CELLULAR & MOLECULAR BIOLOGY LETTERS
Volume 6, (2001) pp 587 – 591

Received 11 May 2001
Accepted 9 August 2001

Short communication

REGENERATION OF *CUPHEA TOLUCANA* PEYR. IN *IN VITRO* CULTURE

ZBIGNIEW PRZYBECKI[1], JAN OLEJNICZAK[2]
and ELŻBIETA ADAMSKA[2]
[1]Department of Plant Genetics, Breeding and Plant Biotechnology, Warsaw University of Agriculture, Nowoursynowska 166, 02-787 Warszawa, Poland,
[2]Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland

Abstract: In order to regenerate *Cuphea tolucana* from hypocotyl, cotyledon and root explants, a solid culture and 8 hormone combinations were used. Only the root explants did not react to any of the media. On most of the media, the other explants formed shoots, roots or callus, or their reaction was more complex. The regeneration probably occurred via direct organogenesis. The regenerants displayed a wide variety of morphological characteristics. However, their offspring did not show any differences from plants which had not undergone culture.

Key Words: *Cuphea tolucana*, *In Vitro* Culture, Direct Regeneration, Somatic Variability.

INTRODUCTION

The authors' earlier attempts to regenerate *Cuphea tolucana* from leaf explants did not succeed, despite the use of a variety of hormone combinations and numerous repetitions [publication in progress]. Thus attempts were made to regenerate this species from cotyledon, hypocotyl and root explants.

MATERIALS AND METHODS

In the experiments, seeds from inbred lines which had been bred for three years were used (*C. tolucana* is a cleistogamic plant). To remove their surface hairs,

the seeds were treated as per Olejniczak and Przybecki [1] with the following modifications: 20% sulphuric acid was used for 20 minutes, then the seeds were rinsed in running water for 24 hours. The rinsed seeds were sterilized with sodium hypochloride diluted 3:1, and then rinsed four times in distilled, sterilized water. Germination took place on a $^1/_2$ MS medium without hormones, with 0.6% agar as the gelling agent. After the cotyledons had fully formed, they were cut into 3 x 3 mm fragments and laid out on the solid agar media right side up. The hypocotyls and roots were cut into 0.5 cm sections, and laid out on the same media as the cotyledons. 5 cotyledon, fragments or 10 hypocotyl, or root fragments were placed on 8 cm diameter Petri dishes. A total of 50 of each explant type (cotyledon, hypocotyl and root) were placed on each media type.

The media used in the experiments were: 1. basic medium + 0.01 mg/l 2.4D + 0.01 mg/l BAP, 2. basic medium + 0.01 mg/l 2.4D + 0.01 mg/l BAP + 10 mg/l $AgNO_3$, 3. $^1/_2$ basic medium + 0.02 mg/l 2.4D + 0.02 mg/l BAP, 4. basic medium + 0.1 mg/l 2.4D + 0.8 mg/l 2IP, 5. basic medium + 0.1 mg/l 2.4D + 1.0 mg/l zeatin, 6. basic medium + 0.1 mg/l 2.4D + 1.0 mg/l kinetin, 7. basic medium + 1.0 mg/l IAA + 0.2 mg/l kinetin, 8. basic medium + 1.2 mg/l 2.4.5T + 0.8 mg/l BAP.

The basic medium was MS + 200 mg/l edamine + 100 mg/l m-inosytol. $AgNO_3$ was added to medium 2 to check if it is a factor which increases morphogenesis, as suggested by Purnhauser ***et al.*** [2].

Transfer to fresh medium was done every 3 - 4 weeks. After two passages, the experiment was stopped. This experiment was repeated twice.

The regenerated shoots were cut off and put into jars with a $^1/_2$ MS + 1 mg/l IAA medium in order to root them. After 7 - 14 days, the rooted plants were transferred in their jars to a greenhouse, and left there until the following morning, when they were potted in a peat substrate. The plants were then kept under beakers for a few days to allow them to gradually adapt to greenhouse conditions.

Seeds were collected from the R_0 plants. The plants were not kept in isolation to ensure self-pollination, as this species is cleistogamic.

To obtain R_1 plants, the R_0 seeds were sown in seed boxes in the greenhouse, and transferred to plastic tents after first leaves appeared. The controls were plants which had not undergone ***in vitro*** culture. Observations of themorphological characteristics of the plants were performed.

RESULTS AND DISCUSSION

The results obtained in the experiments on the reaction of the hypocotyl and cotyledon explants to the media used are presented in Tables 1 and 2. The root explants did not form callus or show shoot or root regeneration - they did not react to the media used.

Tab. 1. The results of experiments on the regeneration of *Cuphea tolucana* from hypocotyl explants.

Medium No.	Callusing explants No.	Callus intensity *	Callus colour	Number of regenerated explants		Number of regenerants	Regeneration yield in shoots/explant **
				shoots	roots		.
1.	27	1	brown	13	0	28	2.1
2.	33	0	black	16	0	35	2.2
3.	15	1	black	1	8	4	4.0
4.	20	2	yellow-white	0	0		
5.	25	2	yellow-white	5	0	8	1.6
6.	31	2	yellow-white	10	20	17	1.7
7.	20	1	brown	3	10		1.3
8.	20	3		0	0		
Total	191			48	38	86	2.15**

*The intensity of callus formation on a three-stage scale
** Average for all the callus-forming explants

Tab. 2. The results of experiments on the regeneration of *Cuphea tolucana* from cotyledon explants.

Medium No.	Callusing explants No.	Callus intensity *	Callus colour	Number of regenerated explants		Number of regenerants	Regeneration yield in shoots/explant **
				shoots	roots		
1.	18	0	brown	8	0	25	3.1
2.	25	0	brown	19	0	22	1.1
3.	20	1	brown	10	5	15	1.2
4.	23	1	yellow	0	0		
5.	28	2	yellow	7	0	8	1.1
6.	2		yellow	11	10	13	1.2
7.	21	1	brown	6	15	10	1.4
8.	31	1		0	0		
Total	181			61	30	91	1.5**

*The intensity of callus formation on a three-stage scale
** Average for all the callus-forming explants

The most important types of reaction of the hypocotyl and cotyledon explants to the culture conditions used were the formation of callus and the regeneration of shoots and roots.

Callus formation

The callus formation was very weak compared to that from leaf explants [publication in progress]. A three-stage scale was used to illustrate the relative intensity of callus formation. It was quite common for the hypocotyls to elongate and very strongly thicken. The same was true for the cotyledons. The callus on the media containing BAP quite quickly turned brown or black.

Shoot and root regeneration

Regeneration occurred on media 6 and 8. This was direct regeneration, probably root or shoot organogenesis - exact microscopic examinations were not performed. Shoots and roots grew in places where there was no callus; and even where they appeared in a callus, it was possible to observe that the initiation of morphogenesis occurred in a place where there was no callus. The regeneration yield was low compared to the leaf regeneration yield of *C. wrightii* [3]. In total, 109 shoots were obtained via regeneration. The average regeneration yield for hypocotyl explants was 2.15 shoots, and for cotyledon explants was 1.5 shoots. The intensity of callus formation (the amount of callus formed and the number of callus-forming explants) and the quality of callus formation did not seem to have any relationship with the intensity of regeneration. This would confirm the idea that the regeneration was direct.

Variability in the post-culture plants

The regenerants obtained were strongly morphologically differentiated, and on the media containing zeatin and kinetin there were many forms so differentiated that they could be acknowledged as teratologic. Many of these plants died in the greenhouse (78%). Seeds were collected from 35 plants. In the R_1 generation, no forms where obtained which were morphologically different from the control plants. It appears that such a culture is not a good method of obtaining new forms, even though it was not checked for R_2 or later generations. Such a method would rather be suitable, after further work, for clonal breeding (i.e. microbreeding).

REFERENCES

1. Olejniczak, J. and Przybecki, Z. Development of new crop - Cuphea as a domestic source of medium chain fatty acid by means of mutagenesis. In **Proc. II Int. Congr. Exper. Mutag.** In Plants Plowdiw, October 26 – 30, (1987) 168 – 174

2. Purnhauser, L., Medgyesy, P., Czako, M., Dix, P.J. and Marton, L. Stimulation of shoot regeneration in *Triticum aestivum* and *Nicotiana plumbaginifolia* Viv. Tissue cultures using the ethylene inhibitor $AgNO_3$. **Plant Cell Rep**. 6 (1987) 1-4.
3. Przybecki, Z., Olejniczak, J. and Szydłowska, E. The in vitro regeneration of Cuphea wrightii. **V Ogólnopolska Nauk. Konf. Kult. Tkan. Roślin**. 7 - 9 wrzesień, Skierniewice (1987).