotic embryogenesis (Minyaka *et al*., 2007). The same study has suggested that to improve cacao somatic embryogenesis expression, culture media might be supplemented with sulfate but the adequate form and concentration were to be determined. In the present paper, we investigate the effect of sulphate supply as K_2SO_4 on cacao somatic embryogenesis expression of five genotypes (IMC67, P7, SCA6, UPA409 and IFC5).

MATERIALS AND METHODS

Plant material: The explants used in this study were made of staminodes and petals from immature flowers buds of five cacao genotypes (IMC67, P7, SCA6, UPA409 and IFC5). Flowers buds from a single tree of each of the five *T. cacao* genotypes were simultaneously harvested (early in the morning in cold water) from CNRA (Centre National de recherche Agronomique) experimental farm at Bengerville (Abidjan, Côte d'Ivoire), surface-sterilized by immersion in 1% (w/v) calcium hypochlorite for 20 min, followed by four times rinsing with sterilized-distilled water to remove all traces of sterilant. Staminodes and petals are extracted from the upper part of surface-sterilized flowers buds after dissection. These explants are then placed on culture media into Petri plates.

Culture media and growth conditions: To test the effect of sulfur supply on cacao somatic embryogenesis, K_2SO_4 salt (used in DKW mineral complex) was used as regulatory factor. Six Primary Callus Growth (PCG) media, different one another in their K_2SO_4 content were defined (Table 1). PCG media were defined using DKW basal salts as described by Driver and Kuniyuki (1984) and Tulecke and McGranahan (1985). Each primary callus growth medium was supplemented with 250 mg L^{-1} glutamine, 100 mg L^{-1} myo-inositol, 1 ml L^{-1} DKW vitamin stock (100 mg mL^{-1} myo-inositol, 2 mg mL^{-1} thiamine-HCl, 1 mg mL^{-1} nicotinic acid and 2 mg mL^{-1} glycine), 20 g L^{-1} glucose, 18 μM 2,4-D and 45.4 nM TDZ. The pH of all PCG media was adjusted at 5.8 by using KOH (0.1 N) or HCl (0.1 N) before adding 0.2% (w/v) phytagel, the gelling agent. Media were finally dispensed into sterilized Petri plates after autoclaving for 20 min at 1 bar pressure and 121°C. The five genotypes explants were cultured simultaneously in the PCG media. Each Petri plate contained 35 staminodes and 35 petals in two separated sets (Fig. 1). Experiments were repeated four times with three replicates Petri plates at each culture initiation (that is, for a giving culture initiation we have, three Petri plates per genotype per PCG medium). Petri plates are then sealed and cultures incubated in darkness at 25±1°C for 14 days.

After 14 days in PCG media, explants are transferred into a secondary callus growth (SCG) medium and incubated in the same conditions as previously for 14 additional days. SCG consisted of DKW basal salts, supplemented with 0.5 ml L^{-1} DKW vitamin, 20 g L^{-1} glucose, 9 μM 2,4-D, 250 μg L^{-1} Kinetine and 0.2% (w/v) phytagel. The pH of the SCG medium was adjusted at 5.7. After staying in SCG, floral tissue-derived callus are then

Table 1: K_2SO_4 content in a given primary callus growth medium (PCG)

	Primaries culture growth media					
	PCG_0 (negative control)	PCG_1 (positive control)	PCG_2	PCG_3	PCG_4	PCG_5
K_2SO_4 (g L^{-1}) content in a PCG medium	0.000	1.559	3.118	6.236	9.354	10.913

transferred for 21 days in Embryos Development (ED) medium. Two additional and successive subcultures of explants were done in ED at intervals of 21 days. ED medium is made of DKW basal salt supplemented with 1 mL DKW vitamin, 30 g L^{-1} sucrose, 1 g L^{-1} glucose and 0.2% (w/v) phytagel. The pH of ED medium was 5.7. Torpedo-shape somatic embryos were then separated from callus and also cultured in ED medium. Mature somatic embryos were selected for embryo conversion and plant regeneration following the protocol described by Li *et al.* (1998) with slight modifications.

Data collection: After 91 days of culture initiation, the number of embryos expressed per genotype per medium, per Petri plate and type of explant (staminode or petals) was collected. The responsive (ratio of staminodes or petals-callus producing embryo over total staminodes or petals-callus in a Petri plate multiply by 100) of embryogenesis of giving explant from a giving PCG medium were also taken down for each genotype. Additionally, the percentage of embryogenesis (PE) and the occurrence probability of embryogenesis (OP) of each type of explant from each PCG medium were also calculated for individual genotype. In order to compare somatic embryogenesis potential of genotypes in a giving PCG medium, a global percentage of somatic embryogenesis (PE) was calculated for each genotype and type of explant in a given PCG medium.

$$\text{Responsive} = \frac{\text{Total staminodes or petals in a giving Petri plate producing embryos}}{\text{Total staminodes or petals in a giving Petri plate}} \times 100$$

$$\text{PE} = \frac{\text{Total somatic embryos from a giving genotype, explant-type and PCG medium}}{\text{Total somatic embryos from a giving genotype, explant-type and the six PCG media}} \times 100$$

$$\text{OP} = \frac{\text{Total Petri plates having somatic embryos from a giving genotype, explant-type and PCG medium}}{\text{Total Petri plates having somatic embryos from a giving genotype, explant-type and the six PCG media}} \times 100$$

$$\text{OP} = \frac{\text{Total somatic embryos from a giving genotype, explant-type and PCG medium}}{\text{Total somatic embryos from the six genotype, explant-type and the six PCG media}} \times 100$$

Statistical analysis: All data analysis was made using SPSS software (version 10.0). The comparison of averages within variables was carried out by the analysis of variances (ANOVA) using the test of Student, Newman and Keuls.

RESULTS

Somatic embryos differentiation and plant regeneration: When cultured in PCG media, staminodes and petals enlarge within the first 7 days of culture. Calli were observed on some explants after 8-14 days of incubation (Fig. 1a). Callogenesis is consistent when explants are transferred on SCG medium for 14 days. Differentiation process (roots differentiation for instance) is appreciable during the first stay of explants in ED medium. The first week of the second stay of morphogenetic structures on ED medium results in differentiation of somatic embryos for the most precocious genotypes, medium or explant but, for the less precocious one, somatic embryos appear later on. These embryos are globular, heart-shape, torpedo or cotyledonary (Fig. 1b-e). Cotyledonary embryos are leaded to plantlets regeneration (Fig. 1g, h).

Evaluation of somatic embryogenesis variables

Responsive: The responsive of explants ranged from 0 to 55.56% depending to the sulfate (K_2SO_4) content in a PCG medium, genotype and the explant (Table 2). In the absence of K_2SO_4 (sulfate) in PCG medium, explants (morphogenetic structures) do not differentiated embryos whatever the genotypes and the explant types (staminode or petal).

In the presence of sulfate, the responsive of the same explant-type from a single genotype is widely variable.

IMC67 seems to be most responsive when staminodes are from PCG_2 (K_2SO_4 = 3.118 g L^{-1}) medium but below or above this sulfate content in the PCG medium, the responsive of explants are reduced. Petals of this genotype also behave like its staminodes.

PCG_1 (1.559 g L^{-1}) and PCG_2 appear to be the most appropriate media for embryos expression for P7 explants however, PCG_2 seems to be more suitable (for P7) than PCG_1 when the responsive of the two types of explant (staminode and petal) is taking together. The absence of embryos expression of P7 explants from PCG_4 (9.354 g L^{-1}) or PCG_5 (10.913 g L^{-1}) might suggest that, these PCG contents in K_2SO_4 inhibit somatic embryogenesis of P7 explants.

Sca6 explants are responsive in PCG_1, PCG_2, PCG_3 and PCG_4 but; PCG5 might be toxic for somatic embryogenesis of Sca6 explants. Between the four above responsive PCG media, PCG3 looks to be the most responsive for the Sca6 explants.

UPA409 and IFC5 respond mostly to somatic embryogenesis stimuli when explants are from PCG_2 medium.

Table 2: Responsive (%) of explants from a given PCG medium

	IMC67		P7		Sca6		UPA409		IFC5	
Media	Staminodes	Petals	Staminodes	Petals	Staminodes	Petals	Staminodes	Petals	Staminodes	Petals
PCG_0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PCG_1	15.38-31.03	6.25	12.5-36.36	8.82-15.15	11.76-38.89	12.9-14.29	5.26-16.13	5.71	5.26	5.71
PCG_2	33.33-42.86	9.1-18.18	18.18-35	9.52-17.39	13.04-27.78	13.04-37.5	22.58-55.56	10.71-6.67	11.76-25	11.11-25
PCG_3	12.3-26.31	9.10	31.58	12.90	25-34.62	35.29-50	0.00	6.67	0.00	0.00
PCG_4	11.11-15.38	5.88-6.06	0.00	0.00	13.33-22.86	17.86-36	30.43	0.00	22.22	3.03
PCG_5	8.5-16	9.68	0.00	0.00	0.00	0.00	30.00	0.00	6.25	11.76-7.86

Fig. 1: Different steps of regeneration via somatic embryogenesis in *T. cacao*, (a) callogenic staminodes, (b) somatic embryos clusters (repetitive secondary somatic embryos), (c, d, e) cotyledonary embryos, (f) somatic embryo with radicle and (g h, i) growing plantlets. Bar = 1 cm

Percentage of embryogenesis (PE): The comparison of the averages values of the percentage of somatic embryogenesis, between the six PCG media, was done for each genotype tested and explant-type.

The results have indicated that, with the staminodes of the five genotypes tested, there is a significant increase in the percentage of somatic embryogenesis from PCG_0 to PCG_2. Above PCG_2 ($K_2SO_4 = 3.118$ g L^{-1}) the percentages of somatic embryogenesis decrease significantly for all genotypes. There was 3.39, 5.14 and 1.19 folds decrease (for IMC67, P7 and Sca6, respectively) in the percentage somatic embryogenesis from PCG_2 to PCG_3. Reversely, there was 1.73, 5, 1.59, 11.28 and 24.35 folds increase (for IMC67, P7, Sca6, UPA409 and IFC5, respectively). Therefore, IMC67 (49.195±1.489%), P7 (71.397±1.945%), Sca6 (32.412±0.585%), UPA409 (82.717±0.850%) and IFC5 (71.760±0.897%) have exhibited highest percentages of somatic embryogenesis when staminodes-derived morphogenetic structures were from PCG_2 (Fig. 2).

Petals of the five genotypes in the same conditions as above have give similar results as those observed with staminodes. The peak of the percentage of somatic embryogenesis was registered at PCG_2 for IMC67 (61.417±0.796%), UPA409 (87.263±1.294%) and IFC5 (57.357±1.248%). But for P7 (30.847±0.835%) and Sca6 (56.850±1.096%) the peak was at PCG_3. However, there is

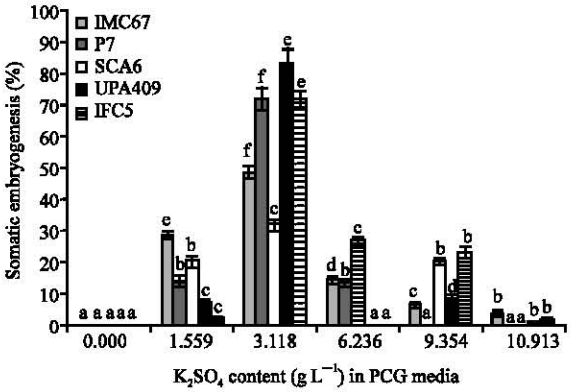

Fig. 2: Percentage of somatic embryogenesis from staminodes for each genotype in the six PCG media. The comparisons of the average percentages are done within a genotype and between the six PCG media (horizontal comparison). Values that are significantly different (at the 5% level of significance) between media for a single genotype, are indicated with different letter(s)

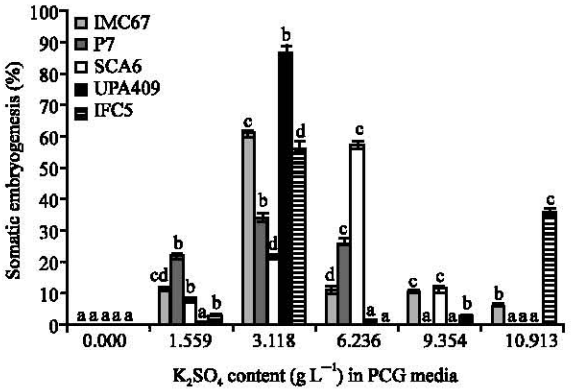

Fig. 3: Percentage of somatic embryogenesis from petals for each cacao genotype in the six PCG media. The comparisons of the average percentages are done within a genotype and between the six PCG media (horizontal comparison). Values that are significantly different (at the 5% level of significance) between media for a single genotype, are indicated with different letter(s)

not a significant difference between the percentage of somatic embryogenesis at PCG_2 and PCG_3 for petals-derived morphogenetic structures of P7 (Fig. 3).

Occurrence probability (OP): The occurrence probability of somatic embryogenesis in six PCG media was evaluated for each genotype and explant-type. For IMC67

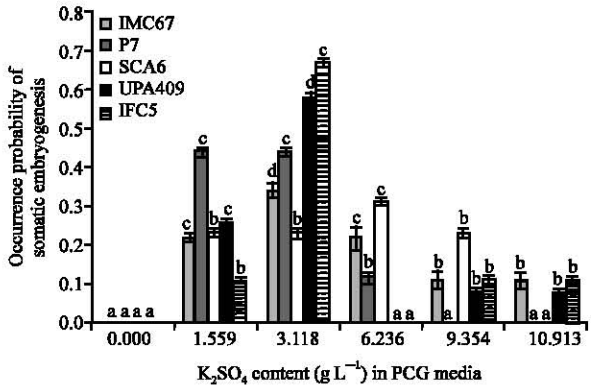

Fig. 4: Occurrence probability of somatic embryogenesis from staminodes for each cacao genotype in the six PCG media. The comparisons of the average percentages are done within a genotype and between the six PCG media (horizontal comparison). Values that are significantly different (at the 5% level of significance) between media for a single genotype, are indicated with different letter(s)

staminodes, somatic embryogenesis occurs mainly when morphogenetic structures are from PCG_2 (OP = 0.345±0.012). There is not a significant difference between the occurrence probability of somatic embryogenesis of PCG_1 (OP = 0.224±0.007) and PCG_3 (OP = 0.222±0.020). The OP of IMC67 staminodes remains constant between PCG_4 (OP = 0.110±0.018) and PCG_5 (OP = 0.11±0.0391). With P7 staminodes, the highest values of OP was obtained when explant were from PCG_1 (OP = 0.44±0.043) and PCG_2 (0.450±0.012). Sca6 staminodes showed the highest value of OP (0.312±0.012) with the explants from PCG_3. However OP of Sca6 staminodes from PCG_1 (0.231±0.016), PCG_2 (0.230±0.021) and PCG_4 (0.232±0.027) were not significantly different. As with IMC67 staminodes, the highest values of OP for UPA409 (0.577±0.017) and IFC5 (0.672±0.028) staminodes were registered at PCG_2 (Fig. 4).

The same analysis conducted with petals of the five cacao genotypes studied has indicated that, somatic embryogenesis mostly occurred when IMC67 (OP = 0.550±0.018), P7 (OP = 0.575±0.031), UPA409 (0.752±0.017) and IFC5 (OP = 0.560±0.021) petals were from PCG_2. But for Sca6 petals, PCG_3 (OP = 0.381±0.008) seems to be the most appropriated medium for somatic embryogenesis to occur (Fig. 5).

Global percentage of somatic embryogenesis (PE): This variable was estimated in order to compare the somatic embryogenesis potential of the five genotypes in a given PCG medium. In PCG_1, the staminodes derived-

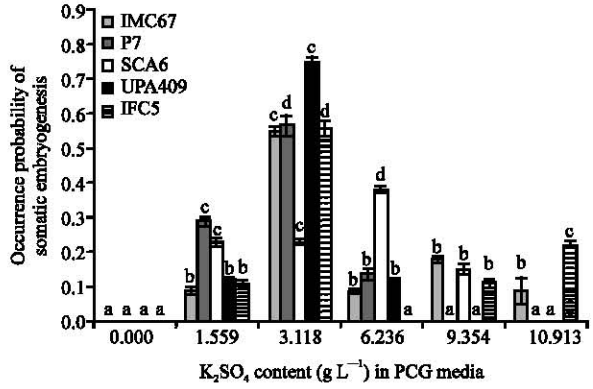

Fig. 5: Occurrence probability of somatic embryogenesis from petals for each cacao genotype in the six PCG media. The comparisons of the average percentages are done within a genotype and between the six PCG media (horizontal comparison). Values that are significantly different (at the 5% level of significance) between media for a single genotype, are indicated with different letter(s)

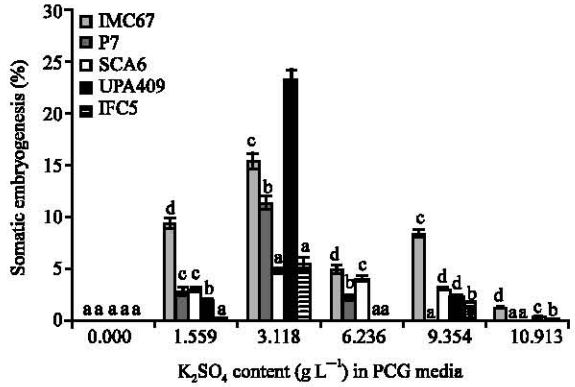

Fig. 6: The evolution of the percentage of embryogenesis from staminodes of a genotype when the five genotypes are taken together in the same PCG medium. The comparisons of the average percentages are done between the five genotypes in a given PCG medium (medium comparison). Values that are significantly different (at the 5% level of significance) between genotypes for a single PCG medium, are indicated with different letter(s)

morphogenetic structures of the five genotypes studied respond together to somatic embryo stimuli. In this medium, IMC67 (PE = 9.375±0.533) exhibited the highest PE and IFC5 registered the lowest value of PE (0.242±0.035). There was no significant difference between

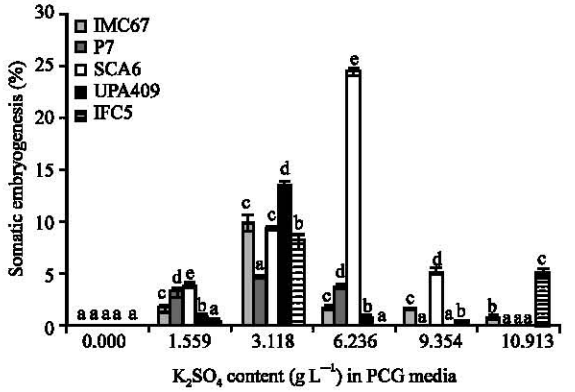

Fig. 7: The evolution of the percentage of embryogenesis from petals of a genotype when the five genotypes are taken together in the same PCG medium. The comparisons of the average percentages are done between the five genotypes in a given PCG medium (medium comparison). Values that are significantly different (at the 5% level of significance) between genotypes for a single PCG medium, are indicated with different letter(s)

the PE values of P7 (2.930±0.49) and Sca6 (3.037±0.180) however, the PE value of the later was significantly higher than that of UPA409 (2.065±0.074). PCG_2 was the most suitable medium for the staminodes explants of the five genotypes since the peak of PE' was registered in that medium. Nevertheless, PE values of the five genotypes were significantly different one another (Fig. 6).

The same variable (PE) was evaluated with petals derived-morphogenetic structures of the five genotypes. In PCG_1 and PCG_2, PE is significantly different between the genotypes in the same medium. Instead to exhibited the peak of PE at PCG_2 (as it was observed with staminodes), Sca6 petals showed the most important value of PE' (24.410±0.334) at PCG_3. As with staminodes, PCG_2 remained the suitable medium for IMC67 (PE' = 9.885±0.865), P7 (PE = 4.745±0.196), UPA409 (PE' = 13.582±0.450) and IFC5 (PE' = 8.272±0.550) petals (Fig. 7).

DISCUSSION

Many studies in the recent years spotlighted on improving media for cacao somatic embryogenesis focused their investigations on organic compounds such as growth regulators (Li *et al*., 1998). But, minerals (macro or micro-element) and particularly sulphate (or sulphur) was never tested as regulated factor in order to optimize cacao somatic embryogenesis expression. This study was

focused on a hypothetical promoting effect of sulphate in cacao somatic embryogenesis expression.

Present results have showed that, somatic embryogenesis and plant regeneration was obtained in vitro with all the five genotypes tested but, somatic embryogenesis expression was differed from one genotype to another and, from one medium to another for a given genotype and explant-type. The absence of sulphate supply has resulted in the absence of somatic embryos differentiation from all tested-genotypes and both types of explants used. This might suggest an importance sulphate (sulphur) for somatic embryos differentiation in *T. cacao*. In higher plants, sulphate is almost always underwent reduction-assimilation pathway that lead mainly to such cysteine, a sulphurous organic compound. According to the results of Xu and Møller (2004), cysteine is a key metabolite to avoid zygotic embryo lethality in *Arabidopsis thaliana*. These authors reported that deficiency in cysteine or AtNAP7 (a protein implicated to the biosynthesis of Fe-S clusters) prevent zygotic embryogenesis to go beyond globular stage. Moreover, sulphur in Fe-S clusters is directly provided by cysteine and, the synthesis of the later depends to the sulphate availability (Urbina *et al*., 2001; Smith *et al*., 2001; Ding *et al*., 2005).

In order to estimate the somatic embryogenesis expression, the variable responsive was calculated. The results obtained showed a wide dispersion of this variable in single medium for given explant-type from each genotype tested. This observation might indicate that, beside the medium composition, physiological statute of explants at cultures initiation might also affects consistently responsive of morphogenetic structures. This suggestion is supported by the fact that, within the same Petri plate, some staminodes or petals-derived morphogenetic structures differentiated somatic embryos and others not. However, we observe an increase of the variable responsive as sulphate content in the Primary Callus Growth medium was increased up to certain value depending to genotype and explant-type. Therefore, some genotypes seemed to be most responsive when the exogenous sulphate content was two folds compared to the positive control but others were most responsive when this content was four fold compared to the positive control. This finding indicates that, sulphur or sulphate supplying raise the responsive of both genotypes and explants-type. But, above certain content, exogenous sulphate seems to repress somatic embryogenesis. This could explain the absence of somatic embryos differentiation from certain genotypes in some high contents of exogenous sulphate.

In addition to responsive, the percentage of embryogenesis which considers the numbers of somatic embryos was also evaluated. This variable was less dispersed. The analysis of the percentage of embryogenesis also reveals the stimulating effect (up to certain threshold) of exogenous sulphate supplying in all genotypes tested. This result shows that during somatic embryogenesis process, exogenous sulphate promotes not only the number of explants-producing-embryos but also, the number of embryos differentiated. When cysteine is synthesized from S-assimilation-reduction process, the sulphur atom of cysteine is directly used in the biosynthesis pathways leading others organic sulphurous compounds such as thiamin and biotin (Begley *et al*., 1999). In a study conducted on date palm (*Phoenix dactylifera* L.) (Jameel and Al-khayri, 2001) thiamin and biotin supplying improve somatic embryogenesis expression in that plant. Additionally these authors reported that, the absence of thiamin results in failure of callusing and, therefore the absence of somatic embryos differentiation. Accordantly, our results therefore suggest that, in *T. cacao*, exogenous sulphate supply might be necessary for the synthesis of cysteine. And this sulphurous metabolite is further used for synthesis of Fe-S clusters, thiamin or biotin which contributes in somatic embryogenesis process in cacao.

The results obtained with Occurrence Probability variable (which show up the frequency of somatic embryogenesis) were similar to those of the percentage of embryogenesis. Thus, sulphate supply not only affects positively the responsive or the percentage of embryogenesis, but also the chance for somatic embryogenesis to happen.

Global percentage of somatic embryogenesis indicates that, beside the promoting outcome of exogenous sulphate in somatic embryogenesis, genetic factor also influence this process in cacao since, in the optimal medium (obtained in this study) there was a significance between genotypes for this variable whatever the explant-type.

In conclusion this has shown that, sulphur content in culture medium is an important factor in cacao somatic embryogenesis. This study additionally highlights the fact that sulphur supply as K_2SO_4 significantly improves cacao somatic embryogenesis for the five genotypes tested. To optimize cacao somatic embryogenesis expression, it seems that the sulphate content of two or three fold, compare to the positive control appear to be most adequate.

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