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Recovering *Taraxacum kok-saghyz* Rodin. via Seed and Callus Culture

K. Uteulin, S. Mukhambetzhanov, I. Rakhimbaev

***Abstract*—**This experiment was performed to optimize the medium for tissue culture of *Taraxacum kok-saghyz* Rodin. Different tissue culture approaches such as shoot regeneration from seed, callus formation from leaf explants and plant regeneration from callus were investigated in this study. All the explants were cultured on MS basal medium supplemented with 20g/l sucrose, 7g/l agar and different plant growth regulators. Seeds of *Taraxacum kok-saghyz*were cultured on media containing different levels of BA and 2,4-D (0.5, 1.0 and 3.0mg/L) to direct shoot regeneration study. Leaf explants were cultured in different combination of BA (at three levels: 0.5, 1.0 and 3.0mg/L) and zeatin (at two levels: 0.5 and 1.0mg/L) to examine callus formation. After the callus formation the formed calli were cultured on different combinations of BA and NAA for shoot regeneration. BA at three levels (0.5 and 1.0 and 3.0mg/L) and NAA at two levels (0.5 and 1.0mg/L) in all possible combinations were used for shoot regeneration from callus. The results showed that the treatment containing 1.0mg/L 2,4-D in combination with 1.0mg/L BA was found to be the best one for shoot regeneration from seeds. The treatment with 1.0mg/L BA in combination with 1.0mg/L zeatin were found to be suitable treatments for callus production from leaf explants, as well. Moreover, 0.5mg/L BA alone or in combination with 1.0mg/L NAA were found to be the best treatments for shoot regeneration from callus.

Keywords*—Taraxacum kok-saghyz* Rodin., shoot regeneration, callus.

I. Introduction

TARAXACUM kok-saghyz Rodin. a species in the genus Taraxacum (family *Asteraceae*) [1]. *Taraxacum kok-saghyz* is commonly known as Russian dandelion was discovered in 1931 in southeastern Kazakhstan, in the valleys of the Tien Shan Mountains [2]. The root is a source of high quality latex, used in making rubber. During the WWII, when hevea rubber from Southeast Asia was not available, kok-saghyz was intensely investigated in Soviet Union, USA, Germany and some other countries as an emergency source for natural rubber [3]. But cultivation of the kok-saghyz as a rubber producing crop was abandoned more than 50 years ago because it could not compete with cheaper hevea rubber and synthetic rubber. The renewed interest in kok-saghyz is due to several factors, including the increasing demand for natural rubber, the disease vulnerability of the hevea plantations and allergy problems associated with health care products made from hevea rubber [4], [5]. Therefore, are strong incentives to develop TKS as a new source of inulin, natural rubber and other products and the potential for doing so is high [6], [7]. But excessive exploitation in the past for the production of rubber and slow growth, poor seed germination ability made this plant endangered [8]. Moreover Kirschner et al. [5] concluded that the condition of most of the ex situ germplasm of kok-saghyz is catastrophic: either the material is misclassified or it is no longer viable.

Methods of in vitro propagation offer highly effective tools for germplasm conservation and mass multiplication of many plant species threatened with extinction. Therefore, microclonal propagation method would be the promising option for rapid propagation, multiplication and preservation of kok-saghyz.

Successful in vitro plant regeneration has been reported in some dandelions, such as the *Taraxacum officinale* [9], [10], *Taraxacum platycarpum* [11], *Taraxacum coreanum* [12] and *Tararacum mongolicum* [13].

Considerable efforts are still required to find out efficient in vitro methods for the rapid recovering of kok-saghyz. Reports in this area, however, are very limited. So far, plant regeneration from leaves has been reported by Luo et al. [14]. While Lin and Wei reported plant recovering from stem and leaf explants [15].

The objective of the study was to induce rapid and repeatable shoot formation and plant regeneration from the mature seed explant of the kok-saghyz, for further use in plant material multiplication and conservation of this rare endemic species.

II. Material and Methods

A. Plant Material and Surface-Decontamination

The mature achenes of kok-saghyz (Taraxacum kok-saghyz Rodin) were collected from their natural habitat in 2012 at Kegen district of Almaty region, Kazakhstan.

Achenes were previously exempt from the pappus. The seeds were treated for 5min in 70% (v/v) ethanol and rinsed with distilled water for 5min. After rinsing with distilled water seeds were surface-sterilized by immersion in a solution of 30% (w/v) sodium hypochlorite for 10min. Two drops of Tween-20 were added. To remove the surfactants, sterilized seeds were rinsed five times with sterile distilled water and blotted on to a sterile Whatman filter paper.

K. Uteulin Institute of Plant Biology and Biotechnology, Bio Tech TKS LLP, 45 Timiryazev str, 050040, Almaty, Kazakhstan (phone: 007-727-3947562; e-mail: gen_uteulink@mail.ru).

S. Mukhambetzhanov Institute of Plant Biology and Biotechnology, Bio Tech TKS LLP, 45 Timiryazev str, 050040, Almaty, Kazakhstan (phone: 007-727-3947562; e-mail: serik_m@list.ru).

I. Rakhimbaiev. C. Institute of Plant Biology and Biotechnology, Bio Tech TKS LLP, 45 Timiryazev str, 050040, Almaty, Kazakhstan (phone: 007-727-3947562; e-mail: rakhim_10@mail.ru).

B. Direct Shoot Regeneration from Seeds

Seeds were cultured on Petri dish containing 20ml MS [16] basal medium supplemented with 6-Benzyladenine (BA) and 2,4-dichlorophenoxyacetic acid (2,4-D) each in three levels (0.5, 1.0, and 3.0mg/L). 20 seeds were considered for each treatment. Moreover, some seeds were cultured on hormone-free MS basal medium as a control. The test tube incubated at 25±2ºC in photo period of 16h light/8h dark. Shoot regeneration percentage was recorded in the third week after culture. The regenerated shoots were transferred to magenta containers to grow more.

C. Callus Formation from Leaf Explants

For callus induction, the leaf explants with the length of about 1.5cm were wounded with scalpel and cultured in Petri dishes containing 20ml MS basal medium supplemented with 20g/L sucrose, 7g/L agar and plant growth regulators. BA (at three levels: 0.5, 1.0, and 3.0mg/L) and zeatin (at two levels: 0.5 and 1.0mg/L) were used as growth regulators sources, respectively. All possible combinations among these levels were considered as treatments so 6 treatments were made. Five replications were considered for each treatment. Eight leaf explants were cultured in each Petri dish. Petri dishes were sealed with parafilm and incubated at 25±2ºC in dark and sub cultured every three weeks.

D. Shoot Regeneration from Callus

After callus formation from leaf explants the formed calli were transferred into magenta containers containing 100ml MS basal medium supplemented with 7g/L agar, 20g/L sucrose and plant growth regulators. BA at three levels (0.5 and 1.0 and 3.0mg/L) and napthaleneacetic acid (NAA) at two levels (0.5 and 1.0mg/L were used as cytokinin and auxin sources respectively. All possible combinations among these levels were considered as hormonal treatments (on the whole 6 hormonal treatments). Five segments of calli (with the dimensions of about 1cm^3) were placed in magenta container. Moreover ten calli with the same dimensions were cultured in two magenta containers (five calli per container) containing 100 ml MS basal medium without any hormones as control. The containers were sealed with parafilm and incubated at 25±2ºC in photoperiod of 16h light/8h dark. The explants were subcultured every three weeks. The number of regenerated shoots per callus was recorded in the sixth week after callus culture.

III. Results and Discussion

A. Direct Shoot Regeneration from Seeds

About a 3-5 days after culture, some seeds in the treatment containing 1.0 mg/L BA initiated to form shoots. The shoots emerged from nodal part of seeds in the form of small leaf clusters. In the next weeks these shoots proliferated rapidly and formed dense cluster of leaves (Fig. 1). Shoot regeneration percentage was determined in the sixth week after culture (see Table I). Shoot regeneration from seeds in the media containing 1.0mg/L 2,4-D occurred lately. Also shoot regeneration percentage in this treatment (32%) was less than treatment containing 1.0 mg/L BA (48%). Shoot regeneration percentages in 3.0 mg/L BA and 3,0 mg/L 2,4-D were 16% and 12% respectively. Lee et al [11] reported that MS media without phytohormones is better for shoot regeneration from seed explants of *Taraxacum platycarpum*, which it not corresponds with our results of seed culture of *Taraxacum kok-saghyz*. We found that low level (0.5mg/L) of these hormones (BA and2,4-D) is not suitable for shoot regeneration from seeds of kok-saghyz.

Fig. 1 Multiple shoot regeneration from a seed explants of *Taraxacum kok-saghyz* in the MS medium containing 1.0 mg/L BA

TABLE I
COMPARING THE DIFFERENT LEVELS OF BAP AND 2,4-D ON SHOOT REGENERATION FROM SEED EXPLANTS OF *TARAXACUM KOK-SAGHYZ*

Hormone	Concentration (mg/L)	Regeneration (%)
BA	0,5	1,2^c
	1,0	48^b
	3,0	16^a
2,4-D	0,5	1,7^c
	1,0	32^b
	3,0	12^a

Different letters within the columns indicate significant differences (Duncan multi range test, P‹=0.05)

B. Callus formation from leaf explants

In most of the treatments callus initiation from leaf explants occurred about two weeks after culture, but the best callus formation and growth rate was observed in the medium containing 1.0 mg/L zeatin in combination with 1.0mg/L BA. The formed calli in these treatments were more friable than those in the others (Fig 2).

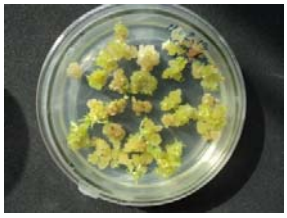

Fig. 2 The formed calli in MS medium containing 1.0 mg/L zeatin plus 1.0 mg/L BA

Generally with neglect of BAconcentration, callus formation in the media containing 1.0mg/l zeatin was much better than those containing 0.5mg/L zeatin. Since callus formation and proliferation in the medium containing 1.0mg/L zeatin and 0.5 or 3.0mg/L BA was better than those containing 0.5 mg/L zeatin in combination with 0.5, or 3.0mg/L BA. Also, callus formation was not suitable when BA used in

higher level than zeatin. It seems that high levels of zeatin are necessary for callus formation and using BA can accelerates this process. We found that the media containing 1.0 mg/L zeatin in combination with 1.0 mg/L BAare suitable for callus induction from leaf explants of *Taraxacum kok-saghyz*. Lin and Wei [15] achieved the best callus production in the media containing 0.3mg/L NAA in combination with 8.0mg/L BA. They reported that the media containing NAA alone is not able to callus formation from leaf explants of *Taraxacum kok-saghyz.* Similar to our results they found that callus formation from leaf explants is not suitable when BA is used in higher concentration.

C. Shoot Regeneration from Callus

After transferring the formed calli to shoot regeneration media, purple or green spots appeared on their surface in some treatments which subsequently converted to shoot primordia (Fig 3) and then to shoots with several leaves. In the next weeks, shoot formation was observed. Shoot regeneration in the medium containing 1.0mg/L BA plus 0.5mg/L NAA was better than the medium containing 1.0mg/L BA alone. Among all the treatments, the treatment containing 3.0mg/L BA alone or in combination plus 0.5mg/L NAA were found to be the best ones for shoot regeneration from callus. Regenerated shoots in these treatments were longer than those in the other treatments and had about 10±3 leaves.

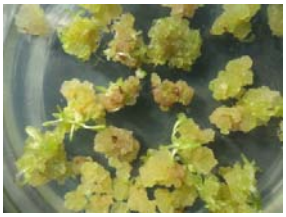

Fig. 3 Regenerated shoot primordia from callus in the medium containing 1.0 mg/L BA plus 0.5 mg/L NAA

Callus proliferation had predominate response on the media containing 0.5mg/L BA alone or in combination with 1.0mg/L NAA. Formed calli in these treatments were green and friable but were not able to regenerate shoots. Luo et al [14] achieved suitable shoot formation in *Taraxacum kok-saghyz*by transferring the leaf-derived callus to MS medium with 2.0 mg/L 6-BA whereas we found that the presence of cytokinin is necessary for shoot regeneration from leaf-derived calli, since the calli which were placed in MS medium without plant growth regulator failed in regenerating shoots. Moreover, we found that in high concentrations (3mg/L) of cytokinins, addition of NAA promotes shoot regeneration from callus, which it corresponds with the results of Lin et al [15]. It seems that high concentrations of BA are necessary to shoot regeneration in *Taraxacum kok-saghyz*. Also it can be deduced that it in higher levels of BA shoot regeneration is promoted by adding of NAA, while in lower concentration of BAP (0.5 mg/L) it helps the callus proliferation. Some researchers have reported that an appropriate combination of NAA and BAP stimulated shoot formation [12], [13], [17], [18]. Our findings confirm their results.

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