Microprogation And Environment Conditions Affecting On Growth Of *In Vitro And Ex Vitro* Of A. *Formosanus* Hay

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SUMMARY

The goal of this research was to develop the effectiveness of in vitro culture method for *A. formosanus* and study the environment *in vitro* conditions affecting on growth.

The first series of experiments were examined to investigate the response of three different basal media, MS (Murashige and Skoog, 1962), Knudson (KC; Knudson, 1946) and modified hyponex on growth and multiplication during *in vitro* culture. Multiple shoot proliferation was induced in shoot tip explants on Hyponex (H3) media supplemented with BA (1 mgl^{-1}) or TDZ (1-2 mgl^{-1}). Addition of activated charcoal (1%) to the TDZ containing medium promoted rapid shoot tip proliferation (11.1 shoots per explant) but the same medium had an opposite effect resulting in poor proliferation in the nodal explants. However, the regenerated shoots had slow growth rate and failed to elongate. This problem was overcome by transferring the shoot clumps to a hormone free H3 media supplemented with 2% sucrose and 0.5% activated charcoal. Using bioreactor culture for scaling up was also shown the best way for multiple shoot induction and growth of this plant.

The second series of experiments was studied to investigate the effect of physical environment factors on growth of *in vitro* plantlets. The Anoectochilus formosanus plantlets were cultured under different air exchange rate (0.1, 0.9, 1.2h^{-1}), without sucrose or supplement 20$g.l^{-1}$ (photoautotrophic or photomixotrophic, respectively), and different photosynthesis photon flux (40, 80, 120 $\mu mol.m^{-2}.s^{-1}$- PPF). Under non-enrichment CO_2 treatment, slow growth was observed in photoautotrophical condition as compared with photomixotrophical condition on shoot height, fresh weigh and dry weight parameters; High air exchange (1.2.h-1) was

found to be inadequate for plant growth in photomixotrophical condition. On the contrary, under CO_2 enrichment treatment, the plant growth parameters were sharply (visibly) improved on photoautotrophic treatments, especially on the treatment with air exchange rate of 0.9.h-1. The growth of plant in photoautotrophic condition was not inferior compared with photomixotrophic, and the best growth of plantlet was observed in treatment with low air exchange rate (0.9.h-1). Raising the PPF level from 80 to 120 $\mu mol.m^{-2}.s^{-1}$ decreased the plant height, particularly at 120 $\mu mol.m^{-2}.s^{-1}$ in photoautotrophic condition, fresh weight and dry weight declined noticeably. At the PPF of 120 $\mu mol.m^{-2}.s^{-1}$, chlorophyll contents lowed compared to those grown under low PPF but time courses of net photosynthesis rate was decreased noticeably.

Light quality mainly affected morphological variables, changes of light quality also positively affected biomass production via changes in leaf area, stem elongation, chlorophyll content. Plant biomass was reduced when *A. formosanus* were grown under red LEDs in the absence of blue wavelengths compare to plants grown under supplemental blue light or under fluorescent light. Stem elongation was observed under red and blue light in the present experiment. Smaller leaf area has found under blue light than with other lighting treatments. Chlorophyll degradation was more pronounced in red and blue light compared with white light or red plus blue light which consequent affected the photosynthetic capacity of the plant.

The third series of experiment were studied to investigate the effect of physical environment factors on growth of ex vitro plants including photosynthesis photon flux (PPF), light quality, growing substrates, electrical conductivity (EC) and humidity conditions. In the present experiments, response of plant on PPF and light quality was similar *in vitro* plants under photosynthesis photon flux 40 μmol.m-2.s-1 and white light or blue plus red lights were the best growth. Substrates testing results were indicated coco-peat or peat moss were good substrates for *A. formosanus* growth under the greenhouse conditions. In case of *A. formosanus* plants, EC is generally maintained in the range 0.7 to 1.5 dS.m-1 was shown best results in growth of this plant. Keeping high humidity over 70% under low radiation enhanced growth rate and mass production.