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Introduction to in vitro culture of isolated Taraxacum kok-saghyz roots

The present study with Taraxacum kok-saghyz was taken up to examine the possibility of establishing in vitro root culture. It were shown that a root development and direct shoot formation (without callus) could be obtained from isolated root culture of Taraxacum kok-saghyz using a basal Knop, White and Murashige and Skoog media without any growth regulator. All the media contained 2% (w/v) sucrose. Knop medium supported maximum root elongation among tested media. MS medium was optimal for direct shoot induction from isolated root culture. Sucrose concentration also affected the elongation of root in vitro. 2% sucrose showed the best root elongation among tested sucrose concentration. Only small differences were found in shoot formation rate between the explants with root apex and those without root apex any type of root explant may potentially have the ability to induce shoots.

Key words: Taraxacum kok-saghyz, in vitro culture of roots, root explants.

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Taraxacum kok-saghyz оқшауланған тамырларын in vitro жағдайына енгізу

Бұл ғылыми зерттеудің басты мақсаты Taraxacum kok-saghyz-дың оқшауланған тамырларының in vitro жағдайында өсу мүмкіндіктерін зерттеу болып табылады. Көрсетілгендей, оқшауланған тамырдан өркеннің тікелей индукциясы мен тамырдың дамуын, негізгі қоректік орталарды Кноп, Уайт және Мурасиге және Скугты (МС) ешбір өсу регуляторсыз пайдаланып, алуға болады. Барлық қоректік орталардың құрамы 2% сахарозадан тұрды. Кноп қоректік ортасы басқа қоректік орталардың ішінде тамырдың максималді ұзындыққа өсуін қамтамасыз етті. МС қоректік ортасы оқшауланған тамырлардан тікелей өркеннің өсуіне қолайлы болды. Сахарозаның концентрациясыда тамырлардың in vitro жағдайында ұзындыққа өсуіне әсер етті. Анықталғандай, 2% сахароза ең қолайлы концентрация болып табылды. Өркен түзудің темпі эксплантардың тамыр апексі бар немесе жоқ түрлері арасында айырмашылық көп емес, ол дегеніміз тамыр эксплантарының әртүрлі типтерінің өркен түзі потенциалы бар.

Түйін сөздер: Taraxacum kok-saghyz, тамырларын in vitro жағдайы, тамыр эксплантары.

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Введение в культуру in vitro изолированных корней Taraxacum kok-saghyz

Настоящее исследование было предпринято с целью изучения возможности введения в культуру in vitro изолированных корней Taraxacum kok-saghyz. Было показано, что развитие корня и прямая индукция побега в культуре изолированного корня может быть получена с использованием базовых питательных сред Кнопа, Уайта и Мурасиге и Скуга без каких-либо ростовых регуляторов. Все среды содержали 2% сахарозы. Среда Кнопа поддерживала максимальное удлинение корней среди испытанных сред. Среда МС была оптимальной для прямой индукции побега из культуры изолированного корня. Концентрация сахарозы также влияла на удлинение корня in vitro. Показано, что 2% сахарозы является лучшей среди испытанных концентраций. Обнаружены лишь небольшие различия в темпах формирования побега между эксплантами с корневым апексом и без корневого апекса т.е. различные типы корневых эксплантов потенциально способны индуцировать побеги.

Ключевые слова: Taraxacum kok-saghyz, культура in vitro изолированных корней, корневые экспланты.

UDC 633.913.322:581.1.035

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INTRODUCTION TO IN VITRO CULTURE OF ISOLATED TARAXACUM KOK-SAGHYZ ROOTS

Introduction

Taraxacum kok-saghyz Rodin (TKS). 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 [3].

The natural regeneration as well as conventional propagation of this plant overcome the several factors like poor seed set, seed germination, seed viability and root initiation [4]. In this reason development of root culture is an alternative method for clonal propagation and germplasm conservation.

One of the pioneering events in the development of tissue culture techniques for dandelion was the successful establishment of actively growing clones of *Taraxacum officinale* roots reported by B.G. Bowes in 1970 [5]. In this research, only callus development from the secondary thickened root occur. Subsequently, a number of successful research on the culture of isolated root for some species of *Taraxacum*. Shoots have been successfully initiated *in vitro* from callus from root cuttings of *T. officinale* [6, 7]. Shoot regeneration in both callus and direct from root cuttings were achieved for *T. officinale, T. platycarpum, T. mongolicum* [6, 8, 9].

Modern methods for culturing excised root tips of *Taraxacum* are basically the same as those described by Bowes. Most methods involve the germination of seed aseptically, removal of the radical upon emergence, and transfer to liquid medium. Once root growth begins and becomes active, the terminal 10 mm of the root and root laterals can be excised and placed on fresh medium.

The present study with *Taraxacum kok-saghyz* was taken up to examine the possibility of establishing *in vitro* root culture.

Material and methods

The mature achenes of TKS were collected from experimental plot in 2014 at Institute of Plant Biology and Biotechnology (Almaty, Kazakhstan). Achenes were previously exempt from the pappus. The seeds were treated for 3 min in 20% (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 20% (w/v) commercial «Belizna» bleach for 3 min.

Sterilized seeds were then planted aseptically in sterile Petri dishes (40 seeds per dish) containing moistened filter paper and kept in dark at 25±1^{0}C.

Apical segments of primary roots (without laterals) from 6 day old seedlings were excised under the aseptic conditions. Segments 10 mm long were placed in 150 ml conical flaks (five per flak and four flaks per treatment) with 20 ml of growth regulators free liquid Knop, White and Murashige and Skoog basal media. All the media contained 2% (w/v) sucrose except for the experiment in which the effect of sucrose concentration (0, 0.5, 1.0, 2.0, 3.0 and 5.0%) was tested using Knop medium. Flaks were kept in darkness (wrapped with writing paper) on a shaker at 100 rpm. under the same conditions as above.

After 6 weeks of culture of root segments in Knop medium, elongated root cultures were cut into 10 mm pieces and laid in MS medium containing 2% sucrose. They were separated into two groups. First was the segment with root apex and second was without root apex. Twelve root minicuttings were cultured for each group of explants. They were maintained at 25±1^{0}C in darkness (wrapped with writing paper) on a shaker at 100 rpm.

Results and Discussion

Elongation of root cultures with root apex differed among basal media tested (Tab. 1). Knop medium yielded the best root elongation and the White medium yielded the lowest. There were no lateral roots in any cultures. However, most root segments turn brown after 4 weeks of culture.

Table 1 – Influence of basal media on elongation of excised root culture of kok-saghyz*

Medium	Mean root length (mm)	
	2 week culture	4 week culture
Knop	15±0.25	60±0.69
White	8±0.25	37±0.25
MS	0±0.00	0±0.00

*Each medium contained 2% sucrose.

Sucrose concentration also affected the elongation of root (Fig. 1). The effect of sucrose concentration was tested on Knop medium. Initial length of explants was 1 cm. Root length was recorded after 2 weeks of culture. As shown in table 2, 1-3% sucrose resulted in better root elongation with maximum root length at 2%, whereas 0.5 and 5% resulted in less elongation.

Table 2 – Effect of sucrose concentration on elongation of excised root culture of kok-saghyz

Sucrose concentration (%)	Mean root length (mm)
0	0±0.00
0.5	2±0.17
1.0	5±0.37
2.0	9±0.38
3.0	7±0.33
5.0	4±0.28

Root cuttings obtained from the root segments without apex showed the response as those obtained from root segments with apex after 2 weeks of culture.

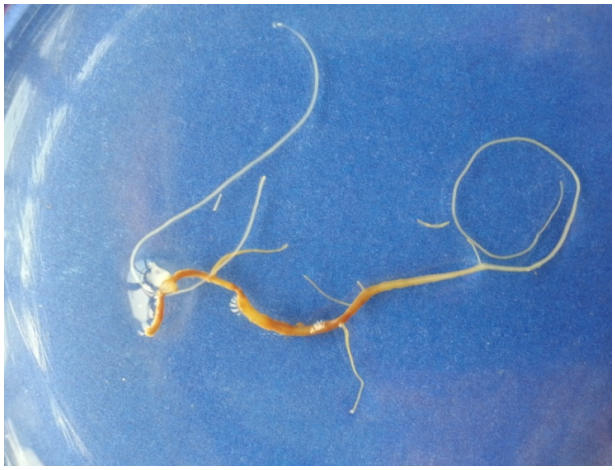

Figure 1 – Excised root elongation on Knop medium with 2% sucrose

Shoots were directly obtained from root culture-derived segments without apex after 6 weeks of culture (Fig. 2), whereas those from root segments with apex took a little longer. The root segments without root apex resulted in a slightly higher shoot induction rate than those with apex. On root cultures without apex, shoot induction rate was 41% (5 out 12), whereas 33.3 (4 out of 12) on those with apex.

The protocols of *in vitro* shoot induction from root explants has previously reported in some *Taraxacum* species [6, 8]. In present study, we

succeeded in the establishment of fast growing root cultures, shoots induction of *Taraxacum kok-saghyz*.

For the elongation of roots in excised root cultures, Knop medium containing 2% of sucrose was the most appropriate among tested media.

Figure 2 – Direct shoot induction from isolated root culture of Taraxacum kok-saghyz on MS basal medium without growth regulators

Presence of sucrose in the nutrient medium can perform regulatory functions [6, 7]. In this study, lower concentration (1-3%) of sucrose promoted root elongation, whereas higher concentration (5%) was rather inhibitory to root elongation. 2% sucrose showed the best root elongation among tested sucrose concentration.

As only small differences were found in shoot formation rate between the explants with root apex and those without root apex any type of root explant may potentially have the ability to induce shoots.

In this preliminary work we have shown that a root development and direct shoot formation (without callus) could be obtained from isolated root culture of *Taraxacum kok-saghyz* using a basal media without any growth regulator. Of the three media tested, Knop medium was the optimal for isolated root growth and MS medium was optimal for direct shoot induction from isolated root culture.

Acknowledgment

This publication is produced as a part of «Obtaining High Productivity Forms of *Taraxacum kok-saghyz* Rodin. – Domestic Producer of Rubber» subproject, funded by Technology Commercialization Project, supported by the World Bank and the Government of the Republic of Kazakhstan. Statements contained herein do not necessarily reflect the official views of the World Bank and the Government of the Republic of Kazakhstan.

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