PLANT REGENERATION THROUGH TISSUE CULTURE OF PEAR MILLET (*Pennisetum Glaucum* (L) R.)

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ABSTRACT.

Many problems were kipt out with immature embryos and shoot explants pieces used ; principally : Calli were initiated in plant flowering periode (every 110 days), and the low percentage of embrogenesis what either the source used. So for *Pennisetum glaucum*, new embryogenic calli sources were explored from calli initiation to fertile plant regeneration achieving. About 90 % of dissected shoot apices, seedlings and seeds developed calli. From meristems position, 6 mm of shoot and 20 mm of root developed 50 to 100 % of calli. No embryogenic calli were obtained from root. More than 80 % of dissected apices led to embryogenic calli. Maintained on MS(1. 1. 2,5), MS(5) and N6(1.100.25) culture media, calli embryogenic potential and fertile plants regeneration were conserved for more than 12 months. Characteristics of regenerated plants were similar to control. It appears that dissected shoot apex was a new appropriate tool in tissue culture.

Key words: Tissue culture, culture medium, callus induction, shoot apex, *Pennisetum glaucum*, Côte d'Ivoire.

RESUME.

REGENERATION DE PLANTE A PARTIR DE LA CULTURE DE TISSUS DE MIL (Pennisetum glaucum (L) R.)

Les difficultés liées à utilisation de l'embryon immature et des explants de tige dans les cultures de tissus sont nombreuses ; dont l'initiation de cal qui ne se fait que pendant la floraison, pour l'embryon immature, soit chaque 110 jours, et le faible pourcentage de l'embryogenèse. Pour Pennisetum glaucum *, de nouvelles sources de cals embryogènes ont été explorées depuis l'initiation de cal jusqu'à l'obtention de plante fertile. Environ 90 % des méristèmes apicaux, des germes et des grains ont développé des cals. A partir des méristèmes, seuls 6 mm de tige et 20 mm de racine ont développé des cals avec un taux de 50 à 100 %. La racine n'a développé aucun cal embryogène. Plus de 80 % des cals de méristèmes apicaux étaient embryogènes. Les cals maintenus sur MS(1.1.2,5), MS(5) et N6(1.100.25), sont restés embryogènes et ont produit des plantes fertiles pendant au moins 12 mois. Les plantes régénérées ont des caractéristiques semblables aux plantes témoins. Il ressort que le méristème apical est un nouveau matériel approprié pour la culture de tissus.*

Mots clés: *culture de tissus, milieu de culture, induction de cal, méristème apical, Pennisetum glaucum*, *Côte d'Ivoire.*

INTRODUCTION

Achieving crop improvement through genetic biotechnology requiered tissue culture with subséquent regeneration of fertile plants. The first investigations, of tissue culture, on Hordeum vulgare (KOBLITZ and Saalbaack 1976 ; DALE and Deambrogio 1979) and on Lolium multiforum (DALE 1977) have shown that graminea dissected shoot apex was not appropriate for tissue culture because it necrotized.

During the last decade, new sources of calli were than explored to improve cereal and grass tissue culture. Today, for the important cereals and grasses, plant regeneration was reported : With somatic embryogenesis from young inflorescences and mature seeds of *Panicum* maximum (CHIN-YI and Vasil 1981), *Triticum aestivum* (OZIAS and Vasil 1982), *Paspalum scrobiculatum* (NAYAK and Sen 1989), *Oryza sativa* (Yao, 1998) and from young leaves of *Pennisetum purpureum* (Haydu and Vasil 1981 ; RAJASE-KARAN *et al,* 1987). Concerning *Pennisetum glaucum*, plant was regenerated with embryoid formation from immature embryos (VASIL and Vasil 1981, 1982a) ; as well as young inflorescences (VASIL and Vasil 1982b), or from excised shoot explant pieces of 4 to 5 days old plants (BOTTI and Vasil 1983 ; Lambé *et al* 1999).

In immature embryos, young inflorescences and shoot explants pieces utilization, many problems were kept out with these most evoked : The waiting of flowering periode befor initiating calli (every 110 days) and the low percentages of embrogenesis of these sources. In the present paper, different explants, principally shoot dissected apex were explored for *Pennisetum glaucum* tissue culture improvement. Since the unsuccessful experiments of KOBLITZ and Saalbaack (1976), DALE and Deambrogio (1979) mention above, no studies from dissected shoot apex was reported yet.

MATERIALS AND METHODS

Plant materials

Four *Pennisetum glaucum* varieties (NE, CN, NW and SR1) cultivated in Côte d'Ivoire were used.

Seeds and seedlings (two to four days old), dissected shoot apex of these seedlings (figure 4 : a), small segments (2-5mm) of main root or shoot of 10 and 20 days old plantlets, immature embryos sampled 5-7 days after pollination and, control, and regenerated plants were used for these studies.

Culture media

MS and N6 media were used with followed modifications. 2,4D (2,4-*dichlorophenoxyacetic acid*), BAP (6-*benzylaminopurine*), NAA (*naphtalene*-1-*acetic acid*), *proline* and *caseine hydrolysat* were added (table 1).

Table 1 : The composition of MS (Murashig and Skoog, 1962) and N6(Chu,1978) Media used in vitro culture of *Pennisetum glaucum*.

Components	Composition of MS and N6 Media	
	MS	N6
Macro-elements (g / l)		
NH4NO3	1,65	-
KNO3	1,9	2,83
CaCl2 2H2O	0,44	0,166
MgSO4 7H2O	0,37	0,185
KH2PO4	0,17	0,4
(NH4)2SO4	-	0,463
Micro-elements (mg / l)		
H3BO3	6,2	1,6
MnSO4 H2O	16,9	3,3
ZnSO4 7H2O	8,6	1,5
Na2MoO4 2H2O	0,25	-
CuSO4 5H2O	0,025	-
CoCl2 6H2O	0,025	0,025
KI	0,83	0,83
FeEDTA (mg / l)		
Na2EDTA	37	37,3
FeSO4 7H2O	27,8	27,8
Vitamines (mg /)		
Thiamine HCl	0,5	1
Pyridoxine HCl	0,5	0,5
Nicotinic Acid	0,05	0,5
Myoinositol	100	100
Other elements (g / l)		
Glycine	0,002	0,002
Sucrose	30	50

Table 2 : Different media used for *Pennisetum glaucum* tissue culture.

medium	NAA mg / l	BAP mg / l	2,4D mg /l	caseine hydrolysat mg / l	proline mM
MS (1. 0,5. 0,5)	1	0,5	0,5	-	-
MS (1. 1. 1)	1	1	1	-	-
MS (0,5. 1. 2)	0,5	1	2	-	-
MS (1. 0,5. 2,5)	1	0,5	2,5	-	-
MS (1. 1. 2,5)	1	1	2,5	-	-
MS (5)	-	-	5	-	-
MS (0,5. 2)	0,5	2	-	-	-
N6 (1. 100. 25)	-	-	1	100	25

Sterilisation

Seeds and immature embryos were surface-sterilised with 95 % (v/v) C2H2OH and 3 % (v/v) H2O2 mixed in a 1:1 ratio for 5 min. Followed, for seeds only, by a 1 and half minite shaking in 50 % (v/v) H2SO4. They were abundantly rinsed with sterile distilled water ; then sterilized one more time with the *ethanol-hydrogen peroxyd mixture* for 6 or 10 min , and abundantly rinsed with sterillized distiled water and used for experiments.

Calli induction

After 20 days, no main root could be distinguished. So, 150 main roots of 10 and 20 days old plants were cut into segments of 5 mm in length from the tip. Each fragments was tested for 7 days on MS (5), MS (1.1.2,5) and N6 (1.100.25) media in PETRI boxes. The percentages of fragments that developped calli were recorded. 150 shoots of 10 and 20 days old plants were cut into segments of 2 mm in length from the meristem and cultured for 7 days on MS(5), MS(1.1.2,5) and N6(1.100.25). The percentages of developping calli fragments were determined. 150 seeds, 60 seedling, 60 dissected shoot apex and 90 immature embryos were tested for 7 days on MS(5), MS(1.1.2,5) and N6(1.100.25) media. The percentages of developping calli explants were also reported.

Embryogenesis design

Two types of calli were observed : The first type, with wet surface, whitish, smooth and translucent, were non-embryogenic calli and led not to plant (figure 4 : b, e (nec)). The second type of calli, yellowish, generally rough and dry, either friable or compact, were embryogenic calli and regenerated plants (figure 4 : c, e (ec)). For each source of calli, embryogenesis and stability improvement were followed. So

100 calli of seedlings, 50 calli of shoots(0-2mm), 70 calli of immature embryos, 90 calli dissected apex, and 100 calli of roots induced on medium

MS(1.1.2,5) were subcultured every 15 days for 5 subcultures because after, no more embryogenesis was observed. The cumulate percentage of embryogenic calli were reported. For the effect of 2,4D on totally embryogenic calli improvement, 50, 81, 84, 85, 57 and 97 dissected apices were cultured for 45 days on MS medium supplemented with respectivly 0, 0,5, 1, 2, 3 and 5 mg/l of 2,4D. The percentages of embryogenic calli and totally embryogenic calli were determined. Concerning the effect of BAP and NAA on totally embryogenic calli improvement, 300 dissected apices were tested on MS (0,5.1.2), MS (1.0,5.0,5), MS (1.0,5.2,5), MS (1.1.1) and MS (1.1.2,5) media (60 dissected apices per medium). The percentage of embryogenic calli and totally embryogenic calli were reported. Embryogenic calli growth was followed with the weight of 60 embryogenic calli cultured for 8 weeks (subculture every two weeks) on MS(1.1.2,5), MS(5) and N6(1.100.25) calli maintaining media ; either 20 embryogenic calli per medium.

Fertile plant regeneration

Embryogenic calli maintained on MS(1.1.2,5), MS(5) and N6(1.100.25) media. They were used for fertile plant regeneration designing. So : The influence of calli age on plant regeneration was studied as followed : after 3, 6, 12 and 15 months, 20 embryogenic calli were sampled and transfered on MS(0,5.2) medium for four weeks. The percentage of calli showing plants were reported. The action of maintaining medium on the cinetic of plant regeneration was determined as followed : 59, 55, and 57 embryogenic calli of three months old sampled respectively from MS (1.1.2,5), MS (5) and N6 (1.100.25) media have regenerated plantlets for 4 weeks on MS(0,5.2). Every week, fresh regeneration medium was used. The cumulate percentage of calli showing plants were reported. For the plant fertility studies, regenerated (30) and control (15) plants of the four Genotype (NE, NW, CN and SR1) were grown, in pot of 10 cm of diameter containing soil (1 / 3) and mould (2 / 3), in greenhouse at 25 °C with 16 h of light/day (120-144 $uEs^{-1}m^{-2}$) for 50 or 60 days. After 60 days, exposition to light was adjusted to 12 h/day to ensure flowering and harvest. Data of some parameters were noted : plant height (cm), bare number, candle length (cm), spike length (cm) and seed number per spike.

Data analysis

For statistical analysis, the chi (X^2) test, percentages comparison and averages comparison methods were used.

RESULTS

Calli induction

Root explants

For the 5 mm from each tip, 100 % of callus was obtained (fig.4 b). This rate decreased gradually along the root (75-10 %) to reach 0% after 30 mm. No significative difference was observed between 10 or 20 days old plants, whatever the callus inducing medium, neither between the tree media.

Table 3: Percentage of induced calli with fragments of roots from 10 and 20 days old plants.

distance of fragment from apex (mm)	Induced root calli from 10 and 20 days old plants, in MS(5), MS(1.1.2,5) and N6(1.100.25) media (%)					
	MS(5)		MS(1.1.2,5)		N6(1.100.25)	
	10	20	10	20	10	20
00-05	100	100	100	100	100	100
05-10	74	70	73	68	77	70
10-15	63	59	65	60	65	62
15-20	48	49	50	50	51	50
20-25	35	21	34	19	37	20
25-30	20	8	20	12	22	12
30-35	00	00	00	00	00	00
35-40	00	00	00	00	00	00

Table 4 : Percentage of induced calli with fragments of shoots from 10 and 20 days old plants.

Distance of fragment from apex (mm)	Induced shoot calli from 10 and 20 days old plants, in MS(5), MS(1.1.2,5) and N6(1.100.25) media (%)					
	MS(5)		MS(1.1.2,5)		N6(1.100.25)	
	10	20	10	20	10	20
00-02	100	100	100	100	100	100
02-04	100	100	100	100	100	00
04-06	61	50	59	49	62	52
06-08	36	32	40	30	43	33
08-10	30	10	28	12	31	10
10-12	15	05	15	04	17	07
12-14	05	00	05	00	05	00
14-16	00	00	00	00	00	00
16-18	00	00	00	00	00	00

Shoot explants

Growing calli were induced up to 12 mm for old plants and up to 14 mm for young plants. A gradiant in callus induction was observed. From 100 % of callus in meristematic region (0-4mm) ; this rate decreased, quickly, from this area (example: 62 to 5 % througth 12 mm). It decreased faster as the plant got older: at 10 mm, 30 % of 10 days old plants produced callus, versus 10 % from 20 days old plants. In any case, no significant difference was observed on the 3 tested media.

Other sources for calli induction

Table 5 : Percentage of induced calli from explants of seed, seedling, dissected apex and immature embryos.

	Induced calli, in each medium (%)		
Origine of explant	MS(5)	MS(1.1.2,5)	N6(1.100.25)
seeds	75	88	82
seedling	99	95	100
dissected apex	95	85	97
immature embryos	50	44	55

Callus induction occured frequently in these other types of materials (80-90 %), except for immature embryos (50 %). We noted the high callus inducing potential of dissected shoot apex (90 %). For each explant used, no significative difference was observed between the three callus inducing media used.

Embryogenic calli and stability

Embryogenic calli harvested through subcultures

Table 6 : Percentage of Somatic embryogenesis of seedling, shoot (0-2mm), immature embryos, dissected apex and root callus followed by five subcultures.

	percentage of somatic embryogenesis for five subcultures				
different explants	1	2	3	4	5
seedling	00	05	15	30	35
shoot(0-2mm)	00	10	50	60	60
immature embryos	00	02	04	08	16
dissected apex	00	50	80	90	90
root	00	00	00	00	00

It appeared that, root did not developp embryogenic calli through subcultures (0%) (fig. 4 b). Embryogenesis induction increased with subcultures whatever the origine of calli. About 16 % of immature calli evoluated into embryogenic calli, followed by 35 % of seedlings' calli, followed by 60 % of shoot's calli and followed by 90 % of dissected apex calli. Embryogenic calli were either totally embryogenic (fig. 4 c), or a mixture (fig. 4 e). Almost 90 % of dissected meristems developed embryogenic calli.

Effect of 2,4D on totally embryogenic calli improvement

Table 7 : Percentage of embryogenic calli with 2,4D concentration (mg/l).

	percentage of embyogenic calli according to concentration(mg/l).					
Types of calli induced	0	0,5	1	2	3	5
embryogenic calli	0	89	92	89	90	91
totally embryogenic calli	0	40	50	65	94	95

No callus was observed without 2,4D. Shoot apex necrosed or developed plantlet. To induce callus, 0,5 mg/l of 2,4D was needed. The rate of embryogenic calli (mixture callus) was high (88 to 92 %) whatever the 2,4D concentration. But totally embryogenic calli rate increased with the increasing of the auxin concentration, to reach 95 % with 3-5 mg/l of 2,4D.

Effect of BAP and NAA on totally embryogenic callo improvement

TAble 8 : Percentage of embryogenic calli of five media with NAA and BAP.

	percentage of embyogenic calli for five media.				
Types of calli induced	MS(1.0,5.0,5)	MS(1.1.1)	MS(0,5.1.2)	MS(1.0,5.2,5)	MS(1.1.2,5)
embryogenic calli	91	95	100	96	90
totally	90	95	90	90	92

NAA and BAP added improved significantly callus embryogenesis. With 1 mg/l and 2 mg/l of 2,4D, 50 % and 65 % of totally embryogenic calli were respectively obtained (table 7), against respectively 95% and 90%, when NAA and BAP were added (MS(1.1.1) and MS(0,5.1.2)). Whatever NAA and BAP concentration, the totally embryogenic calli was high (about 90%) and non significantly different ; so, The effect of NAA and BAP combination was more assigned to their presence than to their concentration. When NAA, BAP and 2,4D were added to a medium, both growth and embryogenic characters were kept through the subcultures.

Embryogenic calli growth

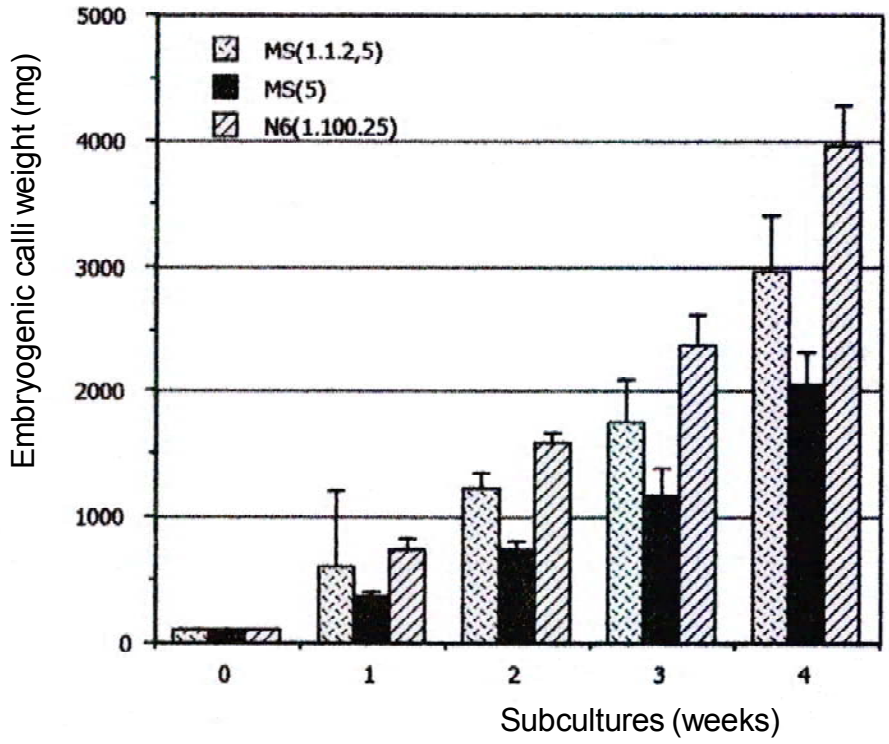

Figure 1 : Embryogenic calli's weight (mg) through subcultures as a function of media.

With four subcultures, embryogenic calli weight passed from 300 mg to 2000 mg, from 500 mg to 3000 mg and from 700 mg to 4000 mg respectvely on M(5), MS(1.1.2,5) and N6 (1.100.25). It was clear that embryogenic calli weight regulary augmented whatever the maintaining medium considered. But this weight augmentation was lightly low on MS(5) medium.

FERTILE PLANT REGENERATION

Age of calli and plant regeneration

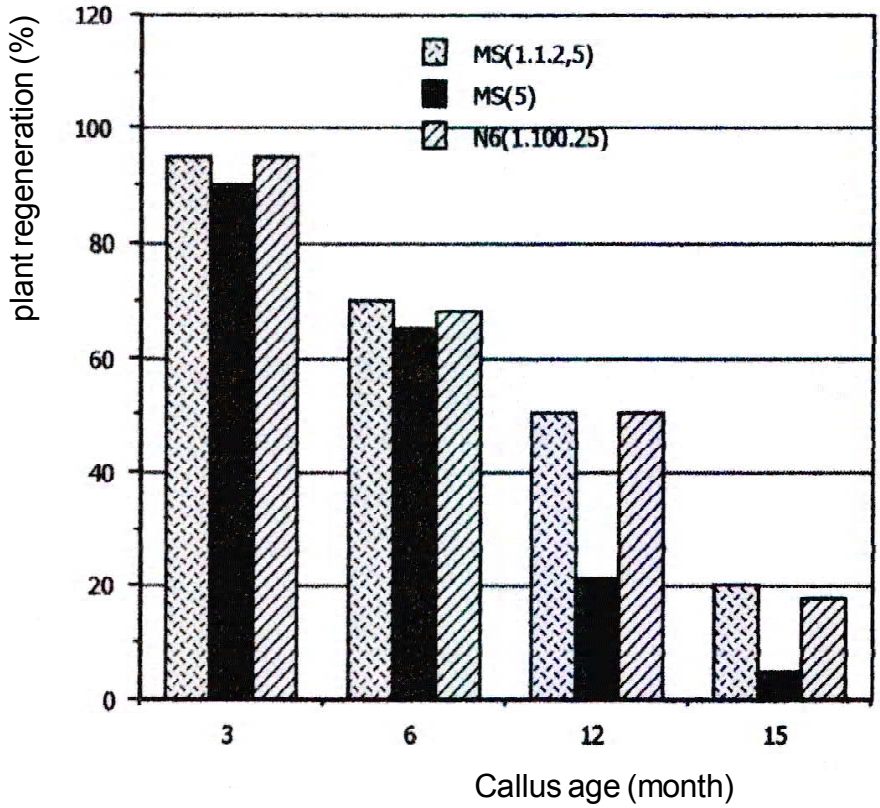

Figure 2 : Evolution of the percentage of plant regeneration as a function of callus age.

The percentage of plant regeneration passed from 95, 70, 50 to 20 respectively with 3, 6, 12 and 15 month old calli deriving from MS(1.1.2,5) or N6(1.100.25). For MS(5), these percentages were 90, 65, 20 to 5 with 3, 6, 12 and 15 month old calli respectively. For each maintaining medium, it was so established that calli developed plants still more than 15 months. The plant regeneration rate was high for calli of about three months old ; but decreased gradually with calli age. For MS (5) medium, the rate was rapidly lowering after six months.

Calli maintaining media and cinetic of plant regeneration

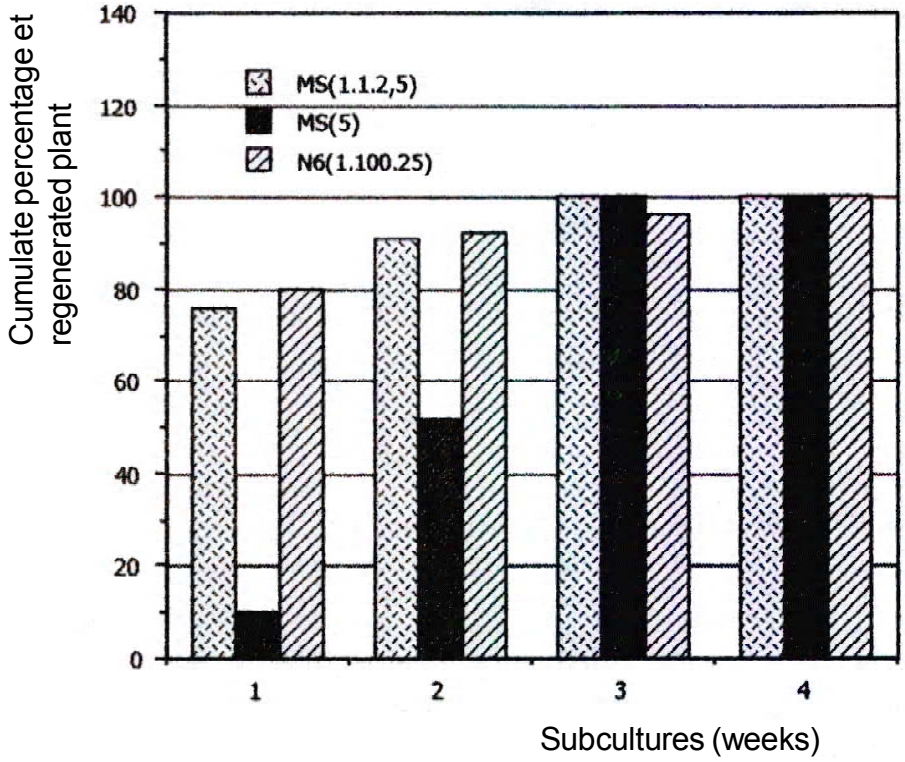

Figure 3 : Cumulate percentage of regenerated plant as a function of media.

The cumulate percentage of calli with plant passed from 80, 90 to 100 respectively after 1, 2 and 3 weeks, with calli deriving from MS(1.1.2,5) or N6(1.100.25). For MS(5), these percentages were 10, 50, 95 to 100 after 1, 2, 3 and 4 weeks respectively. It was clear that one week was needed for majority of calli of MS (1.1.2,5) and N6 (1.100.25) to regenerate plant, versus 3 weeks for calli of MS(5).

Regenerated and control plants data

Table 9 : Some data of mature regenerated and control plants.

Genotype of plant	regenerated and control plants	Data according to genotype and plant used				
		Plant height (cm)	Bare number	Candle length (cm)	Spike length (cm)	Seed number by spike
NE	control	200±4	18±2	31±1	9±2	715±195
	regenerated	180±3	20±4	30±2	12±1	412±278
SR1	control	200±6	16±2	37±1	13±1	681±206
	regenerated	220±4	17±5	33±2	15±1	337±270
CN	control	220±5	17±4	35±1	13±2	431±353
	regenerated	200±3	17±5	30±1	11±1	474±301
NW	control	200±5	17±3	30±1	12±1	488±126
	regenerated	180±4	16±2	30±3	10±1	435±320

Flowering was observed after 55 or 67 days (fig 4 h). Fertile (fig 4 i (fs)) and non fertile (fig 4 i (nfs) spikes were produced. Regenerated plants were heigher (220 cm) than controls (200 cm) for SR1 genotype; but for the other genotypes, controls were heigher (200 cm) than regenerated plants (180 cm). The same seed number for controls and regenerated plants was observed for CN and NW genotypes (about 400); but for NE and SR1 genotypes, this number was more important for controls (about 700) than regenerated plants (about 400). The high standard deviation observed with seed number showed the great varibility of fertility. This variability was probably due to different light variations of culture conditions in green house. No significant statistic, between the controls and the regenerated plants, was observed for the other data : bare number (16 to 20), candle length (30 to37 cm) and spike length (9 to 15 cm).

DISCUSSION

Dale and Deambrogio (1979) reported with *Hordeum vulgare* that root's tip (0-10mm) induced callus. They observed no callus with 1 mg / l of 2,4D in medium ; but with 5 mg / l of 2,4D, lower than 50 % of calli were induced. For *Pennisetum glaucum*, it was establish that 30 mm explants from roots can develop callus and with 10 mm, more than 70 % of calli were obtained. Here 1 mg/l of 2,4D was enough contrary to what was observed for *Hordeum vulgare* by Dale and Deambrogio (1979). Root tip was exclusively with

meristematic totipotent cells, witch cells gave calli, hence root tip's high rate of callus induction. After the root tip, the other root explants contained less and less of meristematic cells, hence their low rate of callus induction. Beyond 30 mm, no callus was observed because, beyond this distance, root explants had no meristematic cells. This experience showed up graminea cell dediffe-rentiation problem : In graminea, diffe-rentiated cells could not retern to meristematic stage even with growth hor-mone. The shoot meristem running followed the same principle as the root as described above. Beyond 10 mm, callus inducing was low with shoot explant, so cell differentiation speed was higher in shoot than in root.

Seed, seedling, dissected apex and immature embryos developed callus because they contained meristematic cells. The sensibility to sterilization method could explain the difference between percentage of seed and seedling callus induction. Factors could explain the low percentage of immature embryos callus induction : The age, for embryos younger than 7 days, the rate was low ; the dissection method, wounded embryos could not lead to callus ; sensibility to sterilization method could low callus percentage too. Botti *et al* (1983) on *P. glaucum* seedling did the same observations. Our rates were better (44-55 %) than those obtained by Lambé *et al* (1999)(12-45 %) in regard to these factors. But for both, immature embryos were less callus inducing than the other callus sources.

For *P. glaucum*, embryogenic callus was the only mean to regenerate plant. After 15-30 days of culture, dissec-tion of non - embryogenic calli of seed and seedling (figure 4 : d) showed a meristem dome at quiescent stage. The dome remained still in the original state ; but free, it led to embryogenic callus (fig 4 : c). After 15-30 days of cul-ture, embryogenic calli of seed and seedling dissected did not show meristem dome. And, the embryogenic callus mass was in the meristem area. It was clear that shoot apex cells were the source of embryogenic calli in *P. glaucum.* Rapid proliferation of non - embryogenic cells slowed down or blocked meristem dome cells develop-ment, and thus could explain the low percentage of embryogenic calli in seed, seedling and immature embryos. For immature embryos, the difference observed by Lambé *et al* (1999) between NE, NW and CN varieties was more due to embryos maturation stage than to the genotype as they pretend (Tiecoura, 1995).

Above 3 mg / l, 2,4D could be toxic for cells growth and so, it slowed down their proliferation ; particulary for non-embryogenic cells and led to a high rate of totally embryogenic callus (Table 7). In combining NAA and BAP with low 2,4D concentrations, embryogenic callus characters were maintained (ta-ble 8). Two facts have led our experiments : 2,4D was callus inducing and growth hormone. Both NAA and BAP led to complete plant regeneration (Nayak, 1989). With NAA alone, callus initiated roots mainly ; with BAP alone, callus led to plants which were rooted with NAA (Tiecoura, 1995). It was clear that conbination of NAA, BAP and 2,4D changed the cell physiology ; and then, interference between plant regeneration and callus growth was observed. The interference of both phenomena could explain the growth and embryogenic characters keeping through subcultures (figure 1).

Concerning green plants rege-neration, the long lasting of the rege-neration potential of calli after a long

period on maintenance media (figures 2 and 3) was observed. MS (5) medium confirmed it's toxicity for cell culture; because at the first week, about 80 % of calli deriving from MS (1.1.2,5) and N6 (1.100.25) regenerated plants versus 10 % from MS (5). For more than 70 % of calli, 7 days were enough to regenerate plant (figure 3) ; so, *P. glaucum* cells moved easily from non-differentiated stage to differentiated one. The number of regenerated plants per callus increased through subcultures and about 4 plants were frequently observed by subculture. So, plant regeneration should be by waves of many cell layers at different stage of development. Each layer could be constituted by many embryos. Thus, the small number of regenerated plants by wave could be due to the diferent physiological development stage of these embryos. So, the synchronized development of cells can be applied to small groups of embryos in a given callus.

On wheat, Kao (1977) noticed a variation of chromosome number after 14 months of culture on medium containing 5 mg of 2,4D (2n-1=27). Karp *et al* (1987) and Winfield *et al* (1993) confirmed this variation for five species of *Triticum* and the negative influence of high concentrations of 2,4D on plant regeneration.

Concerning the fertility of regenerated plants, control and regenerated plants were not significantly different. The important number of seeds obtained by spike was a trump for offsprings and thus genes in stable transformation studies.

CONCLUSION

In conclusion, the fertility of regenerated plants achieved *in vitro* shoot apex culture study. After immature embryos (Vasil, 1981 ; 1982 ; 1987), it appears that shoot apex of *P. glaucum* was a new appropriate tool in tissue culture regarding biotechnology experiments for many reasons :

- shoot apex was available any time and could be used to initiate embryogenic callus ;
- shoot apex embryogenic calli rate was far superior to flowering immature embryos ;
- with maintainance media found, green plants could still be regenerated over 12 or 15 months after callus initiation ;
- cultured in the condition described by Ong (1979) and Tiecoura (1995), regenerated plants were fertile.

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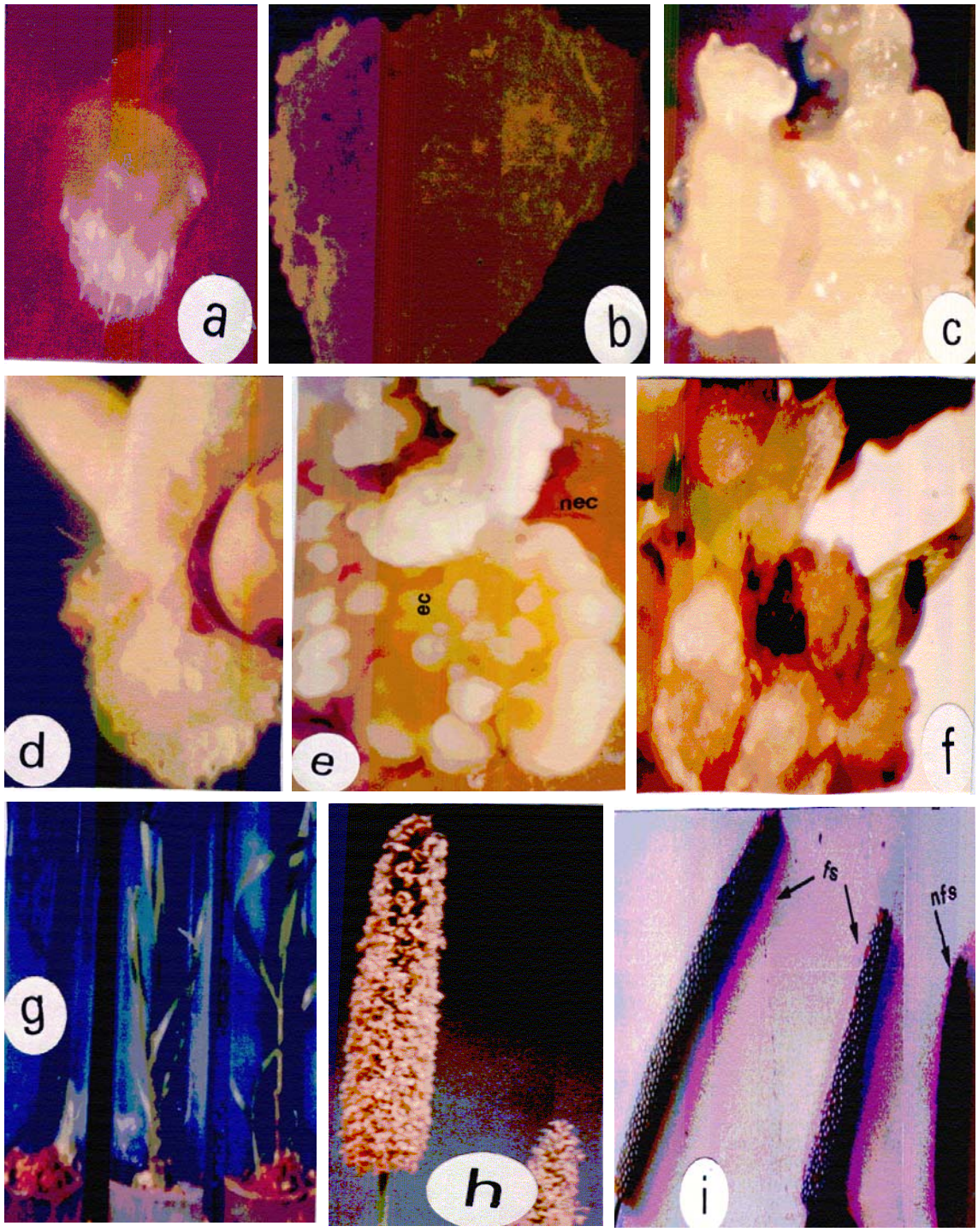

Figure 4 : Somatic embryogenesis and fertile plant regeneration with *Pennisetum glaucum.* a.Excised shoot apex (G : 56 x), b. Root callus (G : 12 x), c. Excised apex callus (G : 27 x) : the callus is totally embryogenic, d. Callus induced from seed (G : 1,25 x), e. callus mixture of seed (G : 12 x) : embryogenic callus (ec) and non-embryogenic callus (nec), f-g. Plant regeneration: organogenesis (f) (G : 12 x) and Regenerated plants (g) (G : 6 x), h. Regenerated plant flowering (G : 6 x),
i. Regenerated plant fertility: fertil (fe) and non-fertil (nfe) spike (G : 6 x).