J. Phytol. Res. 9 (1): 89–93, 1996

MORPHOGENETIC AND BIOCHEMICAL VARIATIONS UNDER DIFFERENT SPECTRAL LIGHTS IN LEAF CULTURES OF *VIGNA ACONITIFOLIA*

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Under the influence of blue, green, red and yellow lights the leaf cultures of *Vigna aconitifolia* displayed morphogenetic and biochemical variations. In the cultures incubated under various monochromatic light rhizogenesis occurred prior to the shoot initiation as compared to control cultures in which rhizogenesis followed shoot initiation. Red spectrum was found to be most supportive for rhizogenesis. Red and yellow spectra enhanced peroxidase, acid phosphatase, GPT and GOT activity and proline and phenolics level during organogenesis.

Keywords: Biochemical variation; Monochromatic light; Rhizogenesis; Organogenesis; *Vigna aconitifolia*.

Introduction

Sachs[1] laid the foundation of research on light influence on physiological process with his experiments on the etiolation of stem cuttings as means of obtaining better rooting. There have been a number of investigations of differential rooting ability of cuttings taken at different seasons[2]. In most investigations of this kind the seasonal fluctuation in root formation could result from changes in light parameters of quality and duration. The effect of light on IAA metabolism which controls free IAA levels could have a profound consequence in plant growth[3]. Parish[4] reported differential effect of light on the specific activity of peroxidase extracted from different tissues of wheat plant; light increases the enzyme activity in the coleoptiles but decreases it in the first leaves. The present investigation was undertaken to compare the effect of different spectral light on the cultures and to identify the suitability of a specific spectrum for a particular response.

Meterial and Methods

The seeds of *Vigna aconitifolia* (Jacq) Marechal var. Jwala were surface sterlized with 0.1% mercuric chloride solution for 2-3 minutes and were rinsed 3-4 times with sterile distilled water. These seeds were transferred aseptically to the paper bridges in the culture tubes (autoclaved) containing distilled water. Leaves from 7-8 day old seedlings were used as explants. The explants were cultured on MS medium[5] containing 3.0 mg/l of kinetin (KIN) and 1.0 mg/l of indole-3-acetic acid (IAA) and were incubated under blue, green, red and yellow spectrum. The light treatments were given by wrapping the culture flasks with cellophane papers. The transmissions maxima of blue, green, red and yellow cellophane papers as checked by Pye Unicam Spectrophotometer were 470, 525, 645 and 595 nm respectively.

For biochemical analysis callus was homogenized in appropiate buffers and centrifuged at 10,000 Xg for 20 min in refrigerated centrifuge. The supernatant thus collected was used for protein estimation and enzyme assay. Peroxidase activity was measured by guaiacol -H_2O_2 method[6], acid phosphatase by using p-nitrophenyl phosphate as substrate[7], glutamate oxaloacetic transaminase (GOT) and glutamate pyruvic transaminase (GPT) by Bergmeyer's[8] method. Protein, proline and phenolics were estimated by the methods of Bradford[9], Bates, Waldren

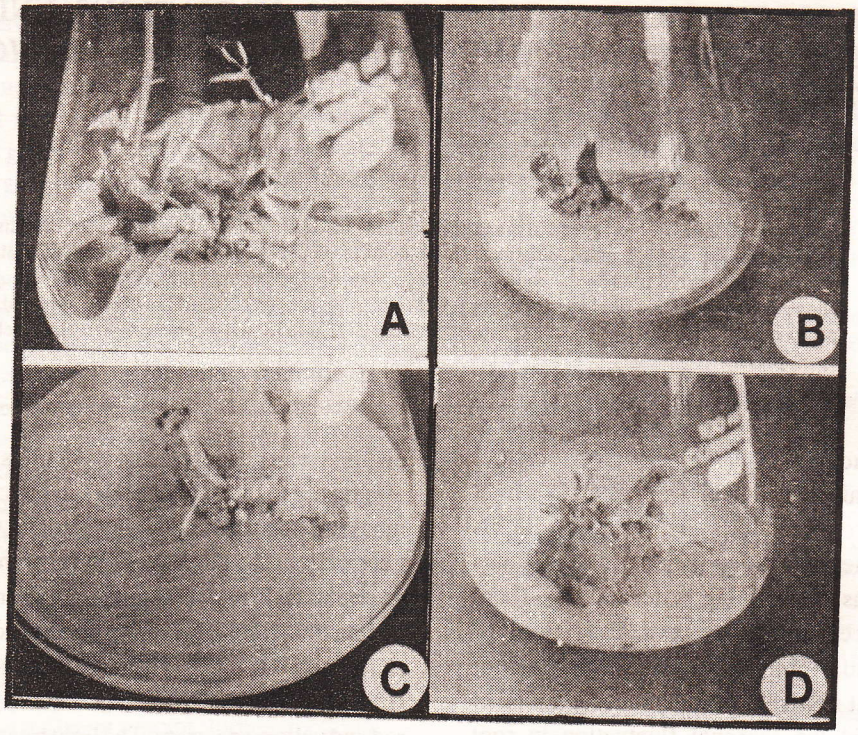

Figure 1. Plant regeneration in leaf cultures of *V. aconitifolia* incubated under : (A) Blue light (B) Green light (C) Red light (D) Yellow light

and Teare[10] and Bray and Thorpe[11] respectively. Results are averages of two extractions each with two replicates.

Results and Discussion

Observations on morphogenesis in leaf cultures revealed that control cultures displayed callus initiation after two weeks of incubation. The callus was green and compact. Shoot buds were initiated during 4th week and root formation during 6th week of incubation. Blue and yellow spectra evoked callus and shoot bud formation similar to control spectrum while the rhizogenesis was initiated in 3rd week. The remarkable difference between the cultures under blue and green lights was that the blue light was supportive for rooting and green for callus formation. Cultures under red spectrum displayed enhanced callus formation and roots were initiated in the 3rd week, while the shoot bud formation was evident in the 4th week of incubation. The roots were nodulated in the cultures incubated under yellow and red spectrum and were thinner in the cultures under yellow spectrum (Fig. 1 C,D).

The enzyme activities for peroxidase and acid phosphatase was highest in cultures under red and yellow lights during advanced root formation and shoot bud regeneration (Fig. 2A,B). In the present investigation red and yellow lights specifically promoted nodulated rooting. This suggests the

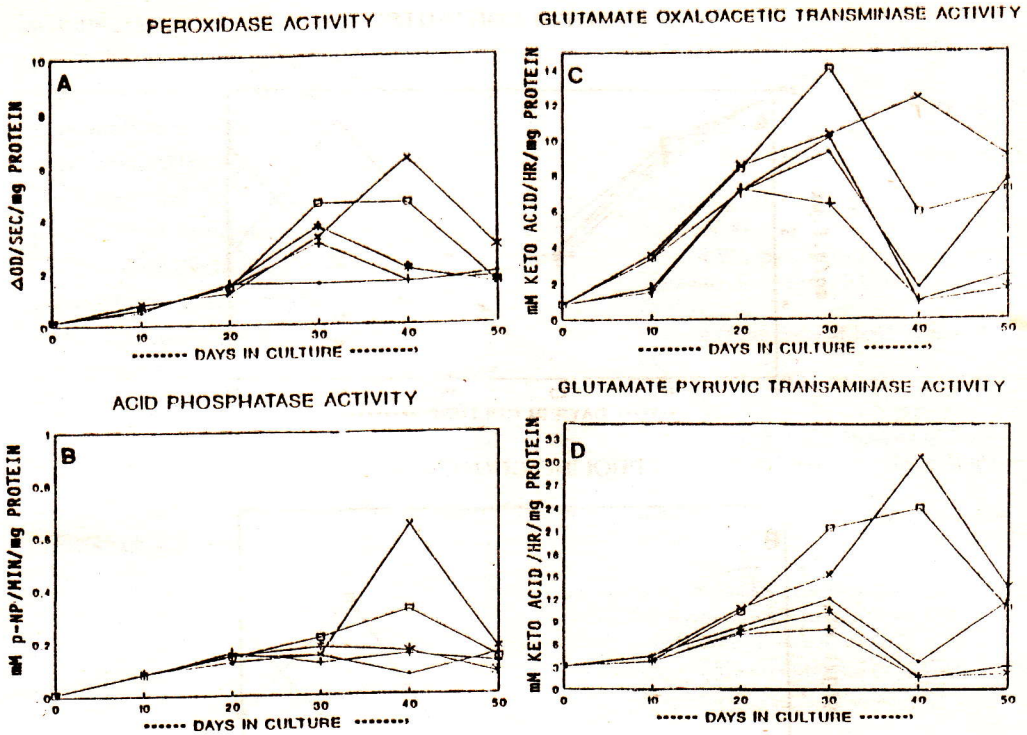

Figure 2. Effect of monochromatic light on enzyme activities under :
(•) Controlled condition (+) Blue light (*) Green light (□)Red light and (×) Yellow light.

involvement of these spectra in promoting rhizogenesis. It was demonstrated by Molnar and LaCroix[12] that increased activity of peroxidase and several other enzymes in the tissues is responsible for root initiation. The enhanced activity of acid phosphatase during root and shoot formation under red and yellow lights could be attributed to its possible involvement in destroying membranes and structural proteins. Its activity was found to be highest in the meristematic tissues. This further supports the role of red and yellow spectra in rooting. However, it is not yet known whether light increases the enzyme levels or activates the enzyme. The activity of GOT was highest in the 4th week in cultures under red and yellow light (Fig 2C). Similar pattern of activity was evident in the 5th week for GPT (Fig 2D). The transaminases, aspartate and alanine are important enzymes as the make amino acids available for protien synthesis. The role of these enzymes in protein metabolism is indicated by their increased activity in the 4th and 5th week during rhizogenesis and shoot formation. The increased level of protein during this period supports this notion (Fig. 3A).

High proline contents in red and yellow light treated cultures indicate the probable involvement of proline during organogenesis in cultured tissues (Fig. 3B). Better root and shoot formation in cultures with high proline

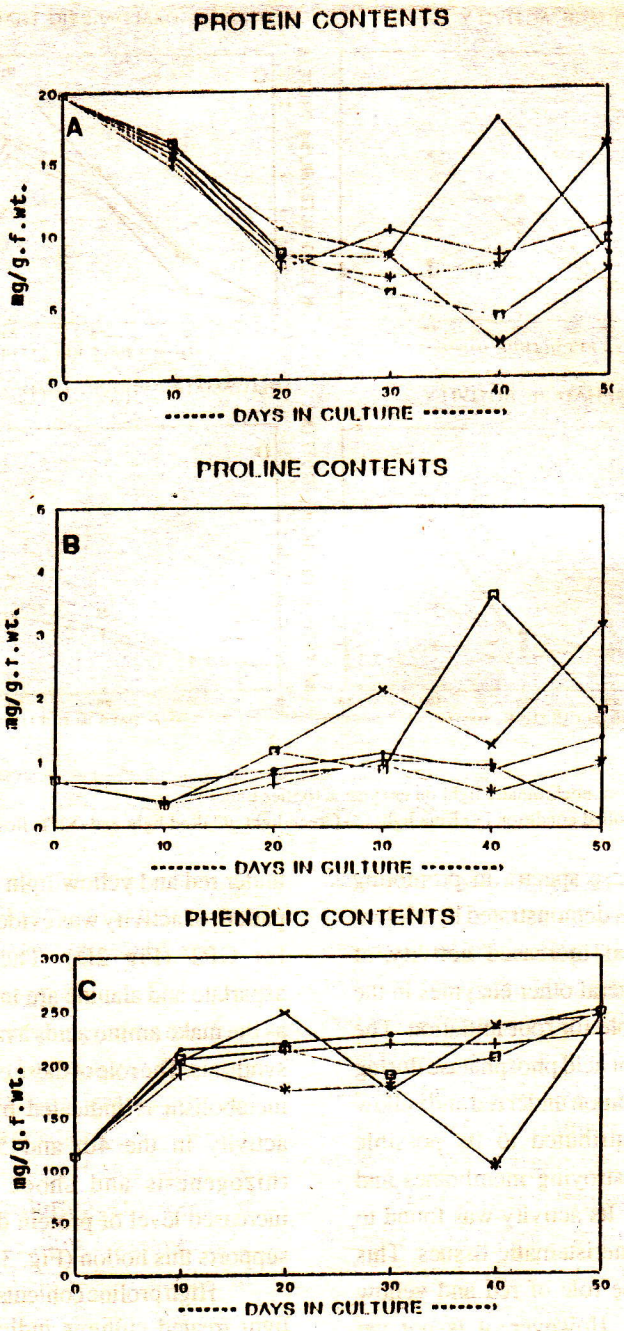

Figure 3. Effect of monochromatic light on metabolites accumulation under :
(•) Controlled condition (+) Blue light (*) Green light (□) Red light and (x)Yellow light.

accumulation support this view. The phytochrome mediated growth response is very sensitive of IAA[3]. Many reports have indicated that the effect of light on IAA oxidase was mediated through the induced changes in cofactors and inhibitors which involved phenolic compounds. In the present investigation phenolic compounds displayed elevated levels under red and yellow spectra which possibly increased rooting and it was minimum under green spectrum where rooting was not so profound (Fig 3C). The influence of light could vary because of complexity and diversity of phenols in plants.

These observations of light quality effects, clearly indicates that key organogenetic processes in tissue cultures are phytomorphogenic phenomena.

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