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Positive Effect of Cefotaxime on Plant Regeneration from *Cichorium intybus* L. Leaf Material

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The use of antibiotics for plant cell and tissue culture has been suggested for inhibiting or removing contaminating micro-organisms. In contrast to their effects on bacteria, certain antibiotics have been shown to stimulate plant growth and development (Fakhrai 1990). Positive effects of the antibiotic cefotaxime on the growth of *Triticum aestivum* were reported by Mathias and Boyd (1986). Mathias and Mucasa (1987) observed a significant improvement of the growth and regeneration of calli initiated from immature embryos from four varieties of barley (*Hordeum vulgare* L.) in the presence of cefotaxime (60-100 mg/l). By contrast in *Antirrhinum majus*, in the presence of cefotaxime at concentrations 250-500 mg/l, no stimulation of callus production was observed and shoot formation was reduced (Holford and Newbury, 1992).

This report describes a reproducible method for the induction of plant regeneration from *Cichorium intybus* leaf midrib explants in the presence of cefotaxime sodium but in the absence of hormones in the culture medium.

Cefotaxime sodium at four different concentrations (0.6, 1.3, 2.6, 5.2 mg/l) was added, either before or after autoclaving of the culture medium, to MS Medium (Murashige and Skoog, 1962), supplemented with 3 % sucrose and 3.4 g/l Gelrite (Roth). No plant hormones were added. The control consisted of MS medium without hormones and antibiotics. The experiments concerning shoot induction were carried out with explant material from plants grown under greenhouse conditions. Two lines from the BGRC (Braunschweig Genetic Resources Collection) have been tested. Leaves (10 cm long) were surface sterilised for 3 minutes in 95 % ethanol, 15 minutes in a mixture of 20 % (v/v) commercial bleach and 0.1 % (v/v) Tween 20 and finally rinsed three times in sterile distilled water. Midrib explants 4 mm in size were placed on culture media. Explants were maintained at 25 ± 1°C (12h light).

The results were evaluated after 15 and 45 days. Cultures were controlled and scored for the percentage of regenerated shoots per explant. On all four media containing cefotaxime shoot induction was observed. After 15 days incubation the highest regeneration efficiency was obtained on C3 Medium containing 2.6 mg/l cefotaxime. After 45 days incubation without replacement of fresh media a dramatic increase in the number of explants, which developed shoots, was observed . High regeneration efficiency occurred also on media C2 containing 1.3 mg/l cefotaxime. Lower efficiency of regeneration was obtained on media C1 and C4. On the phyto-hormone free MS control medium CK, only spontaneous regeneration occurred (Table 1).

Table 1 shows the data of explants which developed shoots. However, multiple shoot formation was induced on the cut surfaces of midrib explants. After 15 days of incubation about 1-3 shoots were induced on each explant (fig. 1a), but after 45 days rapid multiplication occurred and each shoot formed a cluster of up to 3-6 new shoots (fig. 1b).

These shoots were transferred on to a cefotaxime free and phyto-hormone free MS medium. After they reached a size of 3 cm, the shoots were placed on medium for root induction (MS medium, supplemented with 2.5 mg/l IBA and 0.5 mg/l NAA).

Shoot regeneration on media containing BA (benzyladenine) (G. Mix-Wagner, unpubl.) often resulted in vitrification of the regenerated shoots. In experiments containing cefotaxime in the culture media, regenerated plants appeared normal and did not show any vitrification.

In experiments with autoclaved cefotaxime in the culture media, the same regeneration efficiency was obtained as when sterilised by filtration. No difference in the efficiency of shoot proliferation in both tested lines was observed. There was no significant difference in the regeneration response.

These experiments show efficient shoot formation from leaf explants of *Cichorium intybus*. Leaf midribs respond well to in vitro propagation when inoculated onto MS medium containing 2.6 mg/l cefotaxime. Cefotaxime sodium is a highly effective antibiotic against Gram-negative bacteria and to a lesser extent against Gram-positive bacteria. The effect of the antibiotic is based on the inhibition of the bacterial cell wall synthesis, resulting in lysis or deformation of the bacterium. For plant cell and tissue cultures cefotaxime sodium is used during *Agrobacterium* mediated transformation up to a concentration of 250 to 500 mg/l in the medium to inhibit bacterial growth after *Agrobacterium*

Media No.	Cefotaxime concentration (mg/l)	Total number of leaf explants	Shoots regenerated after 15 days		after 45 days	
			number	%	number	%
C1	0.6	150	33	22.0	40	26.6
C2	1.3	150	26	17.3	90	60.6
C3	2.6	150	56	37.3	100	66.6
C4	5.2	150	11	7.3	40	26.6
CK	0.0	150	3	2.0	7	4.6

Table 1: **Effect of different concentrations of cefotaxime on direct shoot regeneration of *Cichorium intybus* leaf midrib explants**

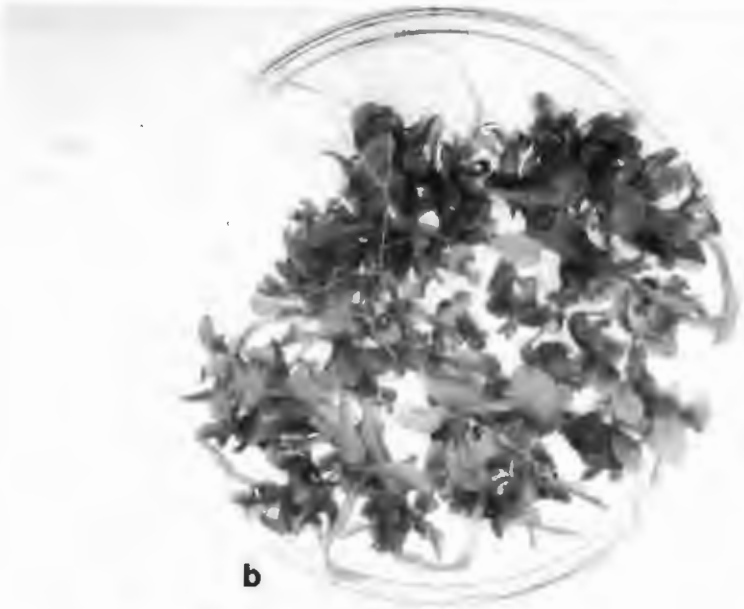

Figure 1: **Shoot induction of midrib explants of *Cichorium intybus* L.**
a) after 15 days
b) after 45 days of incubation

infection and co-cultivation. An explanation for the promoting effect of cefotaxime sodium in the shoot regeneration and morphogenesis could be the reduction of bacterial infection in leaf explants. The presence of epiphytic bacteria in leaves of *Cichorium endivia* was reported by Jacques et al. (1995). The concentrations used in our experiments were 100-fold lower compared to those used for bacterial inhibition (250-500 mg/l). The activity of cefotaxime could possibly be explained by a growth regulatory function. It is possible that a breakdown product of the aminoglycoside has a growth-promoting activity at low concentrations. For *Cichorium*, the initial presence of 2.6 mg/l cefotaxime in the culture media promoted multiple shoot regeneration and growth. The number of regenerated shoots increased dramatically after 45 days without replacement of fresh media and without further addition of antibiotic. There is an expected destruction of the antibiotics at 25°C after 3 weeks. Low concentrations of the antibiotic in the initial media seem to be enough to induce and promote shoot induction in the absence of additional growth regulators. The process of in vitro shoot regeneration might involve, as a first step, an induction of developmental competence (totipotency) of the tissue, which is subsequently responsible for determined formation of tissues and structures.

Summary

Regeneration responses of chicory (*Cichorium intybus* L.) were positively affected by the addition of the antibiotic cefotaxime in low concentrations 1.3 - 2.6 mg/l to the nutrient media. Multiple shoot induction was obtained after 15 days in culture in MS medium with 2.6 mg/l cefotaxime sodium and in the absence of phyto - hormones.

Positiver Einfluß von Cefotaxime auf die Pflanzenregeneration aus *Cichorium intybus* L.-Blattgewebe

Eine Anzahl von Antibiotika zeigen eine stimulierende Wirkung auf Sproßinduktion und Wachstum.

Die Sproßregeneration auf *Cichorium intybus* L. (Wurzelzichorie)-Blattrippensegmenten konnte durch den Zusatz von Cefotaxime zum Nährboden positiv beeinflußt werden. Noch einer Kulturdauer von 15 Tagen hatten 37,3 % und nach 45 Tagen 66,6 % der Segmente auf dem Nährboden mit 2,6 mg/l Cefotaxime-Sprosse gebildet.

Die Erhöhung von Cefotaxime auf 5,2 mg/l im Nährboden hemmte die Sproßbildung (7,3 % nach 15 Tagen). Bei einer niedrigen Cefotaximekonzentration (0,6 mg/l) im Nährboden konnte nach 15 Tagen Kultur zwar eine Regenerationsrate von 22 % erzielt werden, jedoch war nach 45 Tagen nur eine Zunahme von 4,6 % im Vergleich zu 29,3 % (bei 2,6 mg/l Cefotaxime) zu beobachten.

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