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METABOLIC CHANGES IN THE REGENERATING AND NON-REGENERATING *TARAXACUM* ROOT SEGMENTS.*

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Abstract

Metabolic changes in the regenerating and non-regenerating tissues of *Taraxacum* root segments were studied. Early changes in dry weight, proteins, amino acids nucleic acids and a number of enzymes was a result of injury. During regeneration of the root segments, proteins, nucleic acids and enzymes were always at a higher level in the regenerating proximal and distal-end tissues as compared to the non-regenerating tissues. Although the proximal and distal end regenerating tissues were morphogenetically different yet there was no difference in the quantity of proteins, nucleic acids, amino acids and enzymes in them.

Introduction

In the study of regeneration of organ pieces most interest has centered on the intriguing problem of polarity since shoots regenerate only at the morphological shoot-end of the cutting and roots at the root-end. Attempts have been made to identify which of the mobile constituents of the plant body moves, accumulates and promotes the particular new-organ formation. Diffferences in protein contents were found to appear gradually during the polar regeneration in rhizomes of *Lathyrus* and *Agropyron* and that the accumulated proteins did not control the polarity but were probably associated with higher metabolic activity in the regenerating parts of the plant (Schwahnitz ,1936).

A notable feature of the experiments with a three-week regeneration period of *Taraxacum* root segments reported earlier (Khan, 1972-a) was the relatively small difference in the levels of the various constituents found in the three segments during the first few days of regeneration. However, the anatomical study (Khan, 1973) have indicated that as early as 24 hr from the start of regeneration, cell division had commenced at both the proximal and distal-cut ends of the root segments. Thus one might expect to find significant differences of the constituents in the regenerating and non-regenerating tissues as early as 24 hr of regneration. In the three-week experiment (Khan, 1972-a) the sampling of 1 cm long segments necessarily meant that the small volume of dividing tissues were swamped by a large excess of non-dividing tissues resulting into relatively small changes in the metabolites.

The present study was undertaken in order to obtain some additional information underlying the physiology of regnerating tissues which are particularly in the process of organ formation. A prelinimary study related to this has already been published (Khan, 1972-a). In the present study, a different technique of sampling the regnerating and non-regnerating tissues was used. Two consecutive slices were

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excised from each end of the root cutting so that one contained the dividing cells and the other non-dividing. Segments bearing dividing cells were carefully excised in order to minimize the presence of non-dividing cells around them.

Materials and Methods

Root pieces of *Taraxacum officinale* Weber, 2 cm long and 7-10 mm in diameter, were excised and grown as described earlier (Khan, 1972-a). Triplicate samples, of five roots each, were taken after every 12 hr upto a regeneration period of 4 days. Two consecutive slices, 2.1 mm in thickness, were cut from both the proximal and the distal ends of the root pieces using the specially designed razor cutter described earlier (Khan, 1972-b). The proximal-end slices PA and PB and the distal-end slices DA and DB were longitudinally halved, one half was used for the dry weight determination and the other for the extraction and estimation of proteins and amino acids in a manner described below:

The slices were plunged into hot 80% ethanol to kill the tissue quickly. Ethanol was decanted and the tissue was crushed in a glass morter and extracted three times with 80% ethanol at room temperature, using fresh ethanol each time. All the ethanol extracts were pooled and the alcohol removed by distillation at 40°C under reduced pressure. Finally the extract was made upto a fixed volume (generally 20 mg fresh weight per ml) using distilled water. Total free amino acids were estimated by the method of Yemm & Cocking (1955). The alcohol—insoluble residue was used for the extraction and estimation of proteins and nucleic acids as described earlier (Khan, 1972-a).

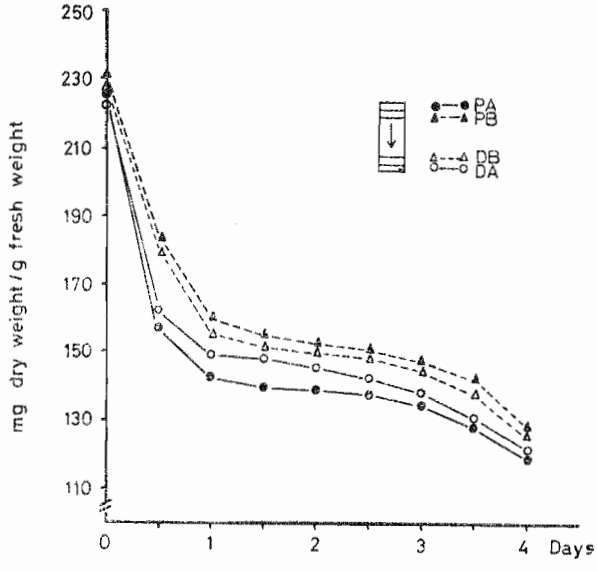

Fig. 1. Changes in dry weight/fresh weight ratio of *Taraxacum* root segments during 4 days of regeneration.

PA, Proximal terminal segement; PB, Proximal sub-terminal segment;

DA, Distal terminal segment; DB, Distal sub-terminal segment.

The slices in which enzymes were to be estimated were crushed in acid — washed sand in an ice-chilled mortar together with 5 ml of cold (5°C) 0.02 M Tris-HCl buffer (pH 7.4) The slurry was filtered through eight layers of muslin and the filtrate centrifuged at 4000 x g for 20 min at 5°C. The clear supernatant kept at 2°C was used for the estimation of enzymes acid phosphatase, malic dehydrogenase peroxidase and IAA-oxidase in a maner described earleier (Khan 1972 d,e).

Results

Dry weight changes during regeneration:

The dry weight changes per unit fresh weight is presented in Fig. 1. It was found that after cutting the roots into 2 cm long segments, the dry weight of the proximal and distal end 2.1 mm thick segments (PA, PB, DA and DB) decresaed to about 70% of original value within 12 hr of regeneration. After this the dry matter decreased slowly upto 4 days. The regenerating portions (PA and DA) always had a less dry matter as compared to the non-regenerating portions (PB and DB). However, the proximal segment PA showed more loss of dry matter as compared to the distal segment DA during 4 days of observations.

In order to compare the dry weight and fresh weight ratios with the change in dry weight per slice (2.1 mm thick), the changes with time in average dry weight of five slices (PA) is plotted against the average dry weight per gram fresh weight of five replicate samples of the proximal slice PA. The result presented in Fig. 2

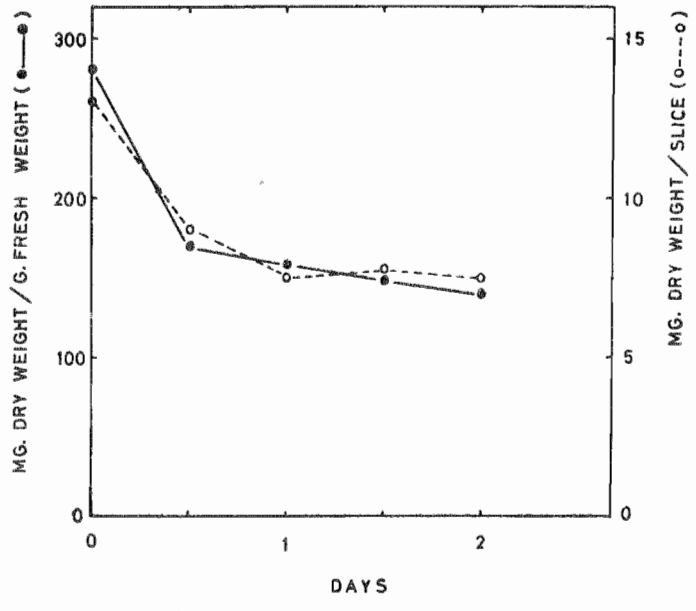

Fig. 2. Comparison of dry weight and fresh weight ratio with change in dry weight per slice (2.1mm thick) of *Taraxacum* root during 2 days of regeneration.

indicates that upto 2 days of regeneration the curves were similar indicating that further results can be presented either on the basis of dry weight or per slice of the root.

Protein and amino acids:

The results for protein changes are presented both on a dry weight and on a per slice basis (Fig. 3). The close similarity between the two sets of curves tend to discount the possibility that the changing values found in the results given on a uuit dry weight basis merely reflects changes in the dry weight of the slices. It was found that during regeneration, the protein level increased in all the proximal and distal slices from the start but later slowed down. The regenerating portions (PA and DA) showed a higher protein level as compared to the non-regenerating portions (PB and DB) throughout the experimental period of 4 days. Close similarity

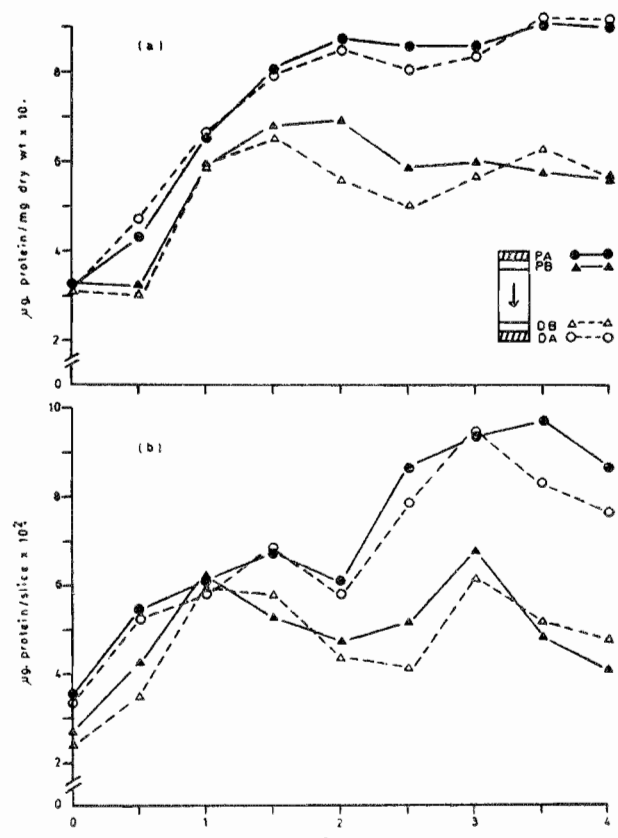

Fig. 3. Changes in the level of total proteins of regenerating (PA, DA) and non-regenerating (PB, DB) portions of *Taraxac m* root segment during 4 days of regeneration.
(a) mg dry weight basis. (b) Per slice basis.

in the level of proteins were observed between the proximal and distal, regenerating and non-regenerating portions. The non-regenerating proximal and distal-end segments (PB and DB) at fourth day of regeneration had 40 % less protein as compared to the levels present in the regenerating segments (PA and DA).

The amount of free amino acids in the regenerationg and non-regenerating *Taraxacum* root segments is presented in Fig. 4. It was found that within 24 hr of regeneration the level of free amino acids decreased to about 30 % of the original value in the non-regenerating segments and 65 % in the regenerating ones. After this the amino acid level change was insignificant upto 4 days of regeneration.

Nucleic acids:

The changes in the levels of RNA in the proximal and distal end slices were on the whole similar to those obtained for proteins and is presented in Fig. 5. As

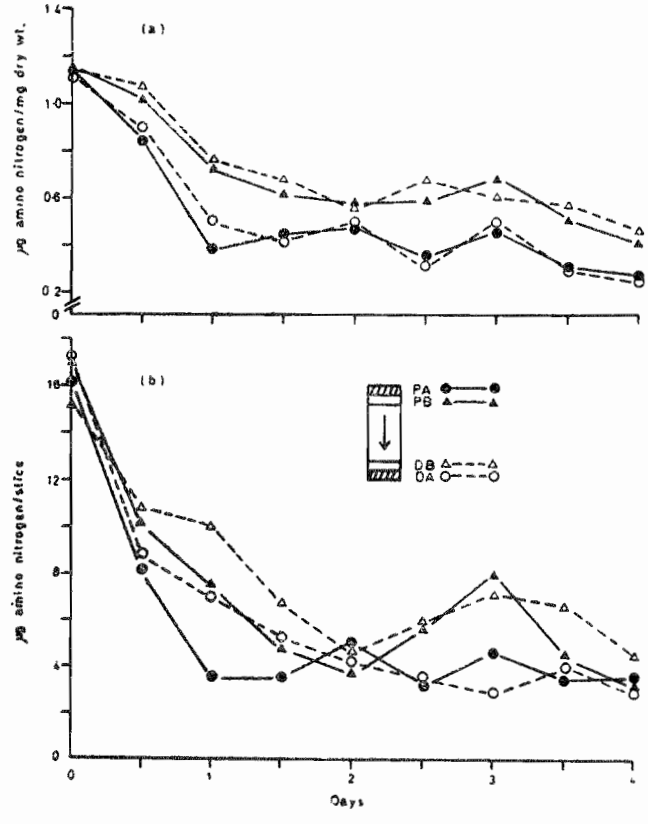

Fig. 4. Changes in the level of free amino acids of regenerating (PA, DA) and non-regenerating (PB, DB) portions of *Taraxacum* root segment during 4 days of regeneration

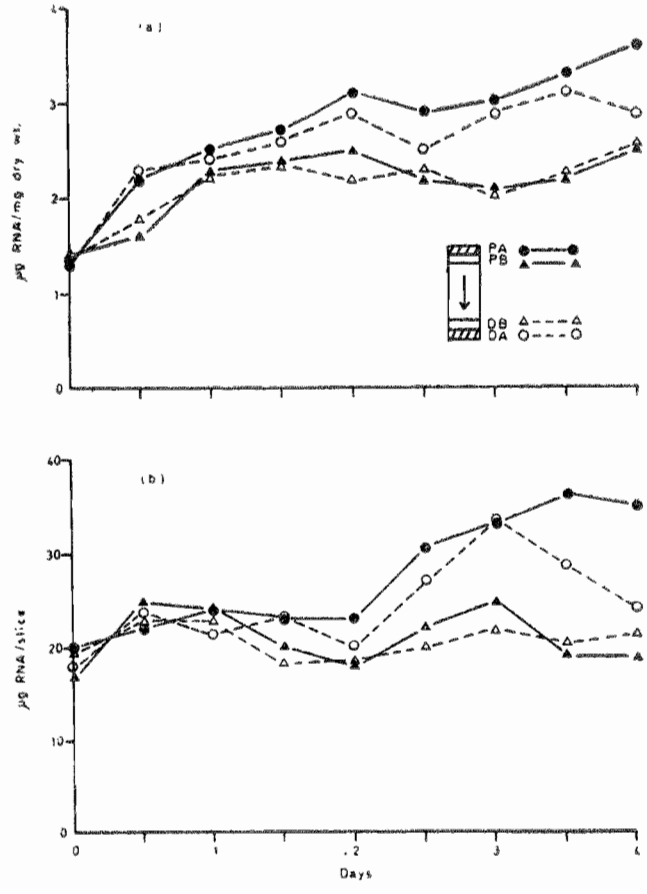

Fig. 5. Changes in the level of RNA of regenerating (PA, DA) and non-regenerating (PB, DB) portions of *Taraxacum* root segment during 4 days of regeneration.

regards DNA, the differences in the regenerating and non-regenerating segments were marked. In the non-regenerating segments the level of DNA remained almost to the original level throughout the whole period of regeneration while a significant increase in the regenerating segments were found from 1 day upto 4 days of regeneration (Fig. 6). Similarly the anatomical study of the regenerating *Taxaxacum* root segments after 1 day of regeneration, as reported earlier (Khan, 1973), has indicated the increased level of DNA in the proximal and distal-end slices.

Enzymes:

The specific activities of acid phosphatease, peroxidase, IAA-oxidase and malic dehydrogenase of regenerating and non-regenerating tissues are presented in

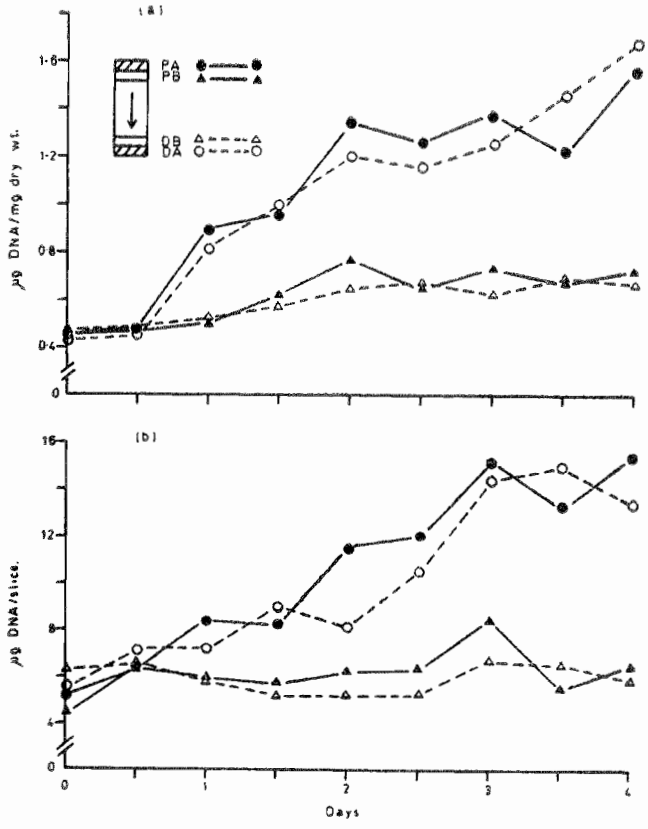

Fig. 6. Changes in the level of DNA of regenerating (PA, DA) and non-regenerating (PB, DB) portions of *Taraxacum* root segements during 4 days of regeneration.

Fig. 7. It was found that immediately after cutting the roots into 2 cm long segments (0 hr), the specific activities of all the enzymes studied increased in both the regenerating and non-regenerating segements reaching a highest value at 12 hr of regeneration. After this enzyme activites decreased rapidly reaching close to the original value at 36 hr and then decreased slowly upto 84 hr of regeneration. Marked differences in the specific activities of enzymes in the inner (B) and outer (A) segments were observed. As regards acid phosphatease and peroxidase, the two outer segments (PA and DA) behaved differently from the inner segments (PB and DB), for acid phosphatase the inner segments showed higher activities whereas peroxidase was higher in the outer segments. Differences between outer and inner segments were less and rather inconsistant for IAA-oxidase and malie dehydrogenase.

Discussion

Mechanical wounding of *Taraxacum* roots induces a localized burst of cell division activity within 24 hr of regeneration and also changed the growth pattern

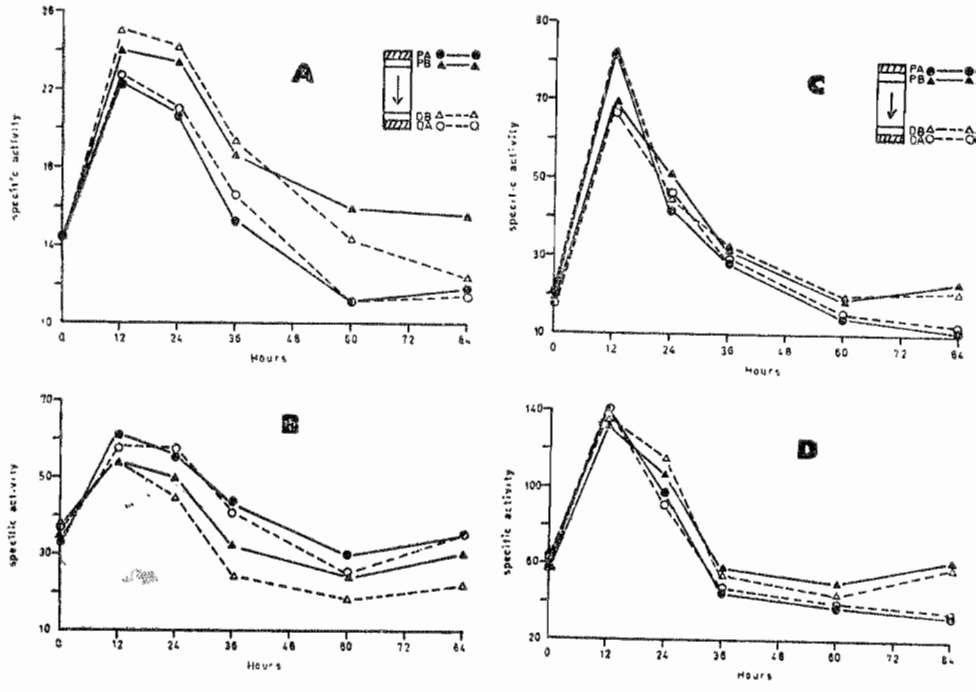

Fig. 7. Changes in specific activities of (A) Acid Phosphatase, (b) Peroxidase, (C) IAA- oxidase and (D) Malic dehydrogenase of re enerating (PA, DA) and non-regenerating (PB, DB) *Taraxacum* root segment during 4 days of regeneration.

by covering the wound cells with the newly formed callus cells (Khan, 1973). The present study has revealed that the early changes in dry weight, protein, amino acids and nucleic acids of *Taxraxacum* root segment was induced by injuring the tissues. These changes may, in turn, cause cell division and callus formation in the regnerating portions of the excised root segments. In fleshy storage tissues, similar to the one used in the present study, synthesis of messenger RNA resulted in an increase in polysome content of ageing carrot tissue (Leavy and Key, 1967) and also increased the incorporation of amino acids into proteins of ageing beet discs (Ellis and MacDonald, 1967).

Protein synthesis has been shown to precede many morphogenetic events, for example, Syono (1965) reported that chloramphenicol, an inhibitor of protein synthesis, inhibited shoot formation in carrot callus. Faskat and Miksche (1966) showed that protein synthesis preceded the first visible sign of wound xylem differentiation in *Coleus* internodal pieces. It is therefore possible that the increase in the level of total proteins which was accompanied by the decrease in the level of free amino acids, may represent increased synthesis of proteins associated with the organ initiation processes.

The difference between organ-forming and non-organ-forming tissue was in the distribution of the DNA which was a consequence of the pattern of cell divi-

sion, and resulted in an increase in DNA content in organ-forming portion of the root segments. Measurement of DNA content of the proximal and distol segements of the root showed that DNA increased after 1 day of regeneration only in the proximal segments (PA and DA). A notable feature of these results was that no clear cut quantitative differences were detected between the level of different metabolites at the proximal and distal regenerating segments at any period of regeneration. This contrasts with the anatomical differences between the two ends from about 48 hr onwards, when shoot primordia were seen at the proximal end whereas root initials were seen at a much later stage of regeneration (Khan, 1972-b).

Although there were no quantitative differences in protein levels at the proximal and distal regenerating ends of the cuttings, one expects to find some qualitative differences in view of the anatomical differences between the two ends. This was investigated and it was found that although spectacular increase in enzyme levels followed cutting, qualitative differences of the enzymes between the two ends of the roots were negligible.

Uritanti et al (1967) reported the development of polyphenols in sweet potato in response to cutting. They also found a correlation between the increase in the level of polyphenols and the increase in the phenylalanine ammonium-lyase activity and that both were inhibited by the application of actinomycin-D. The enzymes studied during regeneration of *Taraxacum* roots also points to the same fact that cutting the roots induces the increase in the activities of a number of enzymes. Moreover histochemical localization of the enzyme peroxidase in the regenerating *Taraxacum* root segments (Khan, 1972-d) clearly indicated the appearance of the enzyme only at the cut surfaces of the roots. It is interesting to note that the increase in the level of enzymes at early period of regeneration was found to be correlated with the increase in metabolic activities of the regenerating proximal and distal-end segments.

The typical picture which emerged from this study was that, irrespective of the anatomical differences present in the proximal shoot-forming proximal segment (PA) and distal root-forming distal segment (DA), no significant differences could be obtained among the level of different metabolites during organogenesis.

Acknowledgement

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