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ORIGINAL ARTICLE

Stem cell induction and plant regeneration are affected by medium components in maca (*Lepidium meyenii* Walp)

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Abstract

Background In medicinal plants, selection, reproduction and preservation of important genotypes are very necessary. Nowadays, using tissue culture and regeneration techniques of medicinal plants under in vitro conditions has been able to proliferate medicinal plants widely, which is much higher than traditional methods of vegetative propagation. Maca (*Lepidium meyenii*), is an industrial plant whose root is the usable part. Maca has valuable medicinal effects such as sexual enhancement and reproductive power, infertility treatment, improved sperm count and quality, anti-stress, osteoporosis prevention and more.

Methods and results This study was conducted to induce callus and regeneration of Maca. First, MS medium supplemented with different concentrations of Kinetin, Naphthaleneacetic acid and 2,4-Dichlorophenoxyacetic acid [0.5, 1 and 2 µM respectively] and control were compared for callus induction from root and leaves. After 38 days of incubation, the first callus appeared, after 50 days of callus induction and after 79 days regeneration occurred. The callus induction experiment was performed for the study of the effect of three explants (leaf, stem and root) and seven hormone levels. The regeneration experiment was carried out by studying the effect of three explants (leaf, stem and root) on eight levels of the hormone. The results of data analysis on callus induction showed that the effects of explants, hormones and their interactions on callus induction percentage were highly significant but not significant on callus growth rate. The results of regression analysis showed that explants, hormones and their interactions had no significant effect on regeneration percentage.

Conclusion Based on our results, the best medium for inducing callus was Hormone 2,4-D [2 µM] and Kinetin [0.5 µM], in which the highest percentage of callus induction was in leaf explants (62%). And the lowest were in stem (30%) and root (27%) explants. According to the comparison of the mean, the best environment for regeneration of the environment was 4 µM 6-Benzylaminopurine 2.5 + Thidiazuron, in which the highest percentage of regeneration was in leaf explant (87%) and stem (69%) and the lowest in root explant (12). %).

Keywords Hormone · Infertility · *Lepidium meyenii* · Maca · Tissue culture

Introduction

Aromatic and medicinal plants have been used for therapeutic, religious, cosmetic, nutritional, and beautification purposes since ancient times and humanity of all civilizations and culture are familiar with their usage. The have numerous active ingredients that has therapeutic effect [1–3]. Global statistics show that the active ingredients of about 50% of the drugs offered on the market are of plant origin and even in some countries, this amount has reached 90% [4, 5]. Maca is a medicinal plant with the scientific name of *Lepidium meyenii*, with ploidy level (2n = 8x = 64) [5, 6] as grows naturally at altitudes above 4500 – 3500 meters in the Andes Mountains in Peru [7]. Maca is one of the few plants

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that can survive in harsh environmental conditions such as high altitudes with scorching sun, cold nights and dry winds and is also well adapted to poor agricultural soil [5, 6]. Thus, in these conditions, a small number of plants are able to survive [8]. Maca naturally repels most pests. The color of maca roots varies depending on the growing area [5, 6]. Colors can be yellow, purple, white, gray, black, and red [9]. In different Maca breeds, most North American and European varieties have been studied. Maca is adapted for large-scale cultivation in other parts of the world, such as China [10]. Maca is better known as a medicinal plant and in addition to its medicinal effects, it has high amounts of protein, carbohydrates, fiber, fats, vitamins and minerals with great nutritional value [11]. Maca is about 13–16% protein and rich in essential amino acids. Fresh varieties contain 80% water and high amounts of iron and calcium [12]. Many secondary metabolites, including macaine and macamide, are found in the root of maca. Macaine and macamide are a group of biologically active components in maca that are involved in improving sexual function [8, 13].

In the sample of dried maca, macaine varies from 0.09 to 0.45 percent and macamide from 0.06 to 0.52 percent [14]. Maca root contains a high medicinal value [11] and valuable effects such as: increasing sperm count, infertility treatment and improving fertility, anti-stress [15], nutrition of the body glands and prevention of osteoporosis [16], improving memory and learning in humans and laboratory animals [17], Hormonal regulation [18], treatment or elimination of rheumatism and endurance enhancer [19], sexual enhancement and increase of fertility and fetal survival [20], improvement of sperm quantity and quality [21], UV protection of the skin [8], increase sports performance and energy [22], effectiveness in combating anemia, leukemia, liver protection, AIDS, anti-flatulence, cancer and is a treatment for depression and fatigue [23]. Maca root powder contains many minerals such as sodium 18.7, iron 16.6, calcium 0.20, copper 7.6, zinc 13.8, potassium 20.5 and manganese 2.8 per 100 grams of dry matter [11, 24]. Leaves are also a source of essential fiber, minerals, vitamins and amino acids [25]. Plant tissue culture is the study of the growth of cells, tissues, and organs isolated (explants) from the mother plant on an artificial culture medium that involves the use of techniques and methods to achieve specific goals in many plant sciences [26, 27].

So far, no study has been done to callus induction in maca plant in IRAN and the purpose of this study was conducted for the first time to investigate the medium components (the type and amount of hormone) as well as the type of explant on maca callus induction and plant regeneration.

Materials and methods

Location and time of the test

This experiment was performed in the plant tissue culture laboratory of Razi University, Faculty of Agricultural Sciences and Engineering, during 2019–2020.

Materials

Chemicals

Chemicals include growth regulators, sucrose, agar, and culture media, including macro and microelements, vitamins, amino acids, myoinositol, iron, and hormones including Kinetin, NAA and 2,4-D [0.5, 1 and 2 µM respectively], TDZ [2, 3 and 4 µM] and BAP [2 and 2.5 µM].

Plant materials and preparation of explants

The plant material used in this study was maca (Lepidium meyenii) which was prepared from Biston Shafa Company. First, Murashige and Skoog medium, which has been used in many studies of plants, was used. Culture media with different concentrations of Kinetin, NAA and 2,4-D [0.5, 1 and 2 µM respectively] were prepared with the control under sterile conditions and explants were prepared from the roots and leaves of the plant and transferred to them. Then place in a growth chamber under controlled conditions at 25°C with a photoperiod of 8.16 (light/dark) to grow.

Cultivation of explants

After the medium preparation, the explants (the callus induction step) were placed on a solid culture medium (containing 7 g/L agar) and then transferred to the growth chamber.

Callus induction medium

This two-factor factorial experiment (7 × 3) was performed by examining the effect of three explants (leaves, stems and roots) on seven levels of hormonal compounds based on a completely randomized design with four replications.

The hormonal compounds

In this study, the following seven hormonal compounds were used to induce callus:

[2 µM 2,4-D], [2 µM 2,4-D + 1 µM NAA], [1 µM NAA], [0.5 µM Kinetin], [1 µM NAA + 0.5 Kinetin], [2 µM 2,4-D + 0.5 Kinetin] and Control (hormone-free).

Samples were cultured in the same medium every two weeks. The normality of the data was also checked. Hormones were optimized and the best hormonal combination was selected for the explant culture medium.

Traits investigated in callus induction experiments

Callus induction percentage In the studied treatments, the percentage of callus induction of explants was calculated by counting the number of calluses obtained divided by the number of cultured explants multiplied by.

Day to callus The time of callus initiation was noted in each explant and it was used as an attribute in comparison to treatments.

Callus growth rate The callus growth rate was calculated as follow:

Callus growth rate (CGR) = Average Callus Diameter/Time.

The CGR rate was measured in meters of diameter per day as follows: Callus diameters from different treatments were measured after 38, 12, 12, 12 and 12 days after explant cultivation (leaves, stems and roots).

This is how the callus diameter was calculated. The length and width of the calluses were measured with a piece of paper and then multiplied and extracted from it. Then, using the average calli in each replication, the average growth rate was calculated using four callus diameters.

Regeneration test

For indirect regeneration through callus, a two-factor (8 × 3) factorial experiment was performed by examining the effect of calli from these three explants (leaf, stem and root) on eight levels of hormonal compounds based on a completely randomized design with four replications.

The hormonal compounds used are as follows In this research, the following eight hormonal compounds were used to induce callus:

[2.5 µM BAP], [2.5 µM BAP + 2 µM TDZ], [2.5 µM BAP + 3 µM TDZ], [2.5 µM BAP + 4 µM TDZ], [2 µM TDZ], [3 µM TDZ], [4 µM TDZ] and Control (hormone-free).

The normality of the data was also checked. Hormones were optimized and the best hormonal combination was selected for the explant culture medium.

The trait examined in the regeneration experiment In this part of the research, the percentage of regeneration was calculated. In this way, in each replication of each treatment, the number of regenerated explants was divided by the total number of cultivated explants and the resulting fraction was multiplied by 100 to obtain the percentage of regeneration of the shoot.

Table 1 Analysis of variance of the effect of explants and hormones on leaves, stems and roots of maca on traits related to callus induction

		MS	
S.O.V.	df	Callus induction percentage	Callus growth rate
Explant	2	0.267 *	0.359 ^{ns}
Hormone	6	0.126 *	0.462 ^{ns}
Explant× Hormone	12	0.018 *	0.448 ^{ns}
Error	60	0.019	0.429
Total	84	-	-
C.V.	-	32	36

*, ** (significant $P < 0.05$ and $P < 0.01$ respectively) and ns (non-significant)

Rooting

For rooting, regenerated samples of hormones with concentrations according to the following were used. A hormone-free culture medium was also used as a control in each experiment. It was also used 0.5 g/l activated charcoal in some compounds. Rooting percentage was recorded for each sample.

Hormonal compounds used in rooting including: [1µM IBA, 2µM IBA], [1µM IBA + 1µM NAA], [1µM IBA + activated charcoal], [2 µM IBA + activated charcoal], [1µM NAA, 2µM NAA], [1µM NAA + activated charcoal], [2 µM NAA + activated charcoal] and control.

Statistical analysis

To determine the effect of treatments, the obtained data were statistically analyzed. Before performing the analysis of variance, the normality of the data distribution was checked using SPSS software (Klomogrof-Smirnov test). If the data distribution was normal, analysis of variance and mean comparison were performed by the Duncan method.

Results and discussion

The results of the analysis of variance are shown in Table 1.

The results of the analysis of variance showed that the effect of explants, hormones and their interactions on callus induction percentage was significant but were not significant on callus growth rate.

Pande et al. (2002) [28] used two combinations of culture medium in *Lepidium sativum* Linn. propagation by callus and through nodal sections of mature plants. In the first combination, the highest regeneration frequency was obtained with the highest number of stems, which means 25 stems in each MS medium with 2.85 µM Indole 3 acetic acid and 6.4 µM kinetin. In the second combination, the

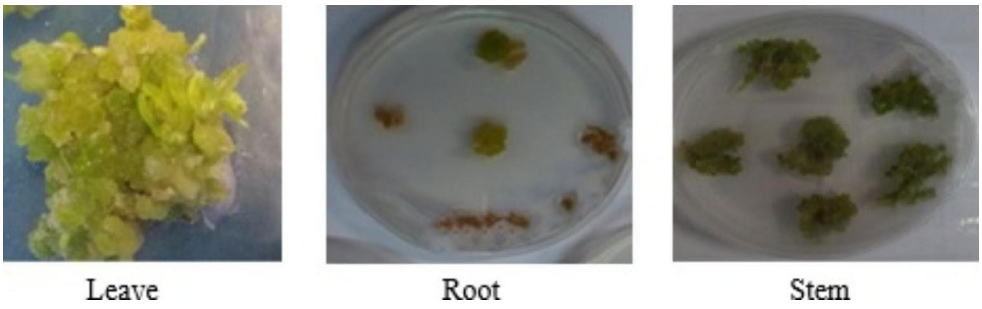

Fig. 1 Callus induction in three explants (Leave, Root, Stem) in maca

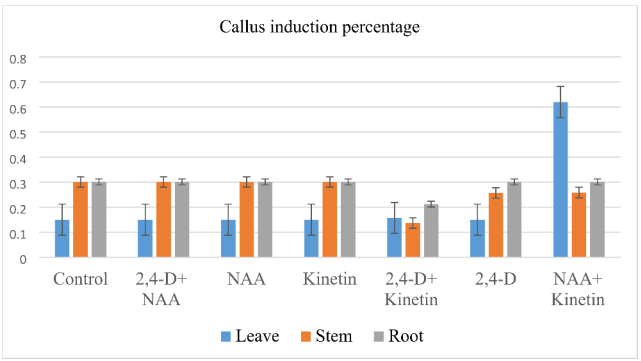

Fig. 2 Comparison of mean interactions of explants and maca leaf, stem and root hormones on callus induction percentage

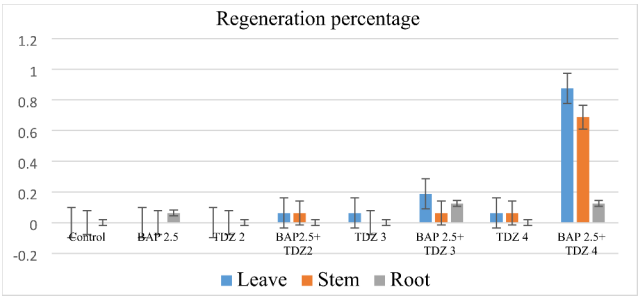

Fig. 3 Comparison of the mean interactions of maca explants (leaf, stem and root) in hormone on regeneration percentage

regeneration rate reached 75% and 5 stems were obtained in each MS medium with 1.14 µM Indole, 3 acetic acid and 23.2 µM kinetin.

Naqvi (2001) [29] in a combined report of 3.3 µM alpha naphthalene acetic acid, 0.058 µM gibberellic acid 3 and 4.9 µM isopentenyl adenine, which accelerated callus proliferation in *Brassica oleracea* and *Brassica napus* have been.

Callus induction in leaf, stem and root explants are shown in Fig. 1. The leaf explants were the most susceptible and root inappropriate explants for callus induction.

Guo et al., (2000) [30] reported that high doses of 2, 4-D [6.22 µM] and BAP [17.7 µM] were used for callus *Brassica rapa* L.

A comparison of the mean interaction effects of explants in the hormone on the percentage of callus induction in the maca plant is shown in Fig. 2.

According to the comparison of the mean, the best medium for inducing callus was Hormone 2,4-D [2 µM] and Kinetin [0.5 µM], which had the highest percentage of callus induction in leaf explants (62%) and the lowest was in stem (30%) and root (27%) explants.

The comparison of the interaction effects of explants in hormones on the percentage of regeneration in the maca plant is shown in Fig. 3.

According to the comparison of the average, the best medium for regeneration was 4 µM BAP 2.5 + TDZ, in which the highest percentage of regeneration was in leaf explant (87%) and stem (69%) and the lowest in root explant was (12%). Images of callus regeneration percentage in three-leaf, stem and root explants are shown in Fig. 4. As can be seen in the pictures, leaf explants are the most susceptible and root unsuitable explants for callus regeneration.

Arshad (2009) showed that for regeneration on MS medium with 9 µM Tidiazuron, 2.6 µM alpha naphthalene acetic acid and 44.1 µM silver nitrate, stem regeneration is obtained directly from the leaves of *Brassica campestris* ssp. Chinensis [31].

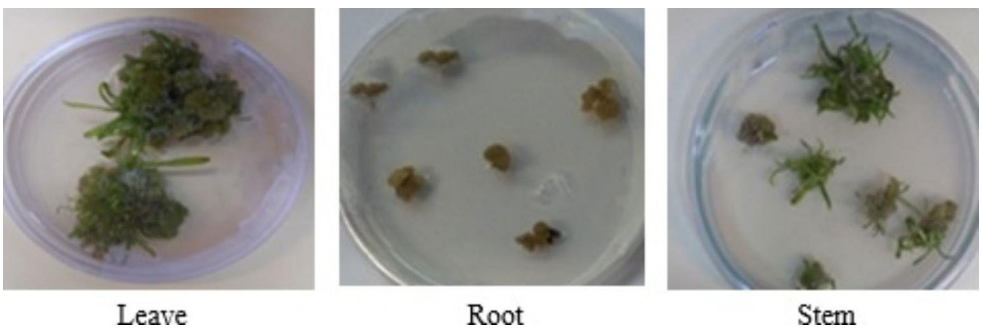

Fig. 4 Regeneration in three explants of leaves, shoot and roots in maca plant

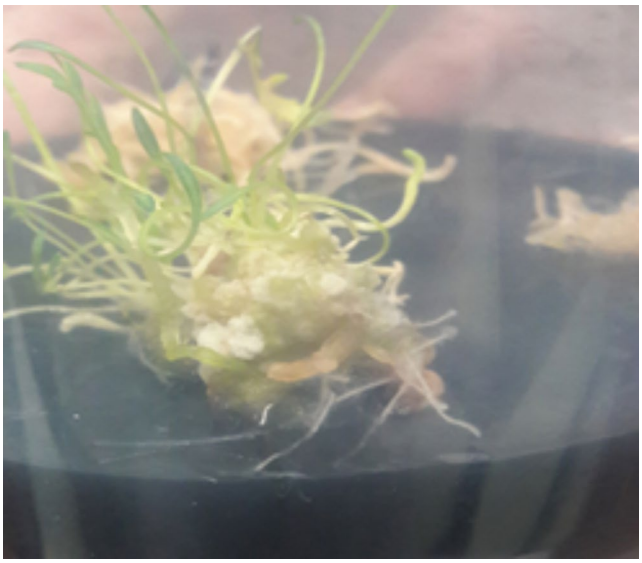

Fig. 5 Maca rooted explant

Osuna et al. (2006) [6] developed a propagation protocol for the medicinal plant *Lepidium virginicum* L. Seeds were used in vitro to prepare cotyledons, hypocotyls and apical buds. The best propagation rate after 15 days of culture was in MS medium containing 0.57 µM indole 3 acetic acid and 13.94 µM kinetin from the apical buds of the explants.

In this experiment, rooting occurred only in a culture medium containing 1 µM IBA with activated charcoal (Fig. 5). For this reason, the decomposition did not take place. The rooting time was very long. It took six months from the transfer of the regenerated plants to the emergence of the roots. One month after root emergence, seedlings were transplanted into the soil. The soil used was light soil, including potting soil in addition to cocopeat, which was disinfected by autoclave. Despite all the necessary things such as moisture retention and irrigation were done for gradual adaptation, the plant was destroyed.

Lebeda et al. (2003) [32] reported that maca plant in 51 days in MS medium without NH_4N0_3 and with 3% sucrose (w/v) and 0.7% agar (w/v) supplemented with different types of plant growth regulators were rooted. Growth regulators include one µM zeatin z1), two µM zeatin Z2), 0.3 µM Gibberellic acid 3 (Z + GA), 2 µM Benzylaminopurine and 0.3 µM Gibberellic acid 3 (BAP + GA), 2 µM were metatopulin and 0.3 µM Gibberellic acid 3 (mT + GA).

Conclusion

The best combination medium for callus induction is the hormonal combination of 2, 4-D [2 µM] and Kinetin [0.5 µM]. The highest percentage of callus induction in the selected medium was in the leaf explant with 62% and also in the root explant with 27%. The best combination of culture medium for regeneration is BAP [2.5 µM] + TDZ [4 µM]. The highest percentage of regeneration in the selected medium was in leaf explant (87%) and the lowest in root explant (12%). The explants did not regenerate in a hormone-free medium (control) and with TDZ 2 (µM).

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Author Contribution **Atefeh Fahimi Far, Zeinab Chaghakaboodi, Mozafar Khazaei**: Execution research project, Experimental design, Data analysis, Manuscript preparation.
Danial Kahrizi, Esra Ucar Sozmen, Hulya Dogan: Experimental design, Data analysis, Manuscript preparation.

Declarations

Conflict of Interest Danial Kahrizi, Atefeh Fahimi Far, Zeinab Chaghakaboodi, Mozafar Khazaei, Esra Ucar Sozmen and Hulya Dogan declare that she does not have a conflict of interest. Danial Kahrizi declares that he does not have a conflict of interest.

Ethical approval This study was approved by the Ethics Committee of Razi University, Kermanshah, Iran.

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