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ELECTROPHORETIC PROTEIN PATTERNS OF NORMAL AND TUMOROUS TISSUES OF BROAD BEAN

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Introduction

In many dicotyledonous plants *Agrobacterium tumefaciens* (Smith et Town) Conn induces crown gall tumours. Regardless of the physiological basis of the autonomous tumour growth and of the manner in which the tumour phenotype is perpetuated in the population of the dividing cells (Meins 1974), there is no doubt that, during the transformation, changes in the degree of the cell differentiation occur (Beiderbeck and Nitsche 1970). Independently of the nature of the TIP (tumour inducing principle) the tumorous transformation may be considered as a process of anomalous differentation (Braun 1969, Meins 1974).

Using cytological and biochemical methods one could state, that tumorous tissue contains more RNA and DNA than the coresponding normal tissue and that the protein content is higher in the former than in the latter (Broekaert and Parijs 1973, Baranowska et al. 1972). The metabolism of aminoacids is changed, so that arginine derivatives (octopine, nopaline) accumulate in the tumorous tissue (Ménagé and Morel 1964a, b, 1965).

On the basis of the differences observed between normal and tumorous tissue it is possible to conclude that the tumorous tissue differs from the normal one by its genetic activity, and that this difference should be reflected also in the protein composition (Bhatia, Buiatti and Smith 1967, Curtis 1970).

The present work has been carried out with the intention to compare, by electrophoresis, the soluble proteins from leaves, stems, and tumours on the stem of broad bean.

Material and Methods

Bacteria and Other Plant Material

Broad bean plants (*Vicia faba* L., Fribo), cultivated in plant chambers with controlled conditions (standardized conditions: temperature 20°C ± 2, relative humidity about 70% ± 10, illumination 9000 lx 16 hours daily), were used for the experiments. The plants for infection were 14 days old and the youngest developed internode was infected (Kupila and Stern 1961, Lippincott and Heberlein 1965, Kupila-Ahvenniemi and Therman 1971).

48 hour old cultures of the strain I_4 of *Agrobacterium tumefaciens* (Smith et Town.) Conn (Krsnik-Rasol 1974) were used for the infection. The concentration of bacteria in the inoculum was $2.4 \cdot 10^9$ bacteria per ml. Stem tumours, 20 — 40 days old of 200 — 400 mg fresh weight, leaves from the same plant and parts of the stem which were wounded and inoculated by the sterile medium were collected for protein extraction.

Total and Soluble Proteins

Soluble proteins were extracted from acetone powder by the method of Ibrahim and Cavia (1975) or from fresh tissue (200 — 400 mg of fresh mass) by the TRIS-HCl buffer (pH — 8.0) after Staples and Stahmann (1964) (see Kluge 1976, 1978)) with an addition to 2% polyvinylpyrolidon. The extraction of the total protein was carried out according to the method by Parthier (1961).

The proteins were quantitatively determined by Lowry's method (Lowry et al. 1951), and the calibration curve was made by means of the leaf protein from broad bean.

Electrophoretic Separation of the Soluble Proteins

For the electrophoretic separation of soluble proteins basic polyacrylamide gel (pH 8.9; separating gel 7,5%, spacer gel 2.5% acrylamide) by Maurer (1971, system 1a) was used. On the gel, 150 μg of protein was layered, and the current of 4 mA per tube was applied. For gel staining, 1% solution of amido-black was used (Kluge 1976).

Results and Discussion

Like the majority of pod plants the broad bean is rich in proteins. Figs. 1 and 2 show the content of total and soluble proteins in various broad bean tissues. The content of proteins in the stem is considerably lower than in the leaves or in the tumorous tissue grown on the same stem. As the tumorous tissue proliferates while its cells are actively dividing, an increase in the protein content in tumorous tissue could be expected (Chen and Venketeswaran 1966, Galston and Novak 1974).

Two methods were used for protein extraction, but only the extraction by means of TRIS-HCl buffer proved to be convenient, because suf-

ficient quantities of protein extract could be obtained from only 200 mg of fresh tissue. For the method by Ibrahim and Cavia (1975) much higher quantities of plant tissue are necessary (more than 3 g), which excludes the possibility of comparing various tissues of one and the same plant. Fig. 3 shows the banding patterns obtained by electrophoretic separation of soluble proteins of leaves, stem and stem tumours of broad bean. In the picture of electrophoretically separated proteins from broad bean leaves there is a dominant broad band of low mobility (Rm 0,06),* i. e. fraction 1 protein, which has been found in leaf homogenates of many plant species and has been identified with the enzyme ribulose biphosphate carboxylase (Curtis 1970, Jones and Lyttleton 1972). In the picture of the protein pattern of the stem (Fig. 3) fraction 1 protein is also dominant, but even the other bands show considerable analogies between the leaf and the stem. In the figure showing the electrophoretic pattern of tumour proteins the band of fraction 1 protein is subdivided into two narrower bands (Rm 0.07 and 0.1). This splitting has been observed in all tumours investigated so far. In the region of fraction 2 proteins interesting bands, characteristic of the tumour (Rm 0.4 and 0.9) were found. These bands, however, vary considerably from tumour to tumour, and even during the development of the same tumour. Up to now this protein fraction has not yet been investigated in all details.

Contrary to the data of Curtis (1970), referring to tumours of primary bean leaves, according to which there are no differences between normal and tumorous tissue, in broad bean the differences in protein patterns have been repeatedly observed. The conspicuous difference in fraction 1 protein is probably due to the reduced number and altered structure of the chloroplasts in tumours tissue (Bopp and Leppla 1964, Beiderbeck and Nitsche 1970, Krsnik-Rasol 1974).

Conclusion

Electrophoretic analysis carried out so far on the broad bean has shown that in fraction 1 protein considerable differences exist between normal and tumorous tissues.

In fraction 2 proteins differences have been observed as well, but they are not yet known in detail.

*

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* Rm — relative mobility

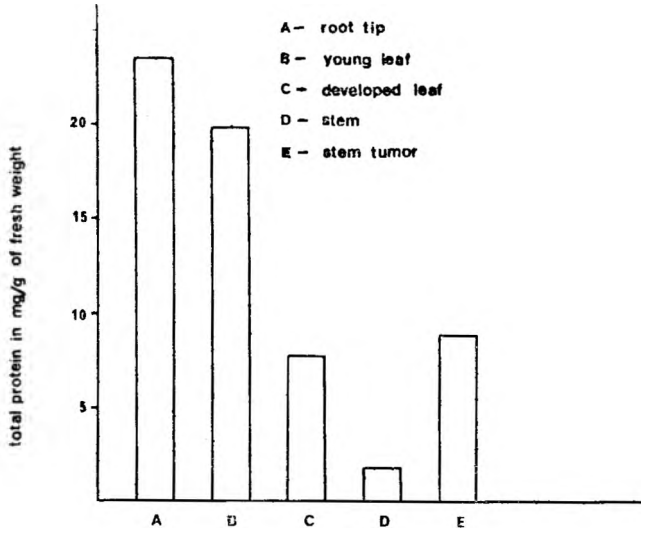

Fig. 1. Content of total proteins in broad bean tissues.

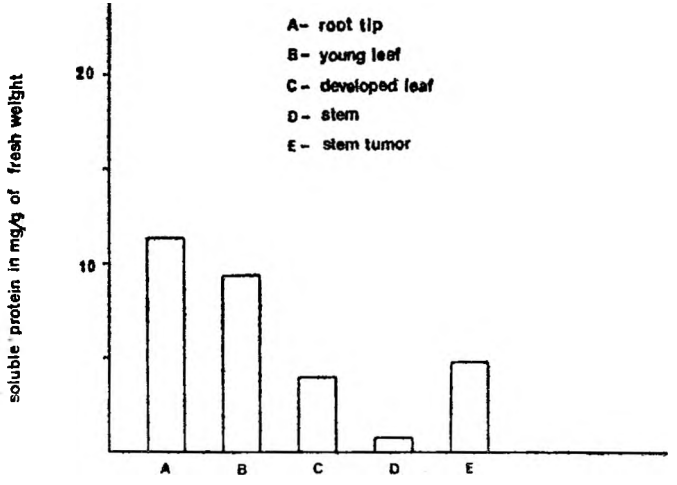

Fig. 2. Content of soluble proteins in broad bean tissues.

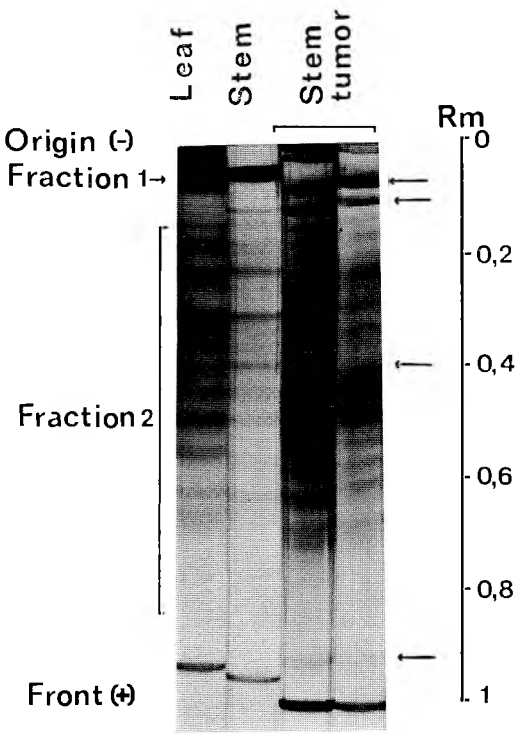

Fig. 3. Electrophoretic patterns of broad bean proteins.

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SAZETAK

ELEKTROFORETSKA SLIKA PROTEINA NORMALNOG I TUMORSKOG TKIVA BOBA

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Iz lista, stabljike i tumora na stabljici boba ekstrahirani su topivi i ukupni proteini te kvantitativno određeni. Proteinima je najbogatiji list, a u tumorskom tkivu znatno ih je više nego u odgovarajućem tkivu stabljike.

Razdvajanje proteina disk-elektroforezom u poliakrilamidnom gelu pokazalo je razlike u proteinskoj slici između normalnog i tumorskog tkiva. Za tumor je karakteristično razdvajanje proteinske frakcije 1 u dvije pruge.

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