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Effect of plant growth regulators on *Fittonia verschaffeltii* regeneration at *in vitro* conditions

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Abstract
We investigated *in vitro* regeneration ability of *Fittonia verschaffeltii* using different explants (leaf, node) and plant growth regulators ((2,4-D, indoleacetic acid, indolebutyric acid (IBA), 6-benzylaminopurine (BAP), thidiazuron)). Percentage of callus induction, number of shoots, shoot length, percentage of rooting and root length were measured. The leaf explant showed higher frequency of callus induction in comparison with the node explant, while node had higher frequency of shoot formation. There were significant differences among plant growth regulators in terms of percentage of callus induction, number of shoots, percentage of rooting and root length, however, the difference was dependent on the type of explant. The highest percentage of callus induction (86.12%) was obtained for the leaf explant at Murashige and Skoog (MS) medium supplemented with 1 mg/L 2,4-D and 1 mg/L IBA. The highest number of shoots (5.9 per explant) and shoot length (7.95 cm) were observed for the node explant at MS medium supplemented with 2 mg/L BAP + 1 mg/L IBA and MS medium containing 1mg/L BAP + 1 mg/L IBA, respectively. The highest percentage of root formation (87%) and root length (10.1 cm) were obtained at MS medium supplemented with 0.2 mg/L IBA and MS medium with no hormones, respectively.

Keywords: Callus induction; Explant; *Fittonia verschaffeltii*; *In vitro* culture; Plant growth regulator

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Introduction

Fittonia verschaffeltii is a tropical plant and native to Peru. It is an ever green plant and known as "mosaic plant" or "nerve plant". *Fittonia* is often used in aquariums but not suitable for this purpose. It is suitable as a house plant (Bercu and Popoviciu 2015).

Plant cell, tissue and organ culture is a strategy to improve genotypes under stress conditions and is useful in breeding programs (Krasensky and Jonak 2012). This technology is used to propagate plants and produce high quality seedlings and valuable secondary metabolites. It is also useful in genetic engineering technology (Mwangi *et al*. 2012; Neelakandan and Wang 2012). Tissue culture is an *in vitro* aseptic culture of cells, tissues, organs and whole plants under controlled conditions using different nutrients, pH, temperatures, etc. (Akin-Idowu *et al*. 2009; Thorpe 2012). Plant tissue culture methods have also been used for pathogen removal (Akin-Idowu *et al*. 2009).

Plant regeneration is performed either indirectly by production of callus or directly

without callus production. Direct regeneration is regarded as a better procedure than indirect regeneration, because the plants regenerated from indirect method may differ from the mother plant and have higher somaclonal variation (Trejgell 1998).

Regeneration of plants in *in vitro* condition is affected by genotype, explant source, plant growth regulator and culture condition. Therefore, studying the effects of these factors is utmost important (Yildiz *et al*. 2002). Explants with the ability to show totipotency such as immature embryos and inflorescence, young leaves, anthers, etc. are suitable for tissue culture. Young tissues and organs have high regeneration capacity (Jha *et al*. 2009). Dhar and Joshi (2005) reported that leaves were the best explant for shoot regeneration of *Saussurea obvallata* (DC) Edgew.

Studies in wheat have shown that the addition of plant growth regulators to culture medium increases shoot regeneration (Rashid *et al*. 2002). Addition of different combinations of plant growth regulators to culture medium has had different results. For example, application of auxins can be beneficial for root development (Akwatulira *et al*. 2011).

For the propagation of Fittonia, different methods are used including root dividing, seeding and stem cuttings, but these methods have encountered with problems such as low propagation rate, dependence on propagation season, inability to frill cuttings, time and space constraints and high costs. Therefore, the aim of this investigation was to optimize the direct regeneration of *Fittonia verschaffeltii* under *in vitro* conditions.

Materials and Methods

Plant materials

Samples of *Fittonia verschaffeltii* were collected and transferred to the laboratory. These samples were surface sterilized as the following: rinsing by tap water for 20 min, soaking in 70% ethanol for 45 seconds and washing with sterile distilled water. Then, they were submerged in mercuric chloride 1% (w/v) for 15 min and rinsed with sterile distilled water three times. After sterilization, the samples were cultured on MS medium (Murashige and Skoog 1962) without plant growth regulators and maintained in a growth chamber at 25 ± 1 ºC and 16 h light/dark photoperiod for 45 days.

Plant regeneration

The node and leaf explants were separated from sterile seedlings and transferred to MS medium with different types and concentrations of plant growth regulators. MS medium, supplemented with different concentrations of 6-benzylaminopurine (BAP), indolebutyric acid (IBA), thidiazuron (TDZ) and 2,4-D (3 mg/L BAP and 1 mg/L IBA, 1 mg/L BAP and 1 mg/L IBA, 1 mg/L TDZ and 1 mg/L IBA, 0.1 mg/L TDZ and 0.1 mg/L IBA, 0.1 mg/L 2, 4-D and 1 mg/L IBA, 1 mg/L 2,4-D and 1 mg/L IBA) was used in this study for plant regeneration. Also, MS medium supplemented with different concentrations of IAA (0, 0.2, 0.5 and 1 mg/L) and IBA (0, 0.2, 0.5 and 1 mg/L) were used for rooting.

Experimental design and data analysis

The shoot regeneration study was performed as a factorial experiment based on completely randomized design with four replications. The

rooting experiment was performed as completely randomized design with four replications. Means were compared by Duncan's multiple range test at the probability level of 0.05. Data analyses were performed using SPSS software.

Results

Effect of explant type and plant growth regulators on percentage of callus induction

Callus induction was observed 3-4 days after culture. Analysis of variance showed that mean squares for the type of explant, plant growth regulator and type of explant × plant growth regulator were significant in terms of percentage of callus induction. Although the leaf explant displayed higher percentage of callus induction (39.85%) than the node explant on the average of different plant growth regulators (Figure 1), but this difference was due to the application if 1 mg/L 2, 4-D and 1 mg/L IBA on this explant, which resulted in 86.12% callus induction (Figure 2). In other plant growth regulators, no significant difference was observed between the two explants (Figure 2).

Effect of explant type and plant growth regulators on number of shoots

According to the ANOVA results, number of shoots was significantly influenced by explant type, different concentrations of plant growth regulators and their interaction (Table 1). Although, the node explant produced higher number of shoots than the leaf explant on the average of different plant growth regulators in *F. verschaffeltii*, but this advantage was only observed at 3 mg/L BAP + 1 mg/L IBA and 1 mg/L BAP + 1 mg/L IBA. (Figure 3). Therefore, the highest number of shoots (5.9 shoots per explant) was obtained at MS medium containing 3 mg/L BAP and 1 mg/L IBA with the node explant (Figure 3).

Effect of explant type and plant growth regulators on shoot length

Shoot length was significantly affected by explant type, different concentrations of plant growth regulators and their interaction (Table 1). The node explant produced higher shoot length than the leaf explant on the average of different plant growth regulators (Figure 4). However, the significant superiority of the node explant was obtained only at 1 mg/L BAP + 1 mg/L IBA and 3 mg/L BAP + 1 mg/L IBA. (Figure 4). The highest shoot length in the node explant was 7.95 cm which was observed in MS medium containing 1 mg/L BAP +1 mg/L IBA (Figure 4).

Effect of explant type and plant growth regulators on percentage of rooting

The results showed that the percentage of rooting was significantly ($p \leq 0.01$) affected by plant growth regulators (Table 2). The percentage of rooting ranged from 31.25% to 87%. Between different concentrations and types of plant growth regulators, the highest percentage of rooting (87%) was obtained at MS medium supplemented with 0.2 mg/L IBA, which differed significantly from the control. By application of 0.2 mg/L IAA, the percentage of rooting increased as compared to the control, but this increase was not significant

Table 1. Analysis of variance of the effect of explant type and plant growth regulators on percentage of callus induction, number of shoots and shoot length of *Fittonia verschaffeltii.*

Source of variation	Degrees of freedom	Mean squares		
		Percentage of callus induction	Number of shoots	Shoot length
Explant (E)	1	247.50 **	32.013 **	56.33 **
PGR	5	247.51 **	13.605 **	24.215 **
E × PGR	5	247.50 **	32.610 **	24.215 **
Error	36	9.311	0.099	5.02

**: significant at p≤ 0.01; PRG: Plant growth regulator.

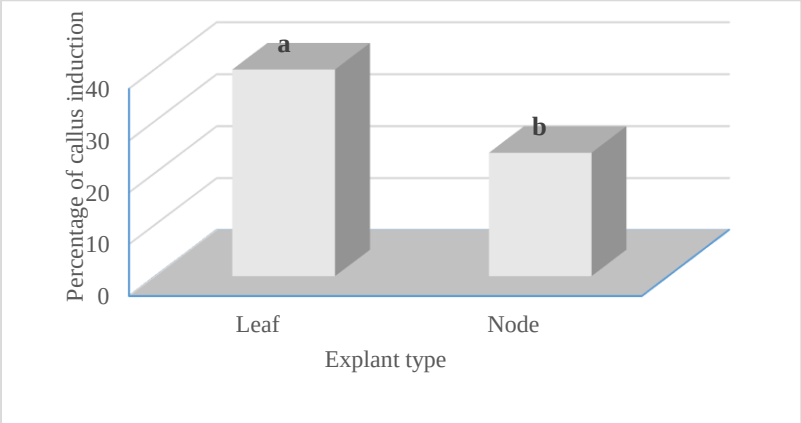

Figure 1. Effect of explant type on percentage of callus induction in *Fittonia verschaffeltii* tissue.

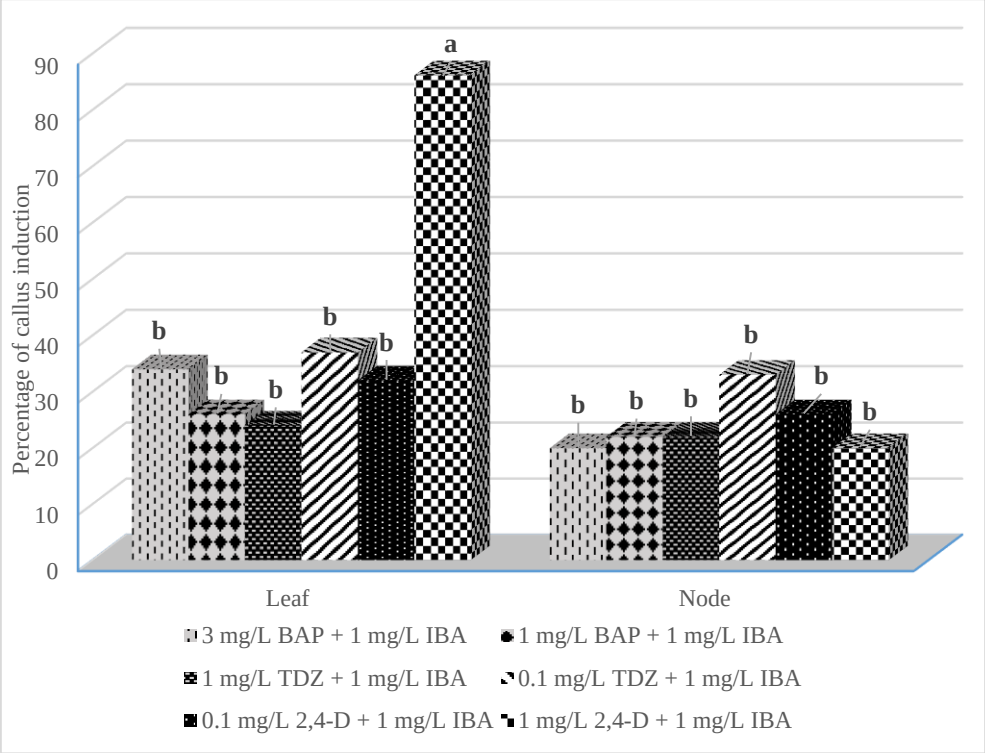

Figure 2. Effect of combination of explant type and plant growth regulators on percentage of callus induction in *Fittonia verschaffeltii* tissue culture.

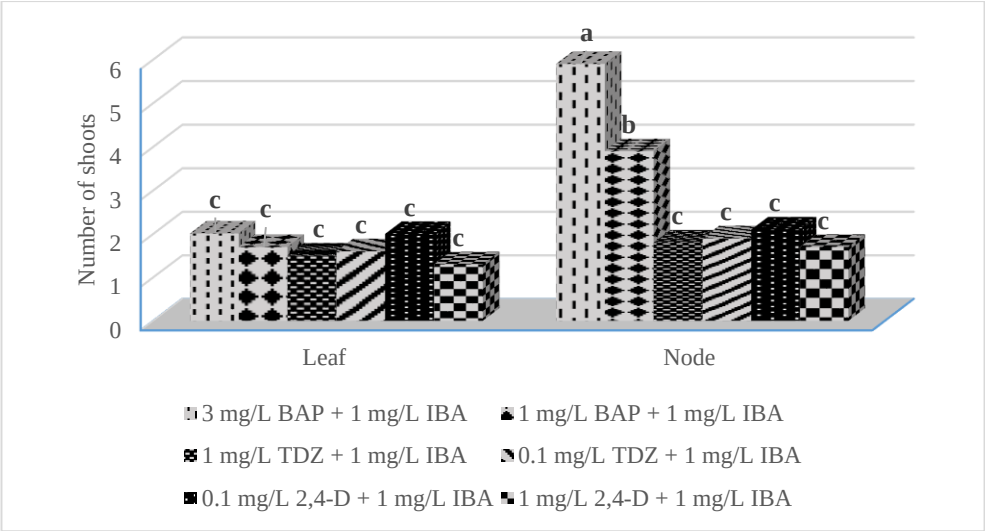

Figure 3. Effect of combinations of explant type and plant growth regulators on number of shoots in *Fittonia verschaffeltii* tissue culture.

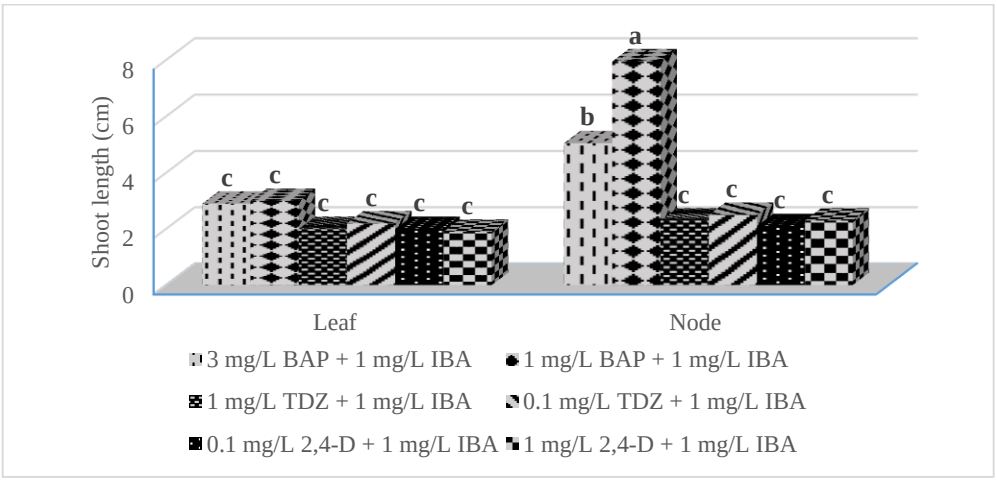

Figure 4. Effect of combination of explant type and plant growth regulators on shoot length in *Fittonia verschaffeltii* tissue culture.

Table 2. Analysis of variance of the effect of plant growth regulators on percentage of rooting and root length of *Fittonia verschaffeltii.*

Source of variation	df	Mean squares	
		Percentage of rooting	Root length
Plant growth regulators	6	1547.62 **	10.51 **
Error	21	156.25	0.275

**: significant at p≤ 0.01.

(Figure 5). Therefore, IBA is suitable plant growth regulator for root production in *F. verschaffeltii* tissue culture.

Effect of explant type and plant growth regulators on root length

Root length was significantly affected by different concentrations and type of plant growth regulators (Table 2). The highest root length was 10.1 cm that was obtained at MS medium without IAA and IBA. Thus, application of different concentrations of IAA and IBA had negative effect on root length. Root length decreased with increasing of IAA and IBA concentrations (Figure 6). Therefore, MS medium without PGRs is ideal for root growth of *F. verschaffeltii*.

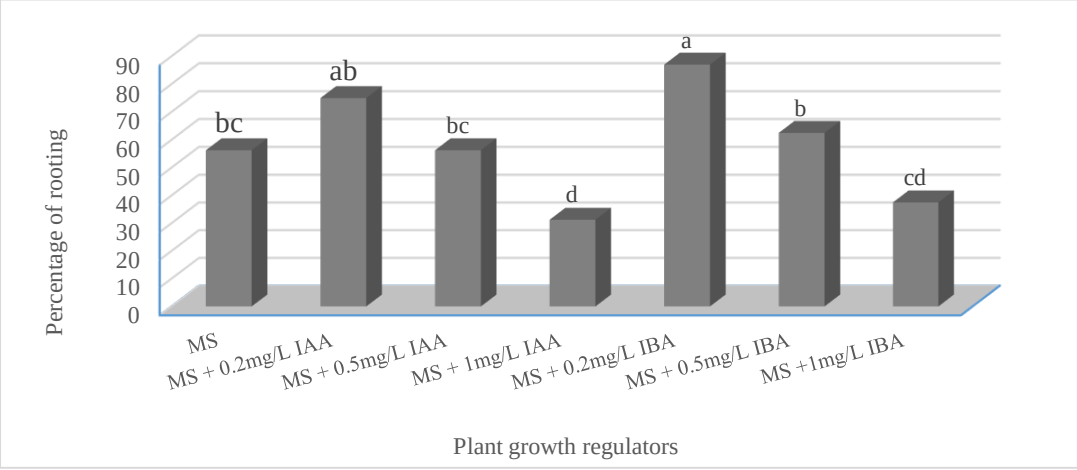

Figure 5. Effect of plant growth regulators on percentage of rooting in *Fittonia verschaffeltii* tissue culture.

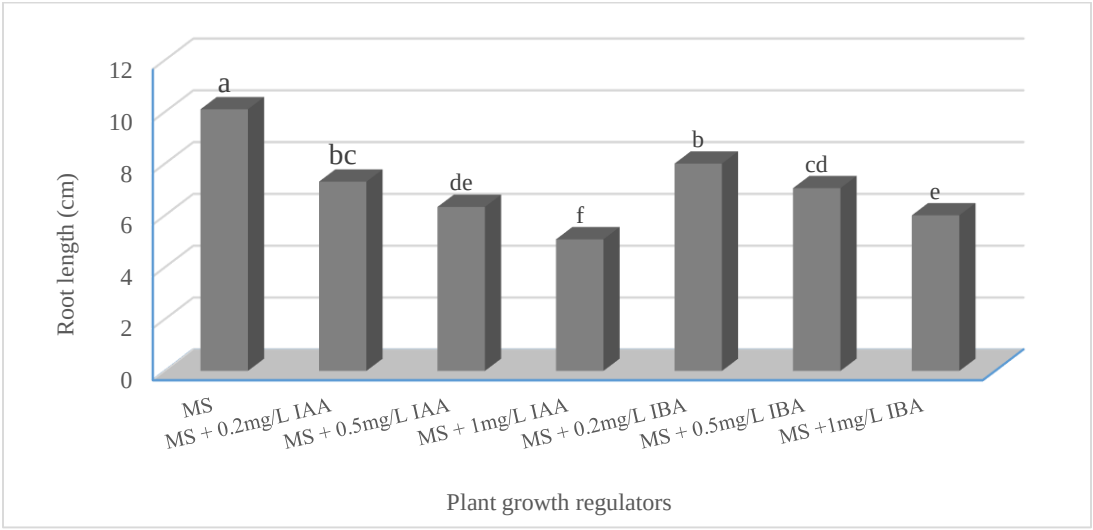

Figure 6. Effect of plant growth regulators on root length in *Fittonia verschaffeltii* tissue culture.

Figure 7. Direct regeneration of *Fittonia verschaffeltii.*

Discussion

It is known that regeneration protocol greatly affect the frequency of callus induction and *in vitro* regeneration of plants (Mendoza and Kaeppler 2002). For a successful genetic transformation of plants, optimization of the *in vitro* regeneration system and its efficiency is a required (Cheng *et al*. 2004). Selection of suitable explant and plant growth regulators significantly affects the callus induction and regeneration (Khawar *et al*. 2005). Therefore, plant growth regulators are important are regarded as an important factor affecting cell growth, differentiation and embryogenesis in plant tissue culture (Ren *et al*. 2010; Farjaminezhad *et al*. 2013). In this study, we used leaf and node explants for culture on media supplemented with different types and concentrations of plant growth regulators. The best result for callus production was obtained in the leaf explant at MS medium containing 1 mg/L 2,4-D and 1 mg/L IBA. Studies have shown that 2,4-D has higher auxin activity than other auxins and induce callus production and somatic embryogenesis (Machakova *et al.* 2008). According to Farjaminezhad *et al*. (2013), the highest percentage of callus induction in *Papaver bracteatum* was obtained on MS medium supplemented with 1 or 2 mg/L 2,4-D, 0.1 or 0.2 mg/L BAP and 15 mg/L salicylic acid. In this study, for direct regeneration and shoot and root production we used lower concentrations of auxins and higher concentrations of cytokinins (Figure 7). The highest number of shoots and shoot length were obtained in the node explant on MS medium supplemented with 3 mg/L BAP and 1 mg/L IBA. Also, the highest percentage of rooting observed on MS medium containing 0.2 mg/L IBA. Although

the presence of 2,4-D is essential for callus induction, but its existence in media reduces the ability of direct regeneration (Rout *et al*. 2006). Chauhan *et al*. (2007) reported that lower concentration of TDZ increases regeneration of *Triticum*. Parmar *et al*. (2012) used 1 mg/L 2,4-D and 1 mg/L TDZ in order to obtain the highest regeneration in wheat. Nas *et al*. (2010) showed that TDZ enhances explant regeneration ability in *Prunus microcarpa*.

Conclusions

The present study, provide an optimized protocol for *Fittonia verschaffeltii* micropropagation. Evaluation of different plant growth regulators and explants to achieve the highest percentage of callus induction, number of shoots and shoot length, has particular importance. Comparing the control treatment with the other media containing plant growth regulators showed that even a small amount of plant growth regulators resulted in higher performance than the medium without hormones. Observing rooting percentage and root length in *Fittonia verschaffeltii* shoots in the medium with plant growth regulators as compared to the non-hormone control indicated that the role of IBA (0.2 mg/L) in rooting percentage of *Fittonia verschaffeltii* is significant. However, the MS medium without plant growth regulator had a favorable effect on root length.

References

Akin-Idowu PE, Ibitoye DO and Ademoyegun OT, 2009. Tissue culture as a plant production technique for horticultural crops. African Journal of Biotechnology 8(16): 3782-3788.

Akwatulira F, Gwali S, Okullo JBL, Ssegawa P, Tumwebaze SB, Mbwambo JR and Muchugi A, 2011. Influence of rooting media and indole-3-butyric acid (IBA) concentration on rooting and shoot formation of *Warburgia ugandensis* stem cuttings. African Journal of Plant Science 5(8): 421-429.

Bercu R and Popoviciu D, 2015. Anatomy of *Fittonia verschaffeltii* (Lem.) Van Houtte (Acanthaceae). Annals of the Romanian Society for Cell Biology, 19(2): 23-28.

Chauhan H, Desai SA and Khurana P, 2007. Comparative analysis of the differential regeneration response of various genotypes of *Triticum aestivum*, *Triticum durum* and *Triticum dicoccum*. Plant Cell, Tissue and Organ Culture 91(3): 191-199.

Cheng M, Lowe BA, Spencer TM, Ye X and Armstrong CL, 2004. Factors influencing *Agrobacterium*-mediated transformation of monocotyledonous species. In Vitro Cellular and Developmental Biology-Plant 40(1): 31-45.

Dhar U and Joshi M, 2005. Efficient plant regeneration protocol through callus for *Saussurea obvallata* (DC.) Edgew.(Asteraceae): effect of explant type, age and plant growth regulators. Plant Cell Reports 24(4): 195-200.

Farjaminezhad R, Zare N, Asghari-Zakaria R and Farjaminezhad M, 2013. Establishment and optimization of cell growth in suspension culture of *Papaver bracteatum*: a biotechnology approach for thebaine production. Turkish Journal of Biology 37(6): 689-697.

Jha P, Yadav C, Anjaiah V and Bhat V, 2009. In vitro plant regeneration through somatic embryogenesis and direct shoot organogenesis in *Pennisetum glaucum* (L.) R. Br. In Vitro Cellular & Developmental Biology-Plant 45(2): 145-154.

Krasensky J and Jonak C, 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. Journal of Experimental Botany 63(4): 1593-1608.

Khawar K, Sarhin E, Sevimay C, Cocu S, Parmaksiz I, Uranbey S, Ipek A, Kaya M, Sancak C and Ozcan S, 2005. Adventitious shoot regeneration and micropropagation of *Plantago lanceolata* L. Periodicum Biologorum 107(1): 113-116.

Machakova I, Zazimalova E and George EF, 2008. Plant growth regulators I: introduction; auxins, their analogues and inhibitors. In: George EF, Hall MA and De Klerk GJ (eds.). Plant Propagation by Tissue Culture. Pp. 175-204. Springer.

Mendoza MG and Kaeppler HF, 2002. Auxin and sugar effects on callus induction and plant regeneration frequencies from mature embryos of wheat (*Triticum aestivum* L.). In Vitro Cellular and Developmental Biology-Plant 38(1): 39-45.

Murashige T and Skoog F, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15(3): 473-497.

Mwangi M, Ogero K, Mburugu G, Ombori O and Ngugi M, 2012. In vitro micropropagation of cassava through low cost tissue culture. Asian Journal of Agricultural Sciences 4(3): 205-209.

Neelakandan AK and Wang K, 2012. Recent progress in the understanding of tissue culture-induced genome level changes in plants and potential applications. Plant Cell Reports 31(4): 597-620.

Nas MN, Bolek Y and Sevgin N, 2010. The effects of explant and cytokinin type on regeneration of *Prunus microcarpa*. Scientia Horticulturae 126(2): 88-94.

Parmar SS, Sainger M, Chaudhary D and Jaiwal PK, 2012. Plant regeneration from mature embryo of commercial Indian bread wheat (*Triticum aestivum* L.) cultivars. Physiology and Molecular Biology of Plants 18(2): 177-183.

Rashid H, Ghani RA, Chaudhry Z, Naqvi SMS and Quraishi A, 2002. Effect of media, growth regulators and genotypes on callus induction and regeneration in wheat (*Triticum aestivum*). Biotechnology 1(1): 49-54.

Ren JP, Wang XG and Jun Y, 2010. Dicamba and sugar effects on callus induction and plant regeneration from mature embryo culture of wheat. Agricultural Sciences in China 9(1): 31-37.

Rout G, Mohapatra A and Jain SM, 2006. Tissue culture of ornamental pot plant: a critical review on present scenario and future prospects. Biotechnology Advances 24(6): 531-560.

Thorpe T, 2012. History of plant tissue culture. Plant Cell Culture Protocols 887: 9-27.

Trejgell A, Tretyn A and Nicos D, 1998. Attempt at regeneration of *Pharbitis nil* from fragments of vegetative organs. Acta Physiologiae Plantarum 20(2): 161-166.

Yildiz M, Özcan S and Er C, 2002. The effect of different explant sources on adventitious shoot regeneration in flax (*Linum usitatissimum* L.). Turkish Journal of Biology 26(1): 37-40.

تأثیر تنظیم‌کننده‌های رشد بر باززایی گیاه فیتونیا (*Fittonia verschaffeltii*) در شرایط درون شیشه‌ای

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چکیده

در پژوهش حاضر به بررسی توانایی باززایی گیاه فیتونیا با به کارگیری ریزنمونه‌های مختلف (برگ، گره) در غلظت‌های متفاوت تنظیم‌کننده‌های رشدی گوناگون ((2,4-D، ایندول استیک اسید، ایندول بوتیریک اسید (IBA)، ۶-بنزیل آمینو پورین (BAP) و تیدیازورون)) پرداخته شد. درصد القای کالوس، تعداد شاخساره، طول شاخساره، درصد ریشه‌زایی و طول ریشه یادداشت‌برداری شدند. تفاوت قابل ملاحظه‌ای میان تنظیم‌کننده‌های رشدی از لحاظ درصد القای کالوس، تعداد شاخساره، درصد ریشه‌زایی و طول ریشه وجود داشت. نتایج حاصل نشان داد که ریزنمونه برگ از بیشترین درصد القای کالوس در مقایسه با گره برخوردار است در حالی که گره بیشترین تعداد شاخساره را داشت. بیشترین درصد القا (۸۶/۱۲ درصد) مربوط به ریزنمونه برگ در محیط کشت MS حاوی یک میلی‌گرم در لیتر 2,4-D و یک میلی‌گرم در لیتر IBA بود. ریزنمونه گره در محیط کشت MS حاوی دو میلی‌گرم در لیتر BAP و یک میلی‌گرم در لیتر IBA دارای بیشترین تعداد شاخساره (۵/۹ در هر ریزنمونه) و در محیط کشت MS حاوی یک میلی‌گرم در لیتر BAP و یک میلی‌گرم در لیتر IBA دارای بیشترین طول شاخساره (۷/۹۵ سانتی‌متر) بود. بالاترین درصد ریشه‌زایی (۸۷ درصد) و طول ریشه (۱۰/۱ سانتی‌متر) به ترتیب در MS غنی شده با ۰/۲ میلی‌گرم در لیتر IBA و محیط کشت MS پایه به دست آمد.

واژه‌های کلیدی: القای کالوس؛ تنظیم‌کننده رشد؛ ریزنمونه؛ کشت درون-شیشه‌ای؛ *Fittonia verschaffeltii*