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Original Research Article

Effect of combination of different hormones on Micro propagation of Mentha sps.

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Abstract: Adventitious bud regeneration from inter node explants of Mentha sps was achieved, shoots from nodal segments grown *in vitro*, were cut in to pieces and used as source of explants. Organogenesis was induced from inter nodal explants cultured on Murashige and Skoog (MS) medium containing different combinations of NAA (1mg/L) and BAP (2mg/L, NAA (1mg/L) and Kinetin (1mg/L) and Kinetin (mg/L) and IBA(1mg/L) under 16 hr photoperiod and at a temperature of 25° ±2. The types of explants markedly influenced organogenesis and growth of the regenerated shoots. The regeneration frequencies were high in internodal explants. A combination of NAA and BAP could influence the best result as it supported elongation of the shoot, multi shooting, root development and callus development of diverse nature. From the study it can be proposed that a low concentration of NAA (1mg/L) and BAP (2mg/L) can be effectively used for micro propagation of *Mentha sps*. The combination of NAA (1mg/L) and Kinetin (1mg/L) and Kinetin (1mg/L) and IBA (1mg/L) does not seem to induce comparable nature of effect as exhibited by combination of NAA and BAP.

Key Words: *Ex plant of wild Mentha sps, Different combination of PGR, Suitability of NAA and BAP.*

Introduction

The *Mentha* genus an important member of family Lamiaceae contains a number of approximately 25 species, different in regard of their ploidy levels (Bhat *et al.,* 2002). Numerous species are cultivated, the plants possess a high content of flavonoids (12%), polyphenols (19%), carotenes, tocopherols, betaine, colines and a volatile oil composed of menthol, menotone, mento furan, carva crol, thymol, are widely used in the cosmetic and pharmaceutical industry. One of the species of *Mentha piperita* is characterized by a sterility which is considered practically total. *Mentha piperita* is a completely sterile hybrid. Because of reduced fertility it is impossible to obtain new varieties with a high production of mint oil by using conventional reproduction techniques. Hence use of modern biotechnological technique becomes important for this plant to increase the yield of biologically important metabolites from this plant. Application of tissue culture technique has come forward in a big way in such attempts. Micro propagation of plants using different growth regulators has been successfully employed. Plant growth regulators are the chemicals which influence the plant growth when applied in very minute quantity.

There are many reports which indicate that application of growth regulators enhanced plant growth and crop yield (Hernandez, 1997; Ashraf *et al.,* 1987, 1989). Lee *et al.,* (1999) reported that GA3 increased stem length and number of flower per plant. Kabar (1990) found that GA3 accelerated bud development and stem elongation but the best results can be achieved if GA3 is applied in combination with kinetin.IAA exerts influence on plant growth by enlarging leaves and increasing photosynthetic activities in plants. It also activates the translocation of carbohydrates during their synthesis (Awan *et al.,* 1999; Ritenour *et al.,* 1996). Cytokinins enhanced the cell expansion in soybean(Makarova *et al.,* 1988) and increased stem thickness while kinetin reduced shoot length but increased the fresh weight by increasing stem diameter in morning glory (Kaul & Farooq, 1994) and in okra (Chaudhry & Khan, 2000). There are also some reports which indicate that kinetin in combination with GA3 enhanced germination and seedling growth in chick pea (Kaur *et al.,* 1998). Thus the use of *in vitro* culture techniques can ensure both conditions for stimulating soma clonal variability and for speedy multiplication of valuable varieties. An efficient work protocols for Mentha sps can be a system of choice for rapid propagation of

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this medicinally important plant for future application. During present study attempt has been made to evaluate the role of various combination of growth hormones on regeneration of ex plants of *Mentha sps*.

Materials and Methods

Source of ex plant

Field grown plants of *Mentha* sps was used as explants for *in vitro* cultivation. Various parts of the plant such as segment of stem, node of the stem, were used as plant source.

Sterilization

The plant material was first washed in continuous running tap water for 2 hours and then in sterilized distilled water mixed with tween 20. Washing in tween 20 was performed with vigorous shaking to remove surface adhered contaminants. The plant sample was once again washed with sterilized distilled water mixed with Bavistin (01%). The plant sample was then washed three times in sterilized distilled water before transfer to laminar flow chamber for transfer to culture media. The plant sample was further disinfected with 0.1 %(w/v) mercuric chloride (HgCl2) for 5 min followed by thorough rinsing in autoclaved distilled water for at least 7 to 8 times. To avoid bacterial contamination the plant sample was treated with Mox solution (500 mg Mox dissolved in 100 ml of sterilized distilled water). The surface sterilized explants contained, nodal segments having length of 0.5 to 1cm containing single node.

Culture medium and condition

MS medium was used for *in vitro* culture of explants (Murashige & Skoog, 1962). This medium was fortified with 3% sucrose, and 0.8% agar-agar (used to solidify the medium). The pH of the medium was adjusted to 5.8 by adding 1N NaOH / 1N HCl and then autoclaved at121^0 C for 20 minutes. The cultures were maintained at 25 ±1°C under 16 h photo-period provided by white fluorescent tubes.

Growth Hormones Used

α–Naphthalene acetic acid-NAA (1mg/L) and 6-Benzylaminopurine-BAP (2mg/L),NAA(1mg/L)and Kinetin(1mg/L) were used to observe its effect on shoot initiation and root initiation. Kinetin (1mg/L) and Indole Butyric Acid-IBA (1mg/L) were also used to monitor its effect on shoot proliferation and root growth.

Results and Discussion

On review of literature concerned with micro propagation of *Mentha* it appears that this plant has been subjected to micro propagation as a novel test plant since a long time. During this practice, Rech & Pires (1986) used auxillary buds and Kukreja (1996) used leaf and nodal segments for the propagation of this plant. During subsequent years various workers successfully carried out micro propagation on different species of this plant (Bhat *et al.,* 2002; Alvi *et al.,* 2004; Goday *et al.,* 2005; Shawl *et al.,* 2006; Senthil & kamraj, 2011; Samantaray *et al.,* 2012; Mehta *et al.,* 2012;Rahman *et al.,* 2013; Biswas *et al.,* 2014). On review of these papers it appears, that workers have proposed supplementation of different growth hormones for callus induction, shoot development and root development. For instance, Samanta ray *et al.*, (2012) while working on *Mentha spicata* have suggested supplementation of MS media with 2, 4-D (2.5mg/L) for callus induction but for shoot induction BAP (2.5mg/L) has been suggested. In this report supplementation with NAA (4mg/L) and IAA (4mg/L) has been found to induce rooting where NAA seems to be more effective as it induces more rooting than IAA. Mehta *et al*, (2012) while carrying out studies on *Mentha piperita* have suggested use of BAP and Kinetin in the range of 1.0-5.0mg/L for shoot proliferation where a concentration of 2.0mg/L yields the best results. During this study it was further observed that shoots when cultured on IBA (2.0mg/L) induced rooting. In an identical study Rahman *et al.,* (2013) while carrying out studies on *Mentha virdis* reported induction of shoot when MS media was supplemented with BAP (2.0mg/L) and IAA (0.5mg/L) but root induction took place when MS media was supplemented with IAA (1.5mg/L).

Considering above described perspective in mind, present study was designed to evaluate the role of different growth hormones in combination on various facets of micro propagation of wild *Mentha sps*.

Effect of NAA and BAP in combination

During this course of study freshly prepared MS media was supplemented with two growth hormones, α–Naphthalene acetic

acid- NAA (1mg/L) and 6-Benzylaminopurine - BAP (2mg/L). Ex plant was collected from fresh and luxuriantly grown *Mentha* sps. The ex plant was allowed to grow for 15 days and different parts of this growing ex plant was used to sub culture for evaluating the effect of different combinations of growth hormones. This step was primarily adopted to ensure the disease free nature of the ex plant and also to confirm the viability of the ex plant on the media in use. Different segments obtained from freshly grown ex plant were inoculated on MS media supplemented with growth hormones in respective culture tubes under strict sterilized condition. The culture tubes were immediately transferred to desired temperature and desired photoperiod. After 15 to 20 days of incubation a vivid account of growth and development could be recorded and this has been shown in Fig-1 and Fig-2.

On observation of the Plates, it appears that a combination of NAA and BAP supports various aspect of micro propagation in the used *Mentha sps*. Some of the notable observed features can be cited below:

1. Multi shooting in the nodal segment (Plate-1, Plate-2, Plate-5, Plate-6 & Plate-7).
2. Multi shooting along with jelly like callus at the surface of the media (Plate-3).
3. Shooting from the auxiliary buds (Plate-4).
4. Green callus at the surface of the media(Plate-8)
5. Greenish white callus at the surface of the media (Plate-9).
6. White compact callus without shooting at the surface of the media (Plate-10).
7. Multi shooting along with rooting (Plate-11).
8. Increase in the period of incubation to 45 days results in development of callus from the inter node as well as from the leaf (Newly developed in the culture tube-Plate-12).

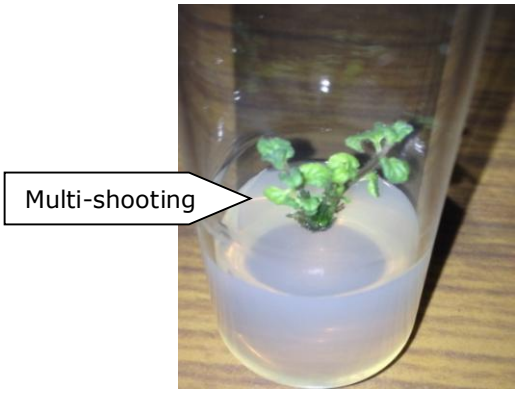

Plate-1

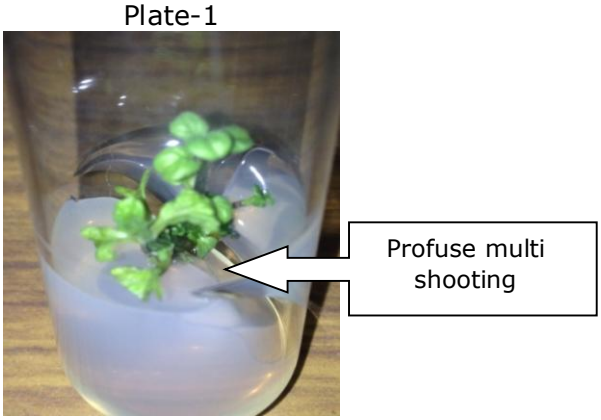

Plate-2

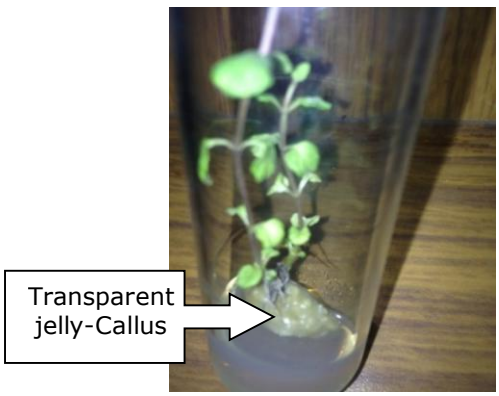

Plate-3

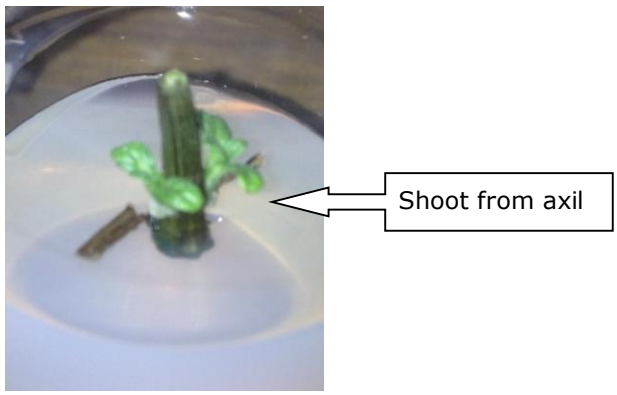

Plate-4

Plate-5

Plate-6

Figure 1: Different type of organogenesis from internode Plate-1 & 2-Multi shooting. Plate3-Shoot proliferation as well as Jelly like callus. Plate4-Axillary bud growth. Plate 5 & 6-Multishooting

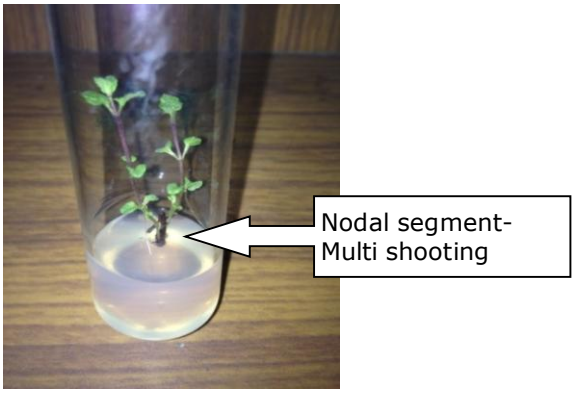

Plate-7

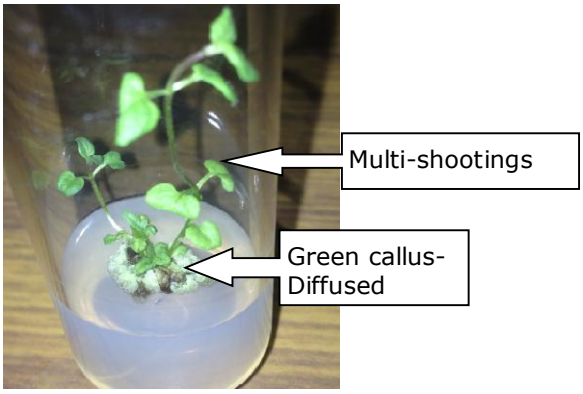

Plate-8

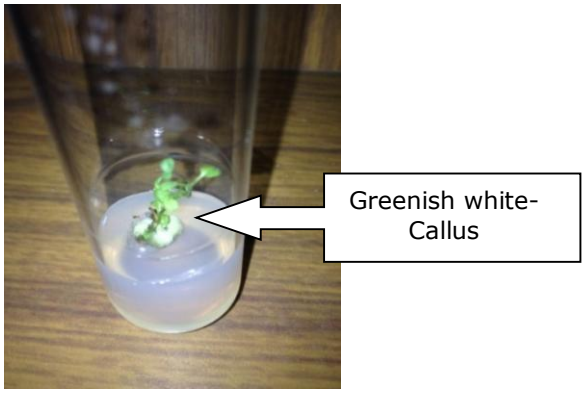

Plate-9

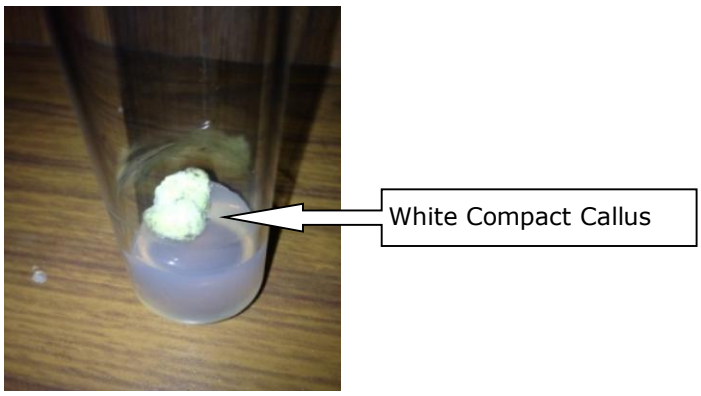

Plate-10

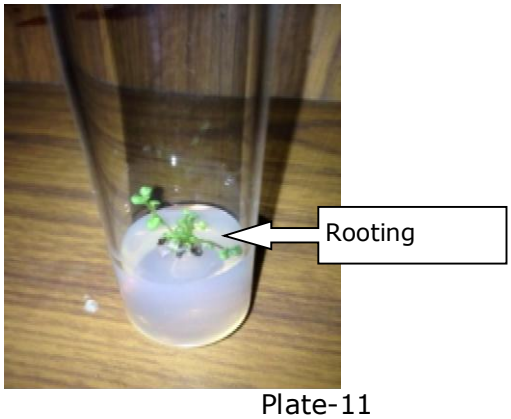

Plate-11

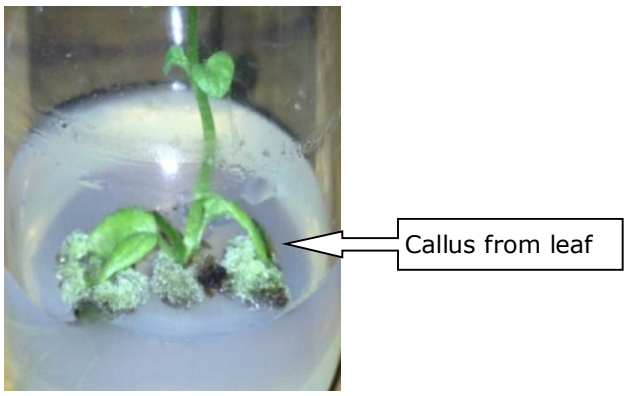

Plate-12

Figure 2: Different type of organogenesis from internode Plate 7-Multishooting from Nodal segment, Plate8-Green callus and Multi shooting from Nodal Segment, Plate 9-

Multishooting and Green white callus, Plate 10-White compact callus with tinge of Green colour, Plate 11-Multishooting with rooting. Plate 12-Callus from inter node and leaf (45 days).

On review of result described above following conclusions can be drawn:

1. In case of *Mentha* a fresh sub cultured ex plant seems to be more useful than a wild plant freshly collected from the garden.
2. A combination of NAA and BAP in a ratio of 1:2 at a concentration of 01mg/L and 02mg/L seems to be quite suitable as this favours callus induction of diverse kind, multi shooting, shoot elongation as well as root development.
3. Nodal segment of the plant as a source ex plant seems to be more viable as it induces propagation more rapidly than other plant part. Nodal ex plants as the source of multiple shoot induction have been suggested in case of medicinal plants such as *Mentha piperita* (Ghanti *et al.,* 2004), *Rauwolfia serpentina* (Roy *et al*,1995) and *Emblica officinalis* (Rahman *et al*,1999).

To extend the work related to evaluation of growth hormones on micro propagation of *Mentha sps*, ex plants were cultured on a combination of NAA and Kinetin.

Effect of NAA and Kinetin in combination

MS media was supplemented with NAA (01mg/L) and Kinetin (01mg/L) on which ex plant was transferred for regeneration. Result obtained has been demonstrated in Plate-13 and Plate-14 respectively.

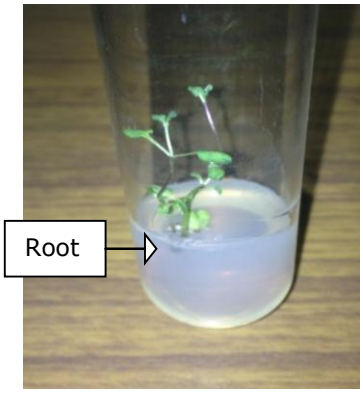

Plate-13

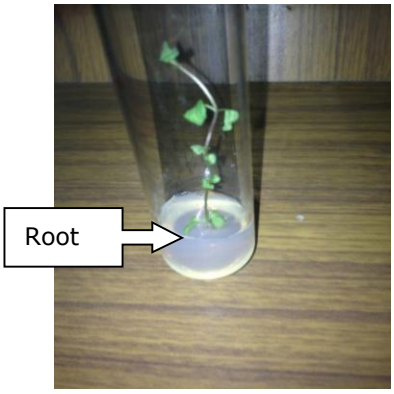

Plate-14

Figure 3: Different type of organogenesis from internode on NAA and Kinetin.Plate12-Multishooting and rooting Plate13-Shoot elongation and rooting

On reviewing the result obtained, it appears that combination of NAA and Kinetin induces multiple shooting, shoot elongation as well as root formation. The remarkable feature of the shoot elongation was that shoot exhibited an etiolating pattern of growth. The growth of the shoot was not that well organized as seen in the combination of NAA and BAP. However, the time period for growth was marginally reduced (from 20 days in NAA-BAP combination to 15 days in NAA-Kinetin combination). Whereas, combination of NAA and BAP favoured auxiliary shooting, Shoot elongation, callus development (both from inter node and leaf) and multi shooting, the combination of NAA and Kinetin favoured, rapid shoot elongation, multi shooting and root development. To evaluate the effect of kinetin and an auxin (IBA) in combination, inter nodal segment of the ex plant was transferred to MS media.

Effect of Kinetin and IBA in combination

MS media supplemented with Kinetin (1mg/L) and IBA (1mg/L)was used as it has been cited in the literature(Mehta *et al.,* 2012;Rahman *et al.,*2013; Biswas *et al.,* 2014; Senthil & Kamraj, 2011) that PGR either alone or in different combination favours rooting and proliferation of shoot in Mentha sps. The result obtained has been depicted in Fig-4, Plate-15 to Plate-18.

The combination of kinetin and IBA seems to affect regeneration in the following manner:

1. Shoot induction initiated much faster after 12 days whereas, in other combinations *viz* NAA - BAP and NAA-Kinetin (20 days and 15 days respectively).

2. Growth along the inter node region was more rapid, this culminates into irregular growth (etiolation) of the plant.
3. Root formation took place from the nodal region also (Fig 4, Plate-16).
4. Multi shooting could also be observed (Fig 4, Plate-15, Plate-17).
5. Delayed incubation of the tube could exhibit establishment of the root in a better way (Plate-18).

Plate-15

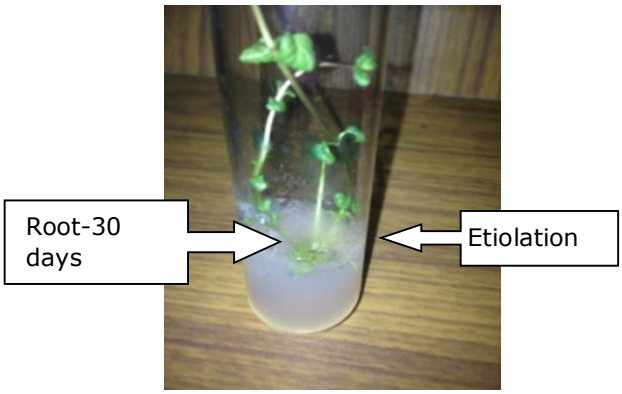

Plate-16

Plate-17

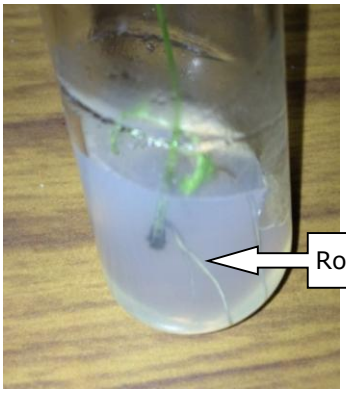

Plate-18

Figure 4: Pattern of growth of *Mentha* in a combination of Kinetin and IBA. Plate-15 Shoot induction Plate-16 Root from nodal region Plate-17 Shoot elongation & Multi shooting Plate-18 Strengthening of the root with lapse of time.

From the study described above it becomes apparent that a combination of Kinetin and IBA works effectively in the organogenesis of *Mentha* sub cultured ex plant. This combination can induce shoot elongation as well as root establishment. Review of literature however suggests separate combination for shooting and rooting in *Mentha sps*. Biswas *et al.,* 2014 used IAA (0.5mg/L) and BAP (1mg/L) for shoot proliferation and IBA (1mg/L) and BAP (0.5mg/L) for root elongation. This combination (Rahman *et al.,* 2013) has been BAP (2mg/L) and IAA (0.5mg/L) for shoot and IAA (1mg/L) alone for rooting. Mehta *et al.,* 2012 used a combination of BAP and Kinetin in the range of 1.0-5.0mg/L for shoot proliferation. BAP at a concentration of 2.0mg/L evoked best response. BAP always remained a better choice than Kinetin. IBA (0.5-2.5mg/L) induced root proliferation, best result could be obtained at a concentration of 2.0mg/L.

Conclusion

From the observations described above and on the basis of interpretation of the result it can be concluded that out of three combination of growth hormones used for organogenesis of the sub cultured ex plant of wild variety of *Mentha sps*, the combination of NAA and BAP evokes the best result. This combination generates shooting, multi shooting, rooting as well as setting up of callus of diverse texture and form.Although other two combinations also effectively supports organogenesis but not exhibiting diverse mechanism of function.

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