INSTITUT DE RECHERCHES
POUR
LES HUILES ET OLEAGINEUX

MISSION TO THE SOCFINDO
VEGETATIVE PROPAGATION LABORATORY

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SUMMARY AND CONCLUSION

I- STATE OF THE PROGRAMME

The programme initially planned (culture of 2 trees per month) is being carried out normally. At the end of February 11 trees have been put to culture. Calluses from the 2 trees (SOC OOA and SOC 4.6) have been placed in embryogenesis cycle and 3 other clones (SOC 4.6, SOC 3.1 and SOC 3.2) show calluses which will be isolated in March 1984.

Several improvements have been proposed concerning cultures and disinfection and modifications of the culture media. It has been agreed that they will be applied as soon as March samplings.

It is recommended to send to IRHO every two months a record of the cultures in order to follow the programme's progress.

II- EQUIPMENT OF THE LABORATORY

Well equipped and operational, the Bangun Bandar laboratory does not show any important problem. However three points must still be improved :

1- The pH of the water supplied to the purification system is too basic and provokes a quick degradation of the filtering membrane. Considering its high cost, we advise to contact MILLIPORE in Jakarta in order to equip the laboratory with a water softener.

2- The air-conditioning system of the lighted culture room is not good. Two improvements can be brought :

- place the regulation sensor on the shelves and not in the air-ducts,

- replace the one-speed engine of the air-conditioning system by a multi-speed engine which would allow to adjust the air flow to requirements. In the meantime the two fans (normal + spare one) must be used simultaneously during the lighting

phase.

3- The horizontal steriliser does not work and the intervention of a LEQUEUX's agent has been asked and planned from April 6th.

I- NOVEMBER 1983 CULTURES - CALLOGENESIS

The 5 trees put to culture in November have given average results for callus production. Practically no neoformation has appeared on 2 clones (< 1 % at 12 weeks) ; the three other trees have given more encouraging results (table 1).

We will simply recall that the media have been prepared with BDH agar, different from the advised SYGMA agar, which gelification is not good at the pH used and at a sterilisation temperature of 120° C. Explants of trees sampled before 1984 have been transferred after 6 weeks culture on a new and more solid medium (Mi 034 - BDH agar sterilisation t° 115° C). This has involved no noticeable browning or contamination which proves the quality of the laboratory and team. Also the parafilm was only available during January and most of the callogenesis stage has been done without parafilm (which has not increased the contamination rate). All these points, as well as yield, have been summed up in table 2. After these precisions, several facts are noteworthy :

I.1- Callogenesis time

The observation of the callogenesis on SOC 00A shows a clear evolution between the 12th and 15th week.

SOC 00A -	8th week	:	0 %
	10th "	:	1.7 %
	12th "	:	5.9 %
	15th "	:	15.3 %

The unexpected evolution of yield between To + 12 weeks and To + 15 weeks can be explained by the subculture at To + 12 weeks, the calluses of clones SOC 03.1, SOC 03.3 will be isolated at To + 15 weeks. For the trees not transferred at To + 6 weeks and cultured on a medium prepared with agar of the required quality, the calluses will continue to be isolated at To + 12 weeks.

I.2- Height of sampling

The detailed observation of callogenesis shows that the first 5 levels (30 to 37.5 cm from the apex) are lower yielding than the 5 levels immediately below. The results given by the first ten cultures will confirm this point. Up to now all samplings have been done between 30 cm and 67.5 cm from the apex and we propose to increase the size of the cylinder in order to know the callogenesis aptitude at the 67.5-75 cm level on the trees of Bangun Bandar (annex 1). This is justified by the fact that the sampling standards have been defined in the La Mé

laboratory on older trees (20 years to 9-12 years) living in a very different ecology, and whose apex has perhaps a different development.

As concerns the behaviour of explants from the level comprised between 30 to 37.5 cm from the apex, two hypotheses can be retained :

- The tissues are too young and are not apt to callogenesis.

- The tissues are apt to callogeensis but do not resist to the hypochlorite treatment.

The observation of the explants leads us to favour the second hypothesis and a particular study will be carried out (cf I.3).

If levels 67.5-75 cm present a good callogenesis, sampling can be done 5 cm farther in order not to use blocks between 30 and 37.5 cm for culture since they do not present an interesting yield.

I.3- Hypochlorite treatment

The hypochlorite used for the cultures is of industrial type and we have as yet no technical specification about it. In these conditions it is possible that the solutions prepared at 45 g/l are too concentrated and damage the explants (mainly the young ones). This seems to be confirmed by the state of the explants in culture and could explain the relative weakness of the callogenesis yield.

In order to solve this problem we advise :

1- To get laboratory calcium hypochlorite at 35 % Cl-BDH.

2- To carry out the trial proposed in annex 2 on a tree in order to use the available calcium hypochlorite at a lower and less damaging concentration until the laboratory product is delivered.

I.4- Emergence of roots

About 40 % of the neoformations present unfavourable structures of root type. The apparition of such structures should decrease with the use of a new callogenesis medium tested in the La Mé Laboratory, the Mi 113 medium.

The composition of this medium has been communicated to Mr KUSNADI who will use it for all cultures from now on.

I.5- Conclusions

Excepting failures recorded on clones SOC 01.2 which remain unexplained, we can consider that the first callogenesis results are encouraging since these works have been done on the starting of the laboratory. The use of a more homogeneous (quality of the agar) and slightly modified medium (Mi 113), a gentler hypochlorite treatment and a somewhat more adapted sampling level would allow to increase noticeably the yield of this stage of process.

II- INVENTORY OF THE CULTURES - FEBRUARY 1984

II.1- Callogenesis stage

CLONE	NUMBER	DATE OF CULTURE	DATE OF ISOLATION
SOC 3.1	1 592	24.11.83	8.03.84
2.3	1 946	12.12.83	26.03.84
3.3	1 999	19.12.83	12.03.84
4.3	1 969	13.01.84	6.04.84
2.7	1 936	31.01.84	23.04.84
3.4	1 994	11.02.84	5.05.84
2.2	2 000	23.02.84	17.05.84

II.2- Embryogenesis stage

CLONE	NUMBER	DATE OF ISOLATION	NEXT SUBCULTURE
SOC OOA	16	24.02.84	20.04.84
SOC 4.6	32	25.02.84	21.04.84

II.3- Embryoids proliferation stage

CLONE	NUMBER	NEXT SUBCULTURE
BC 068	83	9.04.84

IV- TECHNICAL PROBLEMS

The laboratory has been working for 4 months quite satisfactorily. However the following points are still to be solved :

IV.1- Steriliser

Thanks to the demand to Ets LEQUEUX a more complete handbook has been sent to the laboratory, but this is not a maintenance handbook as required. The inflatable joint has been changed on the advice of LEQUEUX, but the sterilization cycle is not satisfactory. The pressure within the chamber does not stabilize during the sterilization phase and it seems from the contacts with LEQUEUX that the thermometer does not work. A technician will bring the necessary parts to the laboratory and solve this problem so that the sterilizer can function at last.

IV.2- Milli Ro 60

It has started to function at the beginning of November 1983 the Milli Ro 60 cartridge containing the filtering membrane has been changed twice in 4 months, and should have lasted 1 year on average according to MILLIPORE. It seems, after the results demanded by Mr KUSNADI that the water supplied to the laboratory is too basic (pH 8) and calcium rich, considering the builder's specifications.

The cost of the Milli Ro 60 cartridge being very high, it is urgent to provide a water softener in order to supply the Milli Ro with a water of pH around 4.5 to 7.5. We advise to ask the characteristics of this apparatus to MILLIPORE Jakarta.

IV.3- Air-conditioning in the lighted room

There has been no change since the november 1983 visit and it is impossible to obtain 27° C constant on 24 hours with 3 fluorescent tubes per shelf. We observe a difference of about 3° C between lighted and dark phase. Since the lighted room has been in use since the end of February , we advise :

- to use only 2 fluorescent tubes to ensure lighting,

- to contact CARRIER in order to modify the positions of the sensor, placing it on a shelf instead of within the air duct. This should allow to get the actual temperature at the shelf level. If this is not enough, a more powerful multi-speed engine must be installed in order to modulate the cold air flow according to the light or obscurity phase. In the meantime, we suggest to use the two fan coils simultaneously.

IV.4- Quality of the media

The use of SYGMA agar has solved the problems met with Oxoid agar. Observations have shown that the sterilisation above 115° C at the pH used prevents the gel from solidifying normally. At lower temperatures, the gel presents better qualities. However

in order to prevent any problem, it is recommended to use only SYGMA agar.

ANNEX I

PROTOCOLE

Aim : Observing the callogenesis on the area between 30 cm and 75 cm below the apex.

Method : Each leaf is cut in 30 blocks of 1.5 cm length. The levels are numbered 1 to 30 and they all will be treated until rinsing, G solution included. Levels 5, 10, 15, 20 and 25 will not be used (25 blocks are cultured according to the usual process).

Culture : Each block is disinfected in a Ca hypochlorite solution (concentration defined for the trial). They are rinsed in a glucose (20 g/l) solution then transferred directly to Mi 113 medium (20 explants per block).

Observations : According to the usual process at To + 8, 10, 12 weeks. Results are expressed in frequency of callogenesis for each block, each level (4 leaves simultaneously) and regrouped for 4 levels, according to the usual way.

This protocole must be used on 4 trees.

ANNEX II

PROTOCOLE

Aim : Disinfection of the blocks - Concentration of hypochlorite

Method : A tree is sampled according to the usual process (see instructions for culture in November 1983 report).

- 1/3 of the blocks is disinfected with a hypochlorite solution at 22.5 g/l,
- 1/3 of the blocks is disinfected with a hypochlorite solution at 34 g/l,
- 1/3 of the blocks is disinfected with a hypochlorite solution at 45 g/l.

The blocks are transferred at To + 20 mn in G solution then in Mi 032. Then they are kept at 27° C for 8 days.

Results : At To + 8 days the presence of contaminations in the flasks indicates the quality of the disinfection. Results are given by the number of contaminated flasks to the number of disinfected flasks for each concentration of hypochlorite.

The lowest concentration giving a good result will be used for the next cultures.

Level (distance from the apex - cm)	SOC OA	SOC 04.6	SOC 03.1	TOTAL
1.5 (30 to 37.5)	3/191 (1.51 %)	4/383 (1.01 %)	27/328 (8.23 %)	34/902 (3.76 %)
6.10 (37.5 to 45)	19/199 (9.5 %)	58/387 (14.98 %)	65/393 (16.53 %)	142/972 (14.50 %)
11.15 (45 to 52.5)	10/186 (5.3 %)	40/391 (10.2 %)	39/397 (9.82 %)	89/974 (9.13 %)
16.20 (52.5 to 60)		49/393 (12.46 %)	31/398 (7.78 %)	80/791 (10 %)
20.25 (60 to 67.5)				
TOTAL	32/576 (5.56 %)	161/1554 (9.72 %)	162/1516 (10.7 %)	345/3646 (9.46 %)

Table 1 : Callogenesis yield according to the distance from the apex at To + 12 weeks.

CLONE	Date of culture	Date of transfer(1)	Date of parafilming	Total yield 12 weeks	% 5 best consecutive levels	Isolation of calluses	
						Date	Number
SOC OOA	9.11.83	24.12.83	25.01.84	5.93 %	10.5 %	24.2.84	16
SOC 01.1	15.11.83	29.12.83	11.01.84	0.14 %	-	-	-
SOC 01.2	17.11.83	30.12.83	17.02.84	0.63 %	-	-	-
SOC 04.6	22.11.83	31.12.83	9.02.84	9.72 %	14.98 %	25.2.84	32
SOC 03.1	24.11.83	3.01.84	3.01.84	10.7 %	16.5 %	not isolated	

Table 2 : Sequence of the operations on the trees put to culture in November 1983 (on poor quality agar).

(1) on SYGMA agar.