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*Research Paper*

# MORPHO-BIOCHEMICAL ASPECTS AMONG ALBINO TO NORMAL PLANTS OF SUGARCANE DEVELOPED IN ITS CALLUS CULTURE

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**Abstract**

In this experiment, a comparative morpho-biochemical analysis was conducted in albino plants when these abnormal forms were regenerated through callus culture of sugarcane (*Saccharum officinarum* L) cv., SPC-79 along normal plantlets. After plant hardening, both albino and normal plants were transferred to green house for further growth. After 3-months, plant growth rate and its biomass was observed higher of normal plants. Chlorophyll contents (a & ab) were also higher in normal, while total carotenoids were significantly higher ($p$<0.05) and chlorophyll b non-significantly high in albino plants. Reducing sugars were higher significantly in albino plants. According to XRF studies Si, Cl, Ca, Mg, Fe, S, Na, Al and Br were remained higher ($p$<0.05) in albino than normal plants, while concentration of Cu also higher in albino plants non-significantly. Differential accumulation of certain elements in the cell could be involved to induce deficiency of green pigments in albino plants. Lake of green pigmentation in albino plants causes mutation in the cell at protein level (epigenetic mutation) due to the occurrence of imbalance concentration of metallic ions in the cell.

## INTRODUCTION

Sugarcane is a major sugar industry as well as important cash crop of domestics. There is constantly tried to increase its output but a number of biotic and abiotic stresses has been causing to reduce yield of this crop. Similarly, changing climate of soil (increasing salinity, drought, temperature and rainfall) has been minimizing crop yields [1]. The crops with limited yields are manageable through reverse genetics approaches [2]. However exploitation of natural or artificial genetic diversity induction is a beneficial strategy for the improvement of major human food crops, while mutagenesis remains a valuable approach to create novel variation in those crops mainly with restricted genetic bottle-neck. Historically mutagenesis has been involved to screen and select individual plants with improved agronomic traits [3]. Although mutation approach has remained very successful for the improvement of potential crops (@ < 0.1%), while

mostly limitations are imposed like as non-detectable novel phenotypes, genetic redundancy due to genetic duplication and polyploidy among the plant species.
Meanwhile aseptic culture is also a source for development of genetically variable clones with characteristics of interest as well as abnormal cultures like as albino plants developed through long term callus culture that are rarely observed among the regenerated plantlets [4,5,6]. Due to lack of biosynthesis of photosynthetic pigments they are unable to prepare their food, normally survive but usually die in their seedling stage [7]. It is therefore difficult to study its growth pattern and biochemical analysis. Much hindrance for the study of growth and development associated with defective chloroplasts [8]. The green plants grew faster than the albinos. The slower growth of albinos apparently was not the result of carbohydrate deficiency, as it could not be corrected by increased sucrose. Growth of the albinos was also not improved by supplementation with various amino acids, growth hormones and trace elements. In early research it was suggested that albinism can be improved only with genetic manipulation (gene insertion).
Albinism among the cultured cells might be happened because of the change in protein structures or interactions among them during the building of the cellular structures. Each genetic makeup direct the accumulation of specific quantity of organic and inorganic characters in the genotype. Similarly, there accumulation of specific ratios of bio-components in the developed plantlets may lead to develop specific phenotypes in the developed individual (epigenetic phenomena). This study was conducted by keeping in mind above mentioned literature. Both albino and normal plantlets were developed unconsciously during the establishment of in-direct plant regeneration culture system. Comparative morpho-biochemical parameters of the regenerated normal or green and albino plants were studied, when plants were reached to 3 months old in green house.

## MATERIALS AND METHODS

Indirect plant regeneration of sugarcane (*Saccharum officinarum* L.) cv., SPC-79 was established. Almost 40 trans-sections of youngest leaf tips from proximal end of five months old open air field grown plant were excised. They were sterilized in 30% Robin bleach on magnet stirrer for 30 minutes than washed with sterile distilled water for three times in laminar air flow cabinet. Sterilized explants were cultured on MS (Murashige and Skoog) medium supplemented with $B_5$ vitamins complex [9, 10], 3 mg $L^{-1}$ 2,4-D for 4-weeks. Multiplied calluses were cultured on plant regeneration medium (MS, 2 mg $L^{-1}$ BA, 1 mg $L^{-1}$ IAA, 2 mg $L^{-1}$ Kinetin).
A large number of shoots were developed and they were counted on the basis of appearance of plantlets (normal or green and albino or chlorophyll mutated). Regenerated plantlets were rooted on MS medium supplemented with 1 mg $L^{-1}$ IBA (indole butyric acid) at 28$^{o}$C with 16/8 day and night photoperiod for 10 days. Rooted plantlets were subjected to plant hardening in a mixture of sand and loamy (3:1) soils in plastic glasses and covered with polythene bags for 1-week. More than 80 % humidity was raised by spraying water to inner side of the polythene bag with syringe. Acclimatized plants were shifted to tissue culture green house for further growth to study various morpho-biochemical studies.
When plantlets were become almost 3-months old than plant height was measured. Two youngest leaves (2$^{nd}$ and 3$^{rd}$) from top to bottom of both normal and albino plants were excised and then weighed. These leaves were dried in electric oven at 80$^{o}$C for 72 hour. Relative water contents were calculated in collected samples [11]. Oven dried leaves were grinded to fine powder, kept in furnace Gold Star- 7122 and muffle furnace

temperature was increased gradually up to 600°C for 6 hours. Obtained sample was weighed than ash contents (%) were measured [12].

Photosynthetic pigments like as chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (chl ab) contents and total carotenoids were determined by following Shabala *et al*., [13] and Lichtenthaler [14] methods respectively. Exact 100 mg of fresh leaf tissues were collected and homogenized in 10 mL acetone (95.5 %) than placed in dark for minimum 6 hrs. The chl a, chl b, chl ab contents and total carotenoids were measured with spectrophotometer (UV-visible) against 95.5 % acetone (blank).

Dried plant material was used for XRF (X-ray fluorescence) analysis by $S_4$ PIONEER spectrophotometer. Exact twenty grams of fine powdered sample was used in fusion beads of 10 g flux of lithium tetraborate (66 %) and lithium metaborate (33 %) fusion matrix. Data of all elements from $S_4$ PIONEER was calculated with computer based Bruker AXS SPECTRA*plus* software package. Some organics were also determined like as total proteins [15], sugars content [16] and reducing sugars [17].

More than 15 replicates per typed plants were selected for data collection. Data was computed for significance by using a computer based COSTAT computer package (CoHort software, Berkeley, USA).

## RESULTS AND DISCUSSION

During indirect plant regeneration, soma-clones had been observed under in-vitro cultures in almost all plant species. This phenomena is more pronounced in sugarcane (*Saccharum officinarum* L.) plants because of high levels of polyploidic (2n=80-205) genome [18]. Some regenerated soma-clones remained superior to parents while others inferior. Less efficient or inferior plants could also be sterile as well as chlorophyll deficient. Such chlorophyll deficient plantlets (11.36±0.54 plantlets per callus) were also observed among the regenerated normal plantlets (4.67±0.86 plantlets per callus) of sugarcane cultivar SPC-79 (Fig 1, Table 1).

Both normal and albino regenerated plantlets were transferred to soil conditions in green house after plant hardening. When they were reached to two months old subjected for morpho-biochemical analysis. Plant height of green plants was significantly higher (82.59±2.11 cm) than albino plants (27.72±4.03 cm). Green plants were also observed with high biomass ($p<0.05$) as well as higher ash contents (36.83±1.57 %) than albino plants but non-significantly (36.79±1.17%). Meanwhile, water contents were measured high ($p<0.05$) in albino plants (35.58±1.80%) than green plants (26.43±1.80).

During present study, not only phenotypic variations in the growth pattern of cultures were observed particularly, while synthesis of pigment also measured differential among the albino and green plants. Among the variants, low contents of chlorophyll a (0.373±0.04 mg $g^{-1}$), while non-significantly higher chlorophyll b (0.668±0.01 mg $g^{-1}$) contents were observed in albino plants than green plants. Overall chlorophyll ab contents were more in green plants than albino significantly. Meanwhile total carotenoids were in albino plants ($p<0.05$). Protein contents and total sugars were present in higher concentrations in green plants, while reducing sugars in albino plants (Table 1). Albino plants grew with less vigor, as well as produced low number of young plants than green plants. Developed phenotypes of the albino plants were remained stable [19,20].

The appearance of altered phenotype in the regenerated plants from callus culture could be accompanied due to different genetic modifications like as disease resistance, herbicide tolerance and antibiotic resistance are reported in past [21]. The

confirmations of mechanisms that involved in induction of altered phenotype had been remained un-known still. It may be resulted because of the change in chlorophyll DNA as well as genomic DNA. In sugarcane, stable and variant phenotypes were observed in texture and colour of leaf so variants have presented much differential and modified morphology [22,23,24]. Meanwhile, ex-vitro showed that variants were faced difficulties to grow further because of their inhibitory or high mortality rate.

Alterations in activities of growth related enzymes like as peroxidases, alcohol dehydroxygenase, amylase and carboxylase causes to slow down growth rate in regenerated variants [25,26]. Similar results like as induction of chromosomal aberration, alteration of protein profiles as well as appearance of altered phenotypes may also be induced by the application of high concentrations of plant growth regulators or delayed in times of subculture of cell populations under aseptic conditions [27,28]. Under such circumstances, alterations in bio-chemical components may also occur due to alteration of differential transportation mechanisms of the transporters located on the cell membrane and also on the tonoplast.

According to XRF analysis, certain substances among the albino and green plants were observed in altered concentrations [18,29]. Like as Si, Cl, Ca, Mg, Fe, S, Na, Br and Al were measured high concentrations in albino plants while, Cu and Br also high but non-significantly than green plants. Similarly, K, P, Zn, and Sr remained higher in green plants ($p<0.05$) while Br and Ti also high but non-significantly (Fig 2). Various elements like nitrogen or others are essential plant nutrient element that involved in the biosynthesis of important nitrogenous compounds in plants. Albinism may also induce reduction or enhances the uptake of various elements in plants. The presented results for various elements may be attributed through an antagonism between their ionic forms [30,31]. Meanwhile such uptake is also going on among the normal or green plants but in balanced form that not in albino plants. Such alterations in the uptake of nutrient elements have also been observed in the environmental stressed plants [32]. Some of the elements are required in largest amounts by the plants that constituent of large number of cell components like as different sugars, amino acids and nucleic acids as well as activation of the enzymes of the concerned biosynthesis [33].

Present study demonstrated that growth rate in albino plants remained low than green plants under in-vitro as well as ex-vitro ($p<0.05$). Reduction in growth rate could be resulted by the deficiency of green pigments that may be affected indirectly because of differential accumulation of various substances in albino plants than green plants. Such alterations induced by in-vitro cultures in biosynthesis of chlorophyll contents and reduction or abnormal transporter's efficiency of different elements on the cell barriers.

**Table I. Different morpho-biochemical attributes of 3-months old albino and normal plants of sugarcane (*Saccharum officinarum* L.) cv., SPC-79 regenerated through callus culture.**

| #s | Characters of plants | Normal plants | Albino plants | Significance |
|---|---|---|---|---|
| ***A. Morphological parameters*** | | | | |
| a. | # of plantlets per callus | 4.67±0.86 | 11.36±0.54 | 0.0028** |
| b. | Plant height (cm) | 82.59±2.11 | 27.72±4.03 | 0.0003*** |
| c. | Fresh weight (g) | 50.07±0.03 | 41.08±1.15 | 0.0014** |
| d. | Dry weight (g) | 36.83±0.91 | 26.43±1.82 | 0.000*** |
| e. | Ash contents (%) | 36.83±1.57 | 36.79±1.17 | 0.975ns |
| ***B. Chlorophyll contents (mg g$^{-1}$ F Wt)*** | | | | |
| a. | Chl a | 0.668±0.01 | 0.373±0.04 | 0.0454* |
| b. | Chl b | 0.522±0.03 | 0.650±0.01 | 0.4485ns |
| c. | Chl ab | 1.190±0.03 | 1.023±0.04 | 0.0233* |
| b. | Crotenoids | 2.374±0.03 | 2.645±0.08 | 0.0298* |
| ***C. Organic contents (mg g$^{-1}$)*** | | | | |
| a. | Protein contents | 3.58±0.13 | 3.48±0.08 | 0.5665ns |
| b. | Sugar contents | 8.18±0.30 | 7.26±0.10 | 0.0429* |
| c. | Reducing sugar contents | 5.61±0.13 | 5.82±0.19 | 0049* |
| d. | Water contents | 26.43±1.82 | 35.58±1.80 | 0.0233* |

*, **, ***, Data significance, ns, Non-significance at 5 % ($p<0.05$),

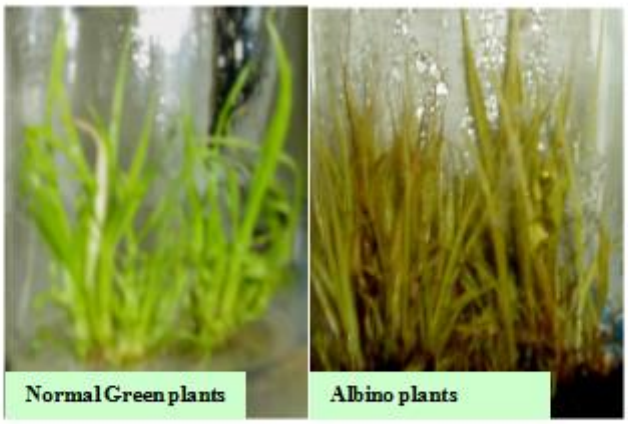


**Figure 1. Albino and normal plantlets of sugarcane (*Saccharum officinarum* L.) cv., SPC-79 regenerated through callus culture.**

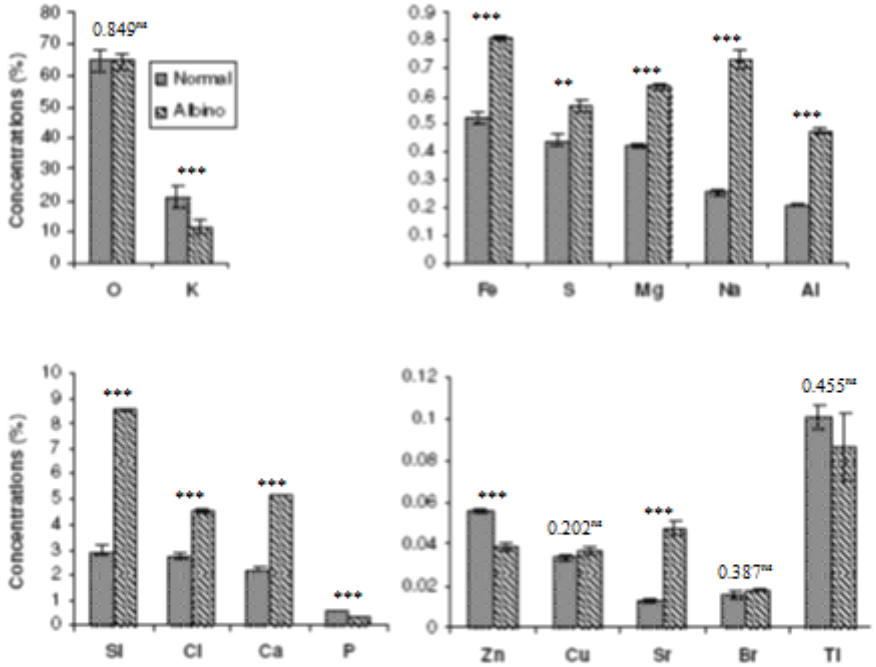


**Figure 2. The XRF analysis of the 3-months old albino and normal plants of sugarcane (*Saccharum officinarum* L.) cv., SPC-79 regenerated through callus culture.**

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