Plant Archives Vol. 18 No. 2, 2018 pp. 2273-2284 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

IN VITRO MICROPROPAGATION OF *TARAXACUM OFFICINALE* MEDICINAL PLANT BY DIRECT AND INDIRECT ORGANOGENESIS

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Abstarct

Taraxacum officinale (Dandelion) is a herbal medicinal plant, which suffers from wide limitations when cultivated in the soil, including environmental limitations as well as the low rate of seed germination. The study involved using different explants (leaves, petioles and roots) of *T. officinale* from two sources, locally collected wild plants from Iraq and plants from *in vitro* germinated seeds. That cultured on Murashige and Skoog (MS) medium supplemented with different combinations and concentrations of growth regulators (NAA, BAP, IAA and BA) with registering the obtained response and periods taken until a response to establish a rapid and efficient protocol for *in vitro* micropropagation of the plant. The study results elucidated the ability for *in vitro* micropropagation of *T. officinale* plant in two ways; through direct and indirect organogenesis. The explants of *in vitro* germinated plants origin are a better source of explants than wild plants origin; moreover, the petioles are better explants than leaves and roots for micropropagation of *T. officinale* plant through direct organogenesis while the roots are the best explants for indirect organogenesis. The highest callus response resulted from petiole explants, while the highest shoot initiation response resulted from root explants and the best root regeneration resulted from leaf explants. The study concluded that complete plantelets can be produced from a single explant of *T. officinale* by tissue culture without requiring for sub-culturing through two ways: indirect and direct organogenesis. Auxins can be used alone in both petioles and roots explant to regenerate complete plants.

Key words: *Taraxacum officinale*, Micropropagation, direct organogenesis, indirect organogenesis, *in vitro* germinated and wild plants.

Introduction

Taraxacum officinale (Dandelion) is a herbal medicinal plant that has phytochemical properties when analyzed as a whole in view of roots, leaves, and flowers (Schütz *et al*., 2006; Ömür *et al*., 2017). It is considered to be a very valuable weed, where both leaves and roots are valued for their medicinal properties. In addition, the plant consumed for its nutritional properties since leaves have a value as a salad crop, while the roots are used in the production of a coffee substitute (Bajaj, 1994). The cultivation and propagation of dandelion are difficult due to low rates of germination and specific environmental requirements (Lee *et al*., 2009; Schippmann *et al*., 2002). In spite of the ability of seeds to germinate with short-period of dormancy, there is difficulty to optimize the environmental conditions required for seed germination (Washitani and Takenaka, 1987; Lee *et al*., 2009). The speed of germination and the percentage of seeds that germinate vary during seasons and largely depending on temperature and moisture (Martinkova *et al*., 2014). Pathogenic infections or physical and physiological damage to seeds could affect germination (Lee *et al*., 2009); also, it needs to be encouraged by the surrounding vegetation during germination (Martinkova *et al*., 2014). Therefore, for all the reasons mentioned above and due to the progressive consumption of its native populations, there is an increased need for the conservation and domestication of Dandelion (Chandra *et al*., 2013). To promote propagation and engineering of desirable traits into the plant, there is a great need for efficient transformation and regeneration protocols for dandelion (Ryu and Lee, 2006). The controlled growth conditions under *in vitro* plant tissue or cell suspension culture systems could allow the control and increase the contents and potency of important medicinal compounds in *T.*

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officinale also can avoid unwanted compounds including soil's heavy metal contaminants, which represent public health risk (Lee *et al*., 2009). Micropropagation would promise technique to conserve the critical genotypes of medicinal plants (Thangavel *et al*., 2014) from a minimum plant material and with low impact on the wild population where its source explants are in limited availability (Thangavel *et al*., 2014; Post *et al*., 2012). it can induce integrate and rapid production of a large number of genetically similar plants (Rezaee and Kamali, 2014; Carvalho *et al*., 2016; Tabatabai, 2011; Isah, 2015; Diasa *et al*., 2015; Pant *et al*., 2014). The study aimed to determine an efficient, rapid, and easy protocol for regeneration and micropropagation of *T. officinale* plant and investigate the possible plant regeneration from leaves, petioles, and roots explants that grown on selected media using different concentrations and combinations of growth regulators.

Materials and methods

The Explants source

The plants of *T. officinale* were obtained from two different sources. The first one was a field from Baghdad city. The second source was seeds that purchased from Seeds Needs Company at the USA; the seeds were germinated in the laboratory on 3% agar (HIMEDIA®, India) medium, and the growing plants were used as explants for tissue culture experiments.

Seed sterilization

The seeds were sterilized with 0.75% Sodium Hypochlorite (NaOCl) for 5 minutes; then washed 3 times with sterilized distilled for 5 minutes each. After that, they were placed on sterilized filter papers to eliminate moisture and be ready for planting.

Explants sterilization:

The explants (leaves, petioles, and roots) were sterilized before culturing according to the results obtained from sterilization experiment. They are treated with 3 different concentrations of Sodium Hypochlorite (NaOCl) (SEHAT, Iran) solution (0.75%, 1%, and 1.5%), then the protocol was accomplished as follow:

The explants were first washed with running tap water for five minutes. The other steps of sterilization were completed inside the laminar air flow; explants were soaked in Ethanol (BDH, England) for 30 seconds, and then washed 3 times with sterilized distilled water for 5 minutes for each one. After that, the explants were surface sterilized by using 1.5% Sodium Hypochlorite (NaOCl) solution for 15 minutes and then washed 5 times with sterilized distilled water for five minutes each once. Finally, to ensure eliminating all of the moisture from the sterilized explants, they were placed on sterilized filter papers for drying before using in tissue culture.

Planting of seeds

The sterilized seeds were planted on 3% Agar medium in sterilized containers; they kept in a growth chamber under light (3500 Lux), and temperature ($25^0C \pm 2$) for germination.

In vitro regeneration of *Taraxacum officinale*

Fresh *in vitro* and *ex vitro* plants of *T. officinale* were cut to different explants including (leaves, petioles, and roots) for using in micropropagation experiment. The explants were cultured on MS media (HIMEDIA®, India) supplemented with different types and concentrations of growth regulators (Sigma Aldrich, USA) for callus induction and regeneration as mentioned in (tables 1, 2, and 3).

Table 1: Type of media and growth regulators used for regeneration of *Taraxacum officinale* from leaves

No.	Basic Medium	Conc. of GRs	Reference
1	MS	BAP + 0.5 NAA	(Emrayanti and Martin, 2011)
2	MS	1 IAA + 1 BA	(Jamshieed e*t al*., 2010)
3	MS	1 IAA + 2 BA	(Jamshieed e*t al*., 2010)

Table 2: Type of media and growth regulators used for regeneration of *Taraxacum officinale* from petioles

No.	Basic Medium	Conc. of GRs	Reference
1	MS	BAP + 0.5 NAA	(Emrayanti and Martin, 2011)
2	MS	2 NAA	(Emrayanti and Martin, 2011)
3	MS	1BAP+1NAA	(Emrayanti and Martin, 2011)

Table 3: Type of media and growth regulatores used for regeneration of *Taraxacum officinale* from roots

No.	Basic Medium	Conc. of GRs	Reference
1	MS	0.5NAA	(Emrayanti and Martin, 2011)
2	MS	0.5BAP+2NAA	(Emrayanti and Martin, 2011)
3	MS	0.5BAP+0.5NAA	(Emrayanti and Martin, 2011)

The cultures were kept in a growth chamber at 25 ± 2^oC, 3500 lux light, and stable relative humidity. Each experiment repeated 28 times; the following data were recorded: frequency of explants producing calli (in addition to the colour, solidity, and time needed for induction), frequency of calli producing shoots and roots, the frequency of roots formation and roots formed directly from explants. Initiation frequency was measured as the number of explants producing calli divided by the total

number of explants cultured, shoots frequency as the number of explants producing shoots divided by the total number of explants cultured, roots frequency as the number of explants producing roots divided by the total number of explants cultured.

Acclimatization

Seven fully morphologically developed plantlets about 5-7cm in length containing both shoot and root regenerated from tissue culture experiment were gradually acclimatized. The tubes containing those plantlets were subjected to the normal environment using successive periods of opening and exposing starting from 0.5 hours and ending with 2.5 hours for 5 days. After that, the plantlets removed from the tubes and washed thoroughly but carefully with ruining tap water to remove media traces attached to root parts without any damaging of the platelet parts.

The removed plantlets were transferred to small plastic pots about 7.5 cm in diameter supplemented with potting material composed of perlite. The pots were then kept in a growth chamber under light intensity equal to 35000 lux at 25+2^{o}C temperature. The pots also covered with polyethylene bags and watered with liquid MS medium to keep humidity and avoid desiccation of the platelet. The polyethylene bags were removed after 10 days to subject the plantlets to the external environment. The plantlets were then transferred to pots containing peatmoss and perlite mixture and watched daily. The percentage of survival plantlets was calculated after 2 weeks.

Statistical Analysis: All the data obtained from micropropagation experiment were analyzed as a complete random design with 20 replications for each treatment using Analysis of Variance (ANOVA) and LSD test by using SPSS 19.0 program.

Results and discussion

The explants (Leaves, Petioles, and Roots) that used in this study showed response for all treatments such as callus induction, shoot and root regeneration. The responses that obtained from the used explants represented their ability to produce complete plantlets from single explant in short time through indirect and direct organogenesis.

The study aimed to produce an efficient and rapid micropropagation system for generating living Dandelion plantlets. The results indicated that explants excised from *in vitro* germinated plants produced a better response to growth regulators combinations than explants excised from wild plants. This could be due to the physiological and biochemical differences among the studied explants as well as to the genetic variability between the two sources of the plants; where the study was accomplished on wild plants collected from Iraq and plants generated from *in vitro* germinated purchased seeds from USA. The differences in genotypes supported by Jamshieed *et al*., (2010) who studied *in vitro* micropropagation of two populations of *T. officinale* plants collected from two regions and reported variable response between them. As well as, these differences can result from the maturity status of the plant, and the healthy status of the explants. The explants of *in vitro* origin are grown under controlled conditions and produce healthy, uncontaminated meristematic tissues that response greatly. Choi *et al*., (2002) mentioned that it is important to select carefully plant source material that is of healthy appearance. Moreover, the meristematic cells are particularly active sites for the biosynthesis and/or the release of natural growth factors favouring cell growth (George *et al*., 2008).

Most of the explants initiate callus that could be regenerative or non-regenerative; the produced callus characterized as green and compact. The regenerative callus proceeded to form shoot or/and root via indirect regeneration; all the explants produced callus within a period of 4-20 days. The callus proceeds to form shoot and/or root within periods less or more than the mentioned period. The explants that respond to direct regeneration take time convergent to time for indirect regeneration as indicated in (table 4). These observations supported by Ermayanti and Martin, (2011) who reported similar observations depending on the used growth regulators. Moreover, the produced callus was green and compact; the green colour could be explained as a result to continuous illumination all the day, where the light is an essential factor for producing green structures like shoots. Where the absence of light may induce white callus or albino shoots from *T. officinale* as demonstrated by Slabnik *et al*., (1986). Although Ermayanti and Martin (2011) elucidated that medium supplemented with (0.5mg/L BAP + 2mg/L NAA) produce yellowish and friable callus, this differences could be explained by differences in the studied genotypes.

There was a difference in response between leaf explants excised from *in vitro* germinated and wild plants, which disagree with Slabnik *et al*., (1986) observations, where he indicated an absence of differences in the morphogenic responses of *T. officinale* due to the age of leaves. Roots originated from wild plant didn't produce any response in spite of the high regenerative ability proved by Booth and Satchuthananthavale(1974), this observation

Table 4: Number of days required by *T. officinale* explants to respond to different growth regulators combinations

Explants Type	Growth Regulators (mg/L)	No. of Days for Response				
		Indirect Regeneration			Direct Regeneration	
		Callus	shoot	Root	Shoot	root
Leaves	1 IAA + 2 BA	7-14	5-8	7-14	-	-
	1 IAA + 1 BA	8-10	9	-	-	-
	1 BAP + 0.5 NAA	6-15	9-25	5-25	12-16	10-16
Petioles	1 BAP + 0.5 NAA	6-10	6-25	5-28	6-11	11-12
	1 BAP + 1 NAA	6-10	6-10	6-10	-	-
	2 NAA	4-18	6-12	6-12	-	-
Roots	0.5 NAA	5-12	5-20	6-27	16-20	18-20
	0.5 BAP + 2 NAA	8-16	5-18	5-18	16-20	12-20
	0.5 BAP + 0.5 NAA	10-20	5-10	5-10	-	-

Table 5: Callus induction from *T. officianle* explants excited from *in vitro* germinated plants with different combinations of growth regulators

Explants Type	GR Combination (mg/L)	No. of Replicates	Callus Induction Response
			Mean ± SD
Leaves	1 IAA+2 BA	**20**	3.25±2.44*
	1 BAP+0.5 NAA	**20**	4.00±1.11
	1 IAA+1 BA	**20**	2.50±2.56
LSD			-
Petioles	1 BAP+0.5 NAA	**20**	4.75±1.11
	1 BAP+1 NAA	**20**	5.00±0.00
	2NAA	**20**	5.00±0.00
LSD			-
Roots	0.5NAA	**20**	4.25± 1.53
	0.5 BAP+0.5 NAA	20	3.50±2.35
	0.5 BAP+2 NAA	20	3.50±2.35
LSD			-

*values represent mean% and standard deviation ($p \leq 0.05$) of each treatment.

was supported by Booth and Bows studies, who noticed that explants of root origin have a low morphogenetic potency in *T. officinale* (Rakhimbaev *et al*., 2013).

Taraxacum officinale explants response to growth regulators

There is a great variability regarding the obtained response from the explants and growth regulators that used in this study through Indirect and direct regeneration; this supported by Ermayanti and Martin, (2011) results who explained the importance of explants type for the response of *T. officinale* to tissue culture. The initial tissue is a fundamental factor to achieve the desired response (Tafazoli, *et al*., 2011; Carvalho *et al*., 2016). The different responses of explants could be explained by the regeneration potential of the explants that resulted from physiological state, ontogenetic ability, age and cellular differentiation abilities, As well as differences in endogenous hormones or some signals related to wounding that play critical role in regeneration process (Ermayanti and Martin, 2011; Slabnik *et al*., 1986; Smulders and de Klerk, 2011; Isah, 2015). Furthermore, Tabatabai (2011) reported significant differences in shoot induction rates based on plant genotype, source, and orientation of explants.

Indirect regeneration

Callus induction response

The highest callus induction (5%) response appeared with petiole explants excited from *in vitro* germinated plants using combinations of (1mg/L BAP + 1mg/L NAA) and using 2mg/L NAA alone, while For leaf explants, the highest callus induction (4%) introduced from *in vitro* germinated plants that cultured on medium supplemented

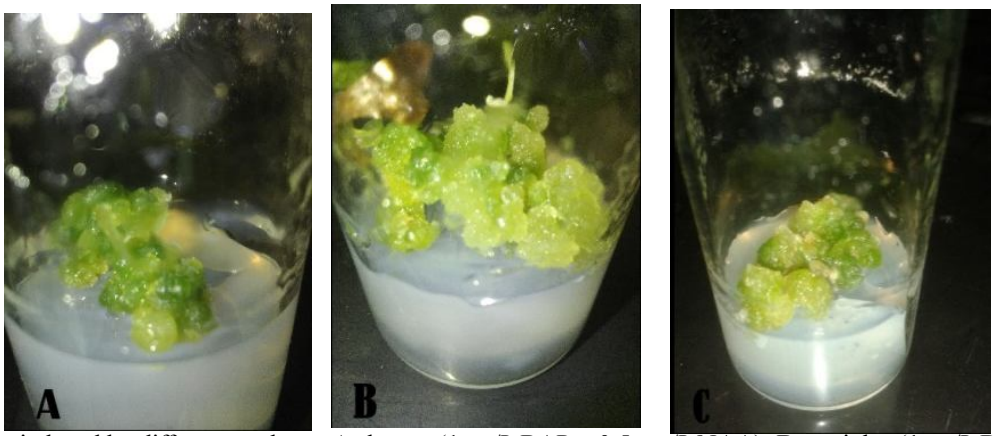

Fig. 1: Callus induced by different explants, **A:** leaves (1mg/L BAP + 0.5 mg/L NAA), **B:** petioles (1mg/L BAP + 1mg/L NAA), **C:** roots (0.5mg/L NAA).

Table 6: Callus induction from *T. officianle* explants excited from wild plants with different combinations of growth regulators

Explants Type	GR Combination (mg/L)	No. of Replicates	Callus Induction Response Mean + SD
Leaves	1 IAA+2 BA	**10**	3.33±5.36*
	1 BAP+0.5 NAA	**10**	0.00±0.00
	1 IAA+1 BA	**10**	4.44±5.73
LSD			-
Petioles	1BAP+0.5NAA	**20**	1.00±2.05
	1BAP+1NAA	**20**	0.50±1.53
	2NAA	**20**	1.00±2.05
LSD			-
Roots	0.5 NAA	**20**	0.00±0.00
	0.5 BAP+0.5NAA	**20**	0.00±0.00
	0.5 BAP+2 NAA	**20**	0.00±0.00
LSD			-

*values represent mean% and standard deviation ($p \leq 0.05$) of each treatment

Table 7: Shoot initiation from *T. officianle* explants existed from *in vitro* germinated plants with different combinations of growth regulators

Type of Explant	GR Combination (mg/L)	No. of Replicates	Shoot Initiation Response Mean + SD
Leaves	1 IAA + 2 BA	**10**	2.25±2.55*
	1 BAP+ 0.5 NAA	**10**	3.75±2.22
	1 IAA + 1 BA	**10**	2.50±2.56
LSD			-
Petioles	1BAP+0.5NAA	**20**	4.25±1.83
	1BAP+1NAA	**20**	3.50±2.35
	2NAA	**20**	1.75±2.44
LSD			**1.62**
Roots	0.5 NAA	**20**	4.50±1.53
	0.5 BAP+0.5NAA	**20**	1.75±2.44
	0.5 BAP + 2 NAA	**20**	1.00±2.05
LSD			**1.49**

*values represent mean% and standard deviation ($p \leq 0.05$) of each treatment.

with (1mg/L BAP + 0.5mg/L NAA, the differences were not statistically significant. At the same manner, the response of root explants showed no significant differences among all the tested growth regulators (table 5). The responses of callus induction from different explants are showed in (fig. 1).

Regarding the response of tissues excised from wild plants, leaf explants produced callus (4.44%) on MS medium plus (1mg/L IAA + 1mg/L BA), Petiole explants generated callus (1%, 0.5%, and 1%) on medium supplemented with (1mg/L BAP + 0.5mg/L NAA), (1mg/L BAP + 1mg/L NAA), and (2mg/L NAA), respectively but the differences were not significant. No response was observed for root explants with any combination of growth regulators (table 6).

Shoot initiation response

The highest shoot initiation response (4.5%) appeared in root explants excised from *in vitro* germinated plants under the treatment (0.5mg/L NAA) significantly. For petioles, the highest shoot imitation (4.25%) was produced on MS medium plus (1mg/L BAP + 0.5mg/L NAA) significantly (table 7). No significant differences were observed among growth regulator combinations that used with leaf explants. Shoot initiation responses from different explants are shown in (fig. 2).

Regarding wild plants, petiole explants respond (0.75%) only to MS medium supplemented with (2mg/L NAA) significantly. The highest shoot response for leaf explants (3.33%) initiated from media supplemented with

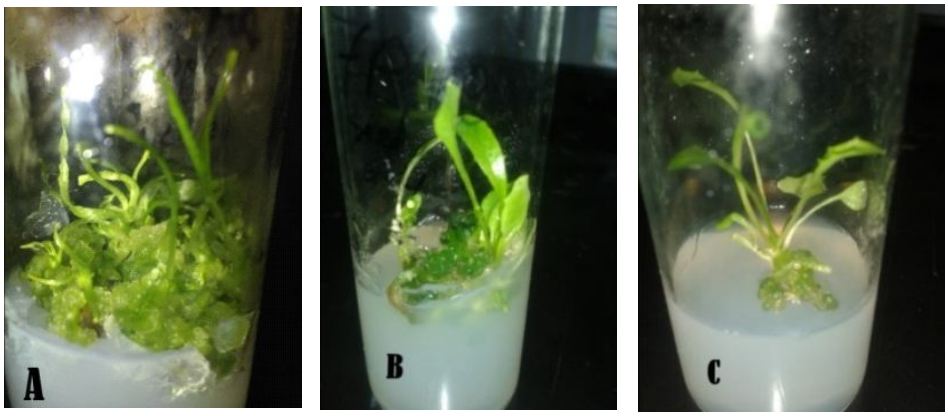

Fig. 2: Shoot initiated by indirect organogenesis from different explants, **A:** leaves (1mg/L IAA + 2mg/L BA), **B:** petioles (1mg/L BAP + 0.5mg/L NAA), **C:** roots (0.5mg/L NAA).

Table 8: Shoot initiation from *T. officianle* explants excised from mature weed plants with different combinations of growth regulators

Type of Explant	GR Combination (mg/L)	No. of Replicates	Shoot Initiation Response Mean + SD
Leaves	1 IAA + 2 BA	**20**	3.33±5.36*
	1 BAP+ 0.5 NAA	**20**	0.00±0.00
	1 IAA + 1 BA	**20**	1.11±3.51
LSD			-
Petioles	1BAP+0.5NAA	**20**	0.00±0.00
	1BAP+1NAA	**20**	0.00±0.00
	2NAA	**20**	0.75±1.83
LSD			**0.75**
Roots	0.5 NAA	**20**	0.00±0.00
	0.5 BAP+0.5NAA	**20**	0.00±0.00
	0.5 BAP + 2 NAA	**20**	0.00±0.00
LSD			-

*values represent mean% and standard deviation ($p \leq 0.05$) of each treatment.

Table 9: Root formation from *T. officianle* explants excited from *in vitro* germinated plants with different combinations of growth regulators

Type of Explant	GR Combination (mg/L)	No. of Replicates	Root Formation Response Mean + SD
Leaves	1 IAA+2 BA	**20**	0.75±1.83*
	1 BAP+0.5 NAA	**20**	4.00±2.05
	1 IAA+1 BA	**20**	0.00±0.00
LSD			**1.006**
Petioles	1 BAP+0.5 NAA	**20**	3.50±2.35
	1 BAP+1 NAA	**20**	0.50±1.53
	2 NAA	**20**	2.75±2.55
LSD			**1.59**
Roots	0.5 NAA	**20**	3.50±2.35
	0.5 BAP+0.5 NAA	**20**	1.50±2.35
	0.5 BAP+2 NAA	**20**	1.75±2.44
LSD			**1.73**

*values represent mean% and standard deviation ($p \leq 0.05$) of each treatment.

(1mg/L IAA + 2mg/l BA), the differences were not significant (table 8). No shoot regeneration was observed from root explants.

Root formation response from different explants

Leaves showed high response (4.5%) on medium supplemented with the combination (1mg/L BAP + 0.5mg/L NAA). Petioles produced roots (3.5%) on the combination (1mg/L BAP+0.5mg/L NAA), followed by (2.75%) on MS medium plus (2mg/L NAA); the responses of rooting are presented in (table 9). Roots regeneration and complete plantlets that induced indirectly from (leaves, petioles, and roots) explants are shown in (fig. 3) and (fig. 4), respectively.

Regarding explants that excised from wild plants, petioles that cultured on a medium provided with 2mg/L NAA produce the highest root formation (1%), the differences were not statistically significant. No rooting was observed from leaves and roots explants with the other treatments (Table 10).

Direct Organogenesis

Some explants that excised from *in vitro* germinated plants showed direct organogenesis bypassing callus stage. The best explants were roots that produced shoots and roots directly at medium supplemented with 0.5mg/L NAA and 0.5mg/L BAP + 2mg/L NAA.

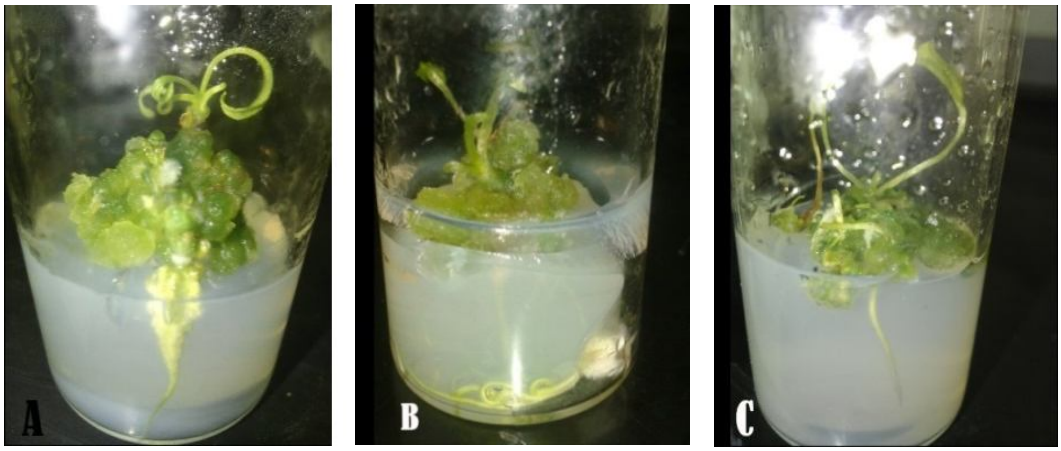

Fig. 4: Complete plantlets of produced by indirect organogenesis from different explants, **A:** Leaves (1mg/L BAP+ 0.5mg/L NAA), **B:** petioles (1mg/L BAP + 0.5 mg/L NAA), **C:** roots (0.5mg/L NAA)

Table 10: Root formation from *T. officianle* explants excited from wild plants with different combinations of growth regulators

Type of Explant	GR Combination (mg/L)	No. of Replicates	Root Formation Response Mean ± SD
Leaves	1 IAA + 2 BA	**20**	0.00±0.00
	1 BAP+ 0.5 NAA	**20**	0.00±0.00
	1 IAA + 1 BA	**20**	0.00±0.00
LSD			-
Petioles	1BAP+0.5NAA	**20**	0.00±0.00
	1BAP+1NAA	**20**	0.25±1.11*
	2NAA	**20**	1.00±2.05
LSD			-
Roots	0.5 NAA	**20**	0.00±0.00
	0.5 BAP+0.5NAA	**20**	0.00±0.00
	0.5 BAP + 2 NAA	**20**	0.00±0.00
LSD			-

*values represent mean% and standard deviation ($p \leq 0.05$) of each treatment.

Table 11. Shoot initiation directly from *T. officianle* explants excited from *in vitro* germinated plants with different combinations of growth regulators

Type of Explant	GR Combination (mg/L)	No. of Replicates	Shoot Initiation Response Mean ± SD
Leaves	1 IAA + 2 BA	**30**	0.00±0.00
	1 BAP+ 0.5 NAA	**30**	0.33±1.01
	1 IAA + 1 BA	**30**	0.00±0.00
LSD			**0.33**
Petioles	1BAP+0.5NAA	**30**	0.22±0.84
	1BAP+1NAA	**30**	0.00±0.00
	2NAA	**30**	0.00±0.00
LSD			-
Roots	0.5 NAA	**30**	0.22±0.84
	0.5 BAP+0.5NAA	**30**	0.00±0.00
	0.5 BAP + 2 NAA	**30**	0.22±0.84
LSD			-

*values represent mean% and standard deviation ($p \leq 0.05$) of each treatment.

Shoot initiation response from different explants

Shoot initiation response (0.33%) occurred directly from leave explants of *in vitro* germinated plants at medium supplemented with 1mg/L BAP + 0.5 mg/L NAA, and the differences were statistically significant (table 11). Petiole explants showed response (0.22%) at MS medium plus the combination (1mg/L BAP + 0.5mg/L NAA). Root explants showed response (0.22%) at medium with 0.5mg/L NAA and medium with (0.5mg/L BAP + 2mg/L NAA) and the differences were not statistically significant as presented in (table 11). The responses of shoot initiation induced from different explants are shown in (fig. 5).

Root formation response from different explants

Root formation (0.77%) from leaf explants of *in vitro* germinated plants occurred at medium supplemented with (1mg/L BAP + 0.5mg/L NAA), and the differences were statistically significant. Petiole explants respond (0.22%) at medium with 1mg/L BAP + 0.5mg/L NAA, while roots respond (0.22%) at medium with (0.5mg/L BAP + 0.5mg/L NAA) and medium with 0.5mg/L NAA. Moreover, no statistically significant differences observed among the treatments as indicated in (table 12). The root regeneration responses and the complete plantlets that produced directly from different explants are presented in (fig. 6) and (fig. 7), respectively.

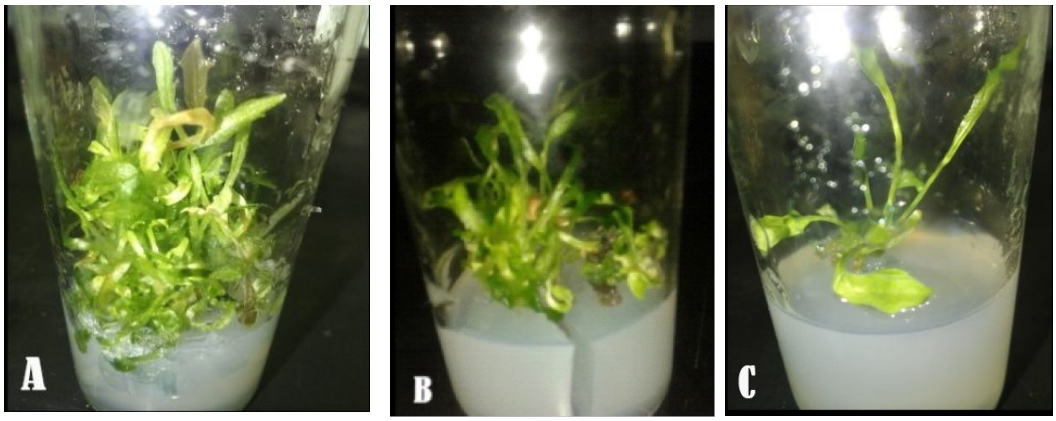

Fig. 5: Shoot induced through direct organogenesis from different explants, **A:** leaves (1mg/L BAP + 0.5mg/L NAA), **B:** petioles (1mg/L BAP + 0.5mg/L NAA), **C:** roots (0.5mg/L NAA)

Table 12: Root formation directly from *T. officianle* explants excited from *in vitro* germinated plants with different combinations of growth regulators

Type of Explant	GR Combination (mg/L)	No. of Replicates	Root Formation Response Mean ± SD
Leaves	1 IAA+2 BA	30	0.00±0.00
	1 BAP+0.5 NAA	30	0.77±1.43
	1 IAA+1 BA	30	0.00±0.00
LSD			**0.48**
Petioles	1BAP+0.5NAA	30	0.22±0.84
	1BAP+1NAA	30	0.00±0.00
	2NAA	30	0.00±0.00
LSD			-
Roots	0.5 NAA	30	0.22±0.84
	0.5 BAP+0.5 NAA	30	0.00±00.00
	0.5 BAP + 2 NAA	30	0.22±0.84
LSD			-

*values represent mean% and standard deviation ($p \leq 0.05$) of each treatment.

Fig. 6: Roots formed through direct organogenesis from leaves explants (1mg/L BAP + 0.5mg/L NAA)

The petioles showed diverse response; they produced the highest response of callus (5%) in addition to high shoot and root regeneration, in comparison to the leaves and roots through indirect organogenesis. This agrees with Ermayanti and Martin, (2011) who reported the high response of petioles for regeneration as well as high viability; they also indicated that presence of endogenous hormones supports the external growth regulators the inducing of regeneration. The petioles produce high callus response using NAA alone or in combination with BAP with both *in vitro* germinated plants and wild plants. The highest shooting and rooting appeared at (1mg/L BAP + 0.5mg/L NAA) and 2mg/L NAA with *in vitro* germinated plants that produce also highest rooting. That supported by Ermayanti and Martin, (2011) who revealed that NAA alone was suitable for root formation from petiole. This result revealing that petiole is more favourable for regeneration of adventitious shoots and root than another part of the leaf. Moreover, regeneration from the petiolar region may result from the increased density of the vascular tissue leading to increased levels of phytohormones and metabolites near the petiolar region (Chang *et al*., 2012).

The leaves followed petioles in response to the growth regulators used in the study. Segments of leaf blades or petioles of seedlings, induce morphogenetic process by many *Taraxacum* spp. including *T. platycarpum, T. mongolicum*, and *T. officinale* (Rakhimbaev *et al*., 2013), where both leaf and petiole explants of *T. formosanum* induce multiple auxiliary shoots, completed with a high frequency of rooting (Chang *et al*., 2012). Leaf explants originated from *in vitro* germinated plants showed high response for callus (4.00%), shoot (3.75%), and root (4.50%) when cultured on MS medium supplemented with the combination (1mg/L BAP + 0.5mg/L NAA) in contrast to leaf explants excised from wild plants; moreover, best rooting produced with this

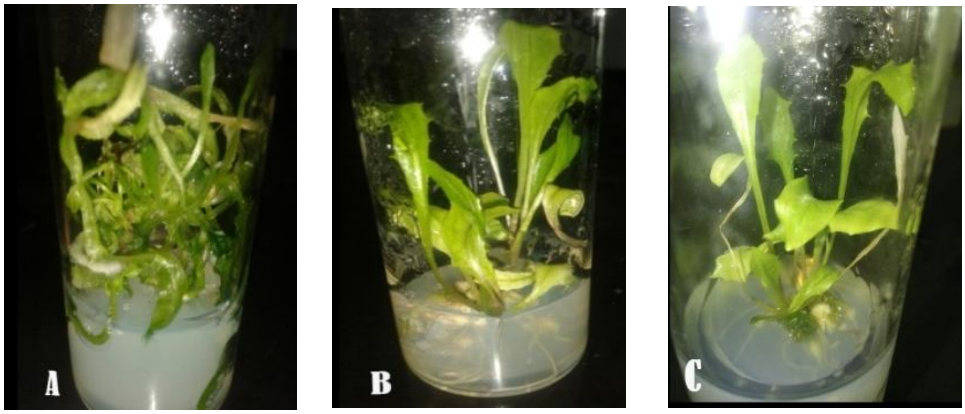

Fig. 7: Complete plantlets of *T. officinale* produced through direct organogenesis from different explants, **A:** leaves (1mg/L BAP + 0.5mg/L NAA), **B:** petioles (1mg/L BAP + 0.5mg/L NAA), **C:** roots (0.5mg/L NAA)

combination. Although, Ermayanti and Martin, (2011) reported an absence of rooting with this combination of leaves as well as petioles and roots. Best shoot initiation for leaves and petioles that excised from *in* vitro germinated plantlets was produced from the combination (1mg/L BAP + 0.5mg/L NAA); these observations supported by Ermayanti and Martin, (2011) who revealed that medium containing 1mg/L BAP separately or in combination with 0.5 mg/L of NAA was the best for shoot regeneration.

The root explants produce high percent of callus in comparing with shooting and rooting responses. Best callus response from roots resulted when culturing explants on medium supplemented with 0.5mg/L NAA. Furthermore; Rajore and Batra mentioned that NAA produced better regenerative callus than either IAA, 2, 4-D or IBA (Tabatabai, 2011; Chang *et al*., 2012) that is reversible with both Booth and Bows observations on the roots of *T. officinale* indicating that the frequency of callus formation was low (Bowes, 1971; Booth and Satchuthananthavale, 1974; Rakhimbaev *et al*., 2013). The Callus produced within 5-20 days for all treatments and the shoot produced after the same period directly or from callus. This agrees with Ermayanti and Martin, (2011) results who demonstrated that callus grown at the first two weeks from roots, and multiple shoots were grown after 3 weeks without rooting. Moreover, callus was induced from roots of *T. formosanum* after two weeks of culturing (Chang *et al*., 2012). Although, Chang *et al*., (2012) reported that response to rooting gradually decreased with increased concentration of the NAA. Moreover, the roots produce the highest shoot initiation response which supported by the findings of Ermayanti and Martin, (2011) results who demonstrated that roots were the best explant for shoot regeneration.

Some roots explants produce shoots and roots directly bypassing callus when culturing on MS medium plus 0.5mg/L NAA or plus (0.5mg/L BAP + 2mg/L NAA); Ermayanti and Martin, (2011) demonstrated direct organogenesis at 1mg/L BAP and (1mg/L BAP + 2mg/L NAA) from root explants. Some leaf explants produced a shoot and/or roots directly that could be resulted from the differences between the leaf pieces location from the entire leaf of the same origin, which affect the endogenous hormonal content, between leaf bases to the apical end of the leaf. A lower proportion of apical segments produce callus proliferation despite the small diameter of leaf, since some segments very near the apical end of the leaf differentiate callus with normal shoots, suggesting that the morphogenic factors are accumulated basipetally (Slabnik *et al*., 1986). The morphogenic responses could be related to the need for an adequate hormonal balance between auxins and cytokinins that would be implicated on tissues for callus proliferation and/or initiation and development of the new organs that would produce normal plants upon transfer to soil. Auxins are critical for cellular division, differentiation and callus induction (Isah, 2015). All the treatments that include auxins (NAA or IAA) induce callusing, shooting and rooting even when used alone, such as medium supplied with 0.5mg/L NAA and 2mg/L NAA in roots and petioles respectively. The cytokinins are added to culture media to induce morphogenesis or rejuvenation of explants (Isah, 2015); these support auxins by elevating the responses. The relative degree of auxins activity in different growth processes is very variable. It differs according to the plant, organ, tissue, cell, as well as the age and physiological state of the tissue (Davies, 2004; George *et al*., 2008). From what mentioned on auxins based treatments in petioles and roots, the treatments that include auxin (NAA) only are capable to produce callus and even shoots in addition to rooting response from petioles as well as roots without application of cytokinin; in spite of the fact that the presence of a cytokinin may not be necessary to induce callusing from explants of monocotyledons but its required to be added with auxins to the media in dicotyledons (George *et al*., 2008). Nevertheless, the high regenerative ability of Asteraceae family, which correlated and its ability to synthesize cytokinins (Hill and Schaller, 2013), could revealed that the low levels of exogenous cytokinins many not be required if the endogenous cytokinins are sufficient for inducing callus (George *et al*., 2008). The original endogenous content of phytohormones may support the provided auxins in the media, which proved to be found in the plant by many researchers such as, Gussakovskaya and Blintsov, (2001) who proved the presence of zeatin in *T. officinale* ovary and ovule, suggesting the presence of this cytokinin in the plant parts. The presence of this cytokinin in the plant parts is known to induce good callus formation in *T. kok-saghyz* that increased with the concentration increasing (Uteulin *et al*., 2014). Moreover, Rakhimbaev *et al*., (2013) suggest presence of sufficient levels of endogenous hormones in the tissues of the plant itself and Warmke and warmke, (1950) indicated that endogenous hormones of *T. officinale* roots could support and control organogenesis in cultures of callus derived from roots as well as inducing shoot initiation from root segments without any treatments, and it didn't induce any roots until treatment with auxins such as Indole butyric acid (IBA), IAA and NAA. Moreover, many compounds of the plants could support explants

regeneration ability by various effects such as phenolic compounds such as chlorogenic acid and *p* coumaric acid which is natural constituent of *T. officinale* and its highest content observed in leaves including petioles (George *et al*., 2008; Jassim *et al*., 2012; García-Carrasco *et al*., 2015).

Combination of BAP and NAA was suitable for regeneration of *T. officinale*. It gave different responses for regeneration into shoots or roots, or for callus formation that supported with the conclusions of Ermayanti and Martin (2011). Moreover, when 0.5mg/L BAP combined with two concentrations of NAA (0.5mg/L and 2mg/L) and used for root explants, it produce the same responses of callus with slightly higher shooting in lowered auxin (0.5mg/L NAA) and slightly higher rooting in higher auxin (2mg/L NAA). Slabnik *et al*., (1986) proved that increasing of BAP concentration lead to gradually inhibiting root formation. They also proved that media supplemented with 0.5mg/L BAP produced organogenic calluses after 10 days in culture and roots formed 10 days later. As BAP represents an important factor for shoot initiation, where Shoot initiation increases with treatment that contained BAP with decreasing of NAA concentration as in petioles. High concentration of BA (2mg/L BA) produce a better response than lower concentration (1mg/L BA) in combination with a constant concentration of IAA (1mg/L IAA) with leaf explants excised from wild plants; it produce regenerative callus that proceed to form shoot and/or root without rooting.

Acclimatization

All the produced platelets survived after 50 days of acclimatization during June and July; they started to produce multiple shoots and stay viable through different stages of acclimatization (Figure 8). The benefit of any micropropagation system can be fully realized by the successful acclimatization of plantlets from tissue-culture vessels to the *ex vitro* conditions (Chang *et al*., 2012). In this study, all the tested plantlets survived after 50 days of acclimatization with (100% of surviving) during June and July 2017. This supported by Furuno *et al*., (1993) who established acclimatized plantlets in about 2 months (June to August), and proceed acclimatization to form complete plants of *T. officinale* with many mature leaves, normal flowers, and fertile seeds that recovered after half a year.

The number of leaves of the produced plantlets started to increase with the different rate from plantlet to another; some of them produced more leaves than others and this could be due to the differences in the development of roots. The roots that grow in agar often lack root hairs,

A: Stage 3, Acclimatization of the produced plantlets in pots containing perlite

B: Stage 4, Acclimatization of the plantlets in pots containing perlite and peatmoss

Fig. 8: Plantlets in 3rd and 4th stages of acclimatization process

fragile and susceptible to mechanical damage; this effect plantlets survival and development by effecting nutrients absorption from the soil leading to slower growth than natural plants. The good development of vigorous root system is a critical factor for the success of acclimatization (Trejgell *et al*., 2013; Chang *et al*., 2012). Thus *T. formosanum* acclimatized better with higher rooting (Chang *et al*., 2012). As well as, the poor growth can result from the differences in the biosynthesis of the epicuticular wax and the development of the internal structure of the leaf. The changes of the relative humidity affect some of the events during the acclimatization of plants in the greenhouse like stomatal opening and closing leading to affects on the plantelets adaptation to harsh *ex vitro* conditions (Ali-Ahmad *et al*., 1998; Chang *et al*., 2012). Through acclimatization, the plantlets are removed from vessel conditions and require overcoming limitations of the external conditions via production of more assimilates with the increased energy necessary for *ex vitro* survival (Isah, 2015).

The survival rate of *T. formosanum* plantlets after five weeks of acclimatization was 100%. They are grown in growth chamber and showed both shoot elongation as well as robust rooting (Chang *et al*., 2012). Moreover, Trejgell *et al*., (2013) reported high survival (82%) of *T. pieninicum* after 8 weeks of acclimatization in non-sterile soil and sand mixture, but it was higher (96.2%) in the

sterile mixture, suggesting a negative effect of pathogenic organisms when plantlets were transferred from aseptic cultures to field conditions; could also affect the development of the cultured plantlets.

Conclussion

The study concluded that complete plantelets can be produced from a single explant of *T. officinale* by tissue culture without requiring for sub-culturing by two ways; direct and indirect organogenesis. The explants of *in vitro* germinated plants origin are better source of explants than wild plants origin; moreover, the petioles are better explants for micropropagation of *T. officinale* plant through direct organogenesis while the roots are the best explants for indirect organogenesis., The regenerative ability of the explant of *in vitro* germinated plants origin is higher than explants of mature plants origin. Auxins can be used alone in both petioles and roots explant to regenerate complete plants.

Abbreviation

NAA: Naphthalene acetic acid

BAP: Benzylaminopurine

IAA: Indol 3 acetic acid

BA: Benzyladenin

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