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IN VITRO SHOOT REGENERATION OF MINT (*Mentha sp.*) USING DIFFERENT EXPLANTS AND GENOTYPES

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Abstract

An experiment was conducted in the Tissue Culture Laboratory of the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during March 2013 to January 2014 to observe the performance of nine mint genotypes (viz. MP-1, MP-2, MP-3, MP-4, MP-5, MP-6, MP-9, MP-19 and MP-22) with three explants (viz. leaf, shoot tip and node) for *in-vitro* shoot regeneration. In this experiment, two growth regulators BAP (1 mg/l) and NAA (1 mg/l) were used for shoot regeneration. Results revealed that the highest number of shoot was produced in shoot tip (1.94) at 55 days after inoculation (DAI), which initiated shoot within 20.09 days. In case of genotype, MP-1 showed the early response for shoot initiation with the highest shoot length but MP-4 (2.44) showed the better performance for number of shoot at 55 DAI. The interaction of MP-1 and node explant produced the highest number of shoots (4.66) at 55 DAI but initiated shoot within 9.67 days. Nodal explants showed the best result for the longest internode (0.98 cm) at 55 DAI. Shoot tip and nodal segment was found the best explants in mint for *in vitro* regeneration of shoot and the genotype MP-1, MP-4 and MP-6 performed better for shoot regeneration *in vitro*.

Key words: Shoot Initiation, mint, In vitro, nodal segment.

Introduction

The genus *Mentha* belongs to the family Lamiaceae, which develops very well in humid areas of the temperate climate, being frequently cultivated in countries of Europa, Asia and USA (Ghanti *et al*., 2004). Mint contains a number of approximately 25 species, different in regard of their ploidy levels (Bhat *et al*., 2002). The major species of mint are peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), wild mint (*Mentha arvensis*), pennyroyal (*Mentha pulegium*) and bergamot mint (*Mentha citrate*). Peppermint and spearmint are the most commercially exploited species of mint (Sharangdhar, 2008) because they have anti-feeding, insecticidal, antiviral, antibacterial, immuno modulating and anti-aging properties (Ali *et al*., 2002). Mint leaves are used in teas, beverages, jellies, syrups, candies and ice creams (Hoque, 2013). Pepperment oil is usually obtained from the leaves of *M. piperita* and *M. arvensis*. Menthol is used in variety of food and medicinal products (Foster, 1996).

In Bangladesh, mint is grown scatterdly all over the country, but there is no statistics available in our country about area and production of mint. Presently, mint oil is imported from abroad for using in industries (Hoque, 2013).

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Most of the commercially important mints are hybrids or amphiploids. *Mentha piperata*, the peppermint, is a sterile first generation hybrid between *Mentha spicata* and *Mentha aquatica* (Hoque, 2013). Because of reduced fertility it is impossible to obtain new varieties with a high production of mint oil by using conventional reproduction techniques. Poor overwintering may occasionally produce an insufficient number of seedlings in the tropical and sub-tropical country like Bangladesh. However, this conventional propagation through stolon is a slow process and susceptible to many diseases (Safaeikhorram *et al*., 2008) like collar rot and wilt disease caused by *Sclerotium rolfsii* (Muthukumar and Venkatesh, 2013). Growth and oil production are affected by *Verticilliun* wilt disease caused by infection of pathogen *Verticillium dahlia* (Nan and Barbara, 2003). Besides these stolon rot caused by *Macrophomina phaseoli* and *Pythium*, rust caused by *Puccinia menthae*, powdery mildew caused by *Erysiphe cichoracearum* also observed in mint crops (Bhat *et al*., 2002).

In this context, the use of *in vitro* culture techniques can ensure speedy multiplication of valuable varieties (Sunandakumari *et al*., 2004) and the possibility to obtain biological material free from pathogens in large-scale (Kane, 2014).

Regeneration of spearmint using cotyledons, hypocotyls (Van Eck and Kitto, 1990) and leaf (Li *et al*., 1999) have had limited success. Plant regeneration from axillary bud, leaf via organogenesis and nodal explants has earlier been reported in *Mentha piperita* (Ghanti *et al*., 2004). Internode had high regeneration capacity in other species of *Mentha* (Bhat *et al*., 2002).

From a previously completed project, a total of 22 germplasm were collected from different part of the country. Out of these twenty two, nine genotypes with high yielding and high flavoring character are used in this experiment (Hoque, 2013). Hence, it is needed to find out the propagation potential of these genotypes in *in vitro* condition. Considering the above mentioned facts the objective of this study was to identify suitable genotype(s) and explant(s) of mint for *in vitro* shoot regeneration.

Materials and Methods

Two experiments were conducted in the Tissue Culture Laboratory of Horticulture Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur-1706, during March 2013 to January 2014.

Experimental materials

Twenty two genotypes were collected from different parts of Bangladesh through a previously completed project and nine genotypes viz. MP-1, MP-2, MP-3, MP-4, MP-5, MP-6, MP-7, MP-19 and MP-22 were selected as promising after a screening trial and were used as experimental materials. Leaves (E_1), tender and actively growing shoot tips (E_2) and nodal segments(E_3) of all genotypes were used as explants for regenerating the shoot *in vitro*.

Preparation of stock solution and media

Full strength MS media supplemented with growth regulators (1mg/l NAA and 1mg/l BAP) was used in this experiment (Hoque, 2013). The stock solutions of two growth regulators (BAP and NAA) were prepared separately by dissolving each of them with a

few drops of appropriate solvents (0.1 N NaOH for BAP and absolute ethanol for NAA). The strength of prepared stock solution was 100.0 mg/l and the solution stored in a refrigerator at 4^0 C. The medium were prepared by mixing required amount of MS powder supplemented with growth regulators and 3% (w/v) sucrose. The mixture was then stirred in an electric stir for dissolving sucrose and MS powder. Then the pH of the medium was adjusted to 5.7 and agar powder was added @ 0.7% (w/v) as solidifying agent. Therefore, the medium was heated into a microwave oven for 3-5 minutes for dissolving the ingredients completely and was autoclaved at 121^0 C, 15 psi for 20 minutes.

Preparation of explants

Shoot tip, first node and upper two expanded leaves were collected from apical part of nine mint genotypes. Explants were then washed under running tap water followed by soaking in 2-3 drops of Tween-20 for 5 minutes. The explants were rinsed in distilled water for 2-3 times followed by surface sterilization using mercuric chloride (0.1% w/v) in laminar flow cabinet and finally rinsed with sterile double distilled water for 3-4 times to remove traces of mercuric chloride. The first and second nodes and shoot meristems (2-3mm) were excised from the sterilized plant parts, while petioles with a small portion of leaves were excised in 3-4 mm pieces.

Inoculation and in vitro culture

The explants were inoculated by inserting their cut ends in the medium. The cultures were kept in growth chambers at 20 ± 2^0 C under 16h light photoperiod using Philips day light florescent lamps under light intensity of 10000 lux. Scoring was done after 15 days of culture by counting all shoots on the explants, genotypes and their 27 combination. After 55 days of culture, the shoots were further cultured for root regeneration.

Design, Data collection and analysis

Completely Randomized Design (CRD) was used for this experiment with five replications. Data were collected on dates taken to shoot initiation, number of shoot, length of longest shoots and length of the longest internode. The regenerated shoots were put in a separate trial with different media and growth regulators for complete plantlet regeneration. Collected data were analyzed using MSTAT-C statistical package. Differences among the means were compared following Duncan's Multiple Range Tests (DMRT) at 1% level of probability (Gomez and Gomez, 1984).

Results and Discussion

Days to shoot initiation: The response of explants for days required to shoot initiation in *in-vitro* regeneration of mint are presented in Fig.1. Shoot regeneration was earlier in E_2 (shoot tip, 20.09 days) followed by E_3 (node, 24.80 days); while E_1 (leaf) produced no shoot at all. This result revealed the superiority of shoot tip and nodal than the leaf explants which might be due to the higher totipotency of shoot tip. In case of genotypes (Fig. 2), early response for shoot initiation was observed in G_1 (9.94 days) which was statistically similar with G_6 (10.39 days) and G_4 (11.50 days). The highest days required for shoot initiation in G_5 (20.28 days) followed by G_3 (18.39 days), G_8 (17.94 days), G_2 (17.83 days), G_9 (15.83 days) and G_7 (15.44). This was in line with the findings of Vasile *et al*. (2011) who

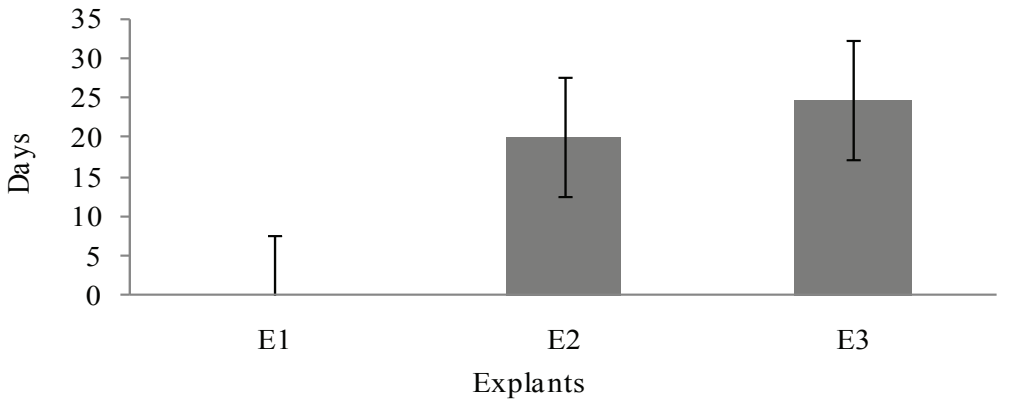

Fig. 1. Response of explants for days to shoot initiation in mint.

E_1=Leaf, E_2=Shoot tip and E_3=Node.

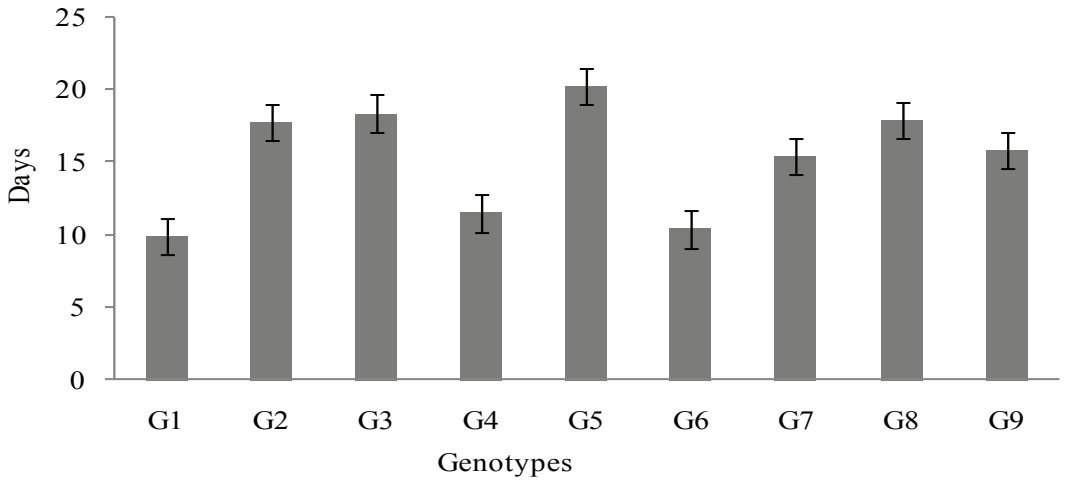

Fig. 2. Response of genotypes for days to shoot initiation in mint.

G_1=MP-1, G_2=MP-2, G_3=MP-3, G_4=MP-4, G_5=MP-5, G_6=MP-6, G_7=MP-9, G_8=MP-19 and G_9=MP-22.

reported that after 14 days of inoculation the appearance of the first tips of shoots was found from the axillary buds of nodal explants. These differences might be due to the variation of explants and culture condition.

In case of interaction of explant and genotypes, days to shoot initiation showed statistically different results (table 1). Shoot tip of the genotype MP-1 (G_1E_2) responded earlier (9.67 days) than the other treatment; whereas, nodal segments of MP-5 (G_5E_3) required more days (33.17 days) to initiate shoot. In every cases, interaction of genotypes and shoot tip showed the early response than the interaction of genotypes and nodal segments. Interaction of genotypes and leaf produced no shoot at all.

Table 1. Interaction effect of explants and genotype on days to shoot initiation in in vitro culture of mint.

Interaction	Days to shoot initiation
G_1E_1	0.00 h
G_1E_2	9.67 gh
G_1E_3	19.33 c-f
G_2E_1	0.00 h
G_2E_2	24.17 a-e
G_2E_3	28.33 a-c
G_3E_1	0.00 h
G_3E_2	25.67 a-d
G_3E_3	28.50 a-c
G_4E_1	0.00 h
G_4E_2	15.67 e-g
G_4E_3	17.83 d-g
G_5E_1	0.00 h
G_5E_2	26.67 a-d
G_5E_3	33.17 a
G_6E_1	0.00 h
G_6E_2	14.33 fg
G_6E_3	15.83 e-g
G_7E_1	0.00 h
G_7E_2	19.83 b-f
G_7E_3	25.67 a-d
G_8E_1	0.00 h
G_8E_2	23.33 b-f
G_8E_3	29.50 ab
G_9E_1	0.00 h
G_9E_2	21.50 b-f
G_9E_3	25.00 a-e
CV (%)	3.68

G_1=MP-1, G_2=MP-2, G_3=MP-3, G_4=MP-4, G_5=MP-5, G_6=MP-6, G_7=MP-9, G_8=MP-19 and G_9=MP-22; and E_1= Leaf, E_2= Shoot tip and E_3= Node.

Means followed by same letter in a column do not differ significantly at 1% level by DMRT.

Number of Shoot

Significant result was found for number of shoots at different days of culture with BAP. Shoot tip was found to be more responsive regarding number of shoot followed by node (Table 2); whereas, leaf produced no shoot at all (Fig. 3). At 15 days after inoculation (DAI), the explant shoot tip (0.09) and node (0.18) produced shoot. But after 25 DAI, the highest numbers of shoots per explants were produced by shoot tip than nodal segment of mint which continued till 55 DAI in *in-vitro* condition. However, at 55 DAI, shoot tip produced the highest number of shoots (1.94) per explant and this was statistically similar to node (1.44). This result agreed with the findings of Ghanti *et al*. (2004), where they recorded the highest success of shoot regeneration in mint from shoot tip followed by nodal explants when the media was supplemented with BAP. Other report have also stated by Afshar *et al.* (2011), who explained that lowest percentage of regeneration was observed in leaf without petiole and petiole without leaf, may be due to these explants had less carbohydrates or endogenous growth hormone contents to produce shoots.

All genotypes performed better at 55 DAI (Table 3). Genotype G_1 showed the best

Table 2. Response of explant on number of shoot in mint.

Explant	Number of shoot				
	15 DAI	25 DAI	35 DAI	45 DAI	55 DAI
E_1	0.00	0.00 b	0.00 b	0.00 b	0.00 b
E_2	0.09	0.61 a	1.03 a	1.44 a	1.94 a
E_3	0.18	0.38 a	0.72 a	1.11 a	1.44 a
CV (%)	-	19.26	15.86	13.87	12.48

E_1= Leaf, E_2= Shoot tip and E_3= Node.

Means followed by same letter in a column do not differ significantly at 1% level by DMRT.

Table 3. Response of genotype on number of shoot in mint .

Genotype	Number of shoot				
	15 DAI	25 DAI	35 DAI	45 DAI	55 DAI
G_1	0.44 a	0.94 a	1.44 a	2.22 a	2.61 a
G_2	0.00 b	0.06 b	0.11 b	0.11 d	0.11 c
G_3	0.00 b	0.06 b	0.11 b	0.28 cd	0.44 bc
G_4	0.17 ab	0.44 ab	0.89 ab	1.61 ab	2.44 a
G_5	0.00 b	0.06 b	0.22 b	0.28 cd	0.28 bc
G_6	0.22 ab	0.67 ab	0.89 ab	0.94 b-d	1.44 a-c
G_7	0.00 b	0.33 b	0.61 ab	0.83 b-d	1.11 bc
G_8	0.00 b	0.06 b	0.11 b	0.11 d	0.17 c
G_9	0.00 b	0.39 ab	0.89 ab	1.28 a-c	1.56 ab
CV (%)	19.30	17.35	11.25	3.83	2.51

G_1=MP-1, G_2=MP-2, G_3=MP-3, G_4=MP-4, G_5=MP-5, G_6=MP-6, G_7=MP-9, G_8=MP-19 and G_9=MP-22. Means followed by same letter in a column do not differ significantly at 1% level by DMRT.

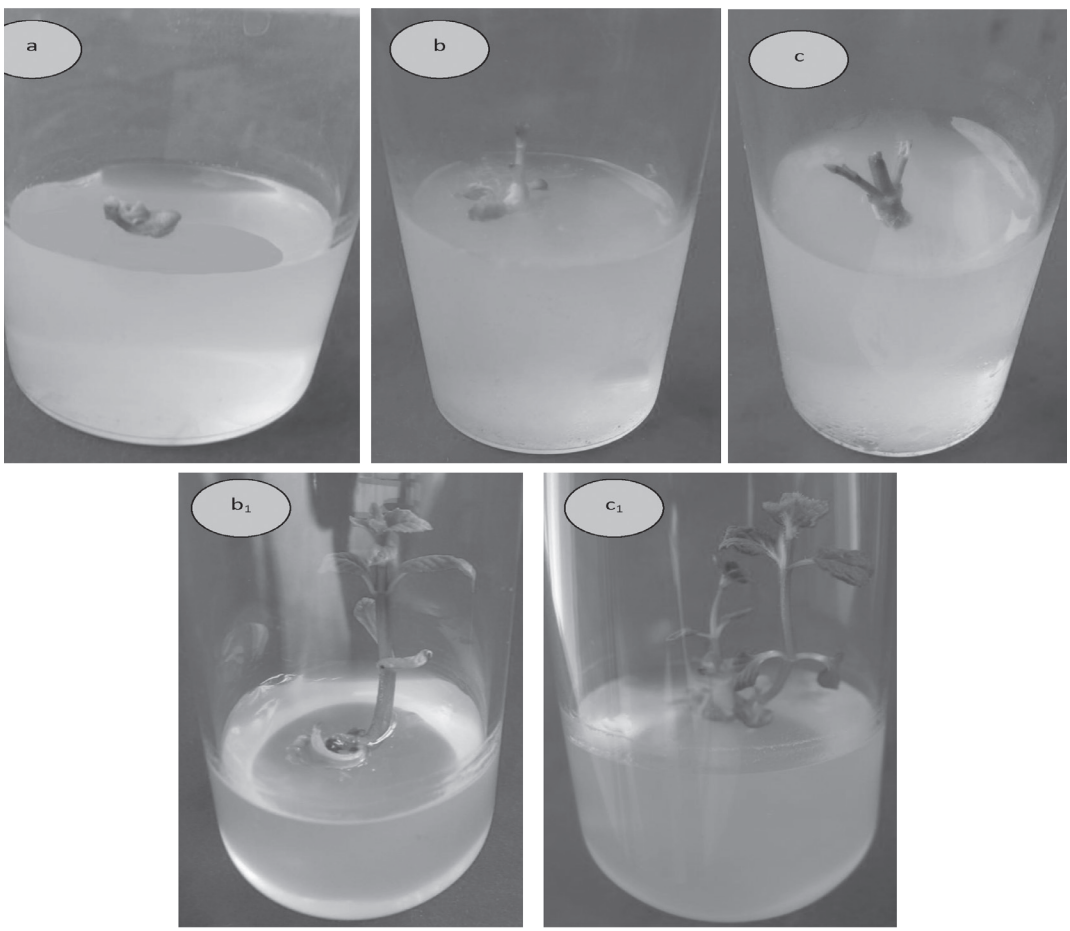

Fig. 3. In vitro regeneration of shoot in mint (a, b and c= explant from leaf, shoot tip and nodal segment at 15 DAI, respectively; Leaf produced no shoot at all, b_1 and c_1 is regenerated shoot from shoot tip and nodal segment at 55 DAI, respectively).

shoot proliferation in all the observation (15-55 DAI). The highest number of shoots per explant was found in G_1 (2.61) at 55 DAI that was statistically identical to G_4 (2.44), G_6 (1.44) and G_9 (1.56). But G_2 (0.11), G_3 (0.17), G_5 (0.28), G_7 (2.44), G_6 (1.11) and G_8 (0.17) showed statistically dissimilar results from other genotypes at all the observations (15-55 DAI). Hoque (2013) reported that shoot regeneration success was different in different genotypes; which might be due to presence of higher amount of vitamins and minerals in the full strength MS media that helped in shoot regeneration.

The number of shoots increased with the increase of the days of culture in most of the interactions. The interaction of G_1E_3 produced the highest number of shoots/explants 1.00, 2.00, 2.66, 3.83 and 4.66 at 15, 25, 35, 45 and 55 days of culture, respectively. Fadel *et al*., (2010) recorded the maximum number of shoot in nodal segment (3.5±1) of *Mentha spicata*, where half strength MS media were supplemented with 0.2 mg/L NAA and 1mg/L Kinetin. *Mentha piperita* (G_1) showed the highest performance in the present experiment. The findings were agreed with Dubois *et al.* (2000) where they noticed, if regeneration capacity depends on sensitivity of tissue to cytokinins, genotypes with high sensitivity increased more explants would form more adventitious buds in a shorter time.

Table 4. Interaction effect of explants and genotype on number of shoot in in vitro culture of mint.

Interaction	Number of shoot				
	15 DAI	25 DAI	35 DAI	45 DAI	55 DAI
G_1E_1	0.00 b	0.00 b	0.00 c	0.00 c	0.00 d
G_1E_2	0.33 ab	0.83 b	1.66 a-c	2.83 ab	3.16 a-c
G_1E_3	1.00 a	2.00 a	2.66 a	3.83 a	4.66 a
G_2E_1	0.00 b	0.00 b	0.00 c	0.00 c	0.00 d
G_2E_2	0.00 b	0.16 b	0.33 c	0.33 c	0.33 d
G_2E_3	0.00 b	0.00 b	0.00 c	000 c	0.00 d
G_3E_1	0.00 b	0.00 b	0.00 c	0.00 c	0.00 d
G_3E_2	0.00 b	0.16 b	0.33 c	0.83 bc	1.33 cd
G_3E_3	0.00 b	0.00 b	0.00 c	0.00 c	0.00 d
G_4E_1	0.00 b	0.00 b	0.00 c	0.00 c	0.00 d
G_4E_2	0.16 b	1.00 ab	1.66 a-c	2.66 ab	4.33 ab
G_4E_3	0.33 ab	0.33 b	1.00 bc	2.16 a-c	3.00 a-c
G_5E_1	0.00 b	0.00 b	0.00 c	0.00c	0.00 d
G_5E_2	0.00 b	0.16 b	0.66 bc	0.00 c	0.83 cd
G_5E_3	0.00 b	0.00 b	0.00 c	0.83 bc	0.00 d
G_6E_1	0.00 b	0.00 b	0.00 c	0.00 c	0.00 d
G_6E_2	0.33 ab	1.16 ab	1.16 a-c	0.00 c	2.00 b-d
G_6E_3	0.33 ab	0.83 b	1.50 a-c	1.16 bc	2.33 a-d
G_7E_1	0.00 b	0.00 b	0.00 c	1.66 bc	0.00 d
G_7E_2	0.00 b	0.83 b	1.00 bc	0.00 c	2.00 b-d
G_7E_3	0.00 b	0.16 b	0.83 bc	1.33 bc	1.33 cd
G_8E_1	0.00 b	0.00 b	000 c	1.16 bc	0.00 d
G_8E_2	0.00 b	0.16 b	0.33 c	0.00 c	0.50 d
G_8E_3	0.00 b	0.00 b	0.00 c	0.33 c	0.00 d
G_9E_1	0.00 b	0.00 b	0.00 c	0.00 c	0.00 d
G_9E_2	0.00 b	1.00 ab	2.16 ab	0.00 c	3.00 a-c
G_9E_3	0.00 b	0.16 b	0.50 c	2.66 ab	1.66 cd
CV (%)	-	19.26	15.86	13.87	12.48

G_1=MP-1, G_2=MP-2, G_3=MP-3, G_4=MP-4, G_5=MP-5, G_6=MP-6, G_7=MP-9, G_8=MP-19 and G_9=MP-22; and E_1= Leaf, E_2= Shoot tip and E_3= Node.

Means followed by same letter in a column do not differ significantly at 1% level by DMRT.

Length of longest shoot

Result presented in Table 5 revealed that shoot length was non-significant in explants (leaf, shoot tip and leaf) at 15 DAI. At 25 DAI, the longest shoot was found in E_2 (shoot tip, 0.4 cm). At the same day, the second longest shoot was found in E_3 (node, 0.23 cm). Statistically similar shoot length was produced by shoot tip (3.05 cm) and nodal segment (2.98 cm) of mint till 55 DAI in *in-vitro* condition. Hoque (2013) reported the longest shoot was found maximum when shoot tips were used as explants which supported the present studies.

Shoot length was significantly varied after 25 days in different genotypes but non significant at 15 days. At 25 and 35 DAI, highest shoot length was found in G_6 (0.45 cm and 0.99 cm respectively), But at 45 and 55 DAI, the highest shoot length was found G_1 (3.00 cm and 4.96 cm, respectively), which was statistically similar with G_4, G_6 and G_9 (1.67 cm and 3.52 cm, 1.99 cm and 3.18 cm, 1.50 cm and 2.98 cm). Depending on species or cultivars, the most important achievement obtained in the propagation of many plant materials through tissue cultures has been frequently based on the successful adjustment of the type and combination of plant growth regulators (Asghari *et al.,* 2012).

In respect of shoot length, the interaction of explants and genotypes showed significant variation at 25 DAI (Table 7). Interaction between genotypes with shoot tip and node showed the best results in all the observations. At 55 DAI, the longest shoot was found in G_1E_3 (8.98 cm) and statistically similar result was also found in G_1E_2 (5.90 cm), G_6E_3 (5.86 cm), G_4E_2 (5.25 cm) and G_4E_3 (5.31 cm).

Length of the longest internode

While studying the effect of explants on length of the longest internode per explants it was observed that length of internode was non significant at 15 DAI (Table 8). At 25 to 45 DAI, the longest inernode / explant was found in E_2 (shoot tip, 0.13cm, 0.28 cm and 0.62 cm respectively). But at 55 DAI, the longest internode / explants was produced by E_3 (0.98 cm) and E_2 (0.90 cm) also produce statistically similar result at same days.

In case of genotype, the highest length of internode was non significant at 15 DAI. The longest internode was observed in G_6 (0.17 cm and 0.35 cm) at 25 and 35 DAI, respectively, At 45 and 55 DAI, G_1 (1.09 cm and 1.47 cm, respectively) produced the highest length of internode.

Table 5. Response of explant on length of longest shoot in mint.

Explant	length of longest shoot (cm)				
	15 DAI	25 DAI	35 DAI	45 DAI	55 DAI
E_1	0.00	0.00 b	0.00 b	0.04 b	0.04 b
E_2	0.04	0.40 a	0.80 a	1.72 a	3.05 a
E_3	0.09	0.23 a	0.58 a	1.60 a	2.98 a
CV (%)	-	19.80	23.1	12.10	9.68

E_1= Leaf, E_2= Shoot tip and E_3= Node.

Means followed by same letter in a column do not differ significantly at 1% level by DMRT.

Table 6. Response of genotype on length of longest shoot in mint.

Genotype	length of longest shoot (cm)				
	15 DAI	25 DAI	35 DAI	45 DAI	55 DAI
G_1	0.16	0.42 ab	0.89 ab	3.00 a	4.96 a
G_2	0.00	0.04 b	0.06 d	0.20 d	0.28 d
G_3	0.00	0.11 b	0.17 cd	0.48 cd	0.97 cd
G_4	0.13	0.41 ab	0.83 ab	1.67 a-c	3.52 ab
G_5	0.00	0.04 b	0.08 d	0.28 cd	0.41 d
G_6	0.12	0.45 a	0.99 a	1.99 ab	3.18 a-c
G_7	0.00	0.17 ab	0.37 b-d	0.86 b-d	1.56 b-d
G_8	0.00	0.03 b	0.09 d	0.14 d	0.33 d
G_9	0.00	0.23 ab	0.69 a-c	1.50 b-d	2.98 a-c
CV (%)	23.15	11.63	9.36	7.51	6.23

G_1=MP-1, G_2=MP-2, G_3=MP-3, G_4=MP-4, G_5=MP-5, G_6=MP-6, G_7=MP-9, G_8=MP-19 and G_9=MP-22.

Means followed by same letter in a column do not differ significantly at 1% level by DMRT.

Table 7. Interaction effect of expalnts and genotypes on length of longest shoot in in vitro culture of mint.

Interaction	Length of longest shoot (cm)				
	15 DAI	25 DAI	35 DAI	45 DAI	55 DAI
G_1E_1	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_1 E_2	0.11	0.45 ab	1.06 a-d	4.05 ab	5.90 ab
G_1 E_3	0.35	0.80 a	1.58 a	4.95 a	8.98 a
G_2 E_1	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_2 E_2	0.00	0.11 b	0.16 d	0.60 de	0.86 c-e
G_2 E_3	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_3 E_1	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_3 E_2	0.00	0.31 ab	0.51 b-d	1.45 c-e	2.91 b-e
G_3 E_3	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_4 E_1	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_4 E_2	0.10	0.81 a	1.51 ab	3.03 a-d	5.25 ab
G_4 E_3	0.30	0.41 ab	1.00 a-d	1.96 b-e	5.31 ab
G_5 E_1	0.00	0.00 b	0.00 d	0.33 e	0.33 de
G_5 E_2	0.00	0.12 b	0.23 d	0.48 de	0.90 c-e
G_5 E_3	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_6 E_1	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_6 E_2	0.15	0.83 a	1.48 a-c	2.10 b-e	3.68 b-e
G_6 E_3	0.21	0.63 ab	1.48 a-c	3.86 a-c	5.86 ab
G_7 E_1	0.00	0.00 c	0.00 d	0.00 e	0.00 ab
G_7 E_2	0.00	0.25 ab	0.65 a-d	1.01 de	2.51 b-e
G_7 E_3	0.00	0.10 b	0.45 cd	1.55 b-e	2.15 b-e
G_8 E_1	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_8 E_2	0.00	0.10 b	0.25 d	0.41 de	1.00 c-e
G_8 E_3	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_9 E_1	0.00	0.00 b	0.00 d	0.00 e	0.00 e
G_9 E_2	0.00	0.61 ab	1.31 a-c	2.40 b-e	4.41 b-d
G_9 E_3	0.00	0.08 b	0.73 a-d	2.08 b-e	4.53 bc
CV (%)	-	18.88	1.31	1.32	1.17

G_1=MP-1, G_2=MP-2, G_3=MP-3, G_4=MP-4, G_5=MP-5, G_6=MP-6, G_7=MP-9, G_8=MP-19 and G_9=MP-22; and E_1= Leaf, E_2= Shoot tip and E_3= Node.

Means followed by same letter in a column do not differ significantly at 1% level by DMRT.

Table 8. Response of explant on length of longest internode in mint.

Explant	Length of longest internode (cm)				
	15 DAI	25 DAI	35 DAI	45 DAI	55 DAI
E_1	0.00	0.00 b	0.00 b	0.00 b	0.00 b
E_2	0.01	0.13 a	0.28 a	0.62 a	0.90 a
E_3	0.03	0.08 a	0.22 a	0.59 a	0.98 a
CV (%)	-	18.93	15.69	13.23	12.11

E_1= Leaf, E_2= Shoot tip and E_3= Node.
Means followed by same letter in a column do not differ significantly at 1% level by DMRT.

Table 9. Response of genotype on length of longest internode in mint.

Genotype	Length of longest internode (cm)				
	15 DAI	25 DAI	35 DAI	45 DAI	55 DAI
G_1	0.05	0.13 ab	0.34 a	1.09 a	1.47 a
G_2	0.00	0.01 b	0.02 c	0.10 c	0.11 d
G_3	0.00	0.02 b	0.04 c	0.16 bc	0.22 cd
G_4	0.04	0.13 ab	0.30 ab	0.57 a-c	1.08 ab
G_5	0.00	0.01 b	0.03 c	0.06 c	0.13 d
G_6	0.03	0.17 a	0.35 a	0.70 ab	1.17 ab
G_7	0.00	0.03 b	0.11 bc	0.33 bc	0.54 b-d
G_8	0.00	0.01 b	0.02 c	0.05 c	0.09 d
G_9	0.00	0.08 ab	0.28 ab	0.58 a-c	0.81 bc
CV (%)	-	16.56	12.32	11.00	10.23

G_1=MP-1, G_2=MP-2, G_3=MP-3, G_4=MP-4, G_5=MP-5, G_6=MP-6, G_7=MP-9, G_8=MP-19 and G_9=MP-22.
Means followed by same letter in a column do not differ significantly at 1% level by DMRT.

In case of interaction effect on length of the longest internode, all combinations of explant and genotypes were increased with the increase of days after culture (Table 10). At 25 and 35 DAI, the highest length of internode was found in G_6E_2 (0.30 cm) and G_6E_3 (0.65 cm), respectively. The combination G_1E_3 produced the longest internode at both 45 and 55 DAI (1.72 cm and 2.52 cm, respectively). But present findings disagreed with the result of Vasile *et al*. (2011) who reported pipermint with the combination of nodal segment produced shorter internodal spaces in presence of Zeatin, which (Zeatin) was totally absent in the present paper.

Table 10. Interaction effect of explants and genotypes on length of longest internode in in vitro culture of mint.

Interaction	Length of longest internode (cm)				
	15 DAI	25 DAI	35 DAI	45 DAI	55 DAI
G_1E_1	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_1E_2	0.05	0.13 a-c	0.45 a-d	1.57 ab	1.90 a-c
G_1E_3	0.10	0.26 ab	0.58 ab	1.72 a	2.52 a
G_2E_1	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_2E_2	0.00	0.05 bc	0.06 d	0.30 d	0.33 e-g
G_2E_3	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_3E_1	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_3E_2	0.00	0.08 a-c	0.13 cd	0.50 cd	0.68 d-g
G_3E_3	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_4E_1	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_4E_2	0.03	0.25 a-c	0.55 a-c	1.05 a-d	1.50 a-e
G_4E_3	0.10	0.16 a-c	0.35 a-d	0.68 b-d	1.75 a-d
G_5E_1	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_5E_2	0.00	0.05 bc	0.10 d	0.18 d	0.42 e-g
G_5E_3	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_6E_1	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_6E_2	0.03	0.30 a	0.42 a-d	0.63 b-d	1.17 c-g
G_6E_3	0.08	0.23 a-c	0.65 a	1.48 a-c	2.35 ab
G_7E_1	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_7E_2	0.00	0.08 a-c	0.18 b-d	0.35 d	0.68 d-g
G_7E_3	0.00	0.03 bc	0.15 cd	0.65 b-d	0.95 c-g
G_8E_1	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_8E_2	0.00	0.03 bc	0.08 d	0.15 d	0.28 fg
G_8E_3	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_9E_1	0.00	0.00 c	0.00 d	0.00 d	0.00 g
G_9E_2	0.00	0.22 a-c	0.58 ab	0.93 a-d	1.13 c-g
G_9E_3	0.00	0.03 bc	0.28 a-d	0.83 a-d	1.32 b-f
CV (%)	-	19.9	1.46	1.42	1.09

G_1=MP-1, G_2=MP-2, G_3=MP-3, G_4=MP-4, G_5=MP-5, G_6=MP-6, G_7=MP-9, G_8=MP-19 and G_9=MP-22; and E_1= Leaf, E_2= Shoot tip and E_3= Node. Means followed by same letter in a column do not differ significantly at 1% level by DMRT.

Conclusion

Genotypes MP-1, MP-4 and MP-6 showed the best performance with shoot tip and nodal segments as explant for *in-vitro* shoot regeneration in mint.

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