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Research Article

Growth and root formation in the microshoots of three basils cultured in vitro on different types and patterns of electrospun nanofibre mats

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Abstract

Nanofibre mats have been mainly used in relation to human health sciences. The application of this material in plant tissue culture was still rare. In this research, using different types and patterns of nanofibre mats, the microshoots (size 2-3 mm) of three basils were cultured in vitro. There were 3 types of materials for fabrication of a nanofibre mat in this study: cellulose (C), polyvinylidene fluoride (F) and polylactic acid (L) and 2 patterns of nanofibre mats: nonwoven (called pattern 1) and a mixture of nonwoven and aligned nanofibres (called pattern 4). Microshoot explants were obtained from aseptic grown holy basil, lemon basil and sweet basil seedlings. These explants were cultured on nanofibre mats that were floated on liquid basal MS medium. The results revealed that holy basil microshoots exhibited the greatest shoot height and number of root formed when cultured on the nonwoven cellulose-based nanofibre mat (C1). The nonwoven nanofibre mats electrospun from polylactic acid (L1), however, supported the greatest shoot height of both lemon basil and sweet basil microshoot explants. In addition, both lemon basil and sweet basil microshoots formed the greatest number of roots when cultured on the nonwoven nanofibre mats electrospun from polyvinylidene fluoride (F1). Thus, the basil microshoot cultures exhibited various responses when cultured on the different types and patterns of nanofibre mats. This innovation of culturing basil microshoots on electrospun nanofibre mats only requires 0.5 ml liquid medium and should also assist plant tissue culturists to save on using the relative expensive agar.

Keywords: holy basil, lemon basil, sweet basil, Murashige and Skoog medium, plant tissue culture

Introduction

An electrospun nanofibre mat is a physical support matrix containing tens to hundreds of nanometer or sub-nanometer fibres which were electrospun and conventionally fabricated into mats with the fibre in random, aligned or patterned arrangements (Bodhipadma et al., 2016; Mahjour et al., 2016; Jian et al., 2018; Kozior et al., 2019). These electrospun ultrafine fibres could be produced from inorganic or organic compounds, for example, polylactic acid, polyvinylidene fluoride (Bodhipadma et al., 2011), polycaprolactone (Chanunpanich & Suwanboon, 2014), fish skin gelatin (Songchotikunpan et al., 2008) and lignin (Hong et al., 2019). So far, there were various applications of electrospun nanofibre mats, including filtrations, affinity membranes and recovery of metal ions, tissue engineering scaffolds, wound healing, drug delivery, composites and templates, catalyst and enzyme carriers, sensors and energy storage (Jian et al., 2008; Wang & Ryan, 2011). All these were primarily used for human care.

Among about 65 species in Lamiaceae (Labiatae) family, three well known basils in Thailand, holy basil (*Ocimum tenuiflorum* L., formerly known as *O. sanctum* L.), lemon basil (*Ocimum* × *citriodorum* Vis.) and sweet basil (*Ocimum basilicum* L.), are just not only the culinary herbs but also the rich sources of natural antioxidants, essential oils and neutraceuticals (Bodhipadma et al., 2012; Wongsen et al., 2015; Lupton et al., 2016; Tangpao et al., 2018). Thus, using these 3 basils for food ingredients in Thai cooking would provide delicious tastes, fragrances and good health for the consumer.

In plant tissue culture, electrospun nanofibre mats have recently been applied in a few experiments, for instance, as a scaffold for callus induction and culture of aromatic chilli and bilimbi (Bodhipadma et al., 2011) and a physical support substrate for germination of the *Artabotrys hexapetalus* pollen (Bodhipadma et al., 2016). A microshoot is an explant comprising the apical meristem with a minimal amount of other shoot tissues. This tiny explant has been used in various applications, including in vitro grafting, in vitro propagation, in vitro selection, and virus eradication (Sarai et al., 2014). It is not known if electrospun nanofibre mats would be suitable for microshoot culture. In this research, we aimed to investigate the growth of microshoots and root production when the microshoot explants of holy basil, lemon basil and sweet basil were cultured on different types and patterns of electrospun nanofibre mats.

Materials and methods

Dry seeds of holy basil, lemon basil and sweet basil were purchased from Chia Tai Seed Co., LTD., Bangkok, Thailand. The seeds of these three basils were first immersed separately in clean tap water for 3 hours. Surface sterilization of the seeds was initiated by transferring the seeds to 10% (v/v) St. Andrews Vegetable and Fruit Washing Liquid [Lion company (Thailand) Ltd., Thailand] for 10 minutes, followed by rinsing them with clean tap water for 10 minutes. The seeds were then soaked in 15% (v/v) Clorox (5.25%, w/w, sodium hypochlorite) and 3-4 drops of Tween 20 for 15 minutes before 3 rinses of 1 minute each in sterile distilled water. For seed germination under axenic conditions, five of the seeds were placed on the surface of basal MS medium (Murashige and Skoog, 1962) gelled with 0.8% (w/v) agar in a jar.

Following seed germination, microshoot explants (size 2-3 mm) of these three basil seedlings were excised and cultured on the surface of different types and patterns of electrospun nanofibre mats: cellulose (C), polyvinylidene fluoride (F) and polylactic acid (L) that were nonwoven (pattern 1) and a mixture of nonwoven and aligned arrangement (pattern 4) (Table 1), floated on 0.5 ml of liquid basal MS medium (Figure 1) placed in polypropylene tubes (size: 100 mm height and 16 mm diameter) with low-density polyethylene screw cap (Daigger Scientific, Inc., USA). For seed germination under axenic conditions and microshoot cultures, all the cultures were kept in a growth room under daylight fluorescent lamps (19.39 $\mu mol/m^2/s$) for 16 hours and 8 hours of darkness at 25±2 °C for 3 and 4 weeks, respectively. All the media used in this study were adjusted to pH 5.7, and autoclaved at 121 °C and 15 psi for 20 minutes. Shoot length and number of root formed were determined and analysed statistically including ANOVA and means comparison by Duncan's Multiple Range Test at a confidence level of 0.05.

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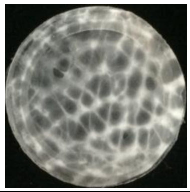
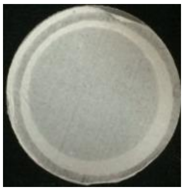
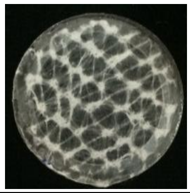
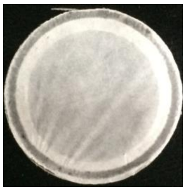
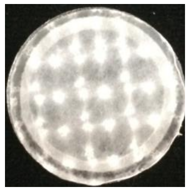
Table 1 Three types and two patterns of electrospun nanofibre mats used in this experiment. Each nanofibre mat was 13 mm diameter.

Type	Pattern 1	Code	Pattern 4	Code
Cellulose (C)		C1		C4
Polyvinylidene fluoride (F)		F1		F4
Polyacetic acid (L)		L1		L4

Note: pattern 1 = nonwoven and pattern 4 = a mixture of nonwoven and aligned nanofibres

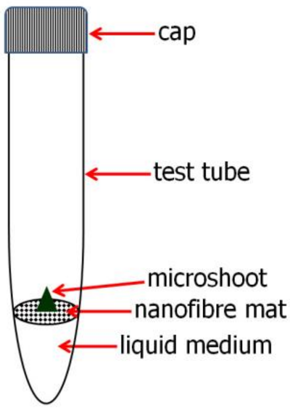


Figure 1 Diagram showing the set-up of an experiment for culturing a microshoot explant on a nanofibre mat floated on the surface of liquid medium in a test tube.

Results and discussion

Generally, microshoot explants could be cultured in vitro on semi-solid medium as well as in liquid medium. For liquid culture, the explants were able to grow when floated in liquid medium under agitation or placed on a filter paper bridge in contact with liquid medium (Grigoriadou et al., 2005). In this research, electrospun nanofibre mats with different chemical compounds and the fibres in different arrangements were used as support materials for culture of holy basil, lemon basil and sweet basil microshoot explants floated on the surface of liquid medium.

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After microshoot explants (2-3 mm) of three basils were cultured on various types and patterns of electrospun nanofibre mats floated on liquid basal MS medium for 4 weeks, it was found that among all the electrospun nanomats used as support materials, the microshoots of holy basil grew particularly well on the C1 nanofibre mats. The microshoots exhibited the highest and lowest shoot heights at 12.75 mm and 6.75 mm when cultured on C1 and F4 nanofibre mats, respectively (Figure 2). There was no significant difference between the shoot heights of the microshoots of three basils cultured on the C1 and L1 nanofibre mats (Figures 2-4). The lemon basil and sweet basil microshoots exhibited greater shoot heights (33.83 mm and 30.9 mm, respectively) than the holy basil microshoots when cultured on L1 nanofibre mats (Figures 2-4). Moreover, the microshoots of these three basils exhibited the lowest shoot heights when cultured on the F4 nanofibre mats (Figures 2-4).

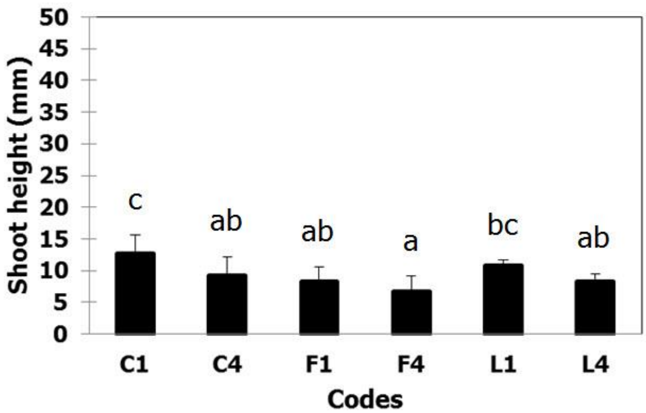

Figure 2 Shoot heights of holy basil microshoots after culturing on the different types and patterns of electrospun nanofibre mats floated on liquid basal MS medium for 4 weeks.

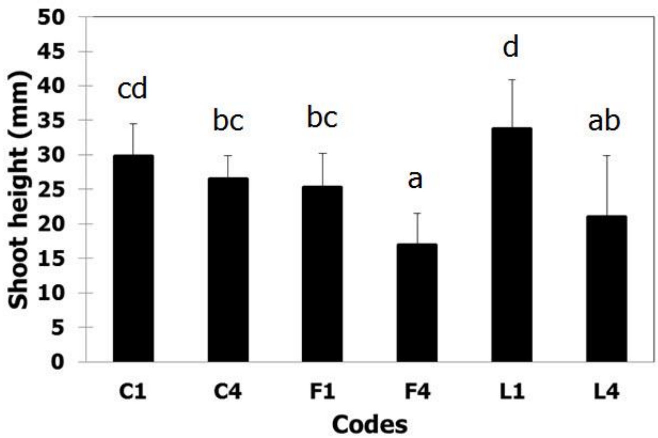

Figure 3 Shoot heights of lemon basil microshoots after culturing on the different types and patterns of electrospun nanofibre mats floated on liquid basal MS medium for 4 weeks.

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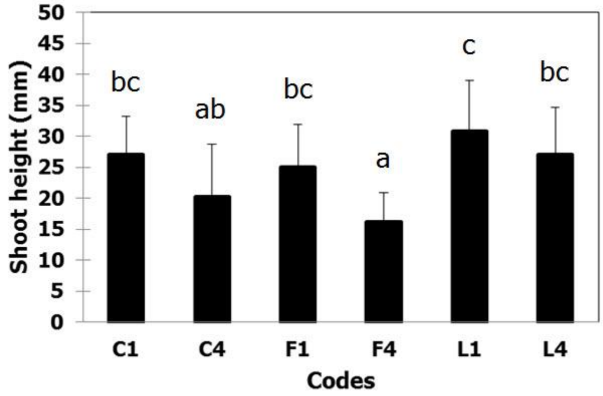

Figure 4 Shoot heights of sweet basil microshoots after culturing on the different types and patterns of electrospun nanofibre mats floated on liquid basal MS medium for 4 weeks.

The holy basil microshoots formed the highest number of roots (3.5) when cultured on the C1 nanofibre mats compared to those on the other nanofiber mats (Figure 5). In contrast, lemon basil and sweet basil microshoots both formed the highest number of roots (12.67) when cultured on the F1 nanofibre mats (Figures 6 and 7). There was no significant difference in the number of roots formed by the microshoots of both basils when cultured on the C1, C4, F1 and L1 nanofibre mats (Figures 6 and 7). Overall, the holy basil microshoots formed fewer roots than the lemon basil and sweet basil microshoots across all the different nanofiber mats used.

From the results obtained, among the different types and patterns of electrospun nanofibre mats used on liquid basal MS medium, differences were found in the shoot growth and root initiation in these three basil microshoots. One of the main reasons of these various responses might be related to the difference in medium adsorption efficiency of each of the nanofibre mats used. Our preliminary results suggested that cellulose (C) nanofibre mats had the highest percentage of medium adsorption, while polyvinylidene fluoride (F) nanofibre mats had the lowest one (data not shown).

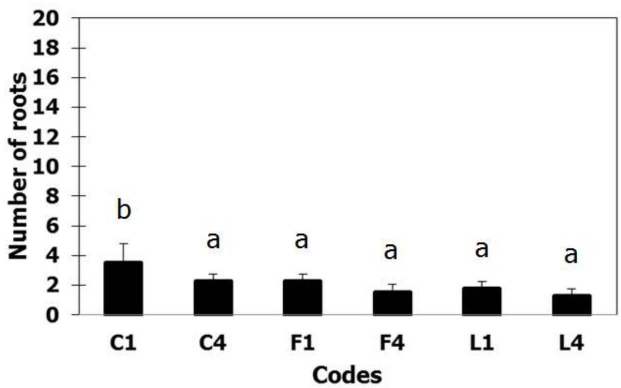

Figure 5 Number of roots formed per holy basil microshoot after culturing on the different types and patterns of electrospun nanofibre mats floated on liquid basal MS medium for 4 weeks.

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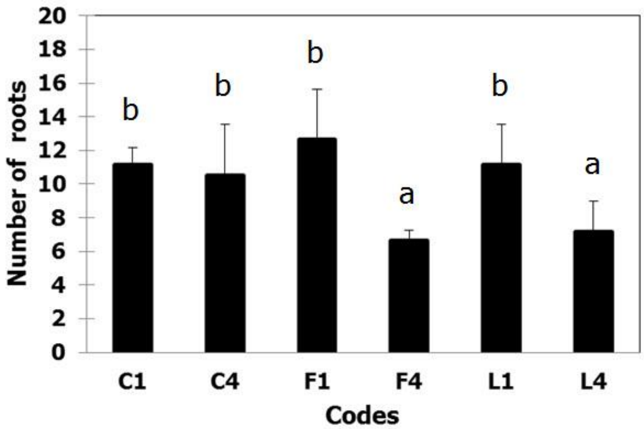

Figure 6 Number of roots formed per lemon basil microshoot after culturing on the different types and patterns of electrospun nanofibre mats floated on liquid basal MS medium for 4 weeks.

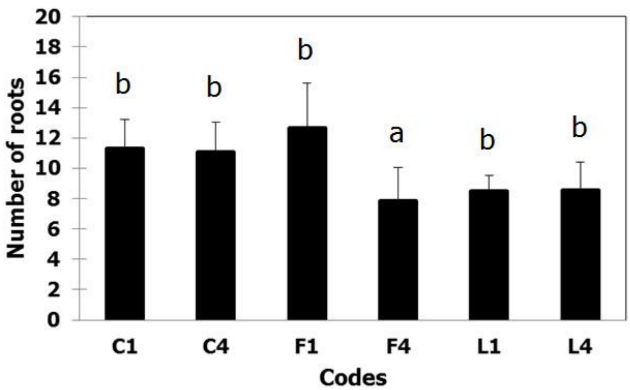

Figure 7 Number of roots formed per sweet basil microshoot after culturing on the different types and patterns of electrospun nanofibre mats floated on liquid basal MS medium for 4 weeks.

It is possible that there might be some influence from the plant source of the microshoot explants on the response of the explants to the different nanofiber mats used. In this study, the growth rate of holy basil microshoots and root formation was about 2.4 and 3.6 times, respectively, lesser than those in the lemon basil and sweet basil microshoots cultured on the nanofiber mats.

The microshoots of these three basils formed the least number of roots when cultured on F4 nanofibre mats. This was probably related to that a mixture of nonwoven and aligned (pattern 4) had inferior medium adsorption percentage than the nonwoven (pattern 1) (data not shown). The precise cause of lower root formation on the F4 nanofibre mats warrants further investigations.

Electrospun nanofibre mats were first used in plant tissue culture for callus induction and subculture (Bodhipadma et al., 2011) and later for pollen germination (Bodhipadma et al., 2016). In both studies, there were also different growth responses on the diverse types and patterns of nanofibre mats as found in the present research. Similar to culture on a nanofibre mat, explants, for example, potato meristem, could be placed on a filter paper bridge as a supporting material standing on liquid medium in a test tube comprising liquid MS medium (Khatun et al., 2015; Blidar et al., 2016). The volume of liquid medium required in the paper bridge support system might apparently be greater than that in the nanofiber mat system. It would be interesting to compare if there are any other differences in these two physical support systems for plant tissue culture.

The liquid culture system has obvious advantages over the semi-solid culture system based on agar. To solidify or form a gel of the medium, agar generally uses as a gelling agent that costs 10-20% of the cultured medium expenditure (Vyas et al., 2008; AlKhateeb & Alturki 2014). Nevertheless, for shoot and root development in vitro, the polarity is an important factor which a semi-solid medium could better provide than liquid medium. Using electrospun nanofibre mats would combine the benefits of both types of medium systems. With this innovation of using nanofiber mats, there is no need to use the more expensive agar and less liquid medium is required. For example, for culturing these three basil microshoots, as little as 0.5 ml of medium was found to be sufficient. Moreover, since the growth of unspecialised cells in callus, haploid pollen cells and tiny organs like microshoots during culture in vitro is likely to depend on the types and patterns of nanofibre mats, it would be possible to select the right types and patterns of electrospun nanofibre mats to use.

Conclusion

Holy basil, lemon basil and sweet basil microshoot explants responded differently on the various types and patterns of electrospun nanofibre mats. In this research, the electrospun nanofibre mats were found to be useful physical support matrices for the growth of the microshoots and root formation in these three basils. The use of nanofiber mats also minimised the volume of liquid medium required for microshoot culture and save on using the relatively expensive agar for the common semi-solidified plant tissue culture medium system.

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