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PROTEIN ANALYSIS OF SHOOT APICAL MERISTEM OF OIL PALM DURING TRANSITION FROM VEGETATIVE MERISTEM TO AN INFLORESCENCE MERISTEM

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Oil palm shoots were grown in vitro on high cytokinin media. Terminal inflorescences were observed after four to fioe months ofgrowth. Two-dimensional polyacrylamide gel electrophoresis was carried out to identify unique proteins in shoot apices producing terminal inflorescence. Three proteins were identified, the absence of which could be associated with the conversion of a vegetative meristem to an inflorescence meristem. Histological studies of the inflorescence revealed that it had no flowers. This showed that in vitro, *under high cytokinin growth condition, the vegetative meristem could be induced to transform into an inflorescence meristem. However, the inflorescence meristems produced are not competent to produce flowers.*

INTRODUCTION

Flowering begins with the transition of the shoot apex from vegetative to reproductive growth. This transition involves a sequence of events beginning from an induced state in the leaves which then transmits unknown signal(s) to the shoot apex which then leads to the floral differentiation (Evans, 1969). Floral induction has been most extensively studied in photoperiodically sensitive plants where evidence have been found of changes in gene expression accompanying floral induction (Bernier, 1988).

Several studies have examined polypeptide changes associated with floral induction. An early study using two-dimensional gel electrophoresis has examined the protein composition of various *Pharbitis nil* organs (Marushige and Marushige, 1962). Clear differences in protein composition were observed between leaves and flowers and between vegetative and reproductive shoot apices. The study, thus, demonstrated that each organ possessed a unique complement of cellular protein. In a separate study done earlier on *Pharbitis nil*, the levels of four small polypep-

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tides decreased during transition of vegetative meristem into floral meristem (Lay-Yee, 1986). Both studies found detectable differences in protein profiles between the vegetative and reproductive meristems.

In this study, total soluble proteins extracted from shoot apices of oil palm grown in high 6-benzylaminopurine (BAP) media were analysed using 2-D gel electrophoresis. They were then compared with total proteins isolated from the shoot apices of oil palm grown on basal media. The presence or absence of protein spots on two-dimensional gels will reflect the conversion of the vegetative meristem to inflorescence meristem. This conversion was confirmed by the morphological changes of the shoot apices.

EXPERIMENTAL

Plant Material

Oil palm shoots grown on basal Murashige and Skoog (MS) media and MS media supplemented with BAP (10^{-4} M) were used. The shoots were maintained on the respective media until terminal inflorescences were observed. The shoots were harvested at monthly intervals up until terminal inflorescences were produced. The shoot apices were dissected out, placed in liquid nitrogen and kept frozen at -80°C until needed,. The shoot apices used for histological studies were dissected out and placed in fixing solution.

Isolation of Total Proteins

Total proteins were extracted according to the method of Mayer et *al.* (1987) and quantified by dot binding assay (Ghosh et al., 1988) using bovine serum albumin as standard.

Isoelectric Focussing Gels and Two-dimensional Polyacrylamide Gel Electrophoresis

The method used was a minor modification of O'Farrell (1975) equilibrium isoelectric focussing protocol. The following components were mixed in a Buchner flask and degassed for one hour: 3.4g urea, 0.9ml 30% acrylamide/bisacrylamide solution, 1.2ml 10% (v/v) Triton X-100 and l.lml deionized water. After one hour, the remaining components of the gel mix were added: 0.42ml Bio-Lyte pH 3-10, 15µl 10% ammonium persulfate and 20µl TEMED. The mixture was taken up into a 10µl syringe with an 18cm, 22 gauge, blunt tip needle attached. The gel solution was delivered so that the gel tubes were filled from the bottom. The gels were poured to three quarter the length of the tubes. The gel solution was allowed to polymerize for 1-2hr in a vertical position. After polymerization, the gel rods were fitted onto the gel tank. The gel was overlaid with 20µl protein extraction buffer plus 9M urea. The lower chamber of the electrophoresis tank was filled with 0.01M phosphoric acid and upper chamber with 0.02M sodium hydroxide. The gels were prefocussed at 200V for 10min, 300V for 15min and 400V for 15min. After prefocussing, the top buffer and any solution overlaying the gel was discarded, the samples introduced and overlaid with 10µl protein extraction buffer. Fresh top buffer was then placed in the gel tank. The gel was run at 400V for 12hr followed by 1500V for one hour. The gels were then removed from the tubes and equilibrated for 30min in SDS sample buffer and stored at -70°C until further use. The equilibrated gels could also be run on a second dimension gel immediately. The second dimension was run on a standard PAGE gel (Laemmli, 1970).

The two-dimensional gels were stained in silver staining solution and photographed for permanent record. The gels were then dried between two sheets of clear cellulose film (Promega). The dried gels were then compared by superimposition over a light source. The dried gels were also divided into squares of 2 x 2cm and a region by region analysis was performed to identify unique protein spots.

Histology

Samples for histology were fixed for 24 to 48hr at room temperature in gluteraldehyde fixing solution (50ml 0.2M phosphate buffer, pH 7.2; 20ml paraformaldehyde; 4ml 25% gluteraldehyde; lg caffeine; and distilled water to a total volume of 100ml). The samples were dehydrated in an ethanol series: 30%, 30min; 50%, 45min; 70%, 45min; 80%, 60min; 90%, 60min; and twice in absolute ethanol for 60min each. The tissues were then ready for impregnation in infiltration solution (Basic resin;

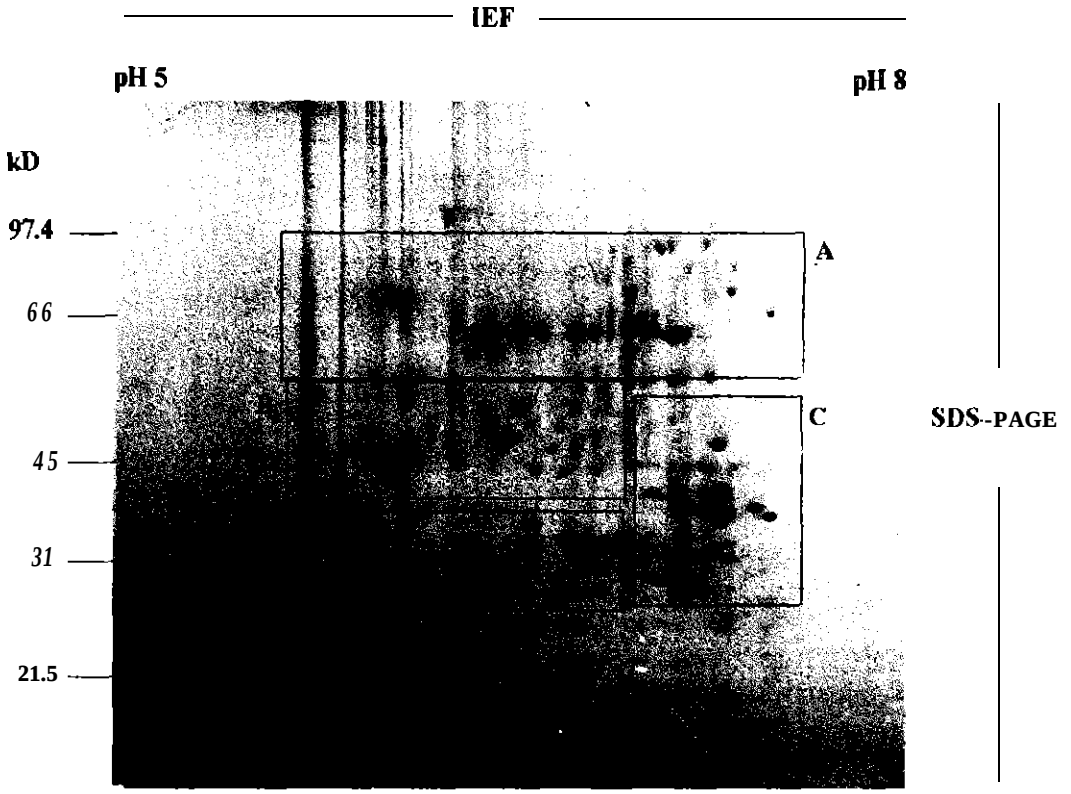

Figure 1. Protein pattern** of **non-induced** oil palm **shoot apex at one month old. This** was **used as standard** for **comparison** of **protein patterns between non-induced and BAP-induced shoot apices** of various **developmental stages.

Leica Instruments) for 24hr at 4°C followed by embedding. Sections of 3.5μm were cut and stained with 0.5% toluidine blue. Tissues that are appropriate for analysis were stained permanently with periodic acid stain.

RESULTS AND DISCUSSION

This study was carried out to examine protein changes that occur during the transition from a vegetative meristem to an inflorescence meristem. Addition of a high concentration of cytokinin induces this transition. Total protein analysis was used as only a small amount of proteins can be extracted from a small number of shoot apices. Analysis of directly extracted proteins also has the advantage of detecting post-translational modification and specific degradation of proteins and unsynthesized or rarely synthesized proteins in translation system in *vitro*. Combined with a powerful technique such as two-dimensional polyacrylamide gel electrophoresis, changes in gene expression during transition of vegetative meristem to inflorescence meristem can be detected.

Two sets of oil palm tissue cultured shoots were grown on two different media. One set was grown on MS media supplemented with 10^{-4} M 6-benzylaminopurine (BAP) and the other was grown in MS media without any added BAP. The shoots were grown under normal conditions (28°C, 12-hour day) and harvested at monthly intervals until a terminal inflorescence was produced by the shoots grown in the BAP supplemented media after about four to five months. The shoot apices were dissected out at monthly intervals for protein analysis and histological studies.

Several hundred protein spots were revealed by silver staining. The pI of the polypeptides ranged from pH 4 to 8 and their molecular weight from 21 to 97kD. Two types ofpolypeptide variation were scored. For qualitative variation, the presence or absence (+/-) of a protein spot was noted, and, for quantitative variation, the intensity of protein spots on both the BAP-induced and non-induced shoot apices was compared. Only distinct differences in spot intensity were considered.

The polypeptides analysed were divided into four regions (A-D) (Figure *1*). A majority of the protein spots in region A appeared in

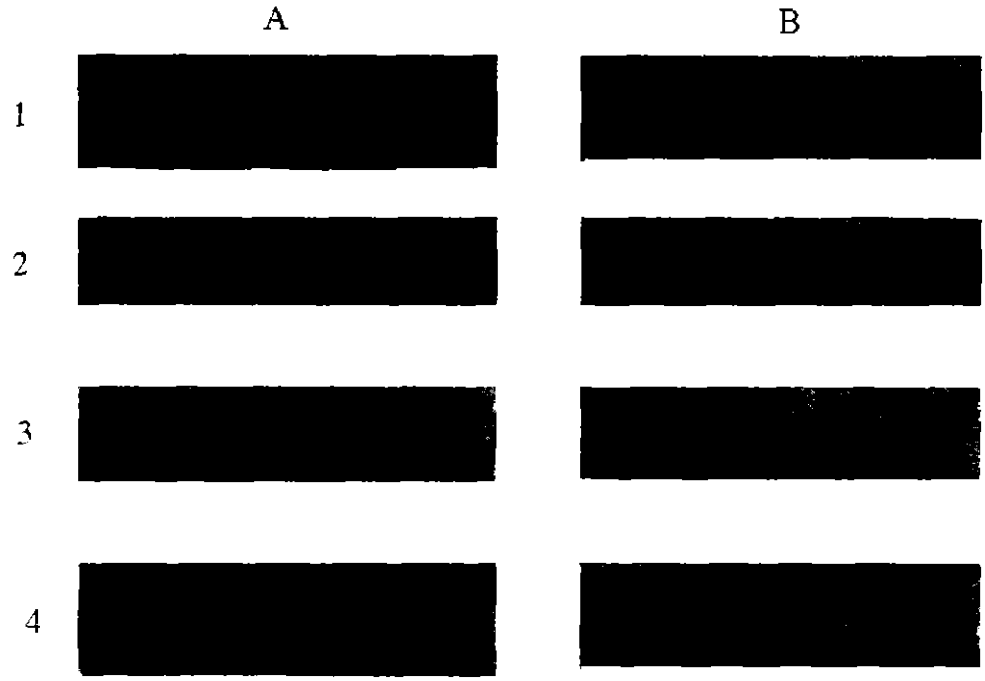

Figure 2. Protein pattern in region A of both the non-induced (A) and BAP-induced (B) shoot apex. 1: one month; 2: two months; 3: three months; 4: four months old shoot apex.

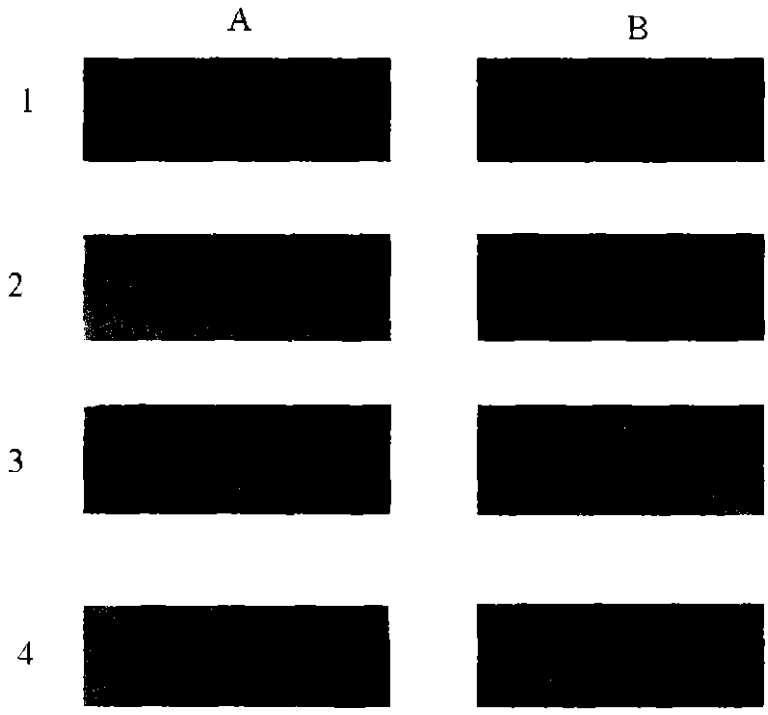

Figure 3. Protein pattern in region B of both non-induced (A) and BAP-induced (B) shoot apex I: one month; 2: two months; 3: three months; 4: four months old shoot apex.

all the shoot apices compared. However, two spots marked a and ***b were*** found only in one-month old non-induced shoot apex (Figure 2). These two protein spots had molecular weight of about 60 to 70kD with a pH of about 5.8. In region B, protein spots marked *c*, *d* and *e* increase in intensity with the age of the vegetative meristem (***Figure*** 3) in both induced and non-induced meristems. These proteins had ***a*** lower molecular weight than a ***or b*** with a range from 45 to 50kD with a pH of about 6.5. The proteins 'identified' could be related to the growth of the meristems since their intensities increased with the development of both types of meristem. In region B also, protein spots marked *f* and g were found consistently in the vegetative meristem but not in the BAP-treated meristem. These proteins had molecular weight of about 45kD and a pH of about 6.2. They could be involved in maintaining the vegetative state of the meristem. Thus, when a vegetative meristem is transformed into inflorescence meristem, production of these proteins is inhibited.

In region C, a protein spot marked I was also found throughout the development of the vegetative meristem but ***was*** absent from the BAP-treated meristems. This protein had a molecular weight of 35kD with a pI of about 7.5 (***Figure 4***). As with proteins *f* and g, protein I could also be involved in maintaining the vegetative state of the meristem. There was no significant difference observed in the pattern of protein spots in region D (Figure 5).

These results showed that there are quantitative and possible qualitative differences in the proteins synthesized in both vegetative and BAP-treated meristems of oil palm. Three different protein spots, *f*, g and I were absent in the BAP-induced samples throughout the development of the meristem. The absence of these spots could indicate either that the proteins are responsible for the vegetative state ***of*** the meristem, ***or*** that the production of ***a*** reproductive meristem, suppressed the production of these proteins. In a study on floral induction in ***Pharbitis nil,*** four major polypeptides were expressed at lower levels as the vegetative meristem converted into a reproductive meristem (Lay-Yee, 1986).

Although there are differences in the pro-

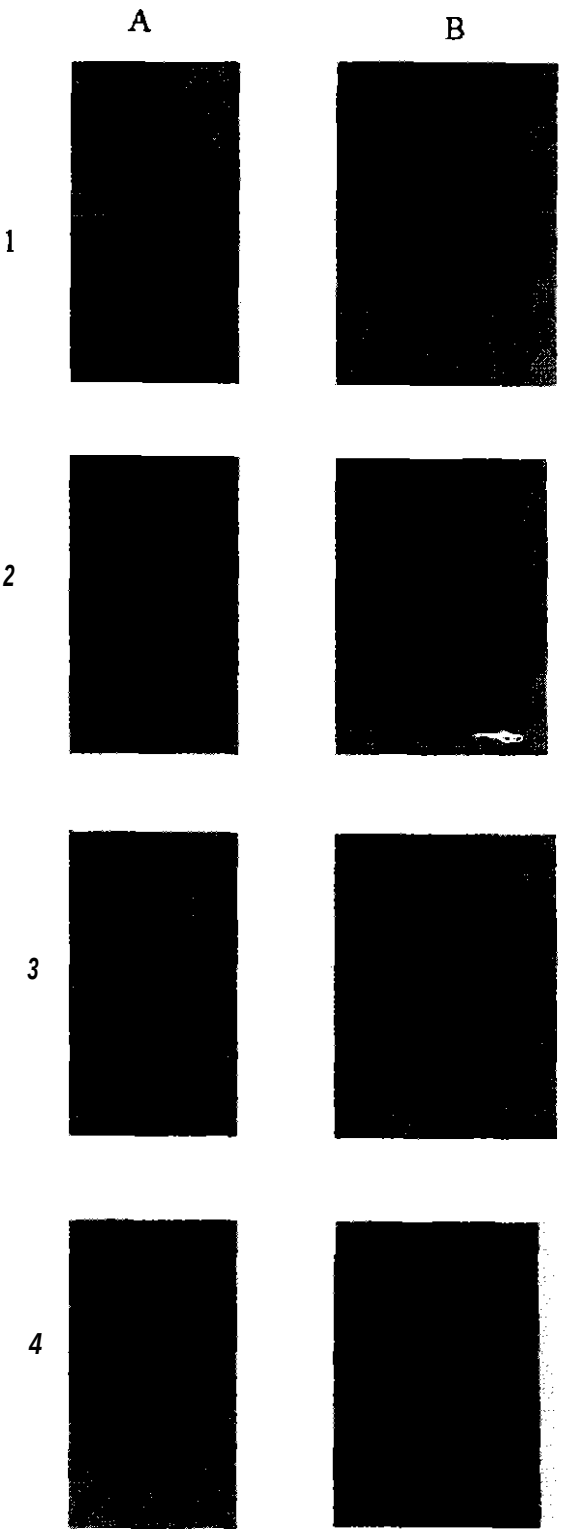

Figure 4. Protein pattern in region C of both non-induced (A) and BAP-induced (B) shoot apex. I: one month; 2: two months; 3: three months; 4: four months old shoot apex.

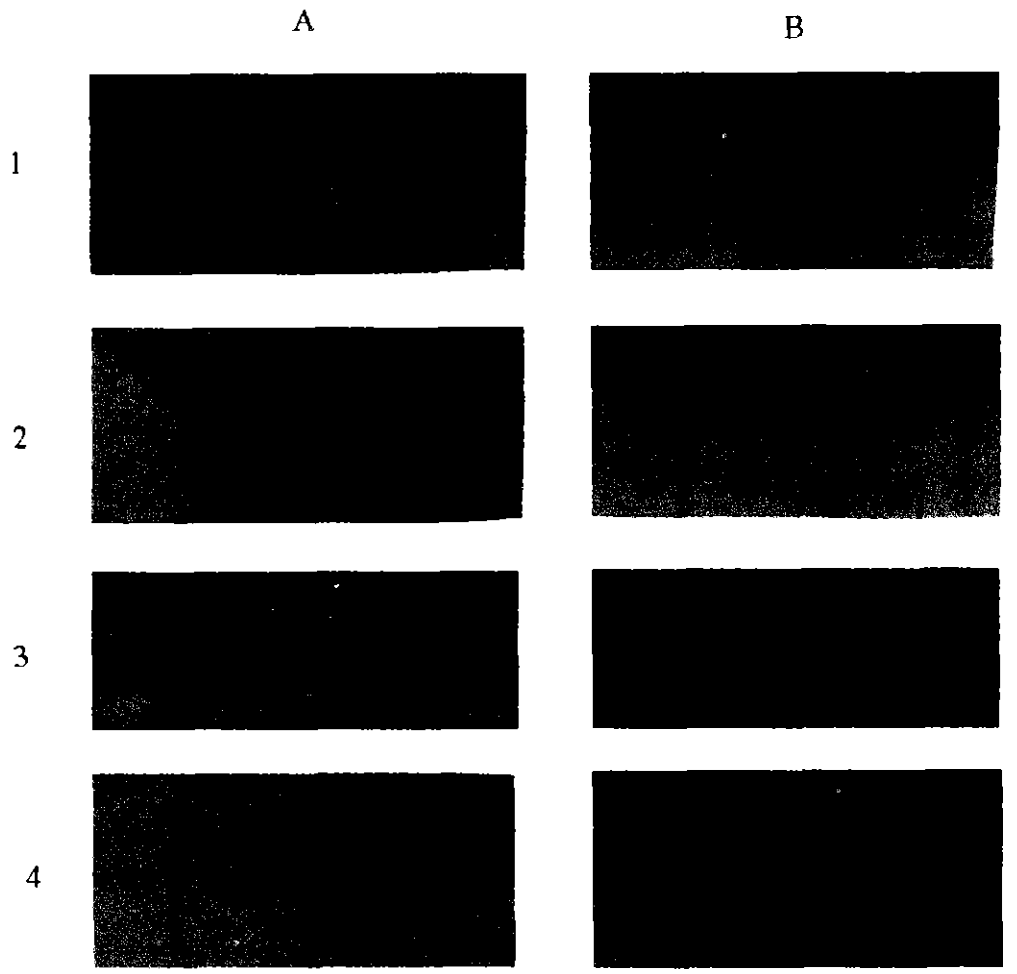

Figure 5. Protein pattern of region D of both non-induced (A) and BAP-induced (B) shoot apex. I: one month; 2: two months; 3: three months; 4: four months old shoot apex.

tein pattern between the BAP-induced and non-induced shoot apical meristem, the differences were only with the minor proteins, not in the major proteins. This observation was also made by other groups where they found the variations in proteins to be very subtle (Bassett and Mothershed, 1986; Lay-Yee, 1986; Bassett et al., 1991). Thus, unless the corresponding polypeptide is stable and/or produced in large amounts, its detection without specific antibodies would be difficult or impossible (Lee et al., 1994).

Changes in the anatomy of the shoot apices during the conversion from vegetative to an inflorescence meristem were also monitored by histology. In the non-induced shoots, the meristem continued to proliferate by producing more leaves (Figure 6). In the BAP-induced shoots, there is a transition of the vegetative meristem to an inflorescence meristem (Figure 7). However, instead of flowers being produced in the axils of the inflorescence bracts, incomplete flower-like structure bearing more bracts were produced. These flower-like structures did not have any reproductive structures. Occasionally, these structures carry carpel-like organs. It was initially thought that these structures resemble the floral abnormalities observed in the fields. However, the phenotype did not reflect this with the in *vitro* flower-like structures the phenotypic changes occurred at the meristematic level where floral abnormalities occur at the floral organ identity level. In fact, it suggested that the cytokinin in the growth medium promotes the transition of vegetative meristem to an inflorescence meristem as this transition is not observed with the shoot grown in basal medium.

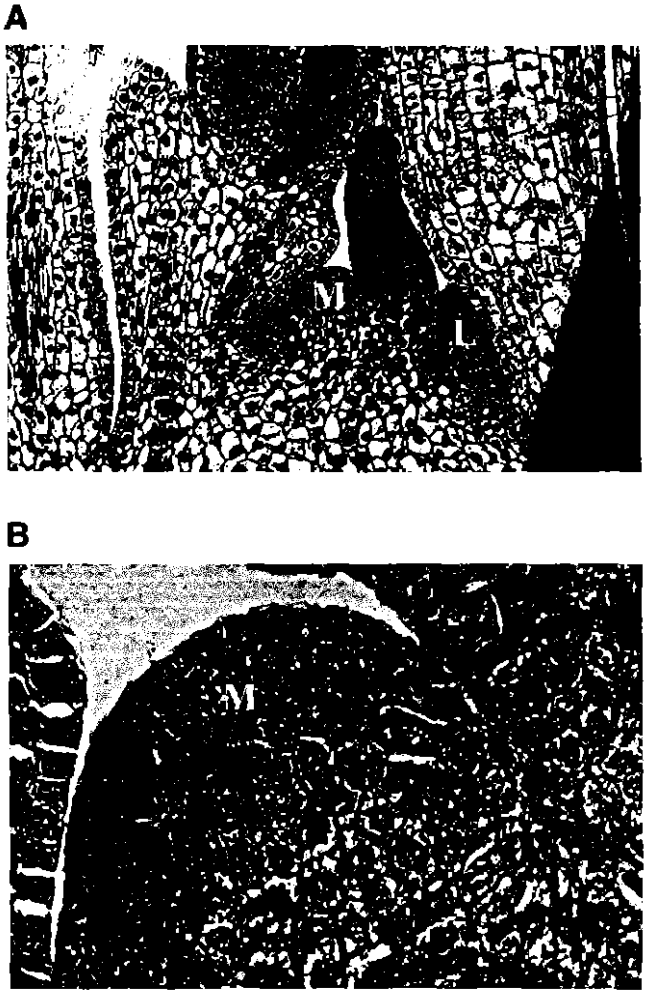

Figure 6. Changes occurring in the shoot apex of non-induced cultures at 3 (A) and 4 (B) months. M: shoot meristem; L: leaf

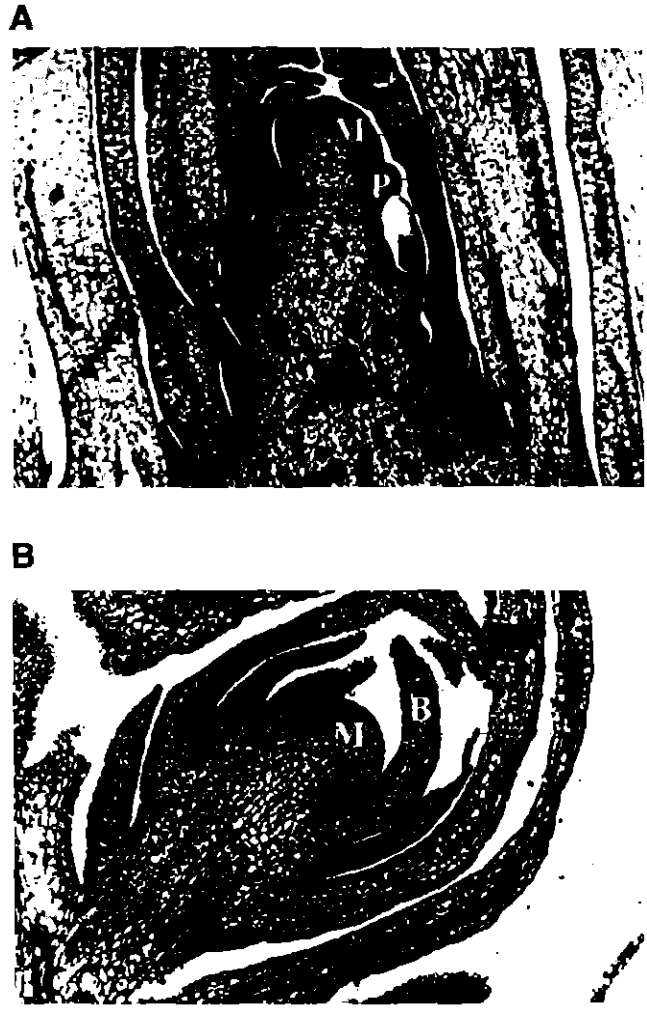

Figure 7. Changes occurring at the shoot apex of BAF-induced cultures at 3 (A) and 4 (B) months. B: bract; M: meristem; P: leaf primordia; L: leaf

CONCLUSION

Protein analysis using two-dimensional polyacrylamide gel electrophoresis revealed three proteins with molecular weights between 35 to 45kD that differed between BAP-induced and non-induced shoot apices of oil palm. These proteins could be involved in the transition from a vegetative meristem to an inflorescence meristem. They were absent in the transition from the vegetative meristem into inflorescence meristem. The protein profile changes may not only reflect changes in the nature of the meristern, it may also be associated with cytokinin treatment. To test these theories, antibody studies can be done on soil-grown wild-type plantlets to see the pattern of protein accumulation. This would verify the roles played by these proteins in the transition from vegetative to reproductive phase in oil palm.

Closer observation of the induced inflorescence confirmed that it did not carry any 'true' flower, merely a structure with all the floral bracts but not without the reproductive structures of a flower. This, however, is a normal property for an inflorescence meristem. This observation suggests cytokinin might play a role in the induction of an inflorescence meristern, although the transition from vegetative meristem appears incomplete.

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