

Direct Multiple Shooting Induction of *Taraxacum*

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Abstract - Plants of the genus *Taraxacum* are well-known as a traditional herbal remedy with a long history, and they have also been extensively used as food, chemicals and cosmetics. In this study, four *Taraxacum* species distributed in Korea (*T. mongolicum*, *T. officinale*, *T. mongolicum* variation and *T. officinale* variation) were utilized for an efficient method for direct multiple shooting induction and regeneration, using leaf blade, transition zone, petiole and root as explants in MS media with various hormone concentration and combination. MS medium containing IAA 0.2 mg/L and TDZ 1.0 mg/L showed the highest induction frequency of all the hormone combinations. Besides, the induction of *T. mongolicum* variation was most effective comparing with the other three species by the average induction frequency of four explants. While the induction effect of leaf blade explant was more obvious than the other three explants. This system exhibited a rapid propagation of shoots from the leaf blade explants and makes it convenient to make use of these *Taraxacum* species to develop their diverse applications in the future.

Key words - Multiple shoots, *Taraxacum*, Explants, TDZ, Vitrification, Organogenesis

Abbreviations

MS : Murashige and Skoog

TDZ : Thidiazuron

IAA : Indole-3-acetic acid

BA : 6-benzyladenine

NAA : α -naphthalene acetic acid

Introduction

The genus *Taraxacum* is a member of the family Asteraceae, subfamily Cichorioideae, tribe Lactuceae and widely distributed in the warmer temperate zones of the Northern Hemisphere (Lee *et al.*, 2004).

As we know, most of the previous researches on *Taraxacum* focused on its apomictic characteristics and effective components (Molinari *et al.*, 2003). Individuals from apomictic heredity are in line with their female parents, so it plays an important role keeping superior characters on breed improvement. Booth and Satchuthanathavale (1974) tried to learn the effects of hormones on regeneration of root cuttings and

genetic transformation in *T. officinale*. And they investigated a suitable method for the production of secondary metabolites based on their stable and high productivity in other systems. The effects of a number of hormonal amendments (IAA, kinetin, NAA, adenine sulfate and cytokinins) on the regeneration of shoots and roots in *T. officinale* (Bowes, 1970; 1971). It was able to generate *T. officinale* callus with teratomatal shoots (non-transgenic), which is predicting a more positive future on the research of *Taraxacum*.

Apart from being used as a pharmaceutical as mentioned above, inflorescences, leaves and roots of *Taraxacum* are processed into different food products high in vitamin A, vitamin C and iron, dried *Taraxacum* leaves and roots are available as herbal teas, and the powdered root is sold in capsule form (William and Harper, 1994). The roasted root is also used as a substitute for coffee. *Taraxacum* root contains high amounts of triterpenes of four different skeletons, namely lupeol, α -amyrin, β -amyrin and taraxasterol, as free alcohols and their esters (Power and Browing, 1912; Burrows and Shimpson, 1938; Masaaki *et al.*, 1999). The extracts are used as flavor components in various food products, including alcoholic beverages and softdrinks, frozen dairy desserts, candy, baked goods, gelatins and puddings and

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cheese (Rivera-Nunez, 1991). Their constituents and pharmacological profiles are also different depending on species. For example, *T. officinale*, has diuretic, laxative, cholagogue, anti-rheumatic, anti-inflammatory, choleric, anti-carcinogenic and hypoglycemic activities. And its crude extract of root (DRE) was found to block invasion of MCF-7/AZ breast cancer cells while DLE blocked the invasion of LNCaP prostate cancer cells. While *T. mongolicum* has some other specific constituents exhibiting corresponding effects. Even for the explants, their discrepancy decides constituents differentiation of different explants (Katrin *et al.*, 2006). For this reason, this direct multiple shooting induction system comprehensively consolidated the superiority of explants, species and hormones at the same time. It supported a reference as a matter of convenience which directly reflected a comparison for researchers to choose what they need for food or pharmacological profiles and so on. For instance, if someone wants to get some specific constituent only existing in *T. mongolicum* he can use the optimal explant and hormone combination.

Materials and Methods

Plant material

This study was based on four *Taraxacum* species: *T. officinale*, *T. mongolicum* and their two variations *T. officinale* variation and *T. mongolicum* variation. Their seeds naturally grown in Korea were sterilized by immersion in 10% sodium hypochlorite for 6 minutes followed by 30 seconds in 70% ethanol. The seeds were washed for four times with sterilized distilled water and then cultured on half-strength MS solid medium with 2% sucrose (Murashige and Skoog, 1962). All media used in this study were adjusted to pH 5.8 and then autoclaved at 121 °C for 20 min.

Culture condition

Fifty seeds were cultured in 90×20 mm Petri dishes containing 30 ml medium in dark in 28 °C culture room (Figure 3A). After 1 week the generating seedlings were transferred into 100×40 mm Petri dishes with 5 seedlings in 50 ml half-strength MS per dish. The culture room was maintained at a temperature of 28 °C, a 16 h photoperiod, and a light intensity of 24 μmol m⁻² s⁻¹ (white-fluorescent tubes). One month later sterilized

plantlets of 6–8 cm height for explants use later came out which was then shown in Figure 3B.

Direct multiple shoot induction

The aseptically plantlets were cut into four parts (Figure 1): leaf blade without midvein, transition zone, petiole and root so as to find out the diversity of induction frequency because of explant discrepancy. First the four explants were respectively plated into MS solid media with four kinds of hormone combination (Figure 3C): MS, MS with 1.0 mg/L BA, MS with 0.2 mg/L IAA and 4.0 mg/L BA, MS with IAA 0.2 mg/L and TDZ 1.0 mg/L (Yang *et al.*, 1996). Ten samples were inoculated in one Petri dish and there would be triplicate of each species in each kind of medium. All these experiments were operated aseptically inside the clean bench. The culture condition was set at 28 °C in light for 12 hours and 28 °C in dark for 12 hours everyday, within 1 month the morphological characteristics of induced direct multiple shoots and callus of different *Taraxacum* species were observed. Here *T. mongolicum* was used for this selection and according to their growing condition we selected the best inducing medium. Then this optimal medium was applied for screening the best species and best explant respectively. Subsequently, the induced direct multiple shoots (Figure 3D) were inoculated in rooting medium, blank MS medium and MS containing 2.0 mg/L NAA, and 2 weeks later the newly regenerated roots from induced shoots came out in the rooting medium as shown in Figure 3E. And the hormone were all sterilized by filtering and saved under 4 °C, which was supplemented to medium under 60 °C after 121 °C, 20 min autoclaving.

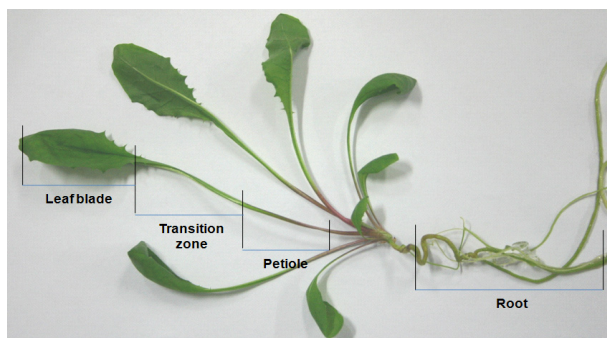


Fig. 1. A complete plantlet used to cut into four kinds of explants, leaf blade, transition zone, petiole and root respectively from left to right.

Plantlets with both shoots and roots were transferred to soil after two months keeping for further growing. The *Taraxacum* plants from adventitious shoots were also finally regenerated (Figure 3F). Ratios of the generated adventitious shoots number to total explants number as four kinds of explants, from the four species, on the four media were used to analyse the data in order to compare the condition and make clear of the influence of hormones on *Taraxacum* direct multiple shooting induction. The ratios of induced adventitious shoots were calculated by dividing the total explants number. The related data processing depended on systat. SigmaPlot 11.1.

Results and Discussion

Altogether four species were used for the establishment of this direct multiple shoots system. The different species direct multiple shoots rates were respectively summarized. And we could also see that the multiple direct shoots of almost each explant knot at more than three as shown in Figure 3D in this research, which meant it was an efficient operation. It also ensured that the subsequent research was worthy to continue.

As for the direct multiple shoots of *Taraxacum*, it is obvi-

ously found that *T. mongolicum* showed the best shoot growth condition in MS medium supplemented with IAA 0.2 mg/L and TDZ 1.0 mg/L (Hoya *et al.*, 2007). The MS with IAA and TDZ among the four compositions had induced high efficient shoots according to Table 1, it means that TDZ had really great induction effect.

As we all know that TDZ is a substituted phenylurea compound and has emerged as a highly efficacious bioregulant of morphogenesis in the tissue culture of many plant species. Application of TDZ induces a diverse array of cultural responses ranging from induction of callus to formation of somatic embryos. TDZ exhibits the unique property of mimicking both auxin and cytokinin effects on growth and differentiation of cultured explants. TDZ is extensively used in plant tissue culture as plant growth regulator now, it can induce types of micropropagation from the material *in vitro* of many kinds plants. Shoot formation occurred only when a low TDZ concentration for cultivation was used. TDZ inducing micropropagation principles in recent years are studied (Xu and Huang, 2003). The research progress that relevant TDZ inducement plant micropropagation and possible principles were briefly described. In this study we can clearly see that TDZ

Table 1. Comparison of different *Taraxacum* species multiple shoot rates of different explants in MS with IAA and TDZ

Species	Explants	Adventitious shoot rates (%)	Average rates (%)
<i>Taraxacum mongolicum</i>	Leaf blade	83.33 ± 5.77	45.833
	Transition zone	10.00 ± 0.00	
	Petiole	66.67 ± 5.77	
	Root	26.67 ± 5.77	
<i>Taraxacum officinale</i>	Leaf blade	50.00 ± 0.00	43.333
	Transition zone	43.33 ± 5.77	
	Petiole	30.00 ± 0.00	
	Root	50.00 ± 0.00	
<i>Taraxacum mongolicum variation</i>	Leaf blade	66.67 ± 5.77	72.503
	Transition zone	100.00 ± 0.00	
	Petiole	56.67 ± 5.77	
	Root	66.67 ± 5.77	
<i>Taraxacum officinale variation</i>	Leaf blade	10.00 ± 0.00	2.500
	Transition zone	0.00	
	Petiole	0.0033	
	Root	0.00	

took effects obviously in inducing direct shoots in *Taraxacum* whichever part it explanted.

Optimal medium was utilized on the explant selection. For the four explants of the seedlings, it was clear that the leaf blade explant had the best induction frequency, besides transition zone also had better growing activities than other explants (Figure 2). From Figure 2 we can see that the induction frequency of leaf blade explant of most species was more than 50%. Besides, only *T. officinale*'s leaf blade explant was induced direct multiple shoots, but nothing generates in the other three species, which means that the leaf blade explant has even higher inducing activity.

And then the selected optimal medium was also applied for screening best species from the four species. The most evident was that, *T. mongolicum* variation, its prevalent direct multiple shoots growth condition is the best of all the four explants including leaf blade, transition zone, petiole and root parts. Its average induction frequency even reached 72.503% (Table 1). At the same time the frequency of *T. mongolicum* and *T. officinale* reached 45.833% and 43.333% respectively. While *T. officinale* showed so little induction effect for its inducing rate reaching only 2.5%, which could even be ignored.

After direct multiple shoots came out in the induction media, two kinds of rooting media (MS and MS with NAA 2.0 mg/L) were designed and the adventitious shoots were transferred into the rooting media and began to induce their roots. Finally the shoots of most species showed better root

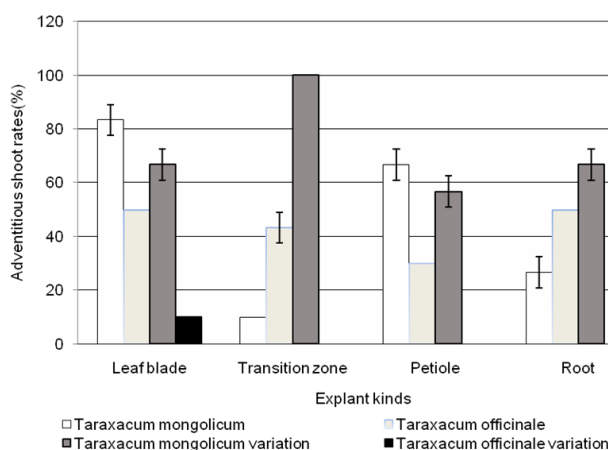


Fig. 2. Comparison of multiple direct shoots rates among different explants of different species in MS medium with 0.2 mg/L IAA and 1.0 mg/L TDZ.

inducing effect in MS with NAA 2.0 mg/L.

Some characteristics of induced multiple shoots knots in different MS media of most species were observed: Some callus of root part appeared big, soft, green were closely together in MS with BA 1.0 mg/L, showing loose morphological structure and in 2 mm granular shape. Most of the callus easily obtained have their texture too compact or compact inside, but soft outside, which were not suitable to subculture. Additionally it is difficult to get shoots but easy to get callus diameter of good growing condition. Some callus of petiole part was soft, full of water but easily dispersed in MS with IAA 0.2 mg/L and BA 4.0 mg/L which was even difficult to generate shoots. Some callus of leaf blade part with bright-coloured condition, proliferated fast, aged slowly, and had a high differentiation frequency. In most media with TDZ *Taraxacum* callus were always inoculated effectively, was integrity (no granules), hard. While, those beside the vein were even bigger, which was easy to get multiple shoots (Wu, 2008). These callus were preferably induced from the medium of in MS with IAA 0.2 mg/L and TDZ 1.0 mg/L, and shoot regeneration were better achieved from them.

Although the direct multiple shoots could be obviously observed in all the species, the vitrification phenomenon seriously inhibited the natural growth of shooting. Growth was substantially enhanced, and vitrification (stunting and epinasty of leaves and hooking of petiole apices) was reduced by increasing the efficiency of ventilation (Zobayed *et al.*, 2001).

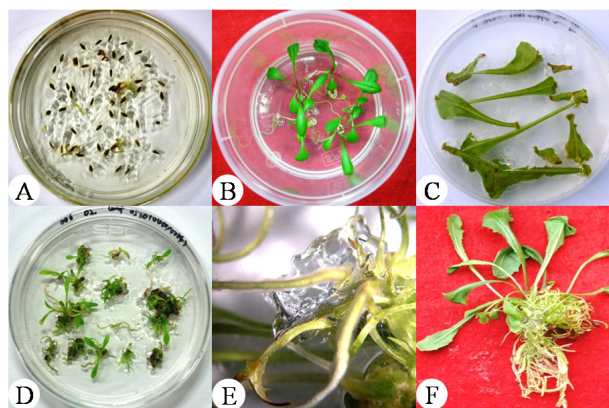


Fig. 3. The course of shooting induction, (A) seedlings from seeds (B) plantlets for explants, (C) inoculated explants, (D) multiple direct shoots, (E) rooting generation, (F) complete new plantlets from induced direct shoot.

Table 2. The prevention effect of AgNO₃ on vitrification of *T. mongolicum*

AgNO ₃ concentration on MS with IAA and TDZ (mg/L)	No. of Explants	Frequency of shoot formation (%)	No. of total shoots	No. of shoots per explant	No. shoots of vitrification	Frequency of vitrification (%)
0	25	63	98	3.9	29	29.6
0.5	30	68	63	2.1	14	22.2
1.0	27	73	59	2.2	13	22.0
1.5	30	77	70	2.3	12	17.1
2.0	30	74	39	1.3	7	18.0

And it was good for alleviating vitrification of direct shoots to increase Gel and decrease sucrose quantity. Besides, AgNO₃ can decrease the browning of explant shoots and increase generation frequency (Qian, 2006). Silver nitrate with the concentration of 2.0 mg/L had better effect on antibrowning and multiplication (Table 2). Proceeding from this point, AgNO₃ prevented vitrification efficiently. Referring to these research, we used AgNO₃ to prevent browning but it appeared even brown medium 1 day after adding 2.0 mg/L silver nitrate. So it needs further analyzing and researching on *Taraxacum*.

This study was carried out for establishment of a direct multiple shoots system for four *Taraxacum* species (*T. mongolicum*, *T. officinale*, *T. mongolicum* variation, *T. officinale* variation). The cultured aseptis *Taraxacum* plantlets were cut into four parts (leaf blade, transition zone, petiole, and root) as explants for being inoculated into corresponding medium. *T. mongolicum* was firstly used to select the optimal medium during the four composition, MS medium, MS supplemented with 1.0 mg/L BA, MS with 4.0 mg/L BA and 0.2 mg/L IAA, and MS with 0.2 mg/L IAA and 1.0 mg/L TDZ. From that MS with 0.2 mg/L IAA and 1.0 mg/L TDZ was selected by its high inducing frequency. Here TDZ plays an important role in inducing the multiple shoots. Its application for further research on shoots induction is recommendable. And then the selected medium was used for the subsequent screening. For the four explants of the plantlets, the direct shooting growth of leaf blade part obviously had advantages of all the species in the media. And they also showed their particularity as different species. Among the different species, direct multiple shoots of *T. mongolicum* variation grewed better than other species in all kinds of the media, for which we can use it for more investigation. Anyway, we expect this direct multiple

shoots inducing system of *Taraxacum* will act as a reference of profound-influence. No matter what we utilize it for, transformation or constituents extract, it's expected that it will offer a certain directive function for the interrelated research in the future.

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