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Plant regeneration from *Cichorium intybus* L. var. *sativum* leaf midrib explants induced by ancymidol supplemented culture medium

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Introduction

In vitro propagation is a way of mass producing offspring with identical genetic background. In our search to improve shoot regeneration of Cichorium we tested ancymidol for its promoting function as a growth regulator.

Ancymidol is a substituted pyrimidine with potent growth regulatory activity in higher plants (Coolbaugh et al., 1978). Ancymidol appears specifically to inhibit series of oxidations in higher plant tissues. As a growth retardant which inhibits GA synthesis, ancymidol promots rooting and shooting in micropropagation of asparagus (Chin, 1982; Desjardins et al., 1987 and Benmoussa et al., 1996) and suppressed abnormalities in the development of somatic embryos, reducing surface callus induction (Chin and Khunachak, 1984). Positive effect on embryo induction of asparagus was reported (Li and Wolyn, 1997).

In this paper we investigate the effect of ancymidol on regeneration efficiency *Cichorium intybus*.

Material and Methods

The cultivar "Fredonia Nova" (FN) and two halfsib families 1 and 2 of *Cichorium intybus* L. var. *sativum* (BGR Collection) were tested for their regeneration response on ancymidol. As explant material midrib leaf segments were used.

Leaves were surface sterilized for 3 minutes in 95 % ethanol, 15 minutes in a mixture of 20 % (v/v) commercial bleach and 0.1 % (v/v) Tween 80 and finally rinsed three times in sterile distillated water. Midrib segments ca. 4 mm in size were cut out and placed onto solid media.

The culture media used consisted of MS Medium (Murashige and Skoog, 1962) supplemented with 30 g/l sucrose and 3.4 g/gelrite (Roth). Media containing ancymidol concentrations, 1.5 mg/l and 3.0 mg/l or benzylaminopurine 3.0 mg/l and 1.5 mg/l were compared. As a control, MS medium without addition of hormones was used.

For shoot induction incubation in dark and under light regime (14h light/10 h dark) at 25° C was tested. Regeneration efficiency was evaluated after four and eight weeks incubation.

Developed shoots were transferred onto root induction medium containing half strength of MS salts, vitamins, 10 g/l sucrose, 3.4 g/l gelrite, 2.5 mg/l indolbutyric acid (IBA) and 0.5 mg/l naphthyl-1-acetic acid (NAA).

Results and Discussion

Incubation of cultures in dark for 5 to 6 days caused browning of all leaf midrib segments. Globular structures appeared here with a delay of 2 to 3 weeks compared to the cultures incubated under photoperiod 14 h light/10 h dark conditions. On each segment up to two globular structures could be observed resulting in shoot differentiation. When cultured under light/dark conditions only a small part of the segments turned brown, the rest remained green. After two weeks of incubation first hard, well formed globular structures appeared on the cut surface of the greenish as well as on the brown segments. One to five shoots initiated directly out of each globular structure.

In all of the treatments on ancymidol containing media no callus induction occurred. Developed shoots were separated and transferred into fresh medium. After 4 weeks of incubation under light conditions shoots were completely developed. In the results light was defined as an obvious parameter enhancing shoot induction and regeneration. This observation could be confirmed for Asparagus cultures by Khunachak et al. (1987).

When cultured on medium containg 1.5 mg/l ancymidol leaf midrib explants resulted in continued multiple shoot regeneration. From all 375 explants incubated, 182 shoots emerged after 4 weeks and 350 shoots after 8 weeks culture time (**Table 1**).

After 8 weeks incubation, promoted shoots on ancymidol containing media appeared shorter and more compact. The size of the plants varied between 5.0 mm and 50.0 mm.

Maximum shoot number per explant was obtained on medium supplemented with 1.5 mg/l ancymidol (1-6 shoots). Only one or two shoots were formed per explant on a medium containg 3.0 mg/l ancymidol.

Increase of ancymidol up to 3.0 mg/l led to a total reduction of shoot induction of 28 % after 4 weeks and 31 % after 8 weeks (data not shown). A range of 1-2 shoots only were developed.

Higher concentration of ancymidol also affected the morphology of the plantlets produced. Shoots appeared strong and thick and reduced in length. Results of *Cichorium intybus* L. were comparable with those reported by application of 4 mg/l ancymidol to Asparagus culture (Khunack et al., 1987). This phenomenon which can be discussed as a kind of inhibition of the GA3 biosynthesis by ancymidol (Coolbaugh et al., 1978) was also documented for *Crinum macowanii* by Slabbert et al., (1993).

Genotypes tested showed different regeneration efficiency, but the same tendency. Variety "Fredonia Nova" showed

with 88 % the highest growth response. Lowest growth response with 41 % was obtained by the genotype 1.

Highest efficiency of shoot production per explant was also investigated. The variety „Fredonia Nova" produced 594 shoots per 150 explants, genotype 1 was only able to initiate 50 shoots (Table 2).

The average of developed shoots per explant varied between the genotypes. The variety „Fredonia Nova" was able to initiate with an average of 4,5 shoots from one explant, genotype 1 only 0,8.

Data in Table 2 present number of shoots and shoots per explant proliferated of the genotypes tested after 8 weeks incubation on culture medium containing ancymidol or BAP. They indicate the stronger positive effect of ancymidol alone on shoot induction compared to the cytokinin BAP.

The efficiency of shoot induction was significantly lower when only BAP was added to the nutrient medium. "Fredonia Nova" developed 116 shoots but the average of developed shoots per explant was only 1.2. Genotype 1 produced approximately 0.4 shoots per shoot explant. An increase of BAP concentration up to 3.0 mg/l improved the shoot induction of 8 % but only of the variety "Fredonia Nova" (Mix-Wagner unpublished). Medium supplemented with ancymidol only as growth regulator allowed high regeneration of shoots per one explant after 8 weeks. This process once induced resulted in continuous shoot formation. Concentration of 1.25 mg/l ancymidol promoted formation of a high number of secondary bulblets from split bullet of *Crinum macowanii* documented by Slabbert and co-worker. The bulbs were better and more vigorous.

Experiments of Khunachak and co-workers (1987) to combine BAP with different auxins demonstrated that all combinations hindered more or less shoot formation compared to ancymidol alone at the same range of concentrations. The enhancing effect of ancymidol for vigorous shoot growth could not be reached by the addition of other cytokinines and/or auxins to the medium. This indicates that the effect of ancymidol does not depend on growth regulators.

Table 1: Effect of Ancymidol on the total number of developed shoots and shoots per explants after 4 and 8 weeks of incubation time/Einfluß von Ancymidol auf die Anzahl gebildeter Sprosse bzw. Sprosse je Explant nach 4 und 8 Wochen Behandlungsdauer

Ancymidol concentration		0.0 mg/l	1.5 mg/l	3.0 mg/l
Incubation time	No. of Explants	375	375	375
4 weeks	Number of developed shoots %	0	182 48.5 %	132 35.2 %
8 weeks	Number of developed shoots %	31 8.26 %	350 93.3 %	252 67.2 %
	Shoots per explant	0-1	1-6	1-2

Table 2: Regeneration response of three genotypes cultured on medium containing 1.5 mg/l Ancymidol (Anc) or 1.5 mg/l Benzylaminopurin (BAP)[1]/Pflanzenregeneration drei verschiedener Genotypen auf ancymidol- oder benzylaminopurinhaltigem Nährboden

	Genotype	Number of Explants	Induced Shoots	average of developed shoots per explant
Anc	1	150	50	0.8
BAP	1	150	21	0.4
Anc	2	150	238	2.2
BAP	2	150	75	1.0
Anc	FN	150	594	4.5
BAP	FN	150	116	1.2

[1] Experiment was carried out under light/dark regime (14 h/10 h).

An important advantage of ancymidol application is the lack of vitrification of regenerated shoots. Vitrification problems often occur when shoots are regenerated on nutrient medium containing BAP. Vitrified shoots were also produced in the experiment presented in Table 2 when BAP was added to the culture medium (Al Atabee et al., 1990; Declerck et al., 1995).

On the control medium without hormone addition all three genotypes showed low shoot proliferation. When replaced on a root inducing medium containg 2.5 mg/l IBA and 0.5 mg/l NAA, 90-100 % of the plantlets showed fast root formation.

The morphological benefit of ancymidol is to promote the shoot proliferation, differentiation and development and the suppression of abnormalities of induced shoots.

The study showed an efficient way of adventitious shoot formation from leaf midribs, avoiding vitrification.

Summary

In our studies efficient continuous regeneration of *Cichorium intybus* L. var. *sativum* Lam et DC. has been investigated, indicating the positive effect of ancymidol on regeneration ability. Multiple shoot induction and rapid regeneration were obtained on MS-Medium supplemented with 1.5 mg/l ancymidol. As explant material leaf midrib segments were used. Plants developed after four weeks of incubation. Regenerated plants were successfully transferred to soil.

Ancymidol-induzierte Pflanzenregeneration aus *Cichorium intybus* var. *sativum*-Blattrippensegmenten

Der positive Einfluß von Ancymidol auf die Regenerationsfähigkeit von *Cichorium intybus* var. *sativum* wurde untersucht. Eine andauernde Mehrfachsproßinduktion und schnelle Regeneration wurde durch die Zugabe von 1,5 mg/l Ancymidol zum Kulturnährboden erreicht.
Drei Genotypen, deren Blattrippen segmentiert wurden, dienten als Ausgangsmaterial. Nach 4 Wochen konnten die ersten bewurzelten Sprosse erfolgreich in Erde überführt werden.

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