African Journal of Biotechnology Vol. 12(7), pp. 670-678, 13 February, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3021
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

In vitro clonal propagation of *Fuchsia magellanica* Lam.

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Accepted 1 August, 2012

Fuchsia magellanica **Lam. is a famous ornamental plant which is rarely found in Pakistan. Its beauty and uniqueness made it an important candidate for tissue culture studies especially for regeneration and multiplication purposes. Here, for the very first time we report a rapid and reliable method for the regeneration of *F. magellanica in vitro*. Axillary buds explants from one year old *F. magellanica* plant were cultured on Murashige and Skoog's (MS) (1962) medium without any hormone supplementation. Multiplication was carried out by using 1 and 0.1 mg/lit., 6-benzylaminopurine (BAP) and α-naphthalene acetic acid (NAA), respectively. For elongation purpose, 0.5 µM gibberellic acid (GA3) was used. 0.1 mg/lit. NAA was used to stimulate extensive root development within one month. After successful regeneration, the plantlets were acclimatized into the soil. This study is undertaken to perform *in vitro* clonal propagation and acclimatization of *F. magellanica* in order to get hundreds of *F. magellanica* plants in the laboratory in comparatively less time. Hence, it would be possible to regenerate *F. magellanica* plants in the laboratory and after successful acclimatization.**

Key words: Axillary buds, clonal propagation, Murashige and Skoog, 6-benzylaminopurine (BAP), α-naphthalene acetic acid (NAA), gibberellic acid (GA3), acclimatization.

INTRODUCTION

The genus *Fuchsia* of flowering plants belong to family Onagraceae and order Myrtales [The Encyclopædia Britannica 11th Edition: Volume XI]. The genus Fuchsia comprises of almost 110 species; including *Fuchsia magellanica* Lam., *Fuchsia denticulate, Fuchsia racemosa* and *Fuchsia corimbiflora*. On the basis of dissimilar patterns of morphological characteristics among *Fuchsia Lam*. species, taxonomists have recognized 11 sections (Berry, 1982; Godley et al., 1995; Munz, 1943). Although, proper research could not be made on the *Fuchsia*'s medicinal properties, in South America it is a routine strategy to use the crushed petals of *Fuchsia La*and the juice from its berries to treat skin ailments, freckles and small blisters and rashes. The flower of *Fuchsia arborescens* are even eaten and being used on bites, scratches and grazes and its juice has a relieve effect on itching and taking away the redness. They are also used to relieve inflamed blisters and sunburn. *Fuchsia* flowers and berries are used to make a superb jelly that include lemon juice, apple juice and a dash of brandy which is used as a remedy for sore throat, tonsillitis and to strengthen the voice (Roberts, 2000).

In vitro regeneration of plantlets from stem nodal explants has been reported as an efficient and rapid strategy for large scale propagation of certain important plants like *Mucuna pruriens* (Faisal et al., 2006; Sathyanarayana et al., 2008), *Jatropha Curcas L*. (Shrivastava et al., 2008), *Stevia rebaudiana Bertoni* (Ahmed et al., 2007), *Azadirachta indica A. juss* (Chaturvedi et al., 2004) and an aromatic medicinal herb, *Ocimum Basilicum L*. (Sahoo et al., 1997).

The callus proliferation and regeneration of *Fuchsia hybrida* was successfully carried out by Chow et al. (1990), which was reported to occur more rapidly than that which was observed in callus derived from ovary tissues (Dabin et al., 1985; Dekeyser et al., 1985). Rapid development of axillary buds from shoot-tips and nodes of 18 cultivars of *F. hybrida* has been obtained on solid Murashige and Skoog (MS) medium with 6-benzylaminopurine (BAP) and an auxin (Kevers et al.,

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2003). Tissue culture and rapid propagation of *Fuchsia alba-coccinea* Hort was also carried out using explants of stem fragments with nodes (Fugen et al., 2006). *F. magellanica* was selected for tissue culture studies and for *in vitro* initiation, multiplication, elongation, rooting and acclimatization.

MATERIALS AND METHODS

Culture and condition

Nodal segments were cultured on ½, ¾ and standard Murashige and Skoog (Murashige and Skoog, 1962) or MS media. After adding the hormones, the pH of medium was adjusted to 5.75. All the culture containing media were autoclaved at 121°C for 20 min. All the cultures were incubated in a culture chamber maintained at a temperature of 23 to 25°C under 16 h photoperiod provided by cool white fluorescent tubes.

Preparation of *Fuchsia magellanica Lam*. explant

The healthy *F. magellanica* plants of an average age of four years were selected as the source of explants, which were kept at 16°C. The plant had eight to 10 shoots and 7 to 11 cm stem length. The plants were systematically washed with running tap water for at least 1 h. Plants were dried out and the lowest part of the stem bearing roots was detached. All the mature leaves present at the nodes were detached with a sterilized pointed blade in such a way that at least ½ to 1 in of the leaf base remained intact with the stem node explant, after incomplete removal of the leaves. A slanting cut was given at the base of the leaf. The cut was given very carefully in such a way that the leave sheath remained intact and enclosed whole of the nodal region under it.

Surface sterilization and explant sizing

All the pre explants were thoroughly washed with sterilized distilled water followed by a dip in solution of 20% bleach and few drops of tween20 (polyoxyethylene (20) sorbitan monolaurate) for 15 min with constant shaking. After this treatment, the pre-explants were given four to five thorough washings with autoclaved double distilled water to remove any trace of the bleach and few drops of tween20. Stem nodal segments of 1.5 cm in size were cut under sterile conditions as per the steps mentioned above. All the explants were cultured in the MS media. The cultures were incubated at 25°C under 16 h photoperiod provided by cool white fluorescent tubes. The cultures were observed periodically.

Initiation strategy

Nodal segments were separately cultured (culture and conditions) on ½, ¾ and standard MS media (Murashige and Skoog, 1962) without addition of any growth regulators.

Multiplication strategy

After successful initiation, the single shoots were independently cultured on ½, ¾ and standard MS media (Murashige and Skoog, 1962) with the addition of α-naphthalene acetic acid (NAA) and BAP. The concentrations of BAP and NAA used were identical to those reported by Kevers et al. (2003) that is, 1 and 0.1 mg l^{-1}, respectively. For control setup, ½, ¾ and standard MS media (Murashige and Skoog, 1962) were used without any addition of hormone.

Elongation strategy

After successful multiplication, the micro shoots were transferred independently on ½, ¾ and standard MS media (Murashige and Skoog, 1962) with the addition of hormone gibberellic acid (GA3). The concentration of GA3 was set to be 0.5 µM. For control setup, ½, ¾ and standard MS media (Murashige and Skoog, 1962) were used without any addition of hormone.

Rooting media

After successful elongation, the isolated shoots with three to four pairs of leaves were individually transferred to ½, ¾ and standard MS media supplement with the addition of hormone NAA. 0.1 mg/liter NAA solution was used to stimulate rooting in *F. magellanica*. For control setup, ½, ¾ and standard MS media (Murashige and Skoog, 1962) were used without any addition of hormone.

Acclimatization

Plantlets with well-developed shoots and roots were removed from the culture medium, washed gently under running tap water and transferred to plastic pots containing sterile garden soil, peat moss under diffuse light conditions. Potted plantlets were covered with a transparent polythene bag to ensure high humidity and watered every three days with ¾ MS salt solution for two weeks. Polythene bag were opened after three weeks to acclimatize plants to field conditions. After five weeks, acclimatized plants were transferred to pots containing standard soil and maintained in a greenhouse under normal day length conditions.

Statistical analysis

It was a case-control study. According to our alternate hypothesis, ¾ MS media is more suitable for optimal development in *F. magellanica Lam* than ½ and standard MS media. We analyzed our results using analysis of variance (ANOVA) test, SPSS-16. The alpha value was set to be 0.05. The mean difference is significant at the 0.05 level.

RESULTS AND DISCUSSION

Initiation of *Fuchsia magellanica* Lam

The shoots of 1.5 cm length were cut in such a way that each shoot contained two axiliary buds. All the shoots were cultured into the initiation media (Figure 1). ½, ¾ and standard MS media was used to check optimal response. After three to four days of incubation, most prominent induction of leaves was observed in ¾ MS. Leaves emerged from each bud and continued to grow during first week (Table 1 and Figure 2). The average shoot length was measured to be 2.06+0.122 cm. After two weeks, one prominent shoot of 4.133+0.152 cm emerged from each bud (Table 1 and Figure 3). At the

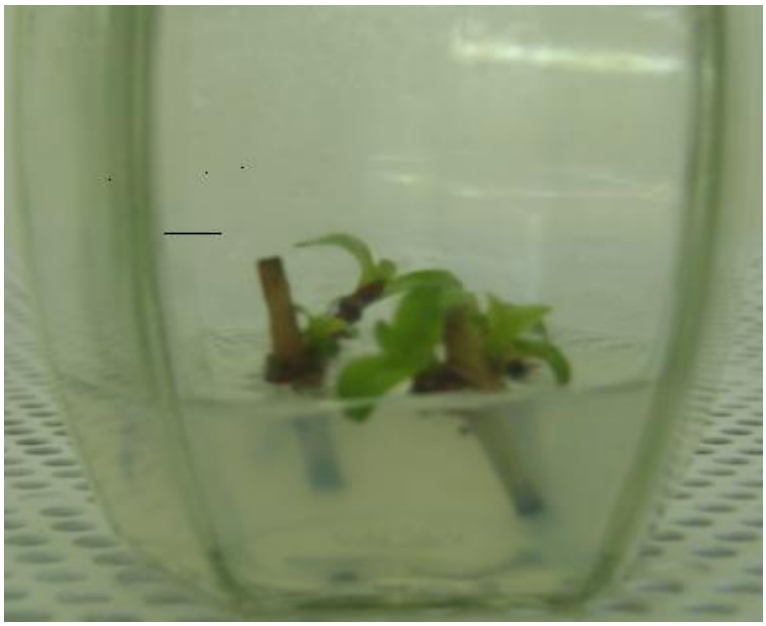

Figure 1. Axillary bud explant of *F. magellanica* cultured in initiation media.

Table 1. Effect of different concentrations of MS media on shoot induction from auxiliary buds.

Shoot length increase (cm)	½ MS test	¾ MS test	Std. MS test
1st week	0.533±0.057	2.06±0.122	0.567±0.057
2nd week	1.067±0.115	4.133±0.152	1.58±0.026
3rd week	1.47±0.047	4.467±0.152	2.066±0.115

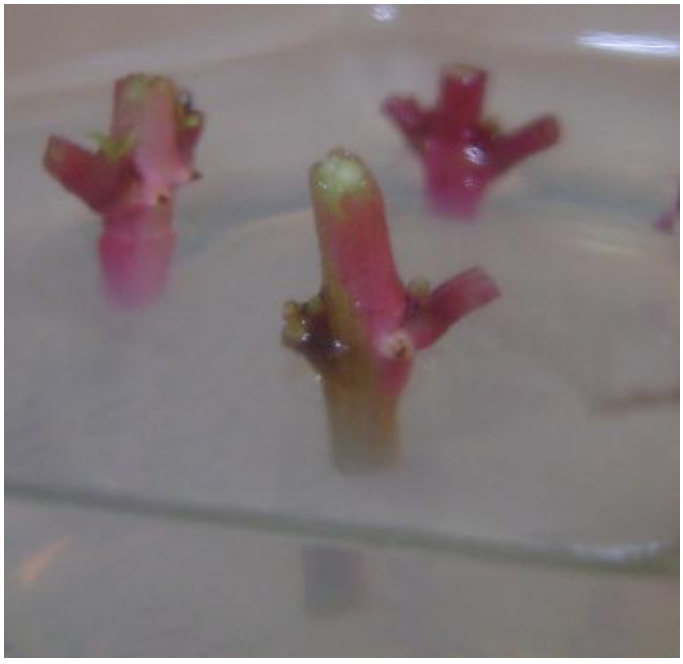

Figure 2. *In vitro* initiation of shoots from axillary bud explant of *Fuchsia magellanica* observed after one week of transfer to initiation media.

Figure 3. *In vitro* initiation of shoots from axillary bud explant of *Fuchsia magellanica* observed after two weeks of transfer to initiation media.

Table 2. Effect of hormones on different developmental stages of *Fuchsia magellanica.*

Developmental stage	Type of observation	½ MS control	½ MS test	¾ MS control	¾ MS test	Std. MS control	Std. MS test
Multiplication							
BAP (1 mg/l)	No. of shoots per explant	1.0±0	2.33±0.577	2.67±0.577	5.33±0.577	1.67±0.577	2.67±0.577
NAA (0.1 mg/l)	Length (cm)	0.45±0.05	1.1±0.113	1.15±0.05	2.5±0.011	0.81±0.036	1.566±0.057
Elongation							
GA_3 (0.5 μM)	Length (cm)	0.496±0.015	1.5±0.02	1.5±0.01	4.1±0.1	1.033±0.057	2.033±0.057
Rooting							
NAA (0.1 mg/l)	No. of roots per explant	3.33±0.577	5.67±0.577	5.33±0.577	11.66±0.577	3.67±0.577	6.67±0.577
	Length (cm)	1.066±0.115	1.983±0.028	3.026±0.064	6.483±0.028	1.526±0.064	3.1±0.1

Figure 4. *In vitro* initiation of shoots from axillary bud explant of *F. magellanica* observed after three weeks of transfer to initiation media.

end of the 3rd week, the shoot length increased up to 4.467+0.152 cm (Table 1 and Figure 4). Since any of the initiation media was not supplemented with growth regulator/ hormone, it means that initiation of *F. magellanica* does not require any hormone. Our results suggest ¾ MS media to be the best media for initiation. The control plant was set by planting a 10 cm long leafless shoot of *F. magellanica* in the soil. The induction of leaves in the control plants could not be observed before 20 days. It suggests that ¾ MS media has enough potential to stimulate rapid initiation in *F. magellanica*. After initiation, 30 plants were obtained using ¾ MS media which were then used for multiplication.

Multiplication of *Fuchsia magellanica* Lam.

After successful initiation of *F. magellanica* on ¾ MS media, we divided the initiated shoot in such a way that each piece should not be more than 1 cm and must contain two nodes. Then the pieces were cultured in sterilized ½, ¾ and standard MS media in the presence of 1 and 0.1 mg l^{-1}, BAP and NAA, respectively. The concentrations of BAP and NAA were used as previously described by Kevers et al. (2003). More extensive multiplication in ¾ MS media as compare to other concentrations of MS media used were observed (Table 2).

After the first week, 2.67+0.577 number of shoots emerged from each node. The shoots continued increasing in length and became 2.5+0.011 cm long at the end of the third week (Table 2 and Figure 5b). It is worth mentioning that the shooting was extensive in all the explants suggesting the suitability of the ¾ MS medium in combination with two hormones that is, BAP and NAA. BAP or cytokinin (KN) helps in rapid induction of multiplication of shoots, buds or meristems (Rajore et al., 2002). Advantage of BAP over KN for multiple shoot induction was established in *Pterocarpus marsupium* (Suresh and Ajay, 2004), *Sapium sebiferum*, (Siril and Dhar, 1997) and *Eclipta alba* (Franca et al., 1995). Sathyanarayana et al. (2008) observed steady increase in the number of shoots of *Mucuna prureins varutilis* while using BAP up to 3.55 *M*. Cytokinin concentrations beyond these adversely affected the shoot growth, as the regenerated shoots became small and thick. The small nature of shoot formation parallel to increased concentration of BAP in the medium was also reported in *Eupatorium* (Martin, 2004) and *Orthosiphon* (Lai-Keng

Figure 5a. Control for multiplication.

Figure 5b. Multiplication of *F. magellanica* observed after 3 weeks of transfer to multiplication media.

and Leng, 2004). For *F. magellanica*, we observed that extensive multiplication was achieved at 0.225 M BAP.

NAA in combination with BAP helps in stem elongation. It has been recognized that the addition of NAA promotes the proliferation and elongation of shoots in *Petasites hybridus* (Wldi et al., 1998), *Mucana pruriens* (Faisal et al., 2006), *Eucalyptus grandis* (Luis et al., 1999) and *Hybanthus enneaspermus* (Prakash et al., 1999). BAP and NAA are effectual for multiplication purpose in tissue culture studies, as described by Kevers et al. (2003). Our results also show that in contrast to the control (¾ MS media without hormone supplementation) which shows no induction of shoot multiplication (Figure 5a), good multiplication of *F. magellanica* was achieved by using a combination of these two hormones in ¾ MS media. This strongly suggests the efficiency of BAP and NAA in promoting extensive lateral branching.

Shoot elongation of *Fuchsia magellanica* Lam.

Elongation of shoots was found to be most excellent on GA3 containing medium over other media supplemented with different hormones. Transferring the micro shoots to MS + GA3 (2.89 - 14.43 *M*) produced better elongation (data not shown), with GA3 (2.89 *M*) producing maximum elongation of 4.0 cm (Sathyanarayana et al., 2008). The positive result of GA3 on shoot elongation is well-established information in plant tissue culture as reported by Jayanand et al. (2003).

The gibberellins are involved in several physiological process regulations such as seed germination, initiation and growth of flowers and shoot elongation (Rkhis et al., 2006). Ben-Nissan et al. (2004) observed that GA3 (gibberellic acid) has role in cell elongation in stems. At this time for shoot elongation purpose, 0.5 cm long shoots were cut and transferred to ½, ¾ and standard MS media supplemented with GA3 (0.5 gm liter). In ¾ MS media, shoots were elongated up to 4.1+0.1 cm within eight days (Table 2 and Figure 6b) while in the two other concentrations of MS media (½, ¾ MS), no prominent response can be achieved. There was a gradual but continuous increase in the length of shoots (Figure 7). The elongation was shown to solely depends on the presence of GA3 hormone because no significant shoot elongation was observed in the control jars (Table 2 and Figure 6a) containing ¾ MS media without GA3 supplementation.

Root development in *F. magellanica*

In vitro rooting of regenerated shoots was achieved on ¾ MS medium equipped with 0.1 mg l^{-1} NAA, though the other concentrations (½ and standard MS media) were also tested. NAA as a key hormone in inducing roots are also reported in several established micro-propagation protocols like *M. pruriens* var. *pruriens* (Chattopadhyay et al., 1995), *J. curcas* (Rajore et al., 2002), *Hyptis suaveolens* (Britto et al., 2001), *Pisonia alba* (Jagadish et al., 1999) and *M. pruriens* var. utilis (Sathyanarayana et al., 2008).

We observed extensive root development in ¾ MS medium in the presence of NAA within two weeks. There were 11.66+0.577 number of roots in each explant. The length was observed to be 6.483+0.028 cm (Table 2 and Figure 8a). Whereas, in control plant (Figure 8b) where no NAA was added, root development was comparatively negligible. It proved the potential of NAA in root development of *F. magellanica.*

Figure 6a. Control for elongation.

Figure 6b. Elongation of *Fuchsia magellanica* observed after eight days of transfer to elongation media.

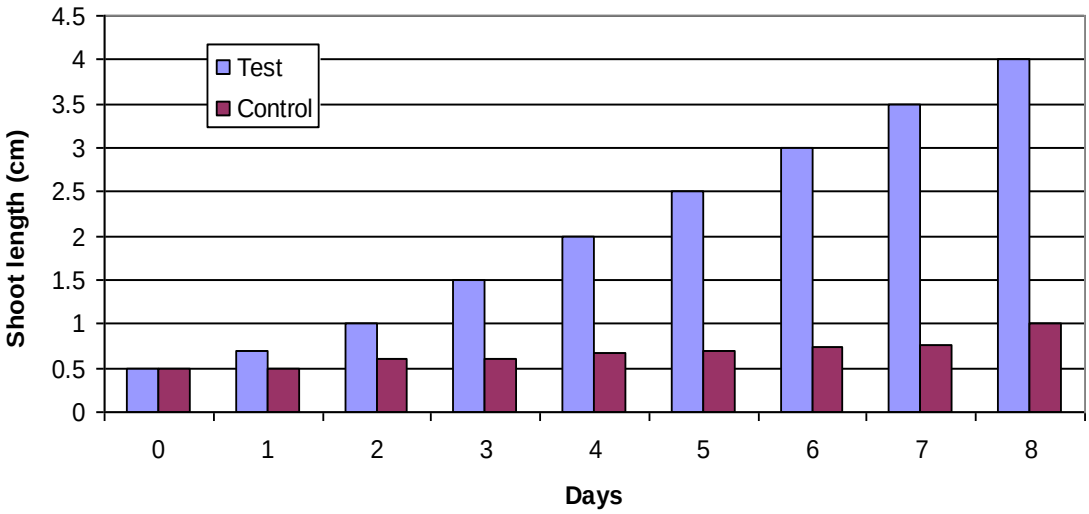

Figure 7. Difference in shoot length of *F. magellanica* in control and test samples over a period of eight days.

Figure 8. Root development in *Fuchsia magellanica* observed after one month of transfer to rooting media (A) as compared to control (B).

Acclimatization of *F. magellanica*

After successful *in vitro* rooting in *F. magellanica*, we performed acclimatization that is, planting the *in vitro* regenerated *F. magellanica* plants into the pots containing normal soil (Figure 9). This study shows that *in vitro* clonal propagation of *F. magellanica* can be more rapidly achieved that is, within three months as compared to the control (Figure 10) which showed only induction of leaves within the same period of time.

Conclusion

We have performed *in vitro* clonal propagation of *F. magellanica* and found that it is easier to grow and propagate *F. magellanica* in tissue culture lab as compared to the soil (control). Here, we also conclude that ¾ MS media is well suited as compared to ½ and standard MS media for the *in vitro* regeneration of *F. magellanica* from stem nodules explant. Additionally, for multiplication, elongation and root development, a

Figure 9. Acclimatization of *Fuchsia magellanica*.

Figure 10. *Fuchsia magellanica* showing initiation of leaf-formation after 25 days of transfer to soil.

combination of NAA and BAP, GA3 and NAA alone are required to be added into the ¾ MS media, respectively. All the results are verified by ANOVA, SPSS-16 as the calculated p-value is less than the alpha value that is, 0.05. Now, it is possible to grow *F. magellanica* rapidly in laboratory conditions with minimum hormonal supplementation.

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