Journal of **A**pplied **B**iotechnology **R**eports

Research Article

Effect of Genotype, Explant Type and 2,4-D on Cell Dedifferentiation and Callus Induction in Cumin (*Cuminum cyminum* L.) Medicinal Plant

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Abstract

Cumin (*Cuminum cyminum* L.), a member of the Apiaceae family, is one of the most important medicinal plants in the world. An experiment was conducted for the evaluation of callus induction optimization in cumin accessions from four different regions: Shahdad, Koohbanan, Badrood, and Afghanistan. A factorial experiment based on completely randomized design was conducted on MS medium supplemented with different concentrations of 2,4-D (0, 0.5, 1 and 2 mg/L) plus 0.1 mg/L Kinetin in different explants (Root, Shoot, Leaf, Embryo and Seed) of cumin accessions. In this experiment the evaluated traits were days to callus induction, callus induction percentage, and callus growth rate. Statistical analysis showed that seed (as the latest) and root (as the earliest) explants require 54 and 11 days for the initiation of callus induction, respectively. Results showed that accession, explant, accession × explant and 2,4-D × explant interactions had statistically significant effects ($P<0.01$) on callus induction percentage and callus growth rate. Furthermore, 2,4-D had a significant effect on callus induction percentage. According to the results of this study, in plants of some regions the root explant is an appropriate explant for large production of callus in some accessions of *Cuminum cyminum*. Also, Koohbananian accession produced callus in shorter time and the Afghanian one produced high number of callus.

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Keywords: 2,4-D, Callus Growth Rate, Days to Callus Induction, Seed Explant

Submission Date: 7/2/2015
Accepted Date: 8/25/2015

Introduction

Cumin (*Cuminum cyminum* L.) is a commercial plant which is valued for its aroma and its medicinal and therapeutic properties. Although India has been the primary exporter of cumin seeds and cumin oil in the world. However Turkey and Iran are providing stiff competition now. India, Turkey, Syria, China, the US, Iran, Indonesia, Sudan, Egypt, Morocco, Algeria, and Libya are the leading producers of cumin in the world. Iran produces 15,000 to 20,000 tons of cumin seeds and stands third in the leading producer's list [1].

Callus culture plays a special role in plant biotechnology area for producing medicinal compounds in large-scale [2]. Moreover, callus masses derived from plant tissues can sometimes produce high amounts of secondary metabolites [3]. Callus production from roots, shoots, and leaves are generally applied to determine the conditions required for the explants to survive and grow, exploit products coming from primary and secondary metabolism, study cell development and obtain cell suspension in propagation [2].

Callus culture of cumin has been previously reported by some researchers using leaf [4-7], embryo [8-10] hypocotyl and cotyledon [5, 6] but there is no comparative study on callus induction of this plant in various explants especially in root and seed explants.

Jha et al., [11] published the first study on the callus induction of cumin. They used the hypocotyl and leaf explants in B5 medium complemented with various plant growth regulators (PGRs). Beiki et al., [12] studied callus induction of three Iranian landraces with the use of embryo explant.

Gupta et al., [13] produced embryogenic and non-embryogenic callus in cumin on Murashige and Skoog (MS) supplemented with 0.5 mg/L 2,4- dichlorophenoxyacetic acid (2,4-D) after 35 days of inoculation from all the four explants viz., root, hypocotyl, cotyledon and shoot apex, and organogenic and non-organogenic callus on MS medium supplemented with 0.1 mg/L TDZ after 40 days of inoculation from hypocotyl explants.

Mansouri et al., [14] studied the effect of length and position of cumin hypocotyl explants on physical and morphological properties of callus were analyzed by machine vision.

In spite of cumin importance in numerous industries such as medical industry, there are a few studies on rapid and efficient callus induction in the world. Therefore, the present experiment was conducted to achieve the various objectives such as large-scale production of callus to ge erate somaclonal variation, application in genetic engineering strategies and extraction of valuable materials from this medicinal plant.

Materials and Methods

The present experiment was conducted in Medicinal Plants Tissue Culture Lab., Agriculture and Natural Resources Campus, Razi University in 2012.

Plant material

The experiments were conducted on four *Cuminum cyminum* seeds which were collected from Shahdad, Koohbanan, Badrood (tree regions in Iran) and Afghanistan. The seeds were surface disinfested following the procedure previously described [7]. One month after seed sowing, when the astatically grown seedlings were about 5 cm in height, different explants include root, shoot and leaf were prepared. The sterilized seeds were cultured directly on MS medium supplemented with different concentrations of 2,4-D. The mature zygotic embryo explants were prepared following Ebrahimie et al., [9] procedure (Fig. 1).

Statistical analysis

All experiments were repeated three times. Before statistical analysis, the normality of distributions of callus induction percentage (CIP) was assessed with the Kolmogorov–Smirnov test and was significant in a value of $p<0.05$, thus normalization by a root square transformation ($y=\sqrt{(x+0.5)}$ was attempted. Three-factor analysis of variance were performed to examine the effect of the genotype, explant type and 2,4-D on callus induction characters in cumin. The differences between means were compared using the LSD at a significance level of 0.05.Studied factors were cumin accessions (Shahdad, Koohbanan, Badrood and Afghanistan), 2,4-D concentrations (0, 0.5, 1 and 2 mg/L) and 0.1 mg/L Kinetin and different explants of cumin accessions (Root, Shoot, Leaf, Mature embryo and Seed). The evaluated traits including days to callus induction (DCI), callus induction percentage (CIP) and callus growth rate (CGR) were calculated as follow:

Days to callus induction (DCI):

Number of days since explant cultivation to callus initiation was recorded and used as a trait for the comparison of treatments.

Callus induction percentage (CIP):

The callus induction percentage was calculated as the number of explants with callus divided by the total number of explants and multiplying by 100.

Callus growth rate (CGR):

The callus growth rate (millimeter per day) was calculated five weeks after explant cultivation, according to the following equation (1):

(1)

$$CGR = \frac{\left(\frac{CD1}{7}\right)+\left(\frac{CD2-CD1}{7}\right)+\left(\frac{CD3-CD2}{7}\right)+\left(\frac{CD4-CD3}{7}\right)+\left(\frac{CD5-CD4}{7}\right)}{4}$$

where CD1-CD5 are the callus diameters of explants after 7, 14, 21, 28, and 35 days, respectively. Callus diameter (CD) for each callus explant was measured as the following procedure [15]:

(2)

$$CD=\sqrt{Length\ of\ callus \times Width\ of\ callus}$$

Due to the delay in callus production of seed explant and discordance with other explants, the CGR was not calculated for seeds.

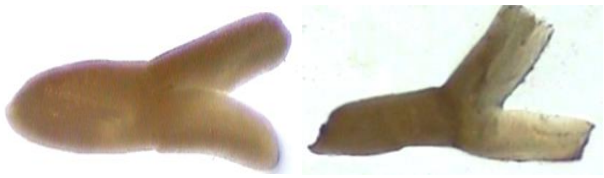

Figure 1. Mature zygotic embryo with a length of 2 mm (left) and embryo-derived explants (right)

Results and Discussion

The efficient callus production method was optimized in various cumin explants of our local cumin accessions of cumin using MS medium supplemented with 2,4-D concentrations. The analysis of variance (ANOVA) for some callus induction characteristics showed that the genotype and explant type had a significant effects on DCI, CIP and CGR traits at 0.01 level of probability and also, 2,4-D had a significant effects on DCI and CIP traits (Table 1). These observations imply that 2,4-D concentration was the callus induction but could not change the callus growth speed.

Days to callus induction (DCI)

Among all landraces, callus induction was initiated more rapidly in Koohbananian plants (Fig. 2). Thus, this genotype can be used for large scale production of callus at minimum time. Although the time required for callus induction is important, however this trait has been neglected in most studies. It is clear that the lower value of this trait is better. Because callus can be produced in shorter time and it is would be commercially valuable. So far, no report has been published on DCI of the callus induction in cumin in detail, however some reports have generally reported the time to callus induction [8, 11].

In general, higher levels of 2,4-D (videlicet 1 and 2 mg/L) has accelerated the callus induction in cumin accessions (Fig. 3). The callus was induced more rapidly in root explant (about 11 days), whereas the longest time for callus induction occurred in seed explants (about 54 days) (Fig. 4). It can be reported that the shoot explant produced the more proper callus for plant regeneration (Fig. 5), because verification was not observed and callus culture was not very bright. Some calli of these experiments were regenerated and reported previously [16].

It seems that callus was induced from mature embryo of the seed explant (Fig. 6). In spite of technically easiness of callus production from seed, since the callus induction in seed takes for a long time, it's recommend to use mature embryo instead of seed explant for sooner callus production.

Results of interaction effects analysis (Fig. 7) revealed the responses of treatments in details, so that roots of Koohbananian landrace produced callus in the shortest time (about 8 days). As shown in figure 8, in roots of cumin accessions on MS medium supplemented with 2 mg/L 2,4-D callus was induced in the lowest time (about 10 days).

Table 1. Analysis of variation for days to callus induction, callus induction percentage and callus growth rate in *Cuminum Cyminum* L

Source of variations	Days to callus induction		Callus induction percentage		Callus growth rate	
	df	Mean squares	df	Mean squares	df	Mean squares
Genotype (G)	3	443.10**	3	8.70**	3	0.032558**
2,4-D	2	140.85**	3	721.91**	2	0.001427^{ns}
Explant type (E)	4	12018.16**	4	165.38**	3	0.077247**
G × 2,4-D	6	5.69^{ns}	9	2.76^{ns}	6	0.003808^{ns}
G × E	12	28.41**	12	7.99**	9	0.012505**
2,4-D × E	8	65.29**	12	19.50**	6	0.010824**
G × 2,4-D × E	24	16.58^{ns}	36	2.49^{ns}	18	0.002165^{ns}
Error	120	10.79	160	1.75	96	0.002104
Coefficient of variation		15.09%		22.40%		17.73%

ns, * and ** means no significant, significant at 0.05 and 0.01 level of probability

Callus induction percentage (CIP)

Callus induction percentage is one of the most important traits of callus induction that has been reported in most callus experiments. Afghanistan accessions produced the most percentage of callus in comparison to other accessions (Fig. 9). Tested levels of 2,4-D had a same effect on CIP trait, which was statistically significant from control (Fig. 10). The seeds of cumin accessions showed the lowest CIP, whereas the root and shoot explants produced the most callus induction percentage (Fig. 11). Roots and shoots in all of genotypes and also leaves of Koohbananian, Badroodian, and Afghanian genotypes had the most CIP (Fig. 12). Mean comparisons for CIP in various explants and different 2,4-D concentrations showed that different levels of 2,4-D (0.5, 1 and 2 mg/L) produced the most CIP in root and shoot explants, but produced the lowest in seeds (Fig. 13).

Callus growth rate (CGR)

The results shown in Figure 14 indicate that Koohbananian, Badroodian, and Afghanian genotypes had the highest CGR with an average of 0.276, 0.268 and 0.277 mm/day, respectively, and the Shahdadian genotype had the least amount (about 0.214 mm/day). The highest and lowest calculated CGR with an average of 0.318 and 0.210 mm/day was obtained in root and embryo explants, respectively (Fig. 15). Altogether the results indicate that the root choice is an appropriate explant for large production of callus, because callus production occurs more rapidly and has the highest callus growth rate. As seen in Figure 16, callus growth rate is a genotype-dependent trait. According to this figure, the highest CGR was observed in Afghanian and Koohbananian accessions (0.378 and 0.359 mm/day, respectively).

The results of explants × 2,4-D interaction (Fig. 17) revealed that the highest CGR obtained in root explants (in all of the 2,4-D concentrations) and also shoot explants on MS medium plus 1 mg/l 2,4-D.

Minaei et al., [17] applied different explants such as seeds for callus induction of chickpea in MS medium supplemented with 2,4-D concentrations and reported the seed as an appropriate explant for callus induction of chickpea.

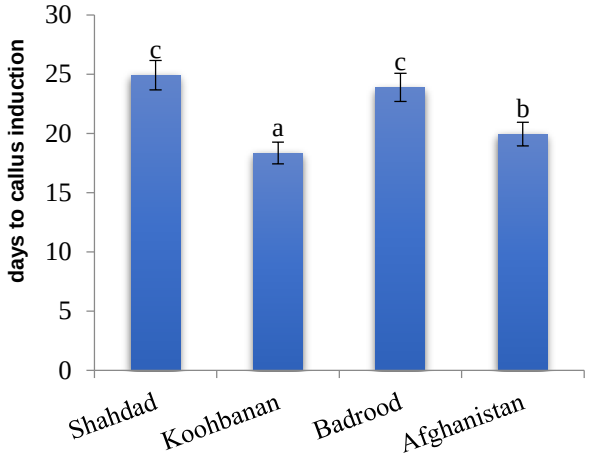

Figure 2. Mean comparisons of days to callus induction in cumin accessions (LSD test, P < 0.05).

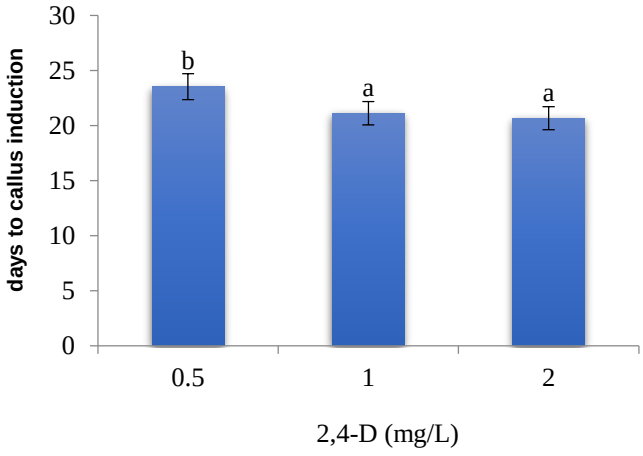

Figure 3. Mean comparisons of days to callus induction in 2,4-D concentrations (LSD test, P < 0.05).

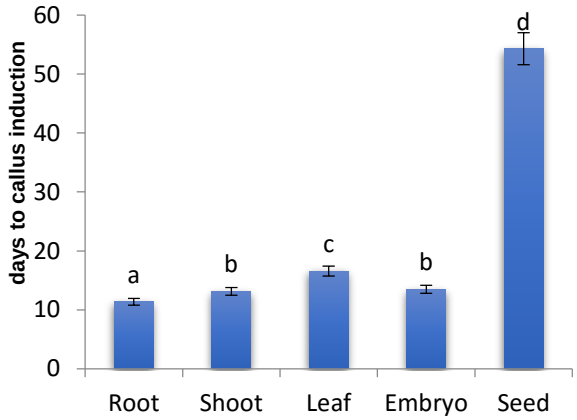

Figure 4. Mean comparisons of days to callus induction in different explants of cumin (LSD test, P < 0.05).

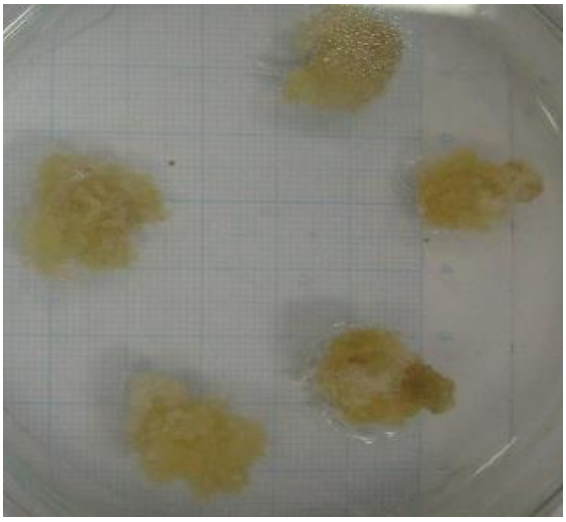

Figure 5. Calli of shoot explant on MS medium supplemented with 0.5 mg/L 2,4-D in Shahdadian accession.

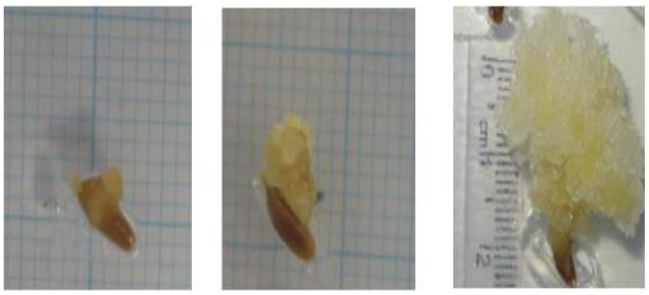

Figure 6. Calli of seed explant on MS medium supplemented with 0.5 mg/l 2,4-D in Shahdadian accession after 50 days.

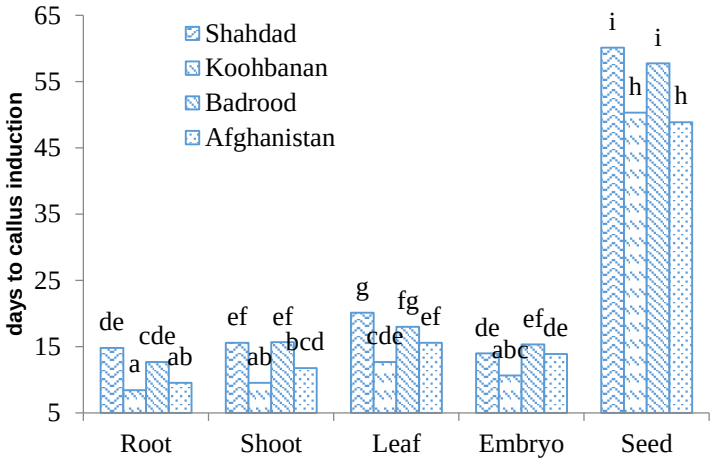

Figure 7. Mean comparisons of days to callus induction in different explants and accessions of cumin (LSD test, P < 0.05).

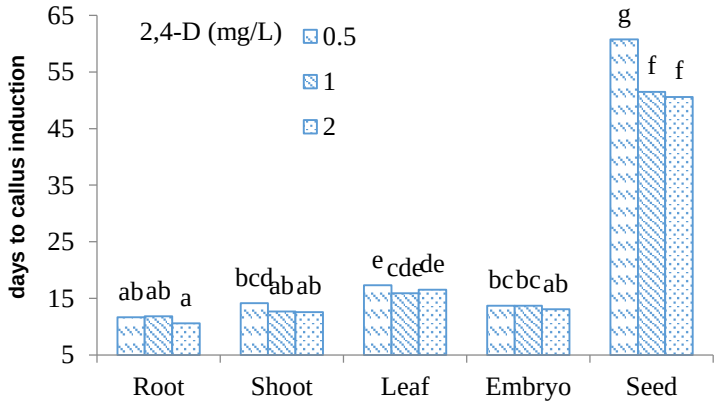

Figure 8. Mean comparisons of days to callus induction in different explants and 2,4-D concentrations in cumin (LSD test, P < 0.05).

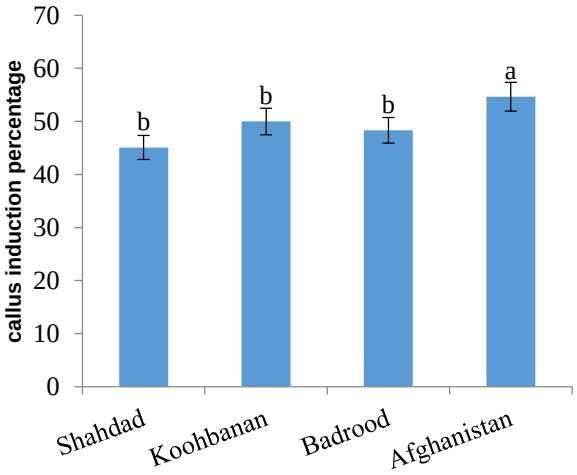

Figure 9. Mean comparisons of callus induction percentage in different accessions of cumin (LSD test, P < 0.05).

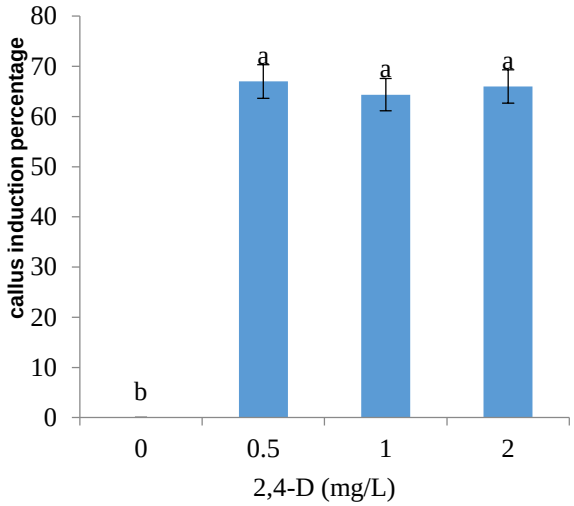

Figure 11. Mean comparisons of callus induction percentage in different 2,4-D concentrations (LSD test, P < 0.05).

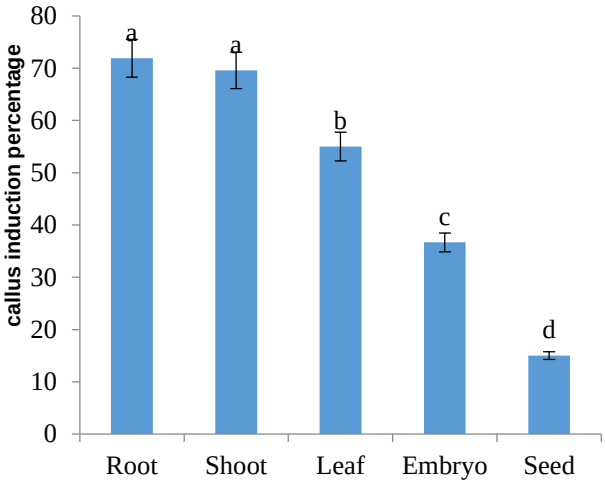

Figure 11. Mean comparisons of callus induction percentage in different explants of cumin (LSD test, P < 0.05).

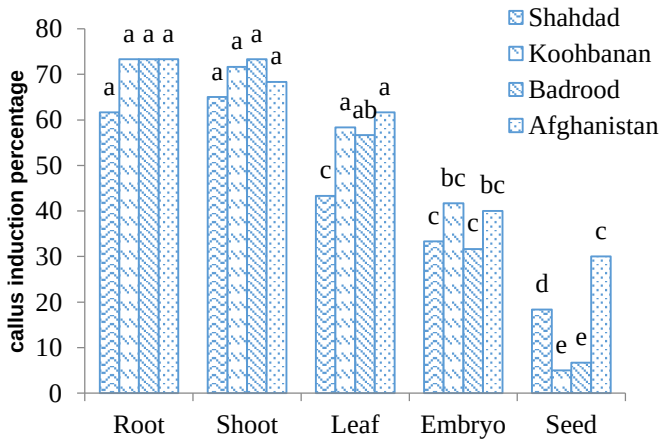

Figure 12. Mean comparisons of callus induction percentage in different explants and accessions of cumin (LSD test, P < 0.05).

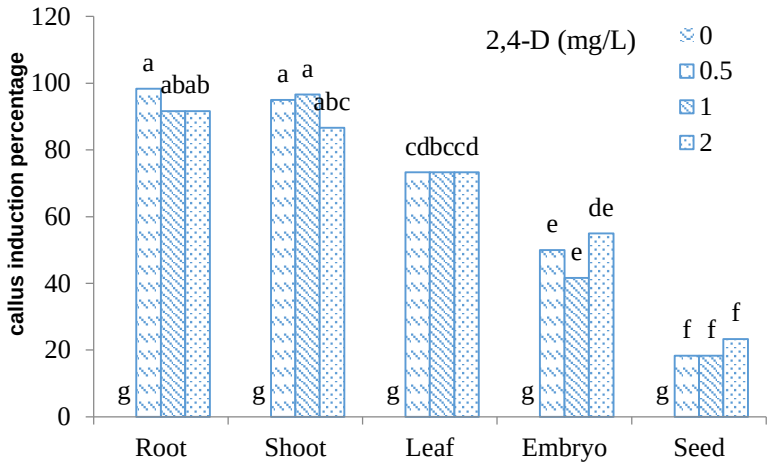

Figure 13. Mean comparisons of callus induction percentage in different explants and 2,4-D concentrations in cumin (LSD test, P < 0.05).

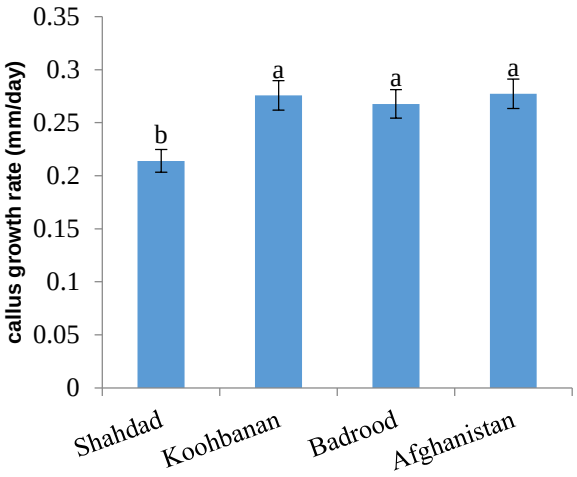

Figure 14. Mean comparisons of callus growth rate in different accessions of cumin (LSD test, P < 0.05).

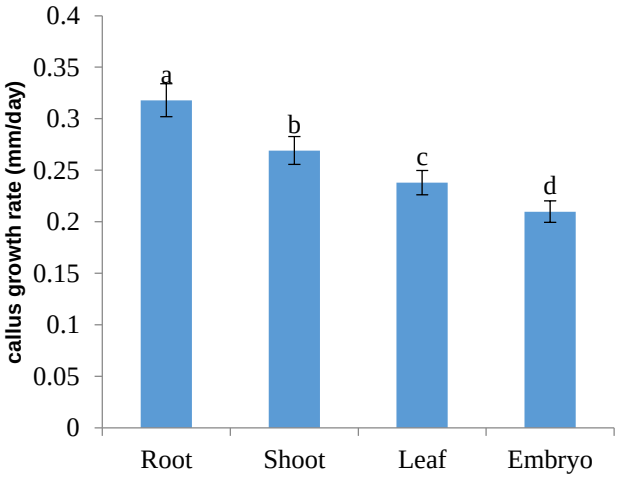

Figure 15. Mean comparisons of callus growth rate in different explants of cumin (LSD test, P < 0.05).

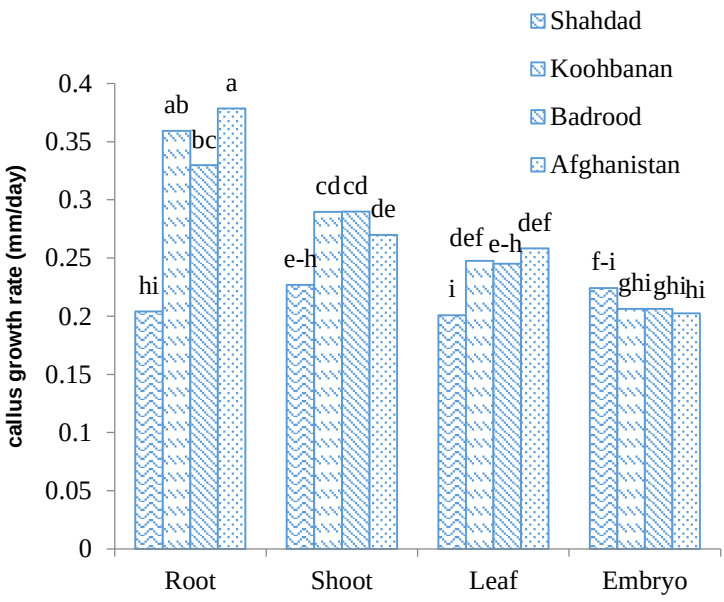

Figure 16. Mean comparisons of callus growth rate in different explants and accessions of cumin (LSD test, P <0.05).

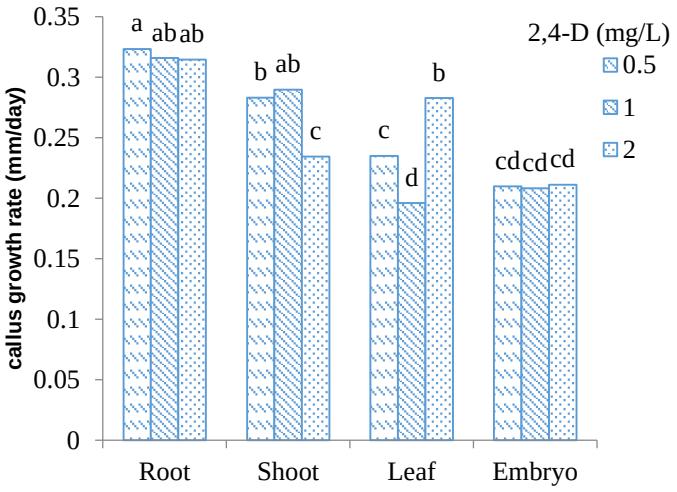

Figure 17. Mean comparisons of callus growth rate in different explants and 2,4-D concentrations in cumin (LSD test, P < 0.05).

Conclusion

Study on callus induction in cumin has very useful applications, such as determination of somaclonal variation, plant regeneration, suspension culture and secondary metabolites studies. Though Koohbananian accession produced callus in the shortest time, but the high percentage of callus was observed in Afghanian accession. According to results of this study, the root explant is appropriate for large production of callus in some accessions of *Cuminum cyminum.*

Acknowledgments

The authors would like to thank all colleagues in the Department of Agronomy and Plant Breeding, Razi University.

References

1. Sowbhagya, H.B., Chemistry, technology, and nutraceutical functions of cumin (*Cuminum cyminum* L.): an overview. *Crit Rev Food Sci Nutr*, 2013, Vol. 53, pp. 1-10.
2. Sen, M.K., Nasrin, S., Rahman, S., Jamal, A.H., *In vitro* callus induction and plantlet regeneration of *Achyranthes aspera* L., a high value medicinal plant. *Asian Pac J Trop Biomed*, 2014, Vol. 4, pp. 40-6.
3. Kirillova, N.V., Smirnova, M.G., Komov, V.P., Sequential Isolation of Superoxide Dismutase and Ajmaline from Tissue Cultures of Rauwolfia serpentine Benth. *Appl Biochem Microbiol*, 2001, Vol. 37, pp. 160-3.
4. Tawfik, A.A., Plant regeneration in callus culture of cumin (*Cuminum cyminum* L.). *Acta Hort*, 1998, Vol. 457, pp. 389-93.
5. Tawfik, A.A., Noga, G., Adventitious shoot proliferation from hypocotyl and internodal stem explants of cumin. *Plant Cell Tissue Organ Cult*, 2001, Vol. 66, pp. 141-7.
6. Tawfik, A.A., Noga, G.J., Differentiation of somatic embryos in suspension cultures and plant regeneration of cumin (*Cuminum cyminum* L.). *J App Botany*, 2002, Vol. 76, pp. 144-9
7. Soorni, J., Kahrizi, D., Molsaghi, M., The Effects of Photoperiod and 2,4-D Concentrations on Callus Induction of *Cuminum cyminum*'s Leaf Explant: an Important Medicinal Plant. *Asian J Biol Sci*, 2012, Vol. 5(7), pp. 378-383.
8. Valizadeh, M., Kazemi-Tabar, S.K., Nematzadeh, G.A., Effect of plant growth regulators on callus induction and regeneration of cumin (*Cuminum cyminum*). *Asian J Agri Res*, 2007, Vol. 1, pp. 17-22.
9. Ebrahimie, E., Naghavi, M., Hosseinzadeh, A., Behamta, M., Mohammadi-Dehcheshmeh, M., Sarrafi, A., Spangenberg, G., Induction and comparison of different in vitro morphogenesis pathways using embryo of cumin (*Cuminum cyminum* L.) as a model material. *Plant Cell Tissue Organ Cult*, 2007, Vol. 90, pp. 293-311.
10. Singh, N., Mishra, A., Joshi, M., Jha, B., Microprojectile bombardment mediated genetic transformation of embryo axes and plant regeneration in cumin (*Cuminum cyminum* L.). *Plant Cell Tissue Organ Cult*, 2010, Vol. 103, pp. 1-6.
11. Jha, T.B., Roy, S.C., Mitra, G.C., In vitro culture of *Cuminum cyminum* regeneration of flowering shoots from calli of hypocotyl and leaf explants. *Plant Cell Tissue Organ Cult*, 1983, Vol. 2, pp. 11-4.
12. Beiki, A.H., Mafavi-fard, M.R., Ahmadi, J., Optimization of two different morphogenesis pathways in three Iranian cumin landraces with the use of an embryo. *Biotechnol Biotechnol Equip*, 2011, Vol. 25, pp. 2228-32.
13. Gupta, D., Studies on Biochemical Markers Associated with Regeneration Potential in *Cuminum cyminum* L. *Res J Chem Env Sci*, 2013, Vol. 1, pp. 63- 65.
14. Mansouri, A., Fadavi, A., Mortazavian, S.M.M., Effects of length and position of hypocotyl explants on *Cuminum cyminum* L. callogensis by image processing analysis. *Plant Cell Tissue Organ Cult*, 2015, Vol. 121, pp. 657-66.
15. Kahrizi, D., Cheghamirza, K., Akbari, L., Rostami-Ahmadvandi, H., Effects of magnetic field on cell dedifferentiation and callus induction derived from embryo culture in bread wheat (*Triticum aestivum* L.) genotypes. *Mol Biol Rep*, 2013, Vol. 40, pp. 1651-1654.
16. Kahrizi, D., Soorni, J., Study on shoot regeneration and somatic embryogenesis in cumin (*Cuminum cyminum* L.) landraces. *Biharean Biol*, 2013, Vol. 7, pp. 37-41.
17. Minaei, H., Kahrizi, D., Zebarjadi, A., Effect of Plant Growth Regulators and Explant Type upon Cell Dedifferentiation and Callus Induction in Chickpea (*Cicer arietinum* L.). *J Appl Biotechnol Rep*, 2015, Vol. 2, pp. 241-244.