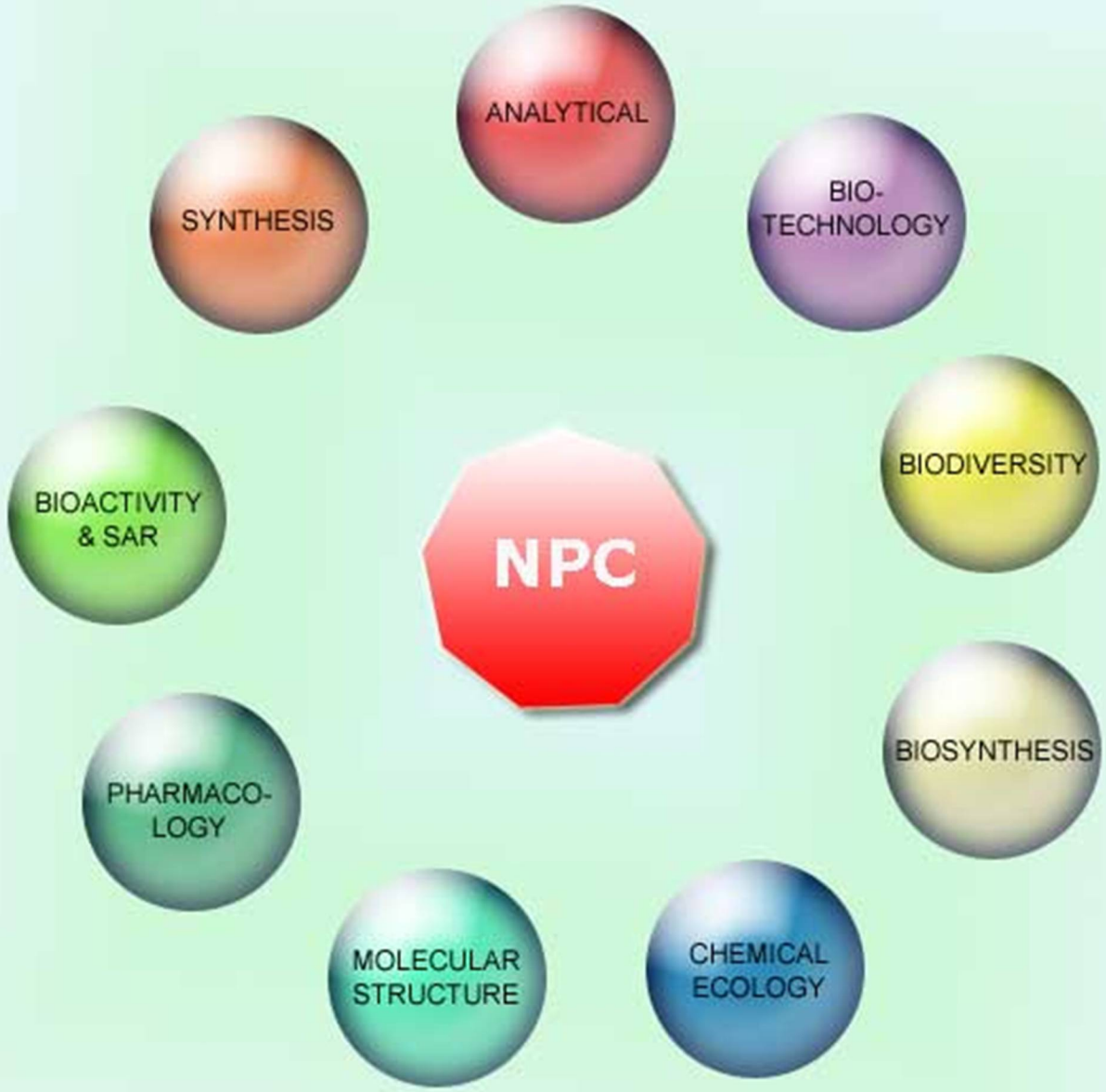
NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all Aspects of Natural Products Research



Volume 5. Issue 12. Pages 1847-2008. 2010
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

NPC Natural Product Communications

INFORMATION FOR AUTHORS

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site http://www.naturalproduct.us.

To Subscribe: Natural Product Communications is a journal published monthly. 2010 subscription price: US$1,695 (Print, ISSN# 1934-578X); US$1,695 (Web edition, ISSN# 1555-9475); US$2,095 (Print + single site online); US$595 (Personal online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.

NPC Natural Product Communications

2010
Vol. 5
No. 12
1945 - 1946

An Efficient Protocol for High-frequency Direct Multiple Shoot Regeneration from Internodes of Peppermint (*Mentha* x *piperita*)

Sanjog T. Thul* and Arun K. Kukreja

Central Institute of Medicinal and Aromatic Plants (CSIR), Lucknow-226015, UP, India

sanjay.thul@gmail.com; akkukreja53@yahoo.com

Received: July 29th, 2010; Accepted: September 24th, 2010

A simple, repeatable and efficient protocol for direct multiple shoot regeneration from internodal explants has been defined in peppermint (*Mentha* x *piperita* var. Indus). *In vitro* regenerated shoots of peppermint were excised into 4 to 8 mm long internodes and cultured on Murashige and Skoog's medium supplemented with different cytokinins. In the hormonal assay, 3.0 mg L^{-1} zeatin or 6-isopentenyl adenine independently supplemented to half strength MS medium exhibited multiple shoot regeneration, while thiaduzorn (0.1-3.0 mg L^{-1}) showed no morphogenetic effect. A maximum of 85% *in vitro* cultured explants showed multiple shoot formation with an average of 7 shoots per explant on MS medium supplemented with zeatin. Multiple shoots were initiated within three weeks of cultivation. Internodes with regenerated multiple shoots were transferred to half - strength MS medium without supplementing with any plant growth hormone for shoot elongation and rhizogenesis. Rooted plants acclimatized and grew to maturity under glasshouse conditions. The plantlets developed were phenotypically identical to the parent plant and exhibited 96 % survival.

Keywords: *Mentha* x *piperita*, plant growth hormones, internodes, multiple shoot regeneration, cytokinins.

Peppermint (*Mentha piperita* L.), an allopolyploid (2n=72) and a natural hybrid of *M. aquatica* x *M. spicata* is cultivated in India and subtropical regions. Its essential oil is valued commercially as an additive to food products, cosmetics and pharmaceuticals [1]. *M. piperita* var. Indus [2] produces high menthofuran (27.2%) and pulegone (15.4%) levels in its essential oil. In the present investigation, we report a simple and efficient method of direct multiple shoot regeneration and formation of complete plantlets from internodal segments of *M. piperita* var. Indus without the production of a callus phase.

Effect of cytokinins on multiple shoot induction: Multiple shoot induction in internodal segments was observed within 3 weeks on half strength MS medium, while a comparatively low regeneration response was observed in full strength MS medium. Of the three cytokinins (Z, 2-iP and TDZ) tested (0.1-3.0 mg L^{-1}), Z at higher levels (>1.0 mg L^{-1}) and 2-iP at all levels (0.1-3.0 mg L^{-1}) tested exhibited the highest (85%) multiple shoot regeneration response (Table 1) and an average of 7 shoots/explant were obtained with Z (3.0 mg L^{-1}), while a comparatively low response (55%) was observed with 2-iP (3.0 mg L^{-1}). None of the explants showed regeneration in medium supplemented with

Table 1: Effect of cytokinins on morphogenetic response in *M. piperita*.

Cytokinin (mgl^{-1})	Morphogenetic response (%)		
	Z	TDZ	2-iP
0.0	–	–	–
0.1	–	–	R (25)
0.5	–	R (35)	R (50)
1.0	MS (25)	–	R (40)
2.0	MS (35)	–	MS (35)
3.0	MS (85)	–	MS (50)

MS - multiple shoots; R - rooting; – no response ; figure in parentheses denotes percentage of cultures showing morphogenetic response

TDZ at any of the levels tested. This is contrary to the earlier reports where regeneration of plantlets in the presence of TDZ has been reported [3-5]. Coconut water (25%), along with either TDZ or BA or 2-iP were evaluated for their effect on organogenesis. Amongst these cytokinins, TDZ was found to be the most effective for inducing shoot formation in peppermint (*Mentha* x *piperita*) leaf explants [4]. These results are in contrast to our present study in which none of the concentrations of TDZ tested induced shoot regeneration. This indicated that the TDZ levels tested alone may not be able to induce multiple shoot induction. Occasionally TDZ and 2-iP at certain low levels also showed root initiation in cultured explants, but shoot regeneration was not observed along with the rooting (Table 1). Leaf disks and petioles of *M. citrata*

Ehrh., *M. piperita* cv. Black mitcham, *M. spicata* L. and *M. gracilis* Sole ex Baker also exhibited high multiple shoot regeneration responses with 2iP [6], as has also been observed in the present study. Coconut water in the MS medium supplemented with 4.5 µM TDZ has been found to affect adventitious shoot formation in callus of *Mentha x gracilis* [3]. Excision of the growing shoots at each sub-culture passage further enhanced the elongation of dormant buds formed during the initial shoot formation.

Shoot elongation and root induction: The half strength MS medium was found to be more effective for rapid and healthy growth of explants. Shoot elongation and rhizogenesis were much more rapid and significant in half strength MS medium in contrast to MS full strength. Although Z and 2-iP stimulated multiple shoot formation, they slowed down the development and elongation of shoots. Simultaneous shoot elongation and rooting was observed in nodal explants and therefore, single shoots separated from multiple shoot clumps were sub-cultured in growth hormone free half strength MS medium. The observed effects of shoot elongation and root initiation on medium devoid of hormones support the reported work [7]. About 90-95% of the single shoots elongated and formed roots within three weeks.

Recovery of plantlets: Of the plantlets transferred to glasshouse conditions, 96% showed survival and grew to maturity. No phenotypic variation was observed among the control (sucker grown parent plants) and *in vitro* raised plants. This study thus demonstrated a simple and efficient protocol for direct multiple shoot regeneration and production of true-to-type plants that could be adapted for study of transgenic *M. piperita*.

Experimental

Establishment of aseptic cultures: Nodal segments were collected from healthy young plants from the National Gene Bank at CIMAP, Lucknow. These were surface sterilized and inoculated on half strength MS medium [8] under reported culture conditions [9].

Shoot induction and multiplication: Internodal segments (4 to 8 mm in size) from pre-cultured mother stocks were placed on half strength MS medium supplemented either with zeatin (Z), thidiazuron (TDZ) or N6-2(2-isopentyl) adenine (2-iP) at 0.1, 0.5, 1.0, 2.0 and 3.0 mg L^{-1} levels. Full and half strength MS basal media were tested to define optimal concentration of cytokinins for multiple shoot induction. Ten replicates of each level of all the 3 cytokinins were used and the experiment was repeated twice. Multiple shoots were separated and sub-cultured on fresh cytokinin free half strength MS medium for further shoot elongation and rooting.

Acclimatization of plantlets: The rooted shoots measuring 4-6 cm in length with 5–7 leaves were taken from the culture vessel, washed gently under running tap water and kept in a culture tube containing water for 5-7 days. After this hardening phase, plants were transferred to plastic trays containing soil and vermicompost mixture (2:1) and acclimatized in a glass house under normal day length conditions.

Acknowledgements - The authors are thankful to the Director, CIMAP, Lucknow and the Council of Scientific and Industrial Research (CSIR), India for providing necessary facilities to carry out the work.

References

[1] Banthorpe DV. (**1996**) *Mentha* species (mints): *In vitro* culture and production of lower terpenoids and pigments. In *Biotechnology in Agriculture and Forestry, Medicinal and Aromatic Plants DC*, Vol. ***37***, Bajaj YPS (Ed.). Springer-Verlag, Berlin. 202-225.

[2] Khanuja SPS, Patra NK, Shasany AK, Kumar B, Gupta S, Gupta MK, Upadhyay RK, Priya TP, Singh AK, Darokar MP, Singh AK, Tomar VKS, Bahl JR, Lal RK, Naqvi AA. (**2005**) High menthofuran chemotype 'CIM-Indus' of *Mentha x piperita*. *Journal of Medicinal and Aromatic Plant Sciences*, ***27***, 721-726.

[3] Wang X, Gao Z, Wang Y, Bressan RA, Weller SC, Li X. (**2009**) Highly efficient *in vitro* adventitious shoot regeneration of peppermint (*Mentha* x *piperita* L.) using internodal explants. *In Vitro Cellular Developmental Biology-Plant*, ***45***, 435-440.

[4] Niu X, Lin K, Hasegawa PM, Bressan RA, Weller SC. (**1998**) Transgenic peppermint (*Mentha* x *piperita* L.) plants obtained by cocultivation with *Agrobacterium tumefaciens*. *Plant Cell Reports*, ***17***, 165-171.

[5] Krasnyanski S, May RA, Loskutov A, Ball TM, Sink KC. (**1999**) Transformation of the limonene synthase gene into peppermint (*Mentha piperita* L.) and preliminary studies on the essential oil profiles of single transgenic plants. *Theoretical and Applied Genetics*, ***99***, 676–682.

[6] Berry C, Van Eck JM, Kitto EL. (**1997**) *In vitro* irradiation and regeneration in mints. *Journal of Herbs, Spices and Medicinal Plants*, ***4***, 17-26.

[7] Diemer F, Jullien F, Faure O, Moja S, Colson M, Matthys-Rochon E, Caissard JC. (**1998**) High efficiency transformation of peppermint (*Mentha* x *piperita* L.) with *Agrobacterium tumefaciens*. *Plant Science, **136***, 101-108.

[8] Murashige T, Skoog F. (**1962**) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, ***15,*** 473–497.

[9] Kukreja AK. (**1996**) Micropropagation and shoot regeneration from leaf and nodal explants of peppermint (*Mentha piperita* L.). *Journal of Spices and Aromatic Crops*, ***5***, 111-119.

Natural Product Communications
2010
Volume 5, Number 12

Contents

Continued inside backcover