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Caulogenic response of *in vitro* raised nodal explants of *Orthosiphon stamineus* to selected auxins

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Auxin,
nodal explants,
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Abstract

The effect of auxins on *in vitro* raised nodal explants of *Orthosiphon stamineus* cultured in MS medium for the study of shoot initiation and elongation. Shoot induction and callus induction development depended on the type of explants (node, internode andleaf) while exposed to MS medium addition with different concentration of 2, 4-D, NAA and IBA plant growth hormones. Nodal explants showed shoot induction at 0.5 mg/l, 1.0 mg/l, and 2.0 mg/l concentration of 2, 4-D, IBA, and NAA. Among these hormones 2, 4-D showed higher (92.8%) shooting response with 18.2 numbers of nodes per shoot and 16.6 cm shoot height followed by NAA and IBA at 0.5 - 3.0 mg/l concentrations. Similarly other explants such as internode and leaf were inoculated in MS medium with the same concentrations of hormones for the identification of morphological changes. After 25 days, leaf and internode explants induced callus. Similarly maximum number of root induction was observed in ¼ MS medium with 1.0 mg/l concentrations of IBA. The present study claims that the effectiveness of hormone concentration and explants efficiency for the mass propagation of the *O. stamineus* plant.

Introduction

Auxins play an important role in stem initiation and elongation which involves in different features of growth and developments in higher plants. The auxins 2, 4-D are strong promoters of callus induction and growth of cell suspensions. There are only a few examples of the shoot and root induction by phenoxy auxins in tree tissue cultures (Zaerr and Mapes, 1982). For shoot induction generally requires the combination of auxin and cytokinin. However, auxin should be used carefully since too much auxin favors callus growth. Moreover, for shoot initiation in some explants, the production of endogenous auxin is sufficient for induction of shoots in larix decidue (Bondga and Von-Aderkas, 1992). The number of shoots induced on MS medium (Murashige and Skoog, 1962). The auxin signal is predictable by plant cells and rapidly converted to a wide variety of responses in the growth and development of plant organs. These comprise alters in the direction of growth, shoot and root branching, and vascular differentiation (Leyser, 2001).

The plant cell division and growth of tissue, cells cultured *in vitro* require an external source of auxin (Petrasek et al., 2002). The proportion of external to internal auxin concentrations is essential for regulation of the different stages of the standard growth cycle. The type of auxin used in the medium influences culture morphology (Hofmann, et al., 2004). Most of the plants naturally contain cytokinins such as 6-furfuryl-aminopurine, Ribosyl zeatin, Zeatin Isopentenyl adenine

and Dihydro zeatin. These endogenous cytokinins interact with exogenous auxin 2, 4-D may lead to shoot induction. Auxin pulse produced a clear effect improving regeneration (Pascual and Marin et al., 2005). Indeed, auxins obviously involved in morphological changes since it regulates plant cell division, elongation, and differentiation (Chen, 2001). The effect of a liquid 2, 4-dichlorophenoxyacetic acid (2, 4-D) influence on the adventitious regeneration of both shoots and roots in the regeneration medium containing 2, 4-D concentration (Pascual and Marin et al., 2005). Numerous alterations occur in plant morphology by lighting conditions and altering hormone composition in the regeneration medium (Gentile et al., 2002).

Orthosiphon stamineus is a medicinal plant belongs to Lamiaceae which is distributed mainly in South East Asian countries. This plant was reported to have secondary metabolites with biological activity properties; therefore, it has a great potential value for the development of this plant through *in vitro* propagation. This may help to develop an alternative documentary repository protocol for this plant. In the past, few micropropagation protocols have been developed using MS medium with an auxin-cytokinin combination of different concentrations for this plant; alternatively, this protocol may influence the ability to develop whole plants. The aim of this study was to develop a new protocol for the development of whole plantlets with more number of nodes in a single shoot using MS medium with different concentrations of auxins such as 2, 4-D, NAA and IBA (0.5 – 3.0 mg/l) on nodal explants of *O. stamineus*.

Materials and Methods

In vitro raised *O. stamineus* plantlets were excised into nodal explants by using sterile scalpel blade and forceps, and then inoculated aseptically on MS medium with 3% sucrose. The devoid of growth regulators were served as control and MS medium with (0.1 - 3.0 mg/l of 2, 4-D, NAA, and IBA separately) different concentration of auxins were served as a hormone-treated medium. The pH was adjusted to 5.7 and medium was solidified by adding 0.6% agar then autoclaved for 20 minutes at 121ºC. *In vitro* cultured nodal explants were used for all the treatments and five replicates were used. All the cultures were grown under a 16 h photoperiod by cool white fluorescent lamps with 48µmol.$m^{-2}s^{-1}$ photon flux density and 8 h dark condition at 25ºC. The maximum number of nodes, height of shootlets and rooting were recorded after 25 days of incubation period.

Data analysis

Shoot induction was recorded based on the percentage of nodal explants development on MS medium supplemented with different auxin concentration in the time period of 25 days of inoculation. A randomized statistical design was performed using SPSS 13 (SPSS Inc., Chicago, IL, USA) and Excel 2007 (Microsoft, Redmond, WA, USA) software. single factor ANOVA data was performed and analyzed the statistical significant and least significant differences (LSD) test was developed to compare means at $p<0.05$ level.

Results and Discussion

Caulogenic response

The *in vitro* raised nodal explants were inoculated on MS medium with a different concentration of each auxins (2,4-D, NAA and IBA) (Table 1). All the three auxins induced the nodal explant to generate single shoots rather than developing clump of multiple shootlets which is a normal response of cytokinin. However the nodal explants gave single shoot the shoot height is significant and remarkable outcome in this experiment. Of the various auxins used maximum shooting response (92.8%) and maximum shoot height (16.6 cm) with 18.6 mean number of nodes per shoot was obtained from the explants cultured on MS + 0.5 mg/l 2, 4-D (Table 1; Fig 1). It is obvious that only at low concentration of auxin regime was suitable for the production of elongated shoots with more number of nodes. Increase in the concentration of auxin reciprocally decrease the production of shootlet with remarkable height and also very few number of node per shootlet proportionally. Hormone free medium containing nodal explants showed less (5%) shoot induction response and at high concentration of auxin (3.0 mg/l) did not induce any shoot development; instead of that callus induction was developed along with small adventitious rootlets.

Table 1: Impact of auxins on caulogenic response of nodal explants cultured in MS medium and data were recorded after 25 days of inoculation.

MS + plant growth regulators	Plant growth regulators concentration (mg/l)	Shooting response (%)	Mean number of node per regenerated shoot ± S.D*	Shoot height (cm) ± S.D*
Control	0	5.0 ± 0.3	2.8 ± 0.5[f]	3.5 ± 0.5
2, 4-D	0.5	**93.0 ± 1.2**	**18.6 ± 0.6[a]**	**16.6 ± 0.4**
	1.0	80.0 ± 1.6	12.2 ± 0.5[b]	14.7 ± 0.4
	2.0	21.0 ± 0.7	6.8 ± 0.4[d]	4.8 ± 0.3
	3.0	-	-	-
NAA	0	4.8 ± 0.2	2.6 ± 0.5[f]	3.5 ± 0.2
	0.5	60.0 ± 0.9	13.0 ± 0.7[b]	4.9 ± 0.1
	1.0	35.0 ± 0.4	7.8 ± 0.4[d]	3.5 ± 0.2
	2.0	10 .0 ± 0.3	4.6 ± 0.5[e]	3.0 ± 0.7
	3.0	-	-	-
IBA	0	4.6 ± 0.5	2.6 ± 0.5[f]	3.2 ± 0.4
	0.5	32.0 ± 0.5	10.4 ± 0.8[c]	3.3 ± 0.2
	1.0	20.0 ± 0.4	7.2 ± 0.6[d]	2.3 ± 0.2
	2.0	5.0 ± 0.3	4.2 ± 0.5[e]	1.9 ± 0.1
	3.0	-	-	-

*Values are mean ± Standard Deviation (n = 5), dissimilar letters indicated significant differences between means within treatments at $p<0.05$ level based on LSD mean separation.

Other explants such as internodes and leaf did not show any shoot induction however callus induction was observed along with few adventitious rootlets. In our studies, shoot induction was observed in the nodal explants under the lower concentration of 2, 4-D. Similarly Popielarska et al., (2006) reported in *Brassica napus*. Cv. and Mello et al., (2001) in *Curcuma zedoaria*. The endogenous auxin produced on the apical shoot tip is used to be transported downward to the basal part of plants like stem and root region (Terasaka et al., 2005). At low concentration of 2, 4-D helps in shoot elongation and also produce more number of nodes per new single regenerated shootlet. Those *in vitro* raised nodes may be explored for the mass propagation of the plant by undergoing many sub or re-culture methods with short interval time.

The morphological changes on cultured tissue may be linked to the composition of the culture medium. It is recognized that culture conditions, as well as the plant genotype, have a high impact on shoot induction frequency (Hu et al., 1999). Shoot development was observed on hypocotyls cultured on 2, 4-D-supplemented media (Popielarska et al., 2006); (Khan et al., 2002). Our observations pointed out that the response of nodal explants in hormone-free medium and hormone (2, 4-D, NAA and IBA auxins) treated medium showed enormous differences in organogenesis for this plant. However, some shoots emerged in the nodal bud and become necrosis after few days of inoculation. Similar results were reported in bean (Angelini and Allavena, 1989). The decline of 2, 4-D concentration may lead to the shoot induction because novel genes are switch on for plant regeneration. This harvest may only be synthesized when exogenous auxin (2, 4-D) is greatly reduced from the medium (Michalczuk et al., 1992a); (Zimmerman 1993). In earlier studies, BAP hormone concentrations were reduced from 1.0 to 0.1 mg/l to avoid the poor effects of shoot induction by long term exposure to high concentrations (Vieitez et al., 1985; San-Jose et al., 1988; Chalupa, 1988). Similarly at low concentration of 2, 4-D hormone favors shoot elongation within short duration of time period.

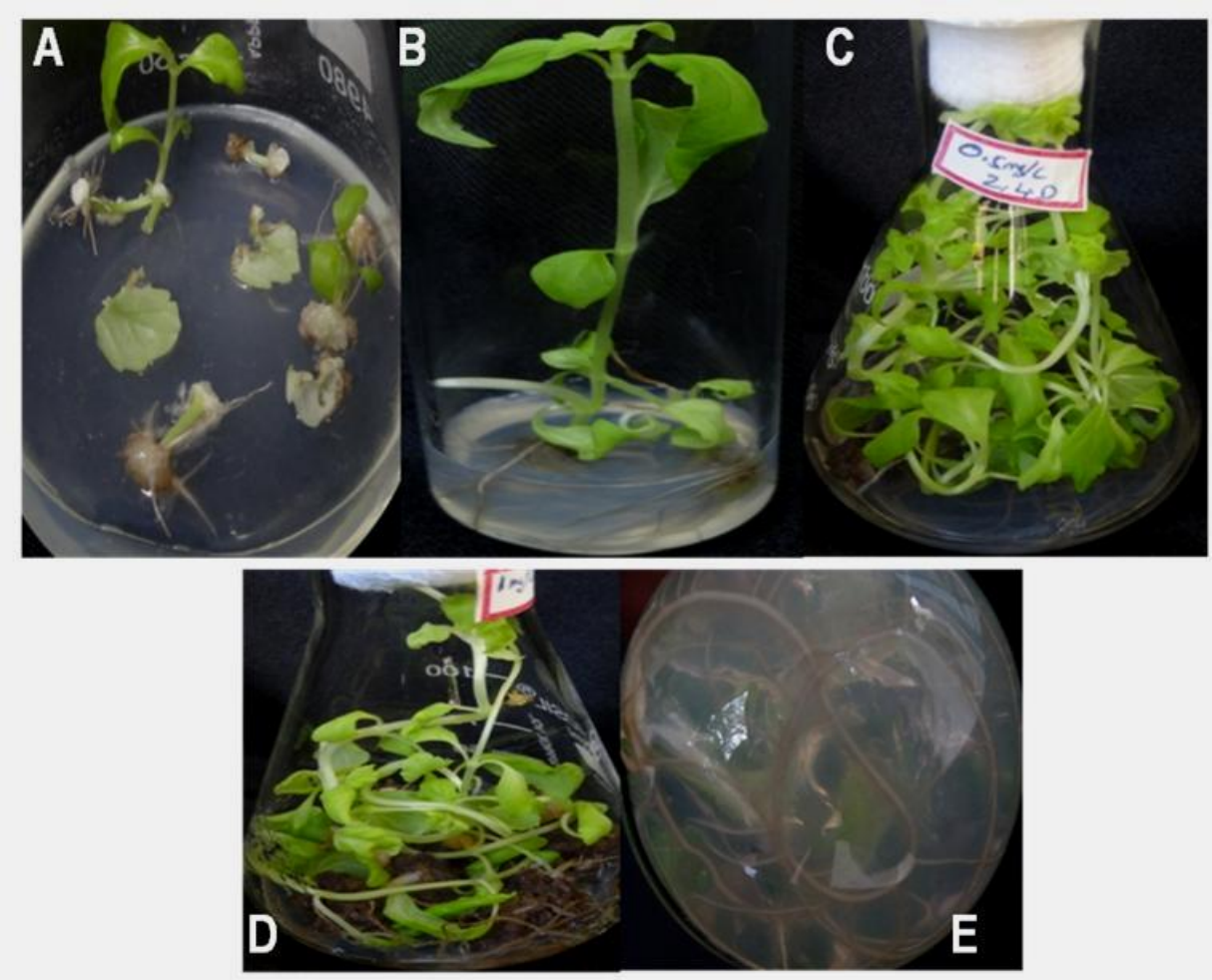

Fig: 1. **(A)** Shoot regeneration in nodal explants and callus induction in internode & leaf explants from *O. stamineus* cultured in MS medium (control). **(B)** Shoot regeneration at 0.5 mg/l 2, 4-D **(C)** Shoot elongation at 0.5 mg/l 2, 4-D **(D)** Shoot elongation at 1.0mg/l 2, 4-D. **(E)** hairy root induction at 1mg/l IBA.

Root induction

The regenerated shoots were transferred to ¼MS medium with auxins at 1.0 mg/l IBA induced 19.4 numbers of rootlets with 94% of rooting response. The role of explants and auxin hormone concentrations take part in an importance in the MS medium for the effectiveness of root induction or inhibition under suitable conditions (Eduardo, 1998). High root induction was developed in the IBA treatment for Ginseng (Choi et al., 1994). Thus, it was suggested that the exogenous supply of IBA induces more number of rootlet induction for this plant specious. Although at high concentration of auxins IBA, IAA and NAA (>3 mg/l) lead to decline of rooting which may be due to the herbicidal activity of auxins at high concentration (Evan et al., 2003).

Table 2: Shows the rooting response of the *in vitro* raised shootlet of *O. stamineus* cultured on ¼ MS medium.

¼ MS + PGRs	Conc. (mg/l)	Percentage of rootlet induction	Mean number of rootlets/ shoot ± S.D*	Root length (cm) ± S.D*
Control	0	21.0 ± 2.2	2.8 ± 0.2^{g}	1.6 ± 0.6
IAA	0.5	68.2 ± 2.4	9.0 ± 0.7^{de}	2.8 ± 0.4
	1.0	84.0 ± 2.2	16.8 ± 0.8^{b}	3.8 ± 0.4
	2.0	69.4 ± 1.9	8.0 ± 0.7^{e}	4.6 ± 0.5
	3.0	45.2 ± 1.4	5.2 ± 0.4^{f}	3.2 ± 0.4
IBA	0	20.4 ± 1.9	2.2 ± 0.4^{g}	1.8 ± 0.4
	0.5	71.0 ± 3.1	11.4 ± 0.9^{d}	4.6 ± 0.5
	1.0	**94.2 ± 2.4**	**19.4 ± 0.5^{a}**	**11.4 ± 0.9**
	2.0	70.4 ± 1.5	15.2 ± 0.8^{b}	8.4 ± 0.5
	3.0	51.2 ± 2.2	10.4 ± 0.6^{d}	5.0 ± 0.7
NAA	0	20.2 ± 1.4	2.6 ± 0.5^{g}	2.2 ± 0.4
	0.5	66.0 ± 2.5	8.2 ± 1.1^{e}	3.2 ± 0.4
	1.0	76.4 ± 3.2	13.6 ± 0.5^{c}	5.0 ± 0.7
	2.0	50.2 ± 1.8	6.4 ± 0.5^{f}	5.2 ± 0.4
	3.0	35.8 ± 2.3	5.0 ± 0.7^{f}	3.6 ± 0.5

*Values are mean ± Standard Deviation (n = 10), dissimilar letters indicated significant differences between means within treatments at $p<0.05$ level based on LSD mean separation.

In conclusion, we found that 2, 4-D involves more effective to develop shoot induction and elongation at an optimized concentration in *O. stamineus*. These studies have shown that the threshold level of 2, 4-D for shoot induction seems to be 0.5-1.0 mg/l. Within this range, the explants cultured in lower concentration of 2, 4-D in MS medium to develop more number of nodes in a single shootlet which favours subculture to enhance the rate of mass propagation within short duration of time.

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References

1. Angelini, R. R. and A. Allavena; 1989. Plant regeneration from immature cotyledon explant cultures of bean (*P. coccineus* L.). Plant cell, tissue and organ cul., 19(2): 167-174.
2. Bonga, J. M and P. Von Aderkas; 1992. *In Vitro* Culture of Trees. Kluwer Academic Publishers., Dordrecht, 236 p.
3. Chalupa V.; 1988. Large scale micropropagation of *Quercus robur* L. using adenine-type cytokinins and thidiazuron to stimulate shoot proliferation. Biologia Plantarum (Praha)., 30(6): 414-21.
4. Chen, J. G.; 2001. Dual auxin signaling pathways control cell elongation and division. Journal of Plant Growth Regulation., 20(3): 255-264.
5. Choi, K. T.; Ahn, J.O. and J.C. Park; 1994. Production of Ginseng Saponin in Tissue Culture of Ginseng. Russian Journal of Plant Physiology., 41: 784-788.
6. Eduardo, S.V.; 1998. In Vitro Root Induction and Plumule Explants of *Helianthus anmus*. Environmental and Experimental Botany., 39: 271-277.
7. Evans, D. E.; Coleman, J. O. D and A. Kearns; 2003. Plant Cell Culture. Bios scientific publishers., London and New York.
8. Gentile, A.; Monticelli S. and C. Damiano; 2002. Adventitious shoot regeneration in peach (*Prunus persica* (L.) Batsch). Plant Cell Rep., 20(11), 1011-1016.
9. Hofmann, N.; Nelson. R. L. and S. S Korban; 2004. Influence of media components and pH on somatic embryo induction in three genotypes of soybean. Plant cell, tissue and organ cult., 77(2): 157-163.
10. Hu, Q.; Andersen. S. B. and L. N Hansen; 1999. Plant regeneration capacity of mesophyll protoplasts from *Brassica napus* and related species. Plant cell, tissue and organ cult., 59(3): 189-196.

11. Khan, M. R.; Rashid, H., and A. Quraishi; 2002. Effect of various growth regulators on callus formation and regeneration in *Brassica napus* Cv. Oscar. Pakistan J. Biol., Sci. 5 (6), 693–695.
12. Leyser, O.; 2001. Auxin signaling: the beginning, the middle, and the end. Current opinion in Plant Biology., 4(5): 382-386.
13. Mello, M. O.; Melo, M. and B. Appezzato-da-Glória; 2001. Histological analysis of the callogenesis and organogenesis from root segments of *Curcuma zedoaria* Roscoe. Brazilian Archives of Biology and Technology.,44(2): 197-203.
14. Michalczuk, L.; Cooke, T. J. and J. D. Cohen; 1992. Auxin levels at different stages of carrot somatic embryogenesis. Phytochemistry., 31(4): 1097-1103.
15. Murashige, T. and F. Skoog; 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15(3): 473-497.
16. Pascual, L and J. A. Marin; 2005. A liquid 2,4-D pulse increased shoot and root regeneration from leaf explants of adult *Prunus* rootstocks. Scientia Horticulturae 106(4):582-592.
17. Petrasek, J.; Elckner, M.; Morris, D. A. and E. Zazimalova; 2002. Auxin efflux carrier activity and auxin accumulation regulate cell division and polarity in tobacco cells. Planta., 216(2): 302-308.
18. Popielarska, M.; Ślesak, H.; and G. Góralski; 2006. Histological and SEM studies on organogenesis in endosperm-derived callus of kiwifruit (Actinidia deliciosa cv. Hayward). Acta Biol Cracov Ser Bot 48(2):97–104.
19. San-Jose M. C.; Ballester A.; A. M. Vieitez; 1988. Factors affecting in vitro propagation of *Quercus robur*. Tree Physiology 4, 281-90.
20. Terasaka, K.; Blakeslee, J. J.; Titapiwatanakun, B.; W.A. Peer.; A.; Bandyopadhyay, A. and S.N. Makam; 2005. PGP4, an ATP Binding Cassette P-glycoprotein, Catalyzes auxin transport in *Arabidopsis thaliana* Roots. Plant Cell., 17: 2922-2939.
21. Vieitez AM, Carmen San-Jose M, Vieitez E. 1985. In vitro plantlet regeneration from juvenile and mature *Quercus robur* L. Journal of Horticultural Science 60(1), 99-106.
22. Zaerr, C and M. O. Mapes; 1982. Action of growth regulators. In: Bonga JM, Durzan DJ, editors. Tissue culture in forestry. Dordrecht: Martinus Nijoff Publishers.,pp. 231–255.
23. Zimmerman, J. L.; 1993. Somatic embryogenesis: a model for early development in higher plants. The Plant Cell., 5(10): 1411–1423.

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