Asian Journal of Agricultural Research 1 (1): 17-22, 2007
ISSN 1819-1894

Effect of Plant Growth Regulators on Callus Induction and Regeneration of Cumin (*Cuminum cyminum*)

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Abstract: A simple method was developed for regeneration of Cumin. This method yielded a large number of shoots within short period of time (30-40 days) without any sub culturing. The effect of different media and various combinations of Plant Growth Regulators (PGRs) on callus formation and shoot regeneration were investigated. Simultaneous callus and shoot regeneration were obtained. The experiment was conducted in a completely randomized design with 30 treatments and 5 replications per treatment. The best response for shoot regeneration was observed on B5 medium containing 0.1 mg L^{-1} α-Naphthalene Acetic Acid (NAA) and 4 mg L^{-1} Kinetin (Kin). The B5 medium containing 2 mg L^{-1} NAA and 2 mg L^{-1} Kin was the best treatment for callus and root induction and regeneration simultaneously.

Key words: *Cuminum cyminum*, embryo explant, regeneration, tissue culture

INTRODUCTION

Cuminum cyminum or Cumin, is one of the oldest and economically most important spices. Production of this plant is limited due to several biotic stresses of which wilt diseases are the most serious.

Generally *Umbeliferae* species including *Cuminum cyminum* have antimicrobial properties (Shetty *et al*., 1994). Potential genetic variability for conventional breeding is limited in *Cuminum cyminum* (Hunault *et al*., 1989). Genetic transformation may enable the development of transgenic plants with enhanced resistance to wilt diseases. However, an efficient micro propagation system with high regeneration frequency is required.

Wakhlu *et al*. (1990) obtained callus from mericarps of *Bunium persicum* on MS (Murashige and Skoog 1962) medium supplemented with 2 mg L^{-1} 2, 4-D and 4 mg L^{-1} Kin. In this report, small white clumps of compactly packed cells developed on the callus on a medium containing 1.0 mg L^{-1} 2,4-D and 0 mg L^{-1} Kin. These cell clumps differentiated into numerous globular embryos on the same medium. Embryo maturation was achieved on the basal as well as on 1 mg L^{-1} Kin supplemented medium.

Sharifi (1995) used hypocotyl and cotyledon explants in *Cuminum cyminum* tissue culture. The callus growth was faster on B5 medium containing 2 mg L^{-1} NAA and 2 mg L^{-1} Kin. Plumule and shoot formation from hypocotyls and somatic embryogenesis were higher on medium supplemented with 0.1 mg L^{-1} NAA and 2 mg L^{-1} Kin and MS medium containing 0.5 mg L^{-1} 2,4-D, respectively.

Tawfik and Noga (2002) used leaflet and hypocotyl explant of Cumin. Calli were obtained on MS medium containing 4 μM L^{-1} 2, 4-D only or together with 4 μM L^{-1} Kin. Embryonic calli were developed on basal medium without hormones and plumules were observed on the treatment with 1 μM L^{-1} after 4 weeks.

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Ebrahimi *et al*. (2003) used embryo explants for cumin tissue culture yielding a large number of shoots within short period of time without any sub culturing. In this report, the best treatments were B5 medium containing 0.2 mg L^{-1} IAA and 1 mg L^{-1} BAP or 0.2 mg L^{-1} NAA and 0.2 mg L^{-1} BAP.

Martin (2004) used stem internode and leaf explants for regeneration of plantlet from *Eryngium foetidum* L. (*Apiaceae*). The callus formation occurred on MS medium supplemented with 5.37-10.74 μM NAA and 2.32 or 4.65 μM Kin. Then, somatic embryogenesis occurred on calli after transfer to half- strength liquid MS with 2.69 μM NAA and 1.16 μM Kin.

To obtain plant material in reported methods for Cumin regeneration, seeds have to be germinated *in vitro* and then hypocotyls and cotyledon leaflets of seedlings used as explant. The reported methods were also laborious and time consuming due to the long callus phase which is associated with using hypocotyl and cotyledon leaflet as explants. Plant regeneration from explants in a short period of time bypassing a long intermediate callus phase could reduce somaclonal variation (Skirvin *et al*., 1994). Availability of a tissue culture protocol with such criteria would be an important step towards the establishment of a genetic transformation system in this species.

The objective of this study was twofold: first, to determine the possibility of using younger explants like embryo for simultaneous callus and root formation and shoot regeneration without any sub culturing; secondly, to optimize growth regulators for increasing regeneration efficiency and reducing the time.

MATERIALS AND METHODS

Cumin seeds were collected from Sarayan at Khorasan province in Iran. Seeds were surface sterilized in 1.5% (w/v) sodium hypochlorite solution for 15 min and rinsed three times with sterile distilled water. Seeds were then soaked for 24 h in sterile distilled water at 4°C in the dark. Embryos were extracted from the turgid seeds by cutting the end of the seeds and pressing the middle of them. The embryos were cut and only the hypocotyls were used. Two different medium formulations-MS (Murashige and Skoog, 1962) and B5 (Gamborg *et al*., 1968) were used. Both were contained full-strength of macro and micro-elements, vitamins and sucrose (30 g L^{-1} in MS and 20 g L^{-1} in B5). The different plant growth regulators, NAA (0, 0.1, 1, 2 mg L^{-1}), 2,4-D (0, 0.1, 1, 2 mg L^{-1}) only or together with Kin (0, 0.5, 1, 2, 4 mg L^{-1}) were added to each medium. Prior to autoclaving at 121°C for 15 min pH was adjusted to 5.7. The media were solidified with 8% (w/v) agar (Sigma). The explant were cultured (one explant per each dish or replication) in sterile dishes (7×12 mm) each containing 15 mL of culture medium, sealed with Para film and maintained at 25±2°C under 16-h photoperiod (30 μmoles m^{-2} s^{-1}). After eight weeks, the number of explants producing callus and regeneration and the number of shoots regenerated from each replication were counted. Then the frequency of callus induction and shoot regeneration were calculated by dividing the number of calli and shoots to the original number of plated explants. The experiment was conducted as a completely randomized design with 30 treatments and 5 replications per treatment. Mean of replications was used for statistical analysis. ANOVA was used to analyze the frequency of callus formation and shoot regeneration. Mean separation was conducted using Duncan's Multiple Range test at 0.05 probability. This study carried out in Khorasan Agriculture and Natural Resources Research Center of Iran in 2005.

RESULTS

Callus initiation and early regeneration were observed on B5 medium, 10 and 20 days after explant transfer to medium, respectively. They were observed on MS medium, 15 days and 4 weeks after explant transfer to medium, respectively (Fig, 1 and 2). The results showed significant effects of treatments on regeneration and callus induction (Table 2). Due to PGR treatments the size of callus

Table 1: The effect of PGRs on shoot regeneration, root induction frequency and shoot, root mean number of Cumin on B5 and MS media

PGRs treatments	Shoot mean number		Root mean number		Shoot regeneration frequency		Root induction frequency	
(mg L^{-1})	B5	MS	B5	MS	B5	MS	B5	MS
0.1 NAA	0b	0b	0b	0b	0c	0b	0c	0b
0.1NAA+0.5KIN	3b	0b	0b	0.6b	0.2bc	0b	0c	0.2ab
0.1 NAA+1KIN	0.4b	0b	0b	0b	0.2bc	0b	0c	0b
0.1 NAA+2KIN	10b	4.4a	0b	1.6b	0.6ab	0.2ab	0c	0.2ab
0.1 NAA+4KIN	19.6a	0b	0b	0b	0.8a	0b	0c	0b
1NAA	0.4b	0.4b	0b	1.2b	0.2bc	0.2ab	0c	0.4a
1NAA+0.5KIN	0b	0b	0b	0b	0c	0b	0c	0 b
1NAA+1KIN	3b	1.4b	0b	1.2b	0.2bc	0.2ab	0c	0.2ab
1NAA+2KIN	9.4b	1b	0b	0b	0.2bc	0.4b	0c	0b
1NAA+4KIN	0.4b	0b	3.6b	0b	0.2bc	0b	0.4b	0b
2NAA	0b	0.25b	0b	2.5b	0c	0.25ab	0c	0.25ab
2NAA+0.5KIN	0.4b	0b	11.4a	4.4b	0.2bc	0b	0.4b	0.2ab
2NAA+1KIN	3.4b	1.25b	0b	8.75a	0.2bc	0.5a	0c	0.5a
2NAA+2KIN	8.8b	0b	3.6b	0b	0.6ab	0b	0.8a	0b
2NAA+4KIN	1.6b	0b	0b	0b	0.2bc	0b	0c	0b
0.1 2,4-D	0b	0b	0b	0b	0c	0b	0c	0b
0.1 2,4-D+0.5KIN	3.2b	0b	0b	0b	0.4abc	0b	0c	0b
0.1 2,4-D+1KIN	0b	0b	0b	0b	0c	0b	0c	0b
0.1 2,4-D+2KIN	0b	0b	0b	0b	0c	0b	0c	0b
0.1 2,4-D+4KIN	0b	0b	0b	0b	0c	0b	0c	0b
1 2,4-D	0b	0b	0b	0b	0c	0b	0c	0b
1 2,4-D+0.5KIN	0b	0b	0b	0b	0c	0b	0c	0b
1 2,4-D+1KIN	0b	0b	0b	0b	0c	0b	0c	0b
1 2,4-D+2KIN	0b	0b	0b	0b	0c	0b	0c	0b
1 2,4-D+4KIN	0b	0b	0b	0b	0c	0b	0c	0b
2 2,4-D	0b	0b	0b	0b	0c	0b	0c	0b
2 2,4-D+0.5KIN	0b	0b	0b	0b	0c	0b	0c	0b
2 2,4-D +1KIN	0b	0b	0b	0b	0c	0b	0c	0b
2 2,4-D +2KIN	0b	0b	0b	0b	0c	0b	0c	0b
2 2,4-D +4KIN	0b	0b	0b	0b	0c	0b	0c	0b

Different letter (s) within each column indicate significant differences

Table 2: Variance analyse of somatic embryogenesis, shoot and root induction frequency, shoot and root mean number in Cumin

		Mean squares					
Source of variation	Degrees ofreedom	Shoot regeneration frequency	Root induction frequency	Somatic embryo frequency	Root mean number	Shoot mean number	Callus induction frequency
Treatment	95.92**	24.86**	0.57**	0.15**	0.22	29	B5
	3.68 ns	13.83*	0.48**	0.08*	0.08*	29	MS
Error	43.26	9.91	0.17	0.03	0.10	118	B5
	3.9	8.53	0.18	0.05	0.05	115	MS

* Significant difference at 0.05 probability level. **Significant difference at 0.01 probability level

was different. Generally, the amount of callus in treatments without cytokinin was lower. There was no significant difference between B5 and MS for callus and root induction. The best treatment for regeneration was B5 medium supplemented with 0.1 mg L^{-1} NAA and 4 mg L^{-1} Kin (Table 1). The B5 medium containing 2 mg L^{-1} NAA and 2 mg L^{-1} Kin was the best treatment for callus and root induction and regeneration simultaneously (Table 1). The highest root induction on B5 and MS were observed in treatments supplemented with 2 mg L^{-1} NAA and 0.5 mg L^{-1} Kin as well as 2 mg L^{-1} NAA and 1 mg L^{-1} Kin respectively (Table 1). In some treatments, simultaneous regeneration, root and callus induction occurred in the same medium (Fig. 3). The above results were obtained eight weeks after explant transfer to media. The number of regenerated plantlets per explant can be significantly increased by sub culturing the regenerated calli.

Fig. 1: Callus formation on embryo explant of Cumin after 10 days

Fig. 2: Early regeneration on embryo explant of Cumin after 20 day

Fig. 3: Simultaneous regeneration, callus and root (arrow) induction

DISCUSSION

It seems that embryo explants have better interaction with low concentrations of macro- and micro-elements because of faster growth on B5 medium. Also, all the measured traits in treatments containing NAA auxin were superior (Table 1).

2,4-D and NAA only or in combination with Kin are essential for continuity of callus induction. Auxin reduction leads to organogenesis and adventitious embryo formation in Cumin. However suitable combination of auxins and cytokinins are important for embryogenesis and organogenesis (Guohua, 1998). The method which has been practiced here revealed some advantages in comparison with others including higher regeneration frequency and regenerated plantlet number, simultaneous regeneration, root and callus induction in the same medium, shortening tissue culture time, less infection, no sub culturing and less chemical consumption. The higher frequency of shoot regeneration from embryo explants compared with previously reports (hypocotyl segments) may be explained by the fact that the former explants are too old to respond to growth regulators rapidly and efficiently, but the later is younger and more sensitive, responding more quickly to PGRs. Embryo culture has been used for olive and iris (Canas *et al*., 1992). In other plant species, embryo explants have been used for micro propagation and genetic transformation (Yong *et al*., 1999).

This method yielded a large number of shoots within short period of time (30-40 days) without any sub culturing. Previously reported methods are consisted of *in vitro* seed germination, obtaining seedling, cutting hypocotyls and culturing hypocotyls in callus formation medium and transferring callus to regeneration medium. These processes are time consuming and laborious to be used as an efficient and economical micro propagation system. The above results show the importance of explant type. The presented method may also be useful for future studies on other spices like *Cuminum setifulium*. As mentioned above, Cumin production is highly limited because of fungal diseases. By application of this method, a large number of desirable and pathogen free genotypes can be propagated. A high frequency of plantlet regeneration increases the chance of obtaining transformed plant on genetic transformation procedures and short tissue culture time is helpful in reducing the time and cost. This method could also be used to produce essence and raw materials for pharmaceutical industry.

Abbreviations

B5-Gamborg *et al*. (1968) medium; BAP-6-benzylaminopurine; 2,4-D-2,4-dichlorophenoxyacetic acid; IAA-indol-3-acetic acid; Kin-kinetin; MS-Murashige and Skoog (1962); NAA-α-Naphthalene acetic acid; PGRs-plant growth regulators.

REFERENCES

Canas, L.A., J. Aliva, M. Vicente and A. Benbadis, 1992. Micro propagation of olive. Biotechnol., Agric. For., 18: 493-505.

Ebrahimi, E., A.A. Habashi B. Ghareyazie M. Ghannadha and M. Mohammadi, 2003. A rapid and efficient method for regeneration of plantlets from embryo explants of cumin. Plant Cell Tiss. Org. Cult., 75: 19-25.

Gamborg, O.L., R.A. Miller and K. Ojima, 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res., 50: 151-158.

Guohua, M., 1998. Effects of cytokinins and auxins on cassava shoot organogenesis and somatic embryogenesis from somatic embryo explant. Plant Cell Tiss. Org. Cult., 54: 1-7.

Hunault, G., P. Desmarest and J.D. Manoir, 1989. *Foeniculum vulgare* Miller: Cell culture. regeneration and the production of anethole In: Bajaj, Y.P.S. (Eds.), Biotechnol. Agric. For., 7: Medicinal and Aromatic Plants II (pp: 185-212). Springer-Verlag, Berlin, Germany.

Martin, K.P., 2004. Plant regeneration through somatic embryi genesis in medicinally important *Eryngium foetidum* L. *In vitro* Cell. Develop. Biol. Plant., 40: 459-463.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth of and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Sharifi, M., 1995. Comparative investigation of essences of *Cuminum cyminum* and *Cuminum cyminum* seed and explant fragments. M.Sc. Thesis, Agricultural College, Tehran University.

Shetty, R.S., R.S. Singhal and P.R. Kulkarni, 1994. Antimicrobial properties of cumin. J. Microbial. Biotech., 10: 232-233.

Skirvin, R.M. K.D. McPheeters and M. Norten, 1994. Source and frequency of somaclonal variation. Hortic. Sci., 29: 1232-1237.

Tawfik, A. A. and Noga, 2002. Cumin regeneration from seedling derived embryogenic callus in response to amended Kinetin. Plant Cell, Tiss. Org. Cult., 69: 35-40.

Wakhlu, A.K., S. Nagari and K.S. Barna, 1990. Somatic embryogensis and plant regeneration from callus cultures of *Cuminum cyminum* Bioss. Plant Cell Rep., 9: 137-138.

Yong, W.K., Y. Yang and C.K. Joon, 1999. Somatic embryogenesis and plant regeneration from immature zygotic embryos of Japanese larch (*Larid eptolepis*). Plant Cell Tiss. Org. Cul., 55: 95-101.