Current Research Journal of Biological Sciences 4(3): 258-260, 2012
ISSN: 2041-0778

Submitted: November 18, 2011 Accepted: December 20, 2011 Published: April 05, 2012

Optimization of Protocol to Enhance the Micro Propagation of Lavender Species

[1]N. Jahan, [3]R. Mustafa, [2]M.A. Zaidi, [1]A. Mansoor, [1]J. Khan and [4]D. Baluch
[1]Faculty of Life Sciences and Informatics, BUITEMS, Quetta Pakistan
[2]Department of Botany University of Baluchistan, Quetta, Pakistan
[3]National Institute of Biotechnology and Genetic Engineering, Faisalabad, Pakistan
[4]Faculty of life Sciences and Informatics, BUITEMS, Quetta, Pakistan

Abstract: Tissue culture is a fascinating tool which allows the rapid production of genetically identical, high yield and disease resistance, long shelf life plants using relatively small amounts of water, space, supplies and time. The present research work involves, shoot induction, multiple shoot formation and callus induction of English and French lavender. In our studies shoot induction response from different explants showed that Murashige Skoog (MS) medium (MS+1 mg/L) IBA, NAA, Kinetin proved best for shoot initiation. Maximum numbers of multiple shoots (12) were obtained in this media at 7th sub culturing. Callus induction response showed that the MS medium supplemented with combination of hormones Naphthalene acetic acid (NAA+ 2, 4-D) prove to be best for callusing in lavender plant. Temperature variations also affected the growth and callus induction from inoculated explants best temperature was 23ºC.

Key words: Callus, explants, kinetin, lavender, Murashige Skoog (MS), Naphthalene Acetic Acid (NAA), tissue culture

INTRODUCTION

The concept of improving plants by tissue culture methods is not new and Steward *et al*. (1970) foresaw the development of micropropagation system and sort of tissue culture genetics.

Micropropagation involves the production of plants from very small plant part tissue or cell grown aseptically. The ability to grow plant organs and tissue such as stem, root, flower and callus has been used in scientific laboratories for many decades as a research tool for genetics, botanists and plant pathologists.

The oil in lavender's small, blue-violet flowers gives the herb its fragrant scent (Chambdon *et al*., 1992). The flowers are arranged in spirals of 6-10 blossoms, forming interrupted spikes above the foliage. Plant tissue culture is a practice used to propagate plants under sterile conditions, often to produce clones of a plant. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation.

Calvo and Segura (1989, 1991) studied two lavender species. Shoot Regeneration has been obtained from various explants (hypocotyls, Cotyledons, roots and leaves), but for lavandin, Attempts to obtain plant regeneration have been less successful.

Chambdon *et al*. (1992) studied *in vitro* morphogenetic potential of various lavandin and lavender clones.

Chambdon *et al*. (1992) Studied on the micro propagation of Lavenders and Lavandins, a tissue culture protocol was established for the mass propagation of disease free plantlets from commercial hybrid stock plants. When regenerates were planted in fields, quality characteristics, including essential oil concentrations, were similar to parent plants. Tissue cultured plantlets could therefore be a good source of disease-free plantlets for regeneration purposes.

Hussein *et al*. (1996) was studied during 2 successive seasons in Egypt that the positive influence of foliar applications of sulfur (1%) and phosphorus (1%) on the yields, volatile oil and minerals of lavender. Foliar applications had positive effects on plant height (P 1%) and dry weight (S 1% + P 1%) of herb. The maximum value of essential oil was obtained with S as a 1% foliar application.

Raev *et al*. (1996) Induced polyploidy in lavender. Bhojwani and Razdan (1996) practice that shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further growth in the greenhouse as normal plants. The aim of this study was to optimize the protocol for lavender species for their micro propagation and conservation.

METHODOLOGY OF TISSUE CULTURING

This research study was carried out in Balochistan University of Information Technology Engineering and Management Sciences Quetta, at the Faculty of life Sciences and informatics. In the Lab of Plant Tissue Culture. The duration of the research study was from Mach 2009 to Jan 2010.

Corresponding Author: N. Jahan, Faculty of life Sciences and Informatics, BUITEMS, Quetta, Pakistan

Table 1: Hormone response using MS media

Name of media	NAA conc. (mg/L)	IBA conc. (mg/L)	Kinetine conc. (mg/L)	2,4-Dconc (mg/L)	Number of. explants	Explants with callus (%)
c1	0	0	0		50	0.0
c2	0	0.2	0	0.00	90	1.1
c3	0.20	0.2	0.2	0.50	100	53.3
c4	0.25	0.2	0.2	0.50	120	60.5
c5	0.50	0.2	0.2	0.75	150	90.8
c6	0.75	0.2	0.2	0.75	200	95.2
c7	0.90	0.2	0.2	0.90	250	98.6
c8	1.00	0.5	0.5	1.00	300	99.6

Table 2: Hormone response for shoot induction using MS media

Name of media	NAA conc. (mg/L)	IBA	KINETIE	2,4-D conc (mg/L)	No of explants	No of shoots per explants	Average proliferation rate	Average shoot length (mm)
c1	0	0.20	0.00	0.0	5	1	1.12	13.41
c2	0.25	0.20	0.20	0.5	10	3	2.35	15.30
c3	0.50	0.20	0.20	0.1	15	4	5.61	16.30
c4	0.50	0.20	0.20	0.0	20	6	4.41	19.64
c5	0.75	0.50	0.50	0.0	30	9	3.71	20.10
c6	0.75	0.75	0.75	0.0	40	10	3.75	20.12
c7	1.00	1.00	1.00	0.0	50	12	9.69	33.39

Selection of the plant:

- Apparently good looking healthy and of good commercial value as well as of expected medicinal important plants were selected.
- The plants were sprayed with the fungicide and insecticide.
- Meristem tips were selected to use as an explant source

Sterilization:

- The explants were washed in running tap water for 10 min.
- Then do the washing in 20% of common house hold bleach few drops of teen 20/200 mL of dist. H_2O as a surfactant and kept on shaker.
- After 20 min plant were washed three times of 5 min. each with sterile dist. Water under aseptic condition.

Initiation:

- The plant meristems of about 2 cm were cut under Laminar Flow Hood and placed vertically on the initiating media.
- All cultures were incubated at 25-28ºC with a 16 h photoperiod (2000 lux) provided by florescence tube lights for 4 weeks.

RESULTS

Effect of callus growth and development in lavender species: Table 1 represents the effect of different hormonal combination on callus formation from the leaf explants of lavender. Results were scored after three week of culture. Values are expressed ±95% confidence interval

Hormone response for shoot induction by using ms medium: Table 2 represent the rate of regeneration was accelerated when MS medium was supplemented with different concentration of IBA, NAA, and kinetin. Maximum increase in shoot number and length was with MS medium at IBA, NAA, and kinetin 1 mg/L.

DISCUSSION

The influence of growth regulator on callus formation was investigated by using different concentration of NAA and 2, 4-D (Fig. 1) with combination of IBA and Kinetin. Explants culture without growth or with IBA alone did not significantly develop callus NAA alone induce callus formation but callogenesis was strongly stimulated by the simutaneouly addition of IBA and an auxin (Table 1). In Calvo and Segura (1989) showed a similar synergetic effect of these two types of growth regulator on callogenesis from various explants likewise in some species In our experiments, callus proliferation was obtained with more than 90% efficiency.

Data represented in Table 1 showed that Ms Media with various concentration of NAA and 2, 4-D use for callus formation. The concentration of NAA and 2, 4-D at 1 mg/L with the combination of IBA and kinetin proved best for callus induction. Increase or decreases in the concentration of these hormones adversely affect on the callus formation and growth. The proliferation response increased with sub culturing and the highest proliferation response obtained in 8th culture. In all the cases callus developed from the cut edges of the leaf, the callus was compact, yellow-white with a few green parts. From the result the media C1 and C2 were no longer used for regeneration experiment calli obtained on C4-C8 were the best C8 shows the 98% of result (Table 1). This callus was transferred onto shoot induction media containing IBA, NAA and kinetin. Percentage of shoot regeneration was scored after 6 week (Table 2). Although shoot regeneration occurred in all the condition tested, this phenomenon was strongly depending on the concentration of auxin. Indeed, only 5% of calli obtained on medium supplemented with 2, 4-D regenerates the shoot, while

Fig. 1: Represents 98% of callus development by using the MS medium supplemented with 1 mg/L NAA and 2,4-D hormone

Fig. 2: Represent shoots induction by using MS medium

about 80% of calli obtained with NAA (C4-C8) were able to develop shoot (Table 2). Therefore we concluded that inducing the callogenesis with 2, 4-D was unsuitable for bud regeneration. Calvo and Segura (1991) reported similar findings in *L. latifola* when the level of 2, 4-D in the callogenesis medium was higher than 0.6uM. Thus the failure to obtained shoot development from leaf explants could be due to the presence of 2, 4-D in their medium.

To determine the best condition for shoot regeneration, best callus was transfer to different concentration of IBA, Kinetin, NAA, results are shown in the Fig. 2 the growth was optimum in (c3-c7) medium. In c7 medium we obtained 33.39 mm length of shoots (Table 2). In contrast C2 and C3 medium which contains 2, 4-D in concentration of 0.5, 0.1 mg/L show the less bud development.

ACKNOWLEDGMENT

The authors are grateful to the Balochistan University of Information Technology Engineering and Management Sciences, (BUITEMS) Quetta, Faculty of life Sciences and informatics and Plant Tissue Culture Lab at BUITEMS, for their financial support and also providing laboratory work facilities.

REFERENCES

Bhojwani, S.S. and M.K. Razdan 1996. Plant Tissue Culture: Theory and Practice (Studies in Plant Science). Elsevier Publishers, Nov 22, 1996.

Calvo, M.C. and J. Segura, 1991. *Lavandula* spp. (*Lavender*): *In vitro* Culture Regeneration of Plants and the Formation of Essential Oils and Pigments. In: Bajaj, Y.P.S., (Ed.), Biotechnology in Agriculture and Forestry, Springer, Berlin, Heidelberg, New York, 15: 283-310.

Calvo, M.C. and J. Segura, 1989. Codenhjhsar. Hort Sci., 24(2): 375-376, ISSN: 0018-5345.

Chambdon, C., A. Poupet, D. Beck, B. Bettachini and J. Touche, 1992. *In vitro* morphogenetic potential of various lavandin and lavender clones: Preliminary observations on the agronomic value of the vitroplants. Agronomie (Paris), 12(2): 173-181.

Steward, F.C., P. V. Ammirato and M.O. Mapes, 1970. Growth and development of totipotent cells; some problems, procedures and perspectives. Ann. Bot., 34: 761-787.

Hussein, M., E. El-Sadie and S. El-Sherbeny, 1996. Yield and quality of lavender in relation to foliar application of sulpher and phosphorus. Egypt. J. Hortic., 23(2): 167-178.

Raev, R., R. Jordanov, V. Zheljazkov, V. Rabotyagov, L. Craker, L. Nolan and K. Shetty, 1996. Induced polyploidy in lavender. International symposium on medicinal and aromatic plants, Amherst, MA (1995). Acta-Horticulturae, 426: 561-572.