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Plant regeneration of wild *Cucumis* species

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Abstract

Plant regeneration from *C. anguria* var. *longipes* A. Meeuse and *C. metuliferus* Naud. leaf explants was investigated. It was found that embryoid-like structures were formed from *C. anguria* leaf explants cultured on media containing 0.3 mg dm^{-3} 2,4-D and 0.8 mg dm^{-3} 2iP. After being transferred to medium with 0.3 mg dm^{-3} 2iP and 1 mg dm^{-3} GA_3 they developed 187 ± 49 shoots per explant. Further growth and root formation proceeded on hormone free medium.

Key words: C. anguria var. longipes, C. metuliferus, leaf explants, plant regeneration

INTRODUCTION

Many genetic-breeding programs use wild species as an attractive source of resistance against diseases, particularly when resistant cultivars are not known. However, in many cases it is impossible to transmit the resistance from wild species to the cultivars due to the barriers which make hybridisation impossible (Visser et al. 1980). Utilization of cellular and genetic engineering is one of the possible solutions. However, this depends directly on the elaboration of cell modification systems and plant regeneration. Plant regeneration of *Cucumis* has been described for *C. sativus* from leaf explants (Malepszy and

Abbreviations: IAA – indole acetic acid, NAA – naphtalene acetic acid, 2,4-D – 2,4-dichlorophenoxyacetic acid, 2,4,5-T – 2,4,5-trichlorophenoxyacetic acid, pCPA – 4-phenoxyacetic acid, 2iP – 6-(3-methyl-2-butenylamino) purine, BAP – 6-benzylaminopurine, GA3 – gibberellic acid.

Nadolska-Orczyk 1983, Nadolska-Orczyk and Malepszy 1985), from cotyledons (Sang-Gu and Joung-Ran 1984), suspension culture (Malepszy and Solarek 1968) and protoplasts (Orczyk and Malepszy 1985) and for *C. melo* from cotyledons (Moreno et al. 1985). In this paper we present the results of studies of plant regeneration of two wild species, *C. anguria* var. *longipes* A. Meeuse and *C. metuliferus* Naud.

MATERIALS AND METHODS

Seeds of *C. anguria* and *C. metuliferus* were sterilized in sodium hypochlorite and placed on medium composed of 1/2 macroelements, microelements and vitamins MS (Murashige and Skoog 1962) supplemented with 15 g dm^{-3} sucrose and 5 g dm^{-3} agar. The seedlings were cultured at 27°C under 12 h white light illumination (4 klx).

After three weeks young and fully developed leaves were cut into 5 × 5 mm explants and put on 9 combinations of the primary media containing 2,4-D and 2iP 0.3, 0.8, 1.5 mg dm^{-3} (each concentration of auxin with each concentration of cytokinin). Explants of *C. metuliferus* were additionally cultured on primary media containing different types of auxins (IAA, NAA, 2,4-D or 2,4,5-T) and cytokinins (2iP or BAP). All media for explant, callus and shoot culture contained MS macroelements, microelements and vitamins, 30 g dm^{-3} sucrose, 250 mg dm^{-3} edamin and 5 g dm^{-3} agar. 60 ± 2 Explants were put on each medium combination. Explants were cultured at 27°C in dark. After 5 weeks, 15 ± 1 explant-derived callus lines were transferred onto the following secondary media: a) medium containing 1 mg dm^{-3} GA_3 (B), b) medium containing 0.3 mg dm^{-3} 2iP (Ct), c) medium containing 0.3 mg dm^{-3} 2iP and 1 mg dm^{-3} GA_3 (G1), d) medium with 0.3 mg dm^{-3} 2iP and 5 mg dm^{-3} GA_3 (G5). The culture was performed in the light (4 klx).

Shoots obtained on hormone-free medium were counted and rooted on hormone and edamin-free MS medium. The experiments were repeated twice.

RESULTS AND DISCUSSION

Our previous investigations proved that plant regeneration from leaf explants of different genotypes of *C. sativus* was possible on media containing three phenoxy derivatives (2,4-D, 2,4,5-T, pCPA) with cytokinin (2iP or BAP). Plant regeneration was not observed on the media containing these cytokinins in combination with other auxins (IAA, NAA, indole-3-butyric acid – IBA) (Malepszy and Nadolska-Orczyk 1983 and unpublished data). The influence of phenoxy derivatives on culture and plant regeneration of selected wild cucumber species was tested on the basis of those preliminary observations.

C. METULIFERUS

After 3 weeks all explants cultured on all primary media containig 2,4-D and 2iP except medium containing 1.5 mg dm^{-3} 2,4-D and 0.3 mg dm^{-3} 2iP developed a light-yellow, loose and granular callus. Callus growing on this medium was compact and granular. Morphogenesis was mainly restricted to the development of roots on all media except those containing the highest concentrations of 2,4-D (1.5 mg dm^{-3}) and lower 2iP. The most intensive root formation occurred on the media containing the highest concentration of

Table 1

Root and shoot regeneration on the calluses after 5 weeks of culture on primary medium and 10 days on secondary medium

	C. metuliferus				*C. anguria*			
Secondary medium / Primary medium mg dm^{-3}	B	Ct	G1	G5	B	Ct	G1	G5
0.3 2,4-D 0.3 2iP	++	+	+	+	+++ 33%	+++ 21%	+ 25%	+++ 10%
0.8 2,4-D 0.3 2iP	+	+	+	+	+++	+++	+++	+++
1.5 2,4-D 0.3 2iP	+	0	+	+	+	+	+	+
0.3 2,4-D 0.8 2iP	+	+	+	+	+ 64%	+++ 40%	++ 33%	++ 22%
0.8 2,4-D 0.8 2iP	++	+	+	+	++	+++	++	++
1.5 2,4-D 0.8 2iP	+	+	+	+	+	+	+	+
0.3 2,4-D 1.5 2iP	+	+	++	++	+	+	+	+
0.8 2,4-D 1.5 2iP	+++	++	++	+	+	+	+	+
1.5 2,4-D 1.5 2iP	+++	+	+	+	++	++	++	++

0 – lack of roots, + – 1-6 roots per explant, ++ – 7 and above roots per explant, +++ – root development on whole explant surface, % – percentage of explants developing shoot. Other abbreviations see Materials and methods.

applied cytokinin. The result was the same in the course of the further culture on primary media and after 10 days of culture on secondary media in light. The process of root formation abated during culture on secondary media. After 15 days of culture on these media green, granular callus originated (Table 1). Formation of embryo-like structures and shoot development were not observed either on the primary or the secondary media.

Our further attempts to obtain regeneration of *C. metuliferus* centered on supplying basic MS medium with different combinations of growth regulators. The hormone concentrations, particularly cytokinins, were determined basing on the previous experiments where regenerable, granular callus originated mainly on the 1.5 mg dm^{-3} 2,4-D, 0.3 mg dm^{-3} 2iP medium and partly on media: 0.8 mg dm^{-3} 2,4-D, 0.3 mg dm^{-3} 2iP or 0.8 mg dm^{-3} 2,4-D, 0.8 mg dm^{-3} 2iP. Also on these media, morphogenesis was mainly restricted to the formation of roots. The most intensive root formation was observed on media containing NAA in combination with 2iP, much lower on medium with higher concentration of 2,4-D compared with BAR. On media supplemented with phenoxy derivatives (2,4-D or 2,4,5-T) and lower concentration of cytokinin, gel-like callus formation was observed. Also, culture on these media and subculture on secondary media did not result in plant regeneration.

Table 2
Callus formation and morphogenesis of *C. metuliferus* leaf explants after 5 weeks of culture on primary media

Primary media, mg dm^{-3}		Explants forming callus, %	Root formation intensity
1 IAA,	0.5 2iP	72	0
2 IAA,	0.5 2iP	59	0
1 NAA,	0.5 2iP	100	+++
2 NAA,	0.5 2iP	100	+++
1 2,4,5-T	0.5 2iP	100	0 g[1]
1 2,4-D,	0.3 BAP	100	+
1 2,4-D,	0.8 BAP	100	0 g[1]
1 2,4-D,	1.5 BAP	100	0

[1] – gel-like callus formation.

C. ANGURIA

On all primary media containing 2,4-D and 2iP a compact, granular and light yellow callus was formed. Additionally after three weeks of culture on media containing 0.3 mg dm^{-3} 2,4-D and 0.8 mg dm^{-3} 2iP, the formation of pale yellow, compact, secondary callus was observed. Morever, on all media, the roots were formed with different intensity. Callus transferred from primary to secondary media developed numerous, very small roots (particularly when cultured on 0.3 mg dm^{-3} 2,4-D and 0.3 mg dm^{-3} 2iP) or developed secondary

Plate I

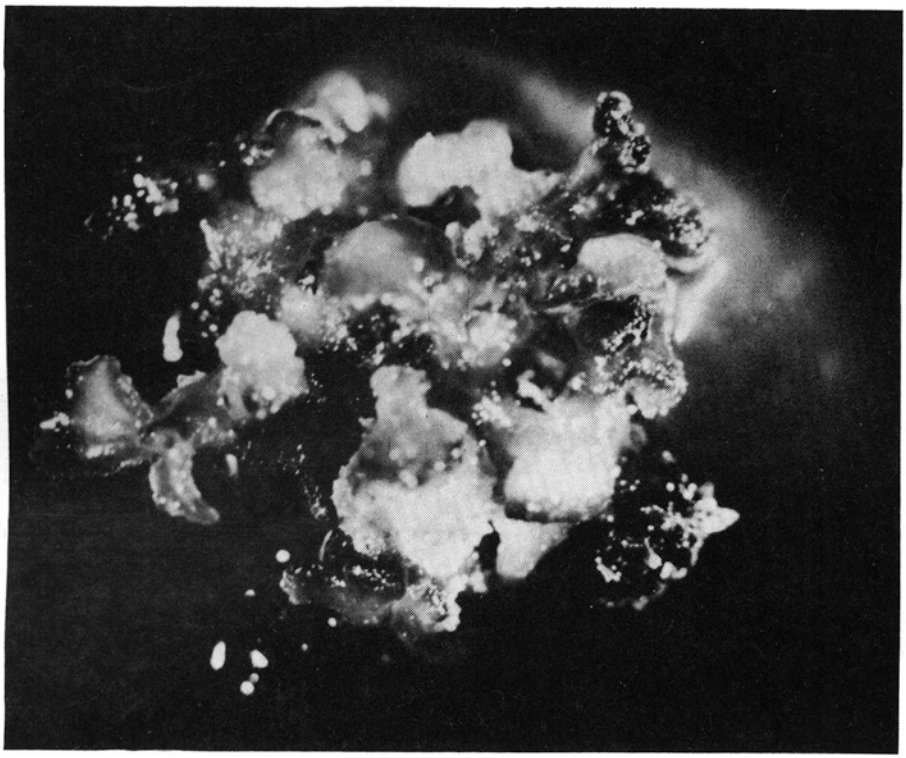

Fig. 1. Plant regeneration of *C. anguria* var. *longipes* after 5 weeks of culture on medium containing 0.3 mg dm^{-3} 2,4-D, 0.8 mg dm^{-3} 2iP and 3 weeks on hormone-free medium, 5×

callus and shoots. Regeneration of shoots was recorded mainly after transferring secondary callus from medium containing 0.3 mg dm^{-3} 2,4-D and 0.8 mg dm^{-3} 2iP to medium with 0.3 mg dm^{-3} 2iP and 1 mg dm^{-3} GA_3 (G1) (Table 1). In this case out of 14 explants placed on secondary medium 9 developed secondary callus. During the next 6 weeks of culture on medium B each callus regenerated 187 ± 49 shoots (Fig. 1).

The previous observations concerning the specific effect of phenoxy derivatives on *C. sativus* leaf explants were found true for cultures of wild *Cucumis* species. The medium containing 2,4-D and 2iP appeared to be the most suitable for plant regeneration of both *C. sativus* and *C. anguria*. Also, a similar sequence of developmental events i.e. the growth of primary callus, secondary callus and shoots was observed.

In spite of the fact that was no plant regeneration of *C. metuliferus*, culture on media containing phenoxy derivatives resulted in gel-like callus formation. In spite of some similarities in callus formation and/or plant regeneration, differences exist concerning mainly intensity of root formation (Table 1) and plant regeneration. There are known examples of plants where regeneration inability was proved to be genotype-dependent (Reisch and Bingham 1980, Charmet and Bernard 1984). The lack of regeneration possibility might be the result of either the genotype or the unapropriate culture conditions.

The elaboration the conditions of *C. anguria* plant regeneration from callus will make it possible to aproximate the culture conditions for protoplasts isolated from *C. anguria*. This will be the next step aiming at obtaining somatic hybrids between *C. sativus* and *C. anguria*.

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Regeneracja roślin z dzikich gatunków Cucumis

Streszczenie

Badano regenerację roślin z eksplantatów liściowych *Cucumis anguria* var. *longipes* A. Meeuse i *C. metuliferus* Naud. Opisano tworzenie struktur embrioidalnych w czasie kultury eksplantatów liściowych *C. anguria* na pożywce zawierającej 0.3 mg dm^{-3} 2,4-D i 0.8 mg dm^{-3} 2iP. Struktury te, po przeniesieniu na pożywkę zawierającą 0.3 mg dm^{-3} 2iP i 1 mg dm^{-3} GA_3, rozwijały się w pędy. Otrzymano 187 ± 49 pędów z eksplantatu liściowego. Kultura eksplantatów liściowych *C. metuliferus* na pożywkach zawierających różne kombinacje auksyn (IAA, NAA, 2,4-D, 2,4,5-T) i cytokinin (2iP, BAP) nie prowadziła do regeneracji pędów.