International Journal of ChemTech Research
CODEN(USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.12pp 141-149,2016

Establishment of in vitro root culture of *Cichorium endivia* subsp*, pumelum* L. –a multipurpose medicinal plant

Ahmed Amer[1], Mona Ibrahim[1], Mohsen Askr[2]

[1]Plant Biotechnology Department, National Research Centre, Dokki, Cairo, Egypt.
[2]Microbial Biotechnology Department, National Research Centre, Dokki, Cairo, Egypt.

Abstract: There is a great interest in the production of biologically active substances from plant origin in today's world. Since chicory roots serve as the major source of valuable secondary metabolites, the aim of this study was to develop an efficient protocol for the in vitro root culture of chicory. Medium supplemented with 0.5mg/l NAA and 1mg/l IBA was found to respond maximum with 91% (leaf), 76% (hypocotyl) and 94% (root) for root induction. The maximum biomass accumulation obtained from leaf, hypocotyl and root derived adventitious culture (4.098g, 3.163g and 4.500g respectively) was obtained on the medium contained 0.5mg/l NAA and 0.8mg/l IBA. While a significant increase in rooting response percentage was recorded for leaf (17%), hypocotyl (15%) and root (8%) cultured on half-strength MS medium compared to that cultured on full-strength MS medium, there was no significant differences recorded for the accumulation of root biomass. There was stimulatory effect of total dark condition on root induction and production in all types of explants. A maximum amount of fresh root biomass (9fold) was produced 6 weeks after culture. Moreover, further analysis showed that inulin obtained from our protocol is closely similar to that obtained from open field cultivation in terms of quality and quantity. This simple reproducible *in vitro* root culture system can be used for the production of adventitious roots and for the subsequent production of useful natural compounds from chicory, thereby providing an efficient alternative method to the field cultivation of intact plants.

Key words: *Cichorium endivia* subsp*, pumelum***,** root culture system, root induction and production, Inulin quantity and quality.

Introduction

Chicory (*Cichorium endivia* subsp, *pumelum* L.) is a medicinally important plant that belongs to the family Asteraceae. Although all parts of the plant are pharmacologically useful, roots are the principle material used in the traditional medicine. The tuberous root of this plant contains number of medicinally important compounds such as inulin, sesquiterpene lactones, coumarins, flavonoids and vitamins[1]. It is used as antihepatotoxic, antiulcerogenic, antiinflammatory, anticancer, antitumor, appetizer, digestive, stomachic, depurative and diuretic[2,3]. Inulin, a polymer of fructose, is reported to be useful for fat and sugar replacement, organoleptic properties, texture improvement[4], improvement of mineral absorption, immunomodulator properties and preventive effect against colon cancer[5]. Due to these properties, food and pharmaceutical industries have found many applications for inulin in the production of functional foods, nutritional composites and medicines[6]. Inulin is present in significant quantities in several fruits and vegetables, such as jerusalem artichoke, salsify, asparagus, onion, garlic, dahlia and chicory[7,8,9], but the main source for commercial scale of inulin production is root chicory [10,11,12].

The *in vitro* production of medicinal substances can be possible through plant tissue culture under precisely controlled physical and chemical conditions[13]. Unlike conventional methods of vegetative propagation, plant tissue culture systems allow higher multiplication rate and permit the production of pathogen-free material. However, most of the earlier studies were limited to develop an efficient *in vitro* regeneration system. Chicory can be multiplied *in vitro* by somatic embryogenesis[14], by direct organogenesis from the shoot apices[15] and by the regeneration of adventitious shoots[16]. On the other hand, development of fast growing root culture system offers unique opportunities for providing root drugs in the laboratory, without having to depend on field cultivation. Moreover, root cultures provide an efficient mean for maximum biomass production of bioactive natural products[17]. They are considered as a promising approach because of their genetic stability, rapid growth rate, easy preparation and maintenance and the capability to be cloned to produce a large supply of experimental tissue[18]. In the field of plant biotechnology, hairy root cultures, induced by Ri plasmid insertion, has been also reported as ideal production system for natural products to achieve industrial-scale production[19]. However, compared with hairy root cultures, normal root culture system is safer and easier for management[20].

Despite these varied useful medicinal uses of the root of chicory, there is lack of information in the literature of chicory on root culture system. In view of the increasing demand of Egyptian chicory for use as herbal medicine and considering the fact that roots are the principle material for herbal preparation which involve destructive harvesting, the aim of this research was to develop a protocol to establish the *in vitro* root cultures of chicory and later on to investigate the production of inulin from the *in vitro* chicory. In the present study, important factors affecting root induction and root growth from leaf, hypocotyl and root explants of Egyptian chicory were evaluated. In addition, inulin, an important medicinal compound presents in higher amount in the root of chicory, quantity and quality was investigated.

Experimental

Plant material and explants preparation

The seeds of (*Cichorium endivia* subsp, *pumelum* L.) were obtained from Agricultural Research Center, Ministry of Agriculture, Egypt. Seeds were immersed in 70% ethanol for 2 to 3 min then, they were rinsed three times in sterile distilled water. Next, the seeds were sterilized for 30 min in 20% commercial Clorox (5% NaOCl) containing 0.5% Tween20. After rinsing three times with sterile distilled water, Aseptic seeds were cultured on MS medium[21]. Leaf, hypocotyl and root pieces from three-week-old seedlings were used as explants for the present study.

In vitro culture conditions and root initiation

Leaf, hypocotyl and root explants from 21 days old seedlings were inoculated on half-strength MS semi solid medium or full-strength MS semi solid medium supplemented with different combinations and concentrations of indole-3-acetic acid (IAA), indole-3- butyric acid (IBA) and 0.5mg/l α-Naphthalene acetic acid (NAA) (Table 1). All media were adjusted to pH 5.8, and 0.7% agar and 3% sucrose were added. The cultures were incubated at 25±2°C under 16/8 h (light/dark) photoperiod with white fluorescent lights or under total darkness for four weeks. The responded explants that succeeded to induce root were scored.

Root production

After four weeks, the adventitious roots were cut to a length of 0.5-1.0 cm and rinsed in sterile water to remove the sticking agar gel. Thereafter, roots (0.5g) were cultured on half-strength MS semi solid medium or full strength MS semi solid medium supplemented with various combinations and concentrations of IAA, IBA and 0.5mg/l NAA (Table 2). All media were adjusted to pH 5.8, and 0.7% agar and 3% sucrose were added. The cultures were incubated at 25±2°C under 16/8 h (light/dark) photoperiod with white fluorescent lights or under total darkness for three weeks. After three weeks, the roots were subcultred to fresh medium containing the same combination and concentration of hormones for another three weeks.The root growth in different auxin regimes was assessed in terms of fresh weight after six weeks of culture.

Inulin extraction

An amount of 1 g of material sample was hot-water extracted (90°C) for 30 min. The volume remaining after the first extraction was topped up with water and the procedure was repeated. The two filtrate portions

were mixed together. A Ca (OH)2 solution was added until pH 8, and the mixture was left at room temperature for 1 h. The precipitated residue was filtered. The filtrate was neutralized with oxalic acid to pH 7, a small amount of activated charcoal was added, and the mixture was then stirred and filtered. The filtrate was precipitated with ethanol 95% overnight at 4°C and centrifuged at 5000 rpm for 20 min. The precipitated amorphous mass was filtered and rinsed twice with absolute 95 % ethanol twice with acetone, once with diethyl ether and dried at 50°C [22].

Determination of inulin by spectrophotometric

Two milliliter of aqueous solution of inulin were mixed with 2 mL of 10 % HCl and heated for 20 min in a boiling water bath. 0.5 mL of Selivanov's reagent (0.5 % solution of resorcinol in 20 % HCl) was added and further heated for 1 min. Absorption was measured at 520 nm using a spectrophotometer UV-visible 2401PC (Shimadzu, Japan) [23].

Determination of molecular weight of inulin

The average molecular weight of inulin was determined on an Agilent 1100 HPLC system equipped with a refractive index detector (RID) and FPl gel particle size (5µm), 3 columns of pore type (100, 104, 105 A°) on series, length 7.5 × 300 mm (1000,5000000) for DMF solvent, 3 µm (7.8 × 300 mm), Water Company Ireland. One column (5000-600000) for water solvent (polyethylene oxide/glycol standard) PL aquagel-OH 7.5 mm and 30um pore type 8um particle size. PL aquagel-OH 7.5 mm, 50 um pore type, 8um particle size, in series Mw from 100-1250000 g/mol. The sample 0.01 g was dissolved in 2 mL of solvent, and then it filtrated by siring filter 0.45 then the sample but in GPC device [24,25].

Determination of reducing sugars

The reducing sugars were determined according to DNS method [26] using fructose as standard.

Statistical analysis

Experiments were setup in a randomized completely blocks designs (RCBD), fifteen explants were used per treatment and each experiment was repeated three times.Means and standard errors (SE) were obtained from analysis for each treatment. Data were presented either as bar graphs with error bars by the use of computer program Microsoft Excel 2010 or as means ± SE and were compared with Duncan's multiple range test [27].

Results and Discussion

Root induction from different explants

Four factors were exploited; type of explants, level and type of auxin, strength of MS medium and photoperiod; for efficient induction of roots from leaf, hypocotyl and root explants. At first, the effect of different auxin regimes was tested. The obtained results in Table (1) showed differences in the rooting response percentage among different types of explants on the same auxin regime. Also, the same explants on the different auxin regimes showed different rooting percentages. In the present study, irrespective of the explants type, roots were produced on all media containing auxins. This result showed that auxins play vital role in the *invitro* root formation of chicory explants .In general, the production of adventitious roots in plant is controlled by growth substances and a key role in this process being played by auxins[28]. Moreover, various plant species exert positive effects on adventitious root formation as a result of auxin treatment[29]. Out of the different concentrations of IAA tested, medium supplemented with 0.5 mg/L NAA and 1mg/mL IAA was found to exert the maximum response with 82% (leaf), 67% (hypocotyls) and 86% (root) root induction frequency. When the different concentrations of IBA were tested, medium supplemented with 0.5 mg/L NAA and 1 mg/L IBA was found to respond maximum with 91% (leaf), 76% (hypocotyl) and 94% (root) for root induction. Thus, it was observed that IBA was more effective than IAA for the adventitious root induction of chicory explants. Similar result was reported by[30] as they indicated the superiority of IBA over IAA in the induction of adventitious roots in chicory.It is noteworthy that IBA have been reported to enhance root formation by initiating cell division, primordium structure and in inducing cell de-differentiation to form root apical meristem[31]. In contrary,[32] reported that the chicory shoots rooted better on MS medium supplemented with IAA rather than IBA. This behavior might be explained by the diversity of genetic background. The different genotypes had different sensitivity to the auxin treatment. It is generally known that genotypes remain one of the major factors affecting root induction and elongation by auxin(s).

Table 1.Effect of different auxins in half-strength MS semi solid media on root induction of leaf, hypocotyl and root explants *Cichorium endiviasubsp, pumelum* L. under total darkness

	Rooting response (%)		
Auxin(mg/ml)	**Leaf**	**Hypocotyl**	**Root**
NAA+IAA			
0.5 + 0.0	28 ± 1.73 [h]	19 ± 1.45 [j]	34 ± 1.86 [g]
0.5 + 0.2	54 ± 1.53 [g]	35 ± 1.15[i]	62 ± 2.00 [f]
0.5 + 0.4	65 ± 2.00[ef]	47 ± 1.76[gh]	70 ± 2.08[ef]
0.5 + 0.6	72 ± 1.15[de]	57 ± 1.15[ef]	77 ± 1.53[de]
0.5 + 0.8	77 ± 1.20[cd]	63 ± 1.53[c-e]	82 ± 1.45[cd]
0.5 + 1.0	82 ± 1.45[bc]	67 ± 1.00 [c]	86 ± 1.33[a-c]
0.5 + 1.2	81 ± 1.86[bc]	66 ± 1.33[cd]	84 ± 2.03[b-d]
NAA+IBA			
0.5 + 0.0	28 ± 1.73 [h]	19 ± 1.45 [j]	34 ± 1.86 [g]
0.5 + 0.2	59 ± 1.76[ef]	40 ± 1.15[hi]	66 ± 1.73 [f]
0.5 + 0.4	72 ± 1.45[de]	51 ± 1.86[fg]	78 ± 2.08[c-e]
0.5 + 0.6	81 ± 1.53[bc]	60 ± 1.20[de]	87 ± 1.45[a-c]
0.5 + 0.8	86 ± 1.15[ab]	69 ± 0.88[bc]	91 ± 1.53[ab]
0.5 + 1.0	91 ± 1.00 [a]	76 ± 1.53[a]	94 ± 1.67 [a]
0.5 + 1.2	89 ± 2.08[ab]	75 ± 1.15[ab]	92 ± 1.86[ab]
Data presented as means ± SE.Means within a column followed by the same letters are not significant at P=0.05 according to DMRT.			

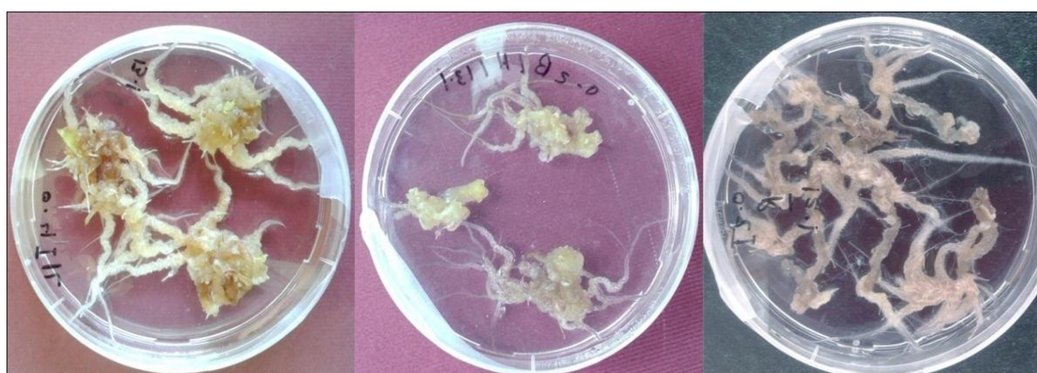

Figure 1. Root induction from different explants. A: leaf explants, B:hypocotyl explants, C:root explants

In addition to auxin, the ability of a plant to produce adventitious roots depends upon a large number of factors including the level of nitrates in the culture medium[33] and the composition of nutrients[34]. Different basal media or different strengths of basal medium respond differently to root induction due to differences in their nutrient composition. Therefore, the influence of the basal MS medium strength on the adventitious root formation of *Cichorium endivia* subsp*, pumelum L.* was examined. Our result clearly indicated that half-strength MS basal medium was better for the induction of roots from all types of explants(Figure1).A significant increase in rooting response percentage was recorded for leaf (17%), hypocotyl (15%) and root (8%) cultured on half-strength MS medium. This result is in accordance with that of [30] who pointed out that leaf and hypoctyl explants of chicory cultured on full-strength MS medium always induced profuse callusing which subsequently turned brownish and hindered initiation of roots. Among the tested explants, the highest percentage of rooting response was shown in root explants (94%), followed by leaf explants (91%) while hypocotyl explants showed the lowest frequency of rooting response 76% (Figure 2). Our results are in agreement with those reported by [35] who obtained higher root biomass from root explants rather than those obtained from leaf explants in *Luffa Acutangula*. Also,[36] reported that adventitious root proliferation and biomass accumulation obtained from root explants were higher than those derived from leaf explants in *Periploca sepium*. In another study, leaf explants of chicory showed rapid induction of roots and higher growth compared to hypocotyl explants[30]. [37] justified that the leaf segments were good starting tissue for root induction may due to the presence of cells associated with the leaf veins (vascular tissue), which can be readily stimulated

by adding auxins and phytohormones can easily be manipulated to direct pluripotent cells to a particular cell fate.

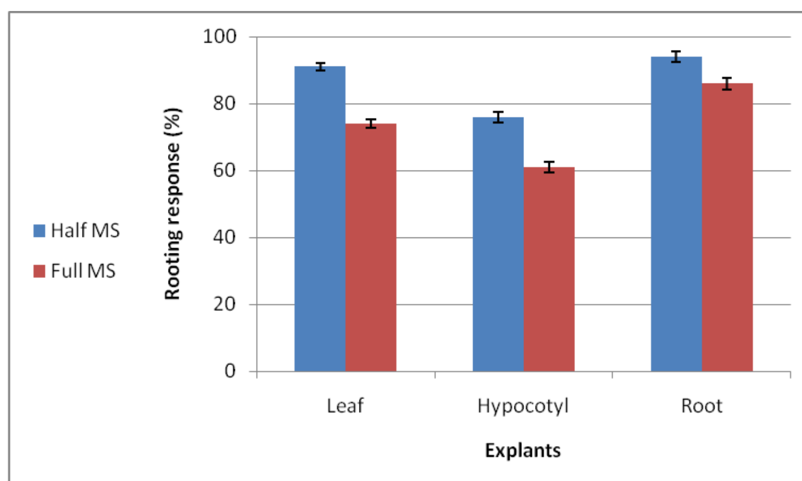

Figure 2. Effect of basal MS strength on root induction

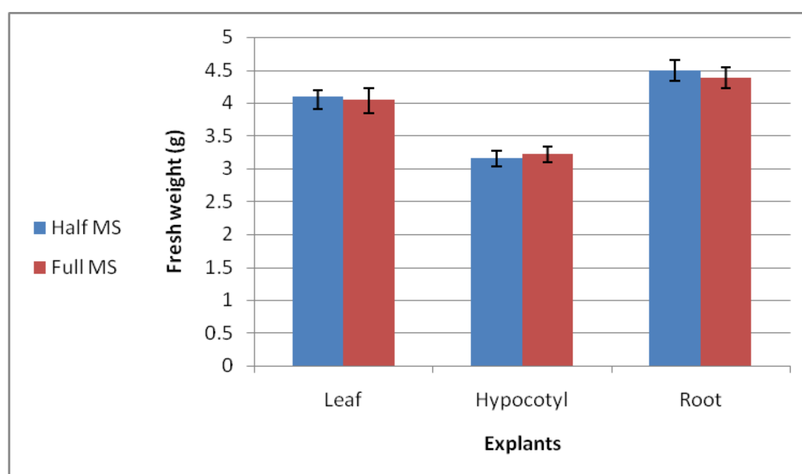

Figure 3. Influence of illumination on root induction

To improve rooting response frequency, the effect of illumination was investigated. Our data in Figure (3) showed that the rooting response under total darkness was more promising than that under light condition (16h). All types of explants showed better performance under total darkness but the magnitude of the rooting response activity was varied. In this respect, leaf and root explants had the highest increasing frequency (9%) while root explants had the lowest increasing frequency (5%). Roots were initiated within one to two weeks in root and leaf explants and within three weeks in hypocotyl explants under total darkness. Whereas rooting, under light treatment (16h) was delayed one week in most cases.This fits with the natural system where roots grow in soil in full darkness underground and far from sun light. The present finding is in consistent with that of [30] who produced more and faster adventitious roots in dark condition than light condition.

Root production

The root growth in different auxin regimes was assessed in terms of fresh weight after six weeks of culture. Differences were observed on the basis of auxin type and concentration and nature of the explants(Table 2).Among the different concentrations of IAA used, the maximum biomass accumulation obtained from leaf and hypocotyl derived adventitious culture (3.794 g and 2.866 g, respectively) was recorded on the medium contained 0.5 mg/L NAA and 1.0 mg/L IAA while the highest biomass accumulation achieved from root adventitious culture (4.301 g) was obtained on the medium supplemented with 0.5 mg/L NAA and 0.8 mg/L IAA. Root biomass accumulation increased from 1.083 g, 0.889 g and 1.243 g (in leaf, hypocotyl and root adventitious culture respectively) to 4.098 g, 3.163 g and 4.500 g with the increasing of IBA concentration

from 0.0 mg/L to 0.8 mg/L. On the whole, though IBA was significantly better than IAA for the induction of roots, it was slightly better than IAA for the production of roots. It was observed also that, the best concentration of IBA (1.0 mg/L in all types of explants) used to initiate roots was higher than that (0.8 mg/L) used to produce maximum root biomass. This result might be explained by the widely accepted theory that high level of auxin promotes the production of adventitious roots, although the auxin inhibits the elongation of root [38]. In the present system, the maximum fresh weight of roots was noticed at the sixth week of cultureand then the growth was slowly declined. Reduced root growth might have been due to the accumulation of endogenous auxins during each subculture. Most likely, loosing root differentiation ability is one of the most important defects of maintaining normal root culture for a long period as observed in chicory plant [30].

Table 2.Effect of different auxins in half-strength MS semi solid media on root production of *in vitro*-derived leaf, hypocotyls and root explants of *Cichorium endivia* subsp, *pumelum* L. under total darkness

Fresh weight (g)			
Auxin(mg/ml)	**Leaf**	**Hypocotyl**	**Root**
NAA+IAA			
0.5+ 0.0	1.083 ± 0.250^{f}	0.889 ± 0.122^{f}	1.243 ± 0.234^{d}
0.5+ 0.2	2.293 ± 0.193^{a}	1.607 ± 0.200^{de}	$0.281^{c}2.437 \pm$
0.5+ 0.4	$2.840 \pm 0.111^{c-e}$	$1.996 \pm 0.101^{c-e}$	$3.362 \pm 0.164^{a-c}$
0.5+ 0.6	$3.304\pm 0.121^{a-d}$	$2.284 \pm 0.136^{e-d}$	3.860 ± 0.132^{ab}
0.5+ 0.8	3.722 ± 0.102^{ab}	2.836 ± 0.140^{ab}	4.301 ± 0.098^{a}
0.5+ 1.0	3.794 ± 0.178^{ab}	2.866 ± 0.169^{ab}	4.224 ± 0.126^{a}
0.5+ 1.2	$3.432 \pm 0.108^{a-d}$	$2.495 \pm 0.117^{a-c}$	3.696 ± 0.142^{ab}
NAA+IBA			
0.5+ 0.0	1.083 ± 0.250^{f}	0.889 ± 0.122^{f}	1.243 ± 0.234^{d}
0.5+ 0.2	2.637 ± 0.133^{de}	1.542 ± 0.202^{ef}	2.762 ± 0.149^{bc}
0.5+ 0.4	$3.225 \pm 0.139^{b-d}$	$2.193 \pm 0.164^{b-e}$	$3.401 \pm 0.272^{a-c}$
0.5+ 0.6	$3.626 \pm 0.129^{a-c}$	2.714 ± 0.105^{ab}	4.480 ± 0.115^{a}
0.5+ 0.8	4.098 ± 0.099^{a}	3.163 ± 0.113^{a}	4.500 ± 0.149^{a}
0.5+ 1.0	3.985 ± 0.131^{ab}	3.102 ± 0.096^{a}	4.369 ± 0.138^{a}
0.5+ 1.2	$3.594 \pm 0.108^{a-c}$	2.826 ± 0.149^{ab}	3.900 ± 0.199^{ab}
Data presented as means ± SE.Means within a column followed by the same letters are not significant at P=0.05 according to DMRT			

In the present research, two different basal media, *i.e.*, full strength MS and half strength MS, were tested for their efficiency for root production. Intriguingly, half-strength MS medium was found to yield the maximum response of root biomass in leaf and root adventitious culture (4.098 g and 4.500 g, respectively) whereas full-strength MS medium appeared to accumulate the highest amount of root biomass in hypocotyl derived adventitious culture (3.222 g)(Figure 4).Comparatively, there were no significant differences recorded in all the three cultures used between using full-strength MS and half-strength MS. This result is in disagreement with that of [30] who favored half strength MS medium for the initiation and production of roots of chicory. This conflict might be illustrated by the physical condition of the culture media used. In some instances, the response of liquid medium on root production of a particular culture may vary with the response of semi solid media even though, they both have the same nutritive requirements. It should be noted that most reports previously have been utilized liquid MS media for the induction and proliferation of roots in various plant species including chicory. Interestingly, in our experiments, semi solid MS proved to be efficient for the successful initiation and production of roots. Generally, semi solid media has distinct advantages over liquid media such as easy operation and less infection by microorganisms. However, this result needs to be verified in future studies.

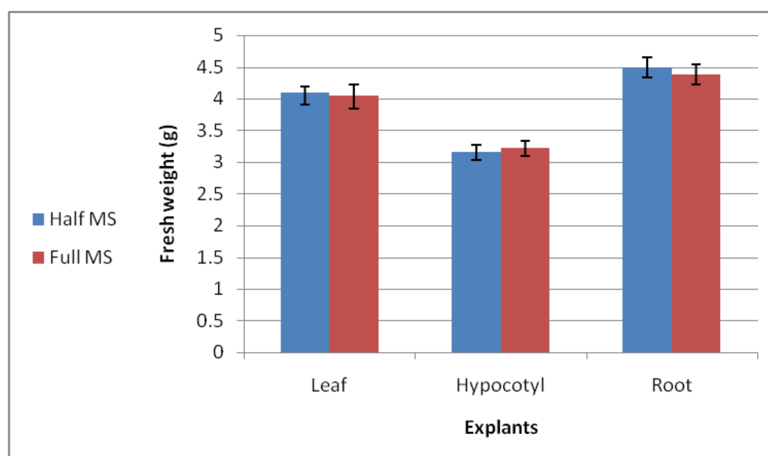

Figure 4. Effect of basal MS strength on root production

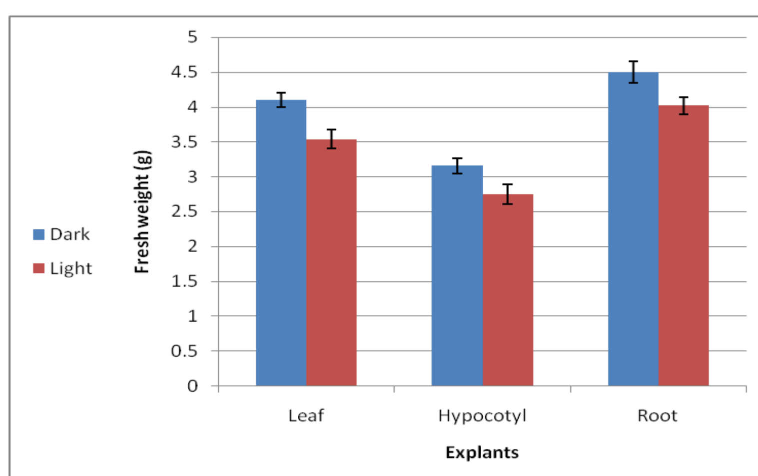

Figure 5 .Influence of illumination on root production

Since plant roots are extremely sensitive to light [39] and this sensitivity is important for their optimal performance [40], further investigation of the impact of photoperiod on root production was accomplished. The results showed that there was stimulatory effect of complete dark condition on root production in all tested samples as shown in Figure (5). Under light condition (16h), the average fresh weight of roots decreased from 4.089 g, 3.163 g and 4.500 g in leaf, hypocotyls and root derived adventitious cultures, respectively to 3.538 g, 2.753g and 4.025g.In accordance with our finding, rooting response reported to be positively influenced by dark condition [20, 41, 42].

Inulin analysis

Inulin, as the most important natural bioactive compound in chicory roots used by industry, was selected for this study. The inulin production was determined in terms of yield, purity, molecular weight and rescuing sugars. The percentage yield of the row extracts were found to contain 17.2% inulin which was 92% in purity and 2.5% free fructose (Table 3).In addition, the molecular weight of inulin extract was 8.09×103 g/mol , indicating it as a medium chain polysaccharide which greatly determines its functional properties. Previously, inulin production was analyzed from the *in vitro* and *in vivo* regenerated plants of chicory [43] but there was no comparative work reported on the production of inulin from tissue culture and open field cultivation. In the present study, no significant differences were recorded between inulin obtained from our protocol and that obtained from open field cultivation. Therefore, our root culture system of chicory could be an alternative method to produce inulin as well as other bioactive compounds in a shorter time with higher quantities and without depending on agroclimatic conditions. Some recent extraction methods were applied for inulin extraction from chicory such as microwave assisted extraction method (MAE) which reported by [44]. In this regard, the yield of inulin content was higher (51.2%) compared to conventional extraction methods. However,

it should be noted that our aim was to compare between the inulin obtained by our protocol and that obtained by open field cultivation with the same extraction method rather than optimize and improve the extraction efficiency. The relatively low percentage of inulin content in the present work might also be explained by the young age of tissue (8 weeks) since it is well known that inulin content depends upon many factors such as plant source, climate and growing conditions, harvesting date, storage time after harvest and extraction and post-extraction processes.

Table 3. Inulin content, purity, molecular weight and rescuing sugars

Sample	Inulin yield (%)	Inulin purity (%)	Mw (g/moL)	Reducing sugars (%)
Open field cultivation	18.7±0.81	95±3.22	8.12×10^3	2.7±0.19
Root culture system	17.2±0.77	92±3.27	8.09×10^3	2.5±0.21

References

1. Varotto S., Lucchin M. and Parrin P., Immature embryos culture in Italian red Chicory (Cichorium intybus), Plant Cell Tiss. Org. Cult., 2000, 62, 75-77.
2. Nandagopal S. and RanjithaKumari B.D., Enhancement of inulinase production from chicory and fenugreek rhizosphere soil, American-Eurasian J. of Agric. & Environ. Sci., 2006a, 3, 225-228.
3. Hazra B., Sarkar R., Bhattacharyya S. and Roy P., Tumour inhibitory activity of chicory root extract against Ehrlich ascites carcinoma in mice, Fitoterapia,2002, 73, 730-33.
4. Franck A., Technological functionality of inulin and oligofructose. Br. J. Nutr., 2002, 87, 287-291.
5. Watzl B., GirrbachS.and Roller M., Inulin, oligofructose and imunomodulation, Br. J. Nutr.,2005, 93, 49-55.
6. Figueira G.M., Park K.J., Brod F.P.R. and Honório S.L., Evaluation of desorption isotherms, drying rates and inulin concentration of chicory roots (Cichoriumintybus L.) with and without enzymatic inactivation, J. Food Eng., 2004, 63, 273.
7. Van Loo J., Coussement P., De Leenheer L., Hoebregs H. and Smits G., On the presence of inulin and oligofructose as natural ingredients in the Western diet, Crit. Rev. Food. Sci. Nutr., 1995, 35,525-552.
8. Rumessen J.J., Bode S., Hamberg O. and Gudmand-Hoyer E., Fructans of Jerusalem artichokes: intestinal transport, absorption, fermentation and influence on blood glucose, insulin and c-peptide responses in wealthy subjects, Am. J. Clin. Nutr., 1990, 52,675-681.
9. Nilsson U., Oste R., Jagerstad M. and Birkhed D., Cereal fructans: in vitro and in vivo studies on availability in rats and humans. J. Nutr., 1988, 118, 1325-1330.
10. Silva R.F., Use of inulin as a natural texture modifier. Cereal Foods World, 1996, 41,792-794.
11. De Leenheer L., Production and use of inulin: Industrial reality with a promising future. In: Bekkum H, Van HR, Voragen AGJ, (eds), Carbohydrates as Organic Raw Materials III, NY: VCH Publishers Inc. New York, 1996, 67-92.
12. Van LaereA.and Van Den Ende W., Inulin metabolism in dicots: chicory as a model system. Plant, Cell and Environment, 2002, 25 (6), 803-813.
13. RamachandraRao S. and Ravishankar G.A. Plant cell cultures: Chemical factories of secondary metabolites, Biot. Advances,2002, 20,101-153.
14. Abdin M.Z and Ilah A., Plant regeneration through somatic embryogenesis from stem and petiole explants of Indian chicory (Cichriumintybus L.), Ind. J. Biotechnol., 2007, 6, 250-255.
15. Previati A., Benelli C., Da Re FDe, Carlo A., VettoriC.andLambardi, M., In vitro propagation and conservation of red chicory germplasm. The role of biotechnology. Villa Gualiano, Turin, Italy, 2005, 5–7 March 207–208.
16. Nandagopal S. and RanjithaKumari B.D., Adenine sulphate induced high frequency shoot organogenesis in callus and in vitro flowering of Cichorium intybus L. cv. Focus- a potent medicinal plant, ActaagriculturaeSlovenica, 2006b, 87, 415 – 425.
17. Choi S.M., Son S.H., Yun S.R., Kwon O.W., Seon J.H. and Paek K.Y., Plot-scale culture of adventitious roots of ginseng in a bioreactor system. Plant Cell Tiss. Organ Cult., 2000, 62, 187-193.
18. Stephen G., Saupe ., Plant organ culture culturing excised tomato roots, Plant Physiol., 2005, 320-324.
19. Georgiev M.I., Pavlov A.I. and BleyT.h., Hairy root type plant in vitro systems as sources of bioactive substances, Appl. Microb. Biotechnol, 2007, 74, 1175–1185.
20. Sudha C.G. and Seeni S., Establishment and analysis of fast-growing normal root culture of Decalepisarayalpathra, a rare endemic medicinal plant. Curr. Sci., 2001, 81, 371-374.

21. Murashige T. and Skoog F., A revised medium for rapid growth and bio assays with tobacco tissue culture, Physiol. Plantarum, 1962, 15, 473-497.
22. Bouaziz M.A., Rassaoui R. and Besbes S., Chemical Composition, Functional Properties, and Effect of Inulin from Tunisian Agave americana L. Leaves on Textural Qualities of Pectin Gel Journal of Chemistry, 2014, Article ID 758697, 11 pages.
23. Gibson G.R., Willis C.L., Van Loo J., Non-digestible oligosaccharides and bifidobacteria- implications for health. Intl, Sugar J., 1994, 96, 1150-1156.
24. Jun L., Jiangguang L., Hong Y., Yi S., Zhaoxin L. and Xiaoxiong Z., Production, characterization and antioxidant activities in vitro of exopolysaccharides from endophytic bacterium paenibacillus polymyxa EJS-3. Carbohydrate Polymers,2009, 78, 275–281.
25. You L., Qing G., Mengying F., Bao Y., Jiaoyan R., Longjian G., Chun C. and Mouming Z., Structural characterisation of polysaccharides from Tricholomamatsutake and their antioxidant and antitumour activities, Food Chemistry, 2013, 138, 2242–2249.
26. Miller G.L., Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 1959, 31(3), 426–428.
27. Duncan D.B., Multiple range and multiple F test. Biometrics, 1955, 11, 1-42.
28. Henselova M., Synergistic effect of benzolinine with IBA and fungicides on the vegetative propagation of ornamental plants, park, and fruit woody species, Zahradnnictvi, 2002, 29 (2), 41-50.
29. Wan X., Landhausser S.M., Lieffers V.J. and Zwiazek J.J., Signals controlling root suckering and adventitious shoot formation in aspen (Populustremuloide) .Tree. Physiol., 2006, 26, 681–687.
30. NandagopalS.andRanjithaKumari B.D., Effectiveness of auxin induced in vitro root culture in chicory, J. Cent. Eur. Agric., 2007, 8 (1), 73- 80.
31. BerlethT.and Sachs T., Plant morphogenesis: long distance Co-ordination and local patterning cell, Opin. Plant Biol., 2001, 4,579-563.
32. Yucesan B., Turker A.U., Gurel E., TDZ-induced high frequency plant regeneration through multiple shoot formation in witloof chicory (Cichoriumintybus L.), Plant Cell Tiss. Organ Cult., 2007, 91, 243–250.
33. Eduardo S.V., In vitro root induction in hypocotyls and plumule explants of Helianthus annuus, Env. Exp Bot., 1998, 39(3), 271-277.
34. Obert B., Dedicova B., Hricova A., Samaj J.and Pretova A., Flax anther culture: effect of genotype, cold treatment and media, Plant Cell Tissue Org. Cult., 2004, 79, 233-238.
35. Umamaheswari1 C., Ambethkar A., Margaret F.S. and Selvaraj N., In Vitro Root Culture From Roots And Leaf Explants Of LuffaAcutangula(L.) Roxb. International Journal of Innovative Research in Science, Engineering and Technology, 2014, 3(10), 16886- 16895.
36. Zhang J., Gao W.Y., Wang J. and Li X.L., Effects of explants types and media salt strength on growth and secondary metabolites accumulation in adventitious roots of Periplocasepium Bunge, Acta. Physiol. Plant, 2011, 33(6), 2447-2452.
37. Kiong Ling A.P., Kok K.M., Hussein S. and Ong S. L., Effects of Plant Growth Regulators on Adventitious Roots Induction from Different Explants of OrthosiphonStamineus, American-Eurasian Journal of Sustainable Agriculture, 2009, 3 (3), 493-501.
38. Blakesley D. G., Weston D. and Hall J.F., The role of endogenous auxin in root initiation. Part I. Evidence from studies on auxin application and analysis of endogenous levels, Plant Growth Regulation, 1991, 10, 341-353.
39. Galen C., Rabenold J.J. and Liscum E., Light-sensing in roots. Plant Signal Behav., 2007a, 2, 106-8.
40. Galen C., Rabenold J.J. and Liscum E., Functional ecology of a blue light photoreceptor: effects of phototropin-1 on root growth enhance drought tolerance in Arabidopsis thaliana, NewPhytol., 2007b, 173, 91-9.
41. Jin-Wook S., Cha-Gyun S. and Yong-Eui C., Mass production of adventitious. root of Eleutherococcussessiliflorus through the bioreactor culture, J Plant Biotechnology, 2003, 5, 187-191.
42. Afshari R. T., Angoshtari R. and Kalantari S., Effects of Light and Different Plant Growth Regulators on Induction of Callus Growth in Rapeseed (Brassica Napus L.) Genotypes, Plant Omics Journal, 2011, 4 (2), 60-67.
43. Rehman R.U., Israr M., Srivastava P.S, BansalK.C.andAbdin M,Z., Invitro cell develop Biol. Plant, 2003, 39, 142-146.
44. Tewari S., Ramalakshmi K., Methra L. and Mahan Rao LJ., Microwave-assisted extraction of Inulin from chicory roots using response surface methodology, J Nutr. Food sci., 2015, 5,342. doi: 10:4172/2155-9600. 10003342.

*****