In vitro Growth Response of *Taraxacum officinale* Weber ex F.H. Wigg Regenerated from Different Type of Explants

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Abstract

Dandelion (*Taraxacum officinale* Weber ex F.H. Wigg) is a medicinal plant species, reported to have some active compounds useful as anti-cancer, anti-inflammatory, antioxidants, and diuretics. Study on *in vitro* secondary metabolic production and tissue culture of these plants has been reported. This research was aimed to investigate plant regeneration from leaf blade, petiole and root as explants grown in selected media. Three different type of explants were cultured in MS solid medium supplemented with BAP (0, 0.5, and 1.0 mg/l) combined with NAA (0, 0.5, 1.0, and 2.0 mg/l) to select the best medium for spontaneous regeneration. Explants were grown for 6 weeks, the viability of explants, and formation of callus, shoots as well as roots were recorded. The results showed BAP at 1 mg/l combined with at 0.5 mg/l of NAA was suitable for shoot formation, whereas NAA alone was suitable for root formation from leaf blade and petiole. Root was the best explant for shoot regeneration, callus was grown at the first two weeks, and multiple shoots were grown after 3 weeks. No roots were found from root explant. The best medium for multiple shoot regeneration was MS containing 1 mg/l BAP without or with addition of 0.5 mg/l NAA.

Keywords: *Taraxacum officinale* Weber ex F.H. Wigg, growth response, plant growth regulators, different explants, regeneration

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Introduction

Plant regeneration can be achieved via somatic embryogenesis, direct regeneration via organogenesis or indirect regeneration via callus formation. Organogenesis and somatic embryogenesis start with one or only a few cells, this type of regeneration is important for plant production and plant biotechnology such as somaclonal propagation, multiplication, and especially genetic transformation (Thobunluepop *et al.,* 2005). Organogenesis is a suitable method for obtaining a large quantity of plantlets grown *in vitro*. In medicinal plants, this method is applicable for selecting the superior plant line subjected for high concentration of secondary metabolite compounds. Some factors affected the organogenesis of explants such as the use of plant growth regulators, type of explants and culture condition need to be investigated in order to have high frequency of organogenesis.

Genus *Taraxacum*, including *Taraxacum officinale* Weber ex F.H. Wigg or commonly called as dandelion (Family *Asteraceae*) grow in subtropical and tropical areas at about 1,700 m above sea level. *T. officinale* is a medicinal plant species having several active compounds useful for choleretic, diuretic, anti-inflammatory (Jeon *et al.,* 2008), anti-oxidative, and anti-carcinogenic activities such as breast and uterus cancer (Bae *et al.,* 2005; Schutz *et al.,* 2006). Besides being used as pharmaceuticals, dandelion plants are consumed as food because this plant contains vitamin A, vitamin C, tannin, alkaloids, pectin, innulin, polysaccharides, beta-carotene, potassium, and flavonoids (Kemper, 1999; de Padua *et al.,* 1999). Different tissues of the plant also reportedly contain flavonoids, coumarins, phenolic acids and their derivatives, triterpenoids, steroids, and sesquiterpene lactones (Kisiel & Barszcz, 2000). Previouse research on *T. officinale* reported that differnt part of plants originated from different field areas gave different antioxidant activity (Ermayanti *et al.,* 2009).

Reports on tissue culture of *Taraxacum* are limited. Adventitious shoot formation from seedling explants of *Taraxacum platycarpum*

was conducted on MS medium containing auxin and cytokinin (Lee *et al.,* 2002). Research on transformation of *T. platycarpum* with *A. rhizogenes* has been done to obtain hairy roots and transgenic plants (Lee *et al.*, 2005). Transformation of this species has also been carried out with *A. tumefaciens* (Bae *et al.,* 2005). The establishment of an efficient transformation method may facilitate the improvement of medicinal plant in terms of the accumulation levels of secondary metabolites. Tissue culture of *T. officinale* was conducted for callus induction using root explant on MS medium containing IAA and NAA, however, the shoot regeneration of this culture was low (Bowes, 1970; Booth & Satchuthananthavale, 1974).

This research was aimed to investigate plant regeneration from leaf blade, petiole, and root of *T. officinale* as explants grown in selected media containing different concentrations of BAP and NAA.

Materials and Methods

Sources of explants. Shoot culture of *T. officinale* was initiated from *in vitro* germination of seeds grown on MS medium (Murashige & Skoog, 1962) with no addition of plant growth regulators. Seeds were collected from Dieng highland, Central Java. After 4 weeks, the shoot tips were isolated and transferred on MS medium containing 1 mg/l BAP for shoot multiplication to study plant regeneration. Shoots were then transferred to medium without plant growth regulators to obtained leaf blade, petiole and roots.

Culture medium. MS medium was used as basal medium. The medium was supplemented with 20 g/l of sugar and solidified with 8 g/l agar. The pH medium was adjusted at 5.8, then the medium was autoclaved at 120°C, 1 atm for 15 min. Cytokinin BAP at 0, 0.5, and 1.0 mg/l in combination with auxin NAA (0, 0.5, 1.0 and 2.0 mg/l) were used as treatments.

Planting and culture incubation. Single leaf blade, petiole, and root were isolated from plantlets grown for 4 weeks on MS solid medium without plant growth regulators, and placed on the treatment medium on Petri dishes. Each treatment had at least 6 replicates. All cultures were incubated in an incubation room at 26-27°C with continuous photoperiod.

Growth assessment. Number of viable explants, regeneration into shoot and root, callus formation, number of leaves from different type of explants i.e. single leaf blade, petiol, and root were recorded until week-6 after culture. Development of explant growth was observed qualitatively every week. All data presented in this research were analyzed by Anova at 5% level, and advanced test used Duncan's Multiple Range Test (DMRT) at 5% level.

Results and Discussion

Leaf blade of *T. officinale* developed into callus, shoots or roots on MS medium containing different level of BAP separately or in combination with NAA. Table 1 shows the growth response of the leaf blade after 6 weeks in culture. On MS medium without addition of plant growth regulators, only a few leaf blade were viable, did not form shoot or root. Addition of NAA stimulated explant to form roots. Addition of BAP in combination of NAA gave shoots regeneration but the number of leaves was low. From previous research on selection medium for shoot multiplication of *T. officinale,* showed that BAP in combination with 2,4-D gave different capability of explant for shoot formation (Al Hafiizh, 2010).

Growth responses of petiole explant of *T. officinale* from petiole explant after 6 weeks on the treatment medium are presented on Table 2. Petiole was more responsive for regeneration. On the medium without addition of plant growth regulators, petioles had high viability and formed callus, and shoot, even at low frequency. All explants formed callus at the beginning of culture, then developed into shoots or roots depending on the concentration of BAP or NAA. Medium containing 1 mg/l of BAP was the best for shoot regeneration, giving the highest number of leaves per explant.

Different explant has different responses to the culture medium for regeneration, and root of *T. officinale* was the best explant for regeneration into shoots. No roots were formed on the medium treatment from this explant (Table 3). Medium containing BAP at

1 mg/l separately or in combination with 0.5 mg/l of NAA was the best for shoot formation. In the field, some species of *Taraxacum*, roots are capable of producing new plants even when the plant is cut at below the soil surface (Schutz *et al.*, 2006). This could be the same characteristic for *T. officinale* when the root culture grown *in vitro*. Addition of exogenous plant growth regulators accelerated regeneration into shoots as well as callus formation.

Table 1. Growth response and development of leaf blade explant of *T. officinale* after 6 weeks cultured on MS medium containing BAP and NAA

BAP (mg/l)	NAA (mg/l)	Viability (%)	Callus (%)	Shoot (%)	Root (%)	No leaves per explant
0	0	11.11 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}
0	0.5	60.00 ^{bc}	0 ^{a}	0 ^{a}	66.67 ^{bc}	0 ^{a}
0	1.0	71.43 ^{c}	0 ^{a}	0 ^{a}	100.00 ^{c}	0 ^{a}
0	2.0	75.00 ^{c}	0 ^{a}	0 ^{a}	83.33 ^{c}	0 ^{a}
0.5	0	100.00 ^{c}	0 ^{a}	33.33 ^{a}	0 ^{a}	1.17 ^{b}
0.5	0.5	20.00 ^{ab}	100.00 ^{c}	0 ^{a}	0 ^{a}	0 ^{a}
0.5	1.0	87.50 ^{c}	42.86 ^{abc}	0 ^{a}	0 ^{a}	0 ^{a}
0.5	2.0	55.56 ^{abc}	80.00 ^{bc}	20.00 ^{a}	40.00 ^{ab}	0.60 ^{ab}
1.0	0	72.73 ^{c}	37.50 ^{ab}	100.00 ^{b}	0 ^{a}	6.50 ^{c}
1.0	0.5	81.82 ^{c}	77.78 ^{bc}	33.33 ^{a}	0 ^{a}	0.89 ^{b}
1.0	1.0	90.00 ^{c}	55.56 ^{abc}	22.22 ^{a}	0 ^{a}	0.67 ^{ab}
1.0	2.0	55.56 ^{abc}	60.00 ^{abc}	0 ^{a}	40.00 ^{ab}	0 ^{a}

Note: Value followed by the same letter on the same column is not sugnificantly different at P. values of 0.05 according to Duncan's Multiple Range test

Table 2. Growth response of petiole explant of *T. officinale* after 6 weeks cultured on MS medium containing BAP and NAA

BAP (mg/l)	NAA (mg/l)	Viability (%)	Callus (%)	Shoot (%)	Root (%)	No leaves per explant
0	0	84.62 ^{a}	72.73 ^{ab}	27.27 ^{abc}	0 ^{a}	1 ^{b}
0	0.5	100.00 ^{a}	7500 ^{ab}	25.00 ^{abc}	50.00 ^{cd}	0.25 ^{a}
0	1.0	86.67 ^{a}	92.31 ^{ab}	0 ^{a}	84.62 ^{e}	0 ^{a}
0	2.0	80.00 ^{a}	66.67 ^{ab}	0 ^{a}	75.00 ^{de}	0 ^{a}
0.5	0	93.33 ^{a}	85.71 ^{ab}	64.29 ^{de}	0 ^{a}	2.79 ^{d}
0.5	0.5	100.00 ^{a}	100.00 ^{b}	38.46 ^{bcd}	15.39 ^{ab}	1.69 ^{c}
0.5	1.0	100.00 ^{a}	58.00 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}
0.5	2.0	91.67 ^{a}	90.91 ^{ab}	9.09 ^{ab}	27.27 ^{abc}	0.09 ^{a}
1.0	0	100.00 ^{a}	91.67 ^{ab}	75.00 ^{e}	0 ^{a}	4.25 ^{e}
1.0	0.5	100.00 ^{a}	100.00 ^{b}	46.67 ^{cde}	0 ^{a}	1.80 ^{c}
1.0	1.0	91.67 ^{a}	81.82 ^{ab}	45.45 ^{cde}	36.36 ^{bc}	0.73 ^{b}
1.0	2.0	90.00 ^{a}	66.67 ^{ab}	0 ^{a}	11.11 ^{ab}	0 ^{a}

Note: Value followed by the same letter on the same column is not sugnificantly different at P. values of 0.05 according to Duncan's Multiple Range test

Table 3. Growth response of root explant of *T. officinale* after 6 weeks cultured on MS medium containing BAP and NAA

BAP (mg/l)	NAA (mg/l)	Viability (%)	Callus (%)	Shoot (%)	Root (%)	No leaves per explant
0	0	100.00	0 ^{a}	75.00 ^{b}	0	4.75 ^{b}
0	0.5	100.00	100.00 ^{b}	66.67 ^{b}	0	3.33 ^{ab}
0	1.0	100.00	100.00 ^{b}	0 ^{a}	0	0 ^{a}
0	2.0	100.00	100.00 ^{b}	66.67 ^{b}	0	5.33 ^{b}
0.5	0	100.00	100.00 ^{b}	100.00 ^{b}	0	19.67 ^{d}
0.5	0.5	100.00	100.00 ^{b}	100.00 ^{b}	0	9.67 ^{c}
0.5	1.0	100.00	100.00 ^{b}	100.00 ^{b}	0	4 ^{b}
0.5	2.0	100.00	100.00 ^{b}	0 ^{a}	0	0 ^{a}
1.0	0	100.00	100.00 ^{b}	100.00 ^{b}	0	29.00 ^{f}
1.0	0.5	100.00	100.00 ^{b}	100.00 ^{b}	0	29.67 ^{f}
1.0	1.0	100.00	100.00 ^{b}	100.00 ^{b}	0	7.33 ^{b}
1.0	2.0	100.00	100.00 ^{b}	100.00 ^{b}	0	24.33 ^{e}

Note: Value followed by the same letter on the same column is not sugnificantly different at P. values of 0.05 according to Duncan's Multiple Range test

Table 4. Growth development of leaf blade, petiole, and root explants of *T. officinale* cultured on MS medium containing BAP and NAA

BAP (mg/l)	NAA (mg/l)	Explant	Viability at week-6	Growth development
0	0	Leaf blade	11.11	All explants did not show any growth; after 1 week in culture; they started to yellowish, dry and dying in 6 weeks
		Petiole	84.62	Some explants started to dry and dying in week 4; after 1 week in culture, some others formed tumour and callus, regenerated into shoots after 3 weeks; maximum leaves was 11 at week-6; no roots were found
		Root	100.00	All roots survived; roots started to form callus and tumour at week-1, then regenerated into shoots after week-2; at the end of week-6 no callus grew anymore; number of leaves ranged 3-10; no roots were found
0	0.5	Leaf blade	60.00	Explants formed roots at the cutting site after 1 week in culture; no callus and shoots were found
		Petiole	100.00	Explants formed roots at the cutting site after 1 week in culture; at week-5 explants started to form shoots with number of leaves was 1-2 per explants
		Root	100.00	Green and compact callus started to grow after 1 week in culture; a week afterward callus started to form shoots with 2-4 leaves; no roots were found
0	1.0	Leaf blade	71.42	All survived explants formed roots 4 weeks in culture; no callus and shoots were found
		Petiole	86.67	Explants formed roots after 1 week culture; roots grew fast; some explants formed callus; no explants formed shoots
		Root	100.00	Explants formed greenish friable callus after 1 week culture, neither shoots nor roots were formed
0	2.0	Leaf blade	75.00	Roots started to grow at the cutting sites 3 weeks after culture; no callus or shoots were found
		Petiole	80.00	One week after culture explants started to form green compact callus; at week-3 roots grew at the site of cutting; at week-6 some explants formed friable callus and growth was maximum; no shoots were found
		Root	100.00	Most explants started to form friable callus after 1 week in culture; some explants formed shoots with 3-13 leaves per explant, no roots were found
0.5	0	Leaf blade	100.00	Growth was slowly at the beginning of culture; after 5 weeks explants started to form shoots slowly. No callus and roots were found, explants directly to form shoots with 3-4 leaves per explant after 6 weeks
		Petiole	93.33	Most explants formed tumour, callus and shoot primordium 1 week after culture; the growth was fast, multiple shoots (with 4-9 leaves) was formed from 2 to 6 weeks; no roots were found
		Root	100.00	Some explants started to form leaves 1 week after culture; other explants started to form callus or tumour 4 week after culture; at week-5 multiple shoots started to grow; after 5 weeks all explants formed multiple shoots with 6-28 leaves; no roots were found
0.5	0.5	Leaf blade	20.00	Few explants survived; at week-3 explants started to yellowish, drying and start to die; only 20% of explants formed callus at the cutting sites; no shoots and roots were formed
		Petiole	100.00	All explants survived and formed callus; after 4-5 weeks in culture some explants started to form callus; shoots started to grow at week-3 with 3-5 leaves, and increased to form roots up to week 6
		Root	100.00	All explants formed callus and shoots started at week 1 after culture; callus was green and friable; at weeks 2 explants started to form shoots; number of leaves was increasing up to week-6 (9-10 leaves) ; no roots were found
0.5	1.0	Leaf blade	87.50	Some explants died at week-3; some survived explants formed callus at the end of culture (week-6); no roots or shoots were found
		Petiole	100.00	All explants survived; some explants started to form callus at week-1; no explants formed roots or shoots
		Root	100.00	All explants survived, formed callus and shoots; callus was compact-greenish or friable-yellowish; shoots started o grow at week 4-5 and formed 5 leaves; no roots were found
0.5	2.0	Leaf blade	55.56	Some explants started to dry after 1 week in culture and started to die; all survived explants formed callus; only few explants formed shoots with 1-2 leaves and roots
		Petiole	91.67	Callus tumour and shoots started to grow after 1 week in culture; more shoots (with single leaf) were formed at week-5; roots started to form at week-3
		Root	100.00	All explants survived and formed callus; greenish-friable callus were formed a week after culture; no roots or shoots were found
1.0	0	Leaf blade	72.73	Some explants dried at week-3 and some others started to form tumour, callus and shoots; at week-4 shoots started to grow faster with 5-11 leaves; callus and tumour formed shoots; no roots were found
		Petiole	100.00	All explants survived; formed callus, tumour and shoot primordium after a week in culture; shoots started to grow faster at week-3 to form 4-13 leaves; no roots were found
		Root	100.00	All explants formed callus, tumour and shoots after 1-2 weeks in culture; after 3 weeks in cultures all tumours formed shoots with 20-37 leaves per explant; no roots were found
1.0	0.5	Leaf blade	81.82	Some explants formed callus after 3 weeks in culture; shoots started to grow after 4 weeks in culture and formed 2-3 leaves per explant; no roots were found
		Petiole	100.00	All explants survived and formed callus; greenish callus started to growth a week after culture, and some of them formed shoots with 2-6 leaves after 3 weeks in culture; no roots were found
		Root	100.00	All explants survived, formed callus and shoots; after 3 weeks in culture all shoots grew faster than formed 22-36 leaves; no roots were found

BAP (mg/l)	NAA (mg/l)	Explant	Viability at week-6	Growth development
1.0	1.0	Leaf blade	90.00	Some explants started to dry after 3 weeks in culture; survived explants started to form callus at week-3; some others slowly to form shoots with 2-4 leaves after 4 weeks in culture ; no roots were found
		Petiole	91.67	Callus and tumour started to grow a week after culture; callus was greenish; after 3 weeks in culture, tumour started to form shoots with 1-4 leaves; roots also started to grow 3 weeks after culture
		Root	100.00	All explants survived and formed callus and shoots; callus was friable-greenish or friable-yellowish; greenish callus started to form shoots with 5-9 leaves after 4 weeks in culture; no roots were found
1.0	2.0	Leaf blade	55.56	Some explants dried and died; few callus was formed after 5 weeks in culture; roots were formed after 6 weeks in culture; no shoots were found
		Petiole	90.00	Callus started to form 3 weeks after culture; no shoots were found; few roots grew at week-6
		Root	100.00	All explants survived, formed callus and shoots; friable-yellowish callus started to grow after a week in culture; greenish started to from shoots 4 weeks after culture; all explants formed shoots with 20-28 leaves after 4 weeks in culture; leaves were small and needle-like; no roots were found

Growth development of *T. officinale* at week-6 after culture of each explant grown in the culture medium without any subculturing (Table 4). The results showed that the type of explant was important when it was grown on the medium containing plant growth regulators. The explants that still develop after 6 weeks were grown on the same culture medium or they were transferred into fresh medium. On MS medium containing IAA and coconut milk, development of *T. officinale* changed after 2 months, but, the growth were back to normal after subculturing (Bowes, 1971).

Regeneration of root, callus, shoot and leaves from different explants of *T. Officinale* is shown in Figure 1. On MS medium containing 2 mg/l of NAA, leaf blade regenerated into roots at the proximal end of the leaf blade (Figure 1A). Callus was formed from roots explant cultured to MS medium containing 0.5 mg/l of BAP in combination with 2 mg/l of NAA (Figure 1B). Callus was yellowish and friable. Root explant was directly regenerated into shoots on the medium containing 1 mg/l BAP (Figure 1C), while on medium MS containing 1 mg/l of BAP in combination with 2 mg/l of NAA, callus was stimulated from roots explant at the beginning of the culture, and formed shoot after 4 weeks in the culture. The different responses of explants in regeneration ability may be due to stimulation by endogenous hormones or some signals related to wounding, which play a vital role during the induction of regeneration, or the ratio of ions present in the medium. Meanwhile, the differences in shoot, callus of root formation, may be a result of differences in the regeneration potential of different explants, which is attributed by the physiological state, age and cellular differentiation among the constituent cells (Beegum *et al.*, 2007).

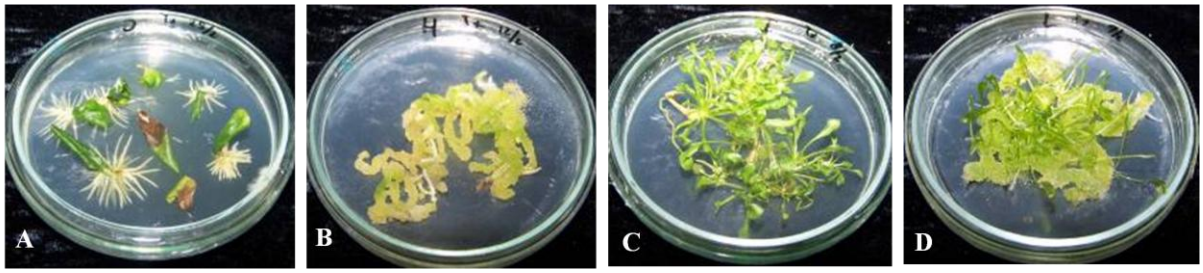

Figure 1. Regeneration of different explants of *T. officinale* grown on various medium cultures after 6 weeks culture. A. Roots regenerated from leaf blade grown on MS medium containing 2 mg/l NAA; B. Callus formation from root grown on MS medium containing 0.5 mg/ BAP + 2 mg/l NAA; C. Shoots regenerated from root explants grown on MS medium containing 1 mg/l BAP; D. Leaves grew after formation of callus from root explants grown on MS medium containing 1 mg/l BAP+ 2mg/l NAA.

Combination of BAP and NAA was suitable for regeneration of *T. officinale*. Depending on the explant type, BAP in combination with NAA or given separately gave different response to regeneration into shoots or roots, or the formation of callus. This plant growth regulators was also suitable for *Dianthus caryophyllus* regeneration using cut

of leaf base. The best medium for direct shoot regeneration was MS medium containing 0.9 mg/l of BAP in combination with 0.9 mg/l of NAA (Iantcheva *et al.,* 2005). In *Ophiorrhiza prostrata*, BAP in combination with NAA were also the best for shoot, root and callus formation (Beegum *et al.*, 2007). BAP was also the best for regeneration into shoots of *Valeriana officinalis*, while NAA was the best for rooting (Abdi & Khosh-Khui, 2007).

Conclusions

Different parts of plant gave different responses of growth when they were cultured on MS medium containing BAP and NAA with various concentrations. Root was the best explant for shoot regeneration. MS medium containing 1 mg/l of BAP separately or in combination with 0.5 mg/l of NAA were the best for spontaneous regeneration of roots into shoots.

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