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IMPROVED MICRO PROPAGATION OF *Cichorium intybus* L. FROM LEAF EXPLANT

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ABSTRACT

Cichorium intybus is edible, medicinal and forage plant. The pharmaceutical raw materials were obtained from wild chicory (var. Maurane). Currently, farmers are increasingly assuming plantations of wild chicory, and breeders are attempting to produce cultivars for medicinal purposes. In the modern breeding of chicory important feature is the ability to propagate in vitro culture.

The aim of our study was to assess capacity of natural population of wild chicory for plant regeneration from leaf explants. In the first was examined the effect of various concentrations of BA and TDZ on the regeneration of shoots from leaf explants (0.5 cm^2). After that, 25 plants were propagated on the medium which was found as optimal. Then, their callus growth and shoots regeneration capacities were compared. The majority of the shoots were regenerated from callus but direct organogenesis was also observed (8%).

Shoot regeneration was found to be the most efficient on MS medium containing 5MµBA and 3MµTDZ –97% of the explants produced shoots, while the average number of shoots was 15.5. The wild chicory shoots rooted easily.

Key words: *Cichorium intybus*, regeneration, TDZ, BA, callus formation and 2, 4-D

INTRODUCTION

Chicory (*Cichorium intybus* L.) is a member of the *Asteraceae* family. According to Vavilov (1951) the species originates from the Mediterranean region. In the historical times, wild chicory and chicory cultivars were popularized across Europe, Africa, Asia, the Americas, Australia and oceanic islands (Bais and Ravishankar 2001, and Tutin *et al*. 2010). The *C. intybus* species is characterized by high diversity with various botanical varieties distinguished. The main use of root chicory is the production of

coffee substitutes and food concentrates from dried roots (Bais and Ravishankar 2001, and Senderski 2009).

Chicory is an important medicinal plant which accumulates various specific organic compounds, such as storage polysaccharide inulin, sesquiterpene lactones, cumarins, phenolic acids and flavonoids (Bais and Ravishankar 2001, and Senderski 2009). Medicinal substances are found in all of its botanical varieties.

In folk medicine, infusions of chicory roots and herb are used to treat gastrointestinal diseases, hepatic cirrhosis and spleen swelling; they are also used externally in eczema treatment.

A rapid propagation of selected genotypes and genetically modified forms of chicory can be carried out by means of *in vitro* culturing. Chicory can be multiplied *in vitro* by somatic embryogenesis (Bellettre *et al*. 1999), by direct organogenesis from the shoot apices (Previati *et al*. 2005) and by the regeneration of adventitious shoots (Rehman *et al*. 2003). Much better results were obtained when the plants were propagated through adventitious shoots.

Studies on the potential for micro propagation through adventitious shoots were performed on wild chicory (var. Maurane) (Marek KOVÁR* and Ivan ČERNÝ 2012, and Yucesan *et al*. 2007) and cultivated forms (var. Sativum and var. Foliosum*)* (Nandagopal and Ranjitha Kumari 2006). In all studies, regeneration of shoots was preceded by the development of callus. Efficiency of callus induction and shoot regeneration depend on the type of explants and growth regulators.

The best results were obtained using leaf explants, callus weaker growth and fewer shoots were obtained from hypocotyls, petioles and roots (Velayutham *et al*. 2006, Yucesan *et al*. 2007). Research Rehman *et al*. (2003) shown that the efficiency of the regeneration of shoots in the chicory can be increased by means of casein hydrolyze (CH). The study Nandagopal and Ranjitha Kumari (2006) observed a beneficial effect of adenine sulphate (ADS). Young shoots of chicory produced roots on essential media without hormones, but the results of rooting were better in auxin application.

Therefore, the aim of our study was to assess the capacity of natural populations of wild chicory for plant regeneration from leaf blade fragments. An attempt was made at finding the optimal concentrations of benzyl-amino-purine (BA) cytokinin and thidiazuron (TDZ) for the production of the number of shoots per explant and the average shoot weight.

Finally, the effect of the NAA and 2, 4-D traits was assessed for the amount of callus.

MATERIALS AND METHODS

*Plant material, sterilization and preparation of explants.*Leaves of (*Cichorium intybus* L.) var. Maurane were collected in the Genetic Engineering and Biotechnology Research Institute (GEBRI), (Egypt) during January 2014 to July 2015.

The leaves were carefully washed with detergent and rinsed with tap water.

Fragments with an area of 0.5 cm^2 were cut off from midrib leaf. We examined the effect of the auxin1-Naphthaleneacetic acid (NAA), 2, 4-Dichlorophenoxyacetic acid (2.4D) (1,2 and 4 mg/L) and cytokinins 6-Benzylaminopurine, (BAP) (1, 3, 5 and 10 µM) and thidiazuron (TDZ) (1, 3, and 10 µM). After four weeks, the number of shoots (0.5-3 mm) and the number of shoots with leaves (> 3 mm) were recorded. In Fig. 1Bregeneration (No. of shoots) and in Fig. 2.The explants showed callus formation on NAA and 2, 4-D respectively.

Culture media.

These explants were then cultured aseptically on basal solid MS-medium with several treatments. The pH was adjusted to 5.7 with 1 N KOH or 1 N HCl before adding gel rite and prior to autoclaving at 121 ˚C (0.1 MPa) for 20 min. The cultures were kept in a growth chamber at 21 ± 1 ˚C, and a photoperiod of 16 h (30 µE m-2s-1, Philips TL 33 light) (Maroufi *et al*, 2012).

The cultivation was done in 300 ml glass jars containing 50 ml of basal MS-medium.

Statistical analysis

Forty to fifty explants were cultured per treatment. Each treatment consisted of 40-50 polypropylene jars (5 cm high) with 1 explant in each jar. In the graphs, we show percentages and means ± SE. The Student *t*-test and the χ2-test were used to evaluate the significance of differences with respect to means and percentages, respectively.

The experiments were carried out at least twice and similar results were obtained. Results were statistically analyzed by a factorial analysis of variance, in completely randomized design according to the procedure by Snedecor and Cochran (1981**)** and means were compared by multiple range tests. The data were illustrated by Origin Lab 8 Program.

RESULTS AND DISCUSSION

In our research, the beginning of callus production by the explants on all media was observed after 15–16 days (Fig. 1A).

The initial of shoot development was observed within 2 first weeks of the experiment. After four weeks from the establishment of the cultures, the percentage of explants which developed shoots counted for individual experimental sets ranged from 26.9 to 99.3%. Regardless of the concentration of BA, the percentage of explants with shoots increased with the increasing concentration of BA. In about 92% of explants, the development of buds and shoots were preceded by callus formation (Fig. 1B).

The remaining explants developed shoots with no previous callus formation (Fig. 1C). The amount of callus depended on the composition of the medium. After four weeks, the mean ratings of the amount of callus ranged from 1.1–8.0°. The most intense callus development was observed in the explants grown on the medium containing 1 mg/L NAA. In the final phase of the shoot regeneration process, some of the explants from each medium were observed to develop roots. The fewest explants (2.7–6.2%) produced roots on the media with the highest concentrations of BA.

Leaf explants of the plant formed new shoots especially at the high concentrations of TDZ (Fig. 1D). After 8 weeks of culture under the photoperiod cultivation and on MS medium, new shoots formed. 10µM TDZ give the highest level of shoots (5.58) comparing to the lower concentrations (Fig. 1E). Higher levels of TDZ caused browning and necrosis (Table 1).

The growth regulators also had a significant impact on the number of shoots produced. The average number of shoots per explant ranged from 4.2 to 15.47 in the whole experiment. At all concentrations of BA, the number of shoots increased with the increasing concentration of BA. In the explants which produced more shoots, the average shoot weight was most often smaller. It was found mainly on the leaves which were in direct contact with the medium. The highest percentage (15.5% and 18%) was observed on the medium containing 5µMBA and 3µMTDZ respectively.

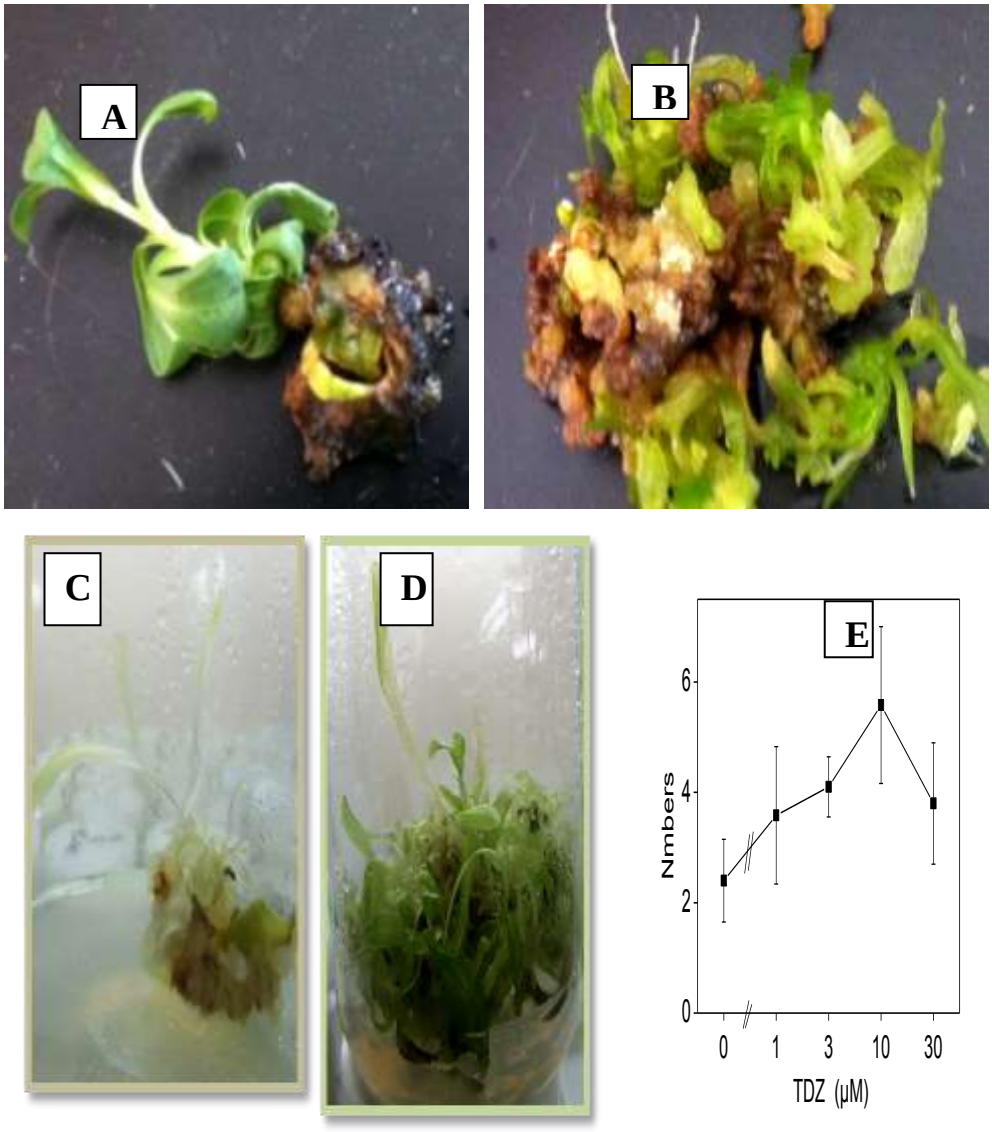

Figure 1. Micro propagation of wild chicory: A – leaf explant on 1M BA with direct shoots regeneration on leaf explant, B – shoots regeneration from callus, C shoots regenerated by organogenesis –, D – shoots formed on 3 M TDZ, E – The formation of shoots from leaves prepared from *C. intybus* leaves at increasing the concentrations of TDZ.

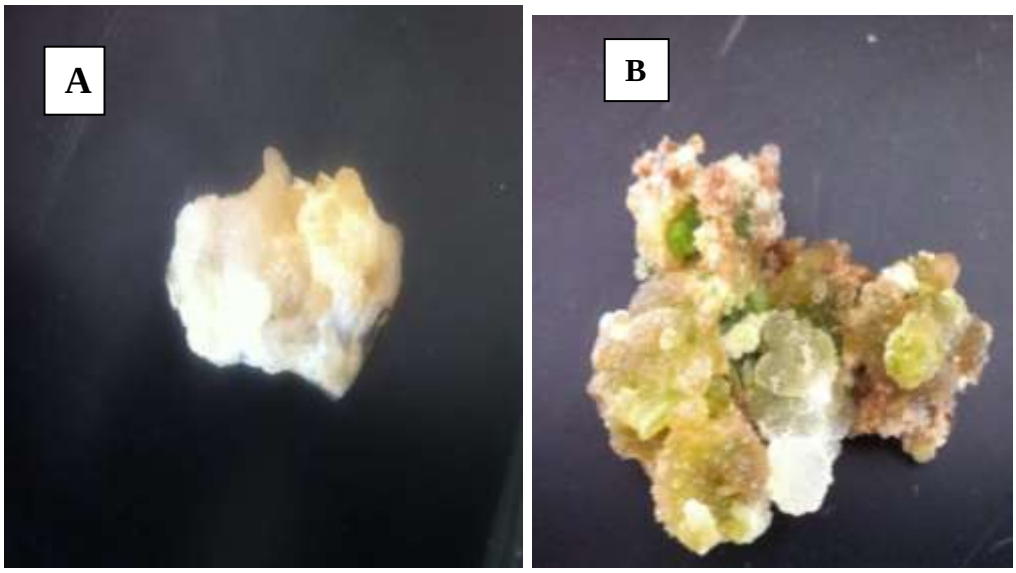

Figure 2. Callus formed on (A) NAA and (B) 2, 4-D.

In the earlier studies, the efficiency of shoot regeneration from leaf explants of *C. intbus* varied. It was depended mainly on the hormonal composition of the media and the material used for the experiments. Rehman *et al*. (2003) were plated wild chicory leaf explants on MS medium supplemented with various concentrations of IAA (0.09–0.35 mg dm-3) in combination with fixed concentrations of 6-benzylaminopurine (BAP – 1.13 mg/L), kinetin (KIN – 1.08 mg/L) and CH (1000 mg/L). The formation of callus was observed after 2 weeks; shoot buds initiated growth in 30-dayoldcallus. The shoots were separated from the 5-week-old callus. The percentage of explants forming callus ranged from 28.8 to 84.4% in individual experimental sets.

Nandagopal and Ranjitha Kumari (2006) were plated chicory leaf explants on medium MS+B5, supplemented with various concentrations of IAA, KIN, BAP and ADS. The development of callus was observed after 10–15 days, while the regeneration of shoots began when the callus was 30-day-old. After 45 days of culturing, the researchers found out that ADS has a positive effect on the percentage of explants which form callus, the percentage of callus which develop shoots and the number of shoots. The best results were obtained on medium containing 1.5 mg dm-3 BAP, 0.5 mg dm-3 IAA and 0.25 mg/L ADS. Callus was produced by 94.3% of the explants; shoots were regenerated by 93.0% of the celli, while the average callus produced 33 shoots. In the study by Yucesan *et al*. (2007), fragments of leaf blades have a little area (0.25 cm^2).

Table 1. Effect of growth regulators on callus induction and shoot regeneration

Treatment	Explants with shoots	No. of shoots/explant	Shoot wt. (mg)	Callus amount (1-9°)
TDZ (µM)				
0	59.7	2.5	25.3	0.3
1	45.5	7.6	32.3	0.7
3	85.0	4.5	18.4	0.4
10	0	0	0	0.8
BA (µM)				
0	26.8	2.6	23.5	0.1
0.5	80.1	3.5	29.4	0.2
5	70.0	5.7	31.5	0.6
10	91.2	2.1	16.46	0.3
2, 4-D (mg)				
0	59.7	2.5	23.4	1.2
1	12.4	0.2	0.5	1.93
2	11.6	0.4	0.9	2.1
4	13.8	0.3	0.4	5.7
NAA				
0	59.7	2.5	25.4	1.3
1	19.4	0.45	0.5	2.53
2	13.6	0.9	1.6	3.7
4	15.8	0.3	2.6	6.9

On the medium with auxins but without cytokinins, callus appeared but no shoots were formed. The development of callus along with the regeneration of shoots was observed on the media with cytokinins (BAP, KIN and thidiazuron – TDZ) no auxins, and on the media with combinations of both hormones. Shoot development began after 9 days culturing and the effects of shoot regeneration was assessed after 4 weeks. The best results were obtained on the medium containing 0.01 mg/L TDZ and 1 mg/L IAA, where 100% of explants produced shoots and the average shoot number was 35.8.

Among the combinations of KIN and BAP cytokinins with IAA and NAA auxins, the best results were obtained on the medium containing 0.3 mg/L IAA and 0.5 mg dm-3KIN (shoot regeneration: 100%, average number of shoots: 19.7).

CONCLUSIONS

1. The wild chicory shoots were regenerated mainly with callus formation, but about8% of the explants developed shoots with no previous callus growth.
2. The efficiency of shoot regeneration depended on the hormone concentrations and the proportion between BA and TDZ. The best results were achieved on the medium containing 5MµBA and 3MµTDZ.
3. The research has shown that selection can be carried out in wild chicory population for the ability to regenerate shoots from leaf explants. On the medium which considered optimal the examined plants were significantly differed in every compared 4feature.
4. The wild chicory shoots rooted easily.
5. A favorable property of the plants obtained was their ability for rapid acclimatization.

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