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IMPACT OF CADMIUM ON SOME CARBOHYDRATE METABOLITES OF DIFFERENTIATING AND NON-DIFFERENTIATING CALLI OF EGG PLANT

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The effect of cadmium on differentiating and non-differentiating calli of egg plant (*Solanum melongena* L.) was studied. The cadmium treatment reduced the accumulation of soluble sugars, starch and a -amylase activity. The cotyledonary and hypocotylar source of calli differed in their metabolic accumulation efficiency.

Key Words: Cadmium, Egg plant, differentiating calli.

Cadmium - a heavy metal pollutant occasionally accumulates in various plant parts which results in various abnormalities in plants (Bingham *et al.*, 1975;Bradshaw, 1984; Chugh *et al.*, 1992). However, in some of the cases the vascular plants have evolved different Cd-tolerance mechanisms (Tomsett and Thurman, 1988). Generally resistance to heavy metals is achieved either by avoidance, i.e. by protecting the plant from the influence of the stress or by tolerance, i.e. plant survives the effect of internal stress by adopting itself to the toxic concentration of heavy metals through various mechanisms (Parsad, 1995).

It is very difficult to understand the modus operandi regarding adaptability to heavy metal resistance in plant grown on the soil medium, because of interaction of the heavy metals with the soil system. So a more logical and realistic assessment of heavy metal resistance in crop system can be made through *in vitro* investigation- a novel technique which can easily be monitored and successfully employed to develop metal resistant lines. Following text is a part of studies regarding the development of metal resistant lines in egg plant (*Solanum melongena*).

It is obvious that during adaptability there are changes in the status of different metabolites both in quantity and quality. To understand these, it becomes imperative to study the metabolic changes which induce resistance to metal toxicity. Among various metabolic processes the carbohydrates are considered to be the primary key which steers the other metabolic processes. Since the quantity of different metabolites varies with the stage of growth, the toxicity of heavy metals is bound to influence the status of various metabolites at different stages of growth. In the present investigation the efforts have been made to find out the changes in various constituents of carbohydrate metabolism of cadmium tolerant calli of egg plant at differentiating and non-differentiating stages of growth.

MATERIALS AND METHODS

Seedlings of egg plant (*Solanum melongena* L.) were aseptically raised on agar medium containing MS (Murashige and Skoog, 1962) micro and macro nutrients at 25±1 °C. Cotyledons and hypocotyl segments were surgically excised from fifteen days old seedling and inoculated on MS medium fortified with 5.38 µM NAA (Naphthyl acetic acid) + 4.44 µM BAP (6-Benzyl aminopurine). The thirty days old calli obtained from these, were subjected to further investigations. These calli were divided into three lots. One of the lots was subcultured and maintained as such on the same medium which served as control. The second lot was subcultured for differentiation on differentiating medium containing MS + 2.86 µM IAA (Indole acetic acid) + 13.65 µM Kn (Kinetin) as reported by Jyoti *et al.* (1994). The third and the final lot was subcultured on the media fortified with 125 µM cadmium chloride. This concentration of cadmium chloride was inhibitory for callus growth (Jyoti *et al.*, 1995). The stability of the altered response in carbohydrate metabolic constituents due to cadmium treatment was tested by growing these calli on the medium devoid of cadmium. A part of such calli was subcultured for differentiation on differentiating medium. While the second part of

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calli was further subjected to cadmium treatment by subculturing the calli on the MS medium fortified with cadmium salt. The process was repeated by bringing the cadmium treated calli back to the cadmium free medium and then subculturing on the differentiating medium. The whole procedure was adopted according to the following plan (Fig. 1). The duration of each subculturing was of 45 days and the samplings of differentiating calli were made prior to the differentiating events.

Determination of starch and soluble sugar : For each samples one hundred and fifty mg of callus was homogenized in 80 per cent ethanol (v/v). The homogenate was refluxed for 15 min on a water bath and centrifuged. The supernatants were combined and volume was made to 5 ml. This supernatant was processed for soluble sugar estimation following the method of Yemm and Willis (1954) and the pellet was processed for starch estimation following McCreddy *et al.* (1958).

Determination of alpha-amylase : One gram of callus sample taken from all the stages were homogenized in chilled glass pestle mortar using acid washed sand as an abrasive. The extraction medium contained 0.1 M Tris HCI pH 7.4; 0.25 mM EDTA; 2.5mM cysteine HCI and 2.5% polyvinyl pyrrollidone. The homogenate was centrifuged at 10,000 X g for 10 min at 4°C in a refrigerated centrifuge. The supernatant was used for estimating the activity of a-amylase following the method of Shuster and Gifford (1962).

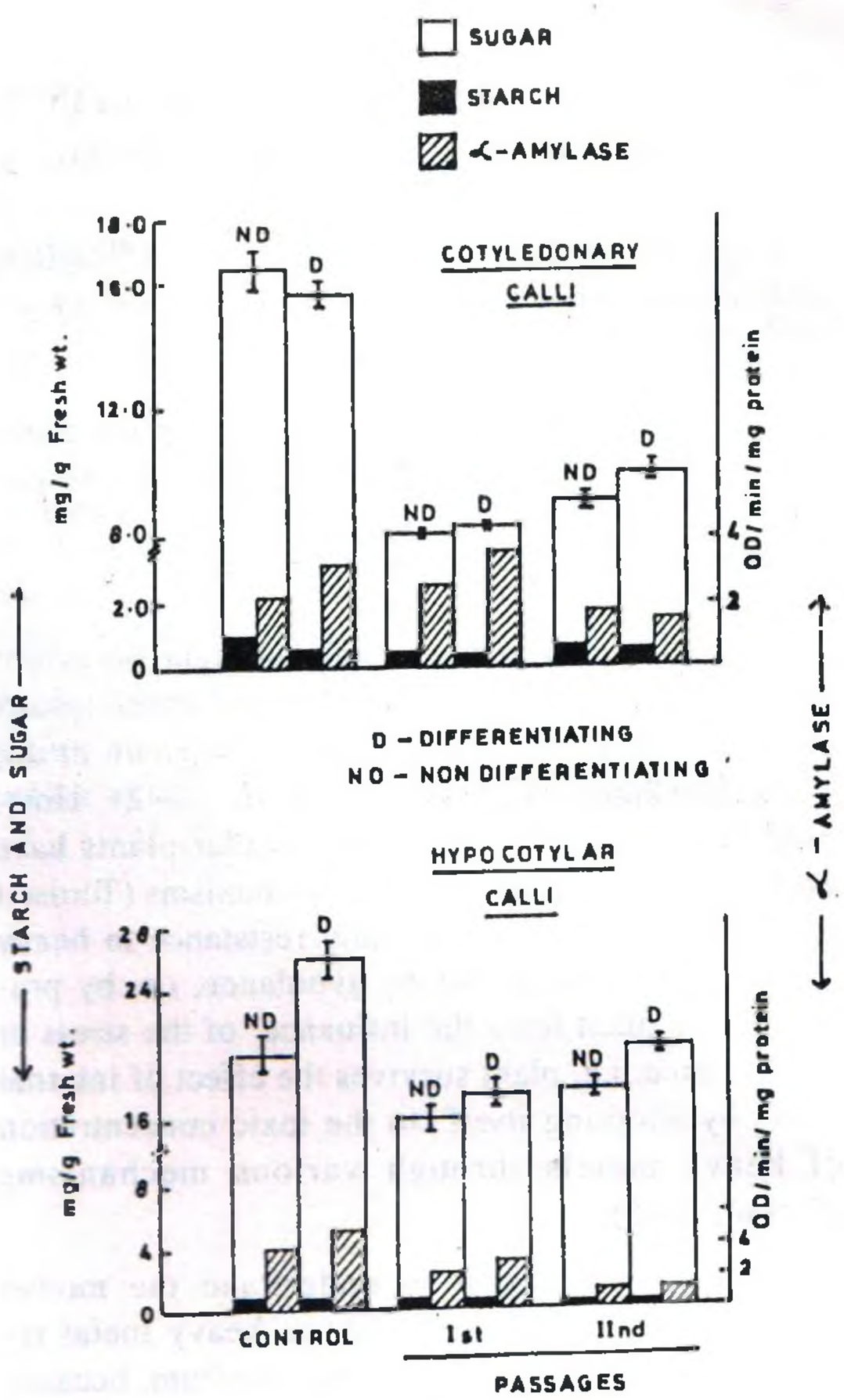

Figure 1. Showing starch and sugar contents and amylase activity in differentiating and non-differentiating calli of cotyledons and hypocotyl.

RESULTS AND DISCUSSION

The decline in soluble sugars in both non-differentiating and differentiating calli was observed in first passage for both the explants, while in the second passage these showed an improvement (Fig. 2). It can be seen from the data in Fig. 2 that the calli of hypocotylar source accumulated more of the soluble sugars than the cotyledonary driven calli. Similarly a comparative study between the two sources of calli also highlights the basic traits of the cells of calli which indicate that practically there was no difference in soluble sugar content of differentiating and non-differentiating calli of cotyledonary source whereas, in the hypocotylar driven calli a significant difference between differentiating and non-differentiating calli was present in both the passages and control. This is perhaps because of the fact that the hypocotyl being a centre of fast and active growth possesses high metabolic activities and eventually the cells derived from it, inherit the same qualities. Studies conducted by Jyoti *et al.* (1994) also indicated that hypocotylar calli of egg plant are more prone to differentiation than cotyledonary one.

The accumulation of starch contents also showed a decline but to a lesser extent than soluble sugars. As the system is heterotrophic and culture growth depends upon the exogenous supply of sucrose, low absorption of sugar perhaps accounts for depressed levels of free sugars and starch in calli. A decline in starch quantity in the cadmium treated calli (Ist and 2nd passage) may be due to several reasons including limited uptake which might be due to the effect of metal on the membrane permeability or direct in-

terference of cadmium with the biosynthetic enzyme of starch. *In vivo* studies on sugar beet also indicated that cadmium indirectly depressed the soluble sugars and starch (Greger and Bertell, 1992; Greger and Linderg, 1986). Although reports of Sheoran *et al.* (1991) indicated that in pigeonpea under low and high concentration of cadmium (i.e. 3 mM and 6 mM) the level of soluble carbohydrate and starch was not affected, yet sucrose content and activity of its allied enzyme decreased.

In the present studies, generally the activity of the a-amylase declined with the treatment of the cadmium. This perhaps is because of the binding of the metal ions to enzymes resulting in the alteration of catalytic functions (Clijsters and Van Assche, 1985; Eicchan *et al.*, 1969) which results into a decline. One of the striking feature of the present study was that in control as well as in passage I despite of metal toxicity there was an increase in a-amylase activity in all the differentiating stages as compared to non-differentiating stage which perhaps may be due to the fact that during differentiation/organogenesis carbohydrate demand become high which involves increase in a-amylase activity (Kumar and Meherchandani, 1988; Swaranker *et al.*, 1986; Thorpe and Meier, 1974).

The aforesaid studies indicate that heavy metal toxicity affects plant metabolism which can be directly correlated with the duration/number of exposure.

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