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BRIEF COMMUNICATION

Changes in proteins and RNA during storage of *Curcuma longa* L. rhizome

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Abstract

Changes in RNA and protein contents, and protein profiles were studied in the rhizomes of turmeric (*Curcuma longa* L.) from the day of harvest to the commencement of sprouting. Protein and RNA contents increased gradually in the initial stages and rapidly in the final stages of storage period. SDS-PAGE analysis of storage proteins revealed synthesis of 56, 52 and 47 kDa proteins during the later stages of storage with concomitant disintegration of 23 and 18 kDa proteins.

Additional key words: dormancy, SDS-PAGE, turmeric.

Rappaport and Wolf (1968), Waring (1982) and Wickham *et al.* (1984) have reported the hormonal control of resting buds and tubers. The role of proteins and nucleic acids in regulating dormancy is another question which requires further investigation. Proteins and nucleic acids are considered to be essential for the sprouting of potato buds (Tuan and Bonner 1964, Madison and Rappaport 1968). New proteins have been synthesized in yam *(Dioscorea alata)* tubers at the time of sprouting (Jayakumar *et al.* 1993). In this study we report the changes in protein and RNA contents and in protein profiles during different storage periods in *Curcuma longa* rhizomes under controlled atmosphere.

Undamaged healthy turmeric (*Curcuma longa* L.) rhizomes were selected after harvest and they were stored in a controlled environment cabinet (*Lab Line Instruments*, USA) under a 13-h photoperiod (fluorescent and incandesent bulbs; irradiance of 120 μmol m^{-2} s^{-1}). The temperature was 28 ± 2 °C (day) and 24 ± 2 °C (night). The relative humidity was maintained at 70 %. The total storage period was 75 d. Samples were drawn at an interval of 15 d from the harvest until sprouting.

Protein content was measured by using the method of Lowry *et al.* (1951). RNA content was estimated by the method of Smille and Krotkov (1960). One g of rhizomes were crushed in 10 cm^3 of 50 mM Tris-HCl (pH 7.5) buffer and the extract was centrifuged at 2 500 *g* for 3 min at 4 °C . The pellet was washed in ice cold acetone and precipitated at the above speed and repeatedly washed in cold acetone until the pigments are completely removed. The final pellet was dissolved in 50 mM Tris-HCl (pH 7.5) buffer and 1 mM $MgCl_2$ solution and dialysed in the same buffer for 12 h. The dialysed sample was centrifuged at 2 500 *g* for 10 min and the supernatant was used as the source of soluble proteins.

A known aliquot is taken from the sample and assayed for protein content following the method of Lowry *et al.* (1951). The extracted proteins were analysed on uni-dimensional SDS-PAGE according to Laemmli (1970) using 1.5 mm thick 9 - 18 % acrylamide gradient slab gel. The samples containing 150 μg proteins were loaded in each slot. Electrophoresis was carried out at 20 °C for 12 h at 20 mA with the initial 1 h at 5 mA. Protein bands were visualized by staining with 0.2 % Coomassie Brilliant Blue in 50 % ethanol and 7 % acetic acid for 4 - 5 h and de-stained in 25 % ethanol containing 7 % acetic acid for 1 h. The gel was stored in 7 % acetic acid and photographed.

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Abbreviations: SDS - PAGE - sodium dodecyl sulfate polyacrylamide gel elcetrophoresis.
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The trend of total protein showed a gradual increase during the initial stage of the storage period. As much as 31 % increase was showed after 45 d. During the late period of storage (45 - 75 d) there was a rapid increase in the protein content. The rhizomes of *C. longa* exhibited as much as 90 % increase in protein content from the date of harvest to the end of dormancy (Fig. 1).

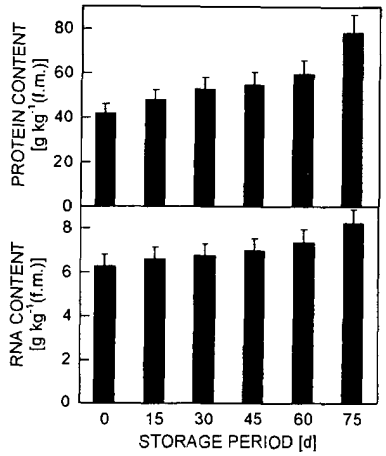

Fig. 1. Changes in the contents of protein and RNA during the storage period of *Curcuma longa* rhizomes.

The increase in RNA content was gradual during the initial stage of the storage period. Similar to the change in protein the latter storage period demonstrated a rapid increase in RNA (Fig. 1). 66, 52 and 47 kDa proteins have not been synthesized till 30 d of storage and prominently appeared after 45 d of storage. In samples collected after 45, 60 and 75 days after storage, both 23 and 18 kDa proteins have disappeared (Fig. 2).

Similarly Levitt (1954) has observed an increase in protein content near the end of the dormant period of potato tubers, and Okagami (1978) reported that some proteins are involved in the release of dormancy in *Dioscorea* tubers. Jan *et al.* (1984) have reported an increase in protein synthesis in *Agrostemma* seeds during the breakage of dormancy. According to Okagami (1978), sprouting might be promoted when the synthesis of dormancy-induced proteins decreased. These proteins, however, serve as a useful biochemical marker for dormancy (Wisniewski *et al.* 1996).

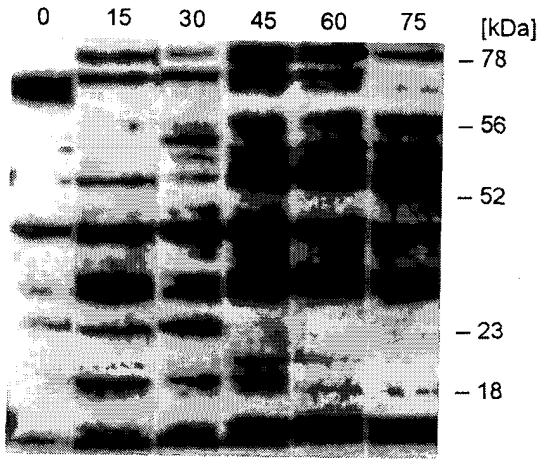

Fig. 2. SDS-PAGE polypeptide profiles of *Curcuma longa* rhizomes during storage period (0 - 75 indicates the days after harvest).

In the case of seed dormancy, a net synthesis of nucleic acids occurs during the storage period and various types of RNAs are concerned with the control of protein synthesis (Tao and Khan 1977).

The results of the present study convincingly reveal the fact that the protein synthesis precedes sprouting and terminating the dormancy in *Curcuma*. It is also evident that in the rhizomes of *C. longa* the definite/stable dormant period is only 30 d although sprouting takes place on 75th day after harvest. Thus termination of dormancy in *Curcuma* seems to be initiated by an endogenous mechanism after a definite time period, which is suggestive of the fact that this process is genetically controlled rather than environmentally influenced.

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