ANTHRAQUINONES FROM LEAVES OF *CASSIA PODOCARPA*

Cassia podocarpa (Fam. Ceasalpinaceae) is indigenous to savannah regions of West Africa[1]. *C. Podocarpa* is closely related to officinale senna[2]. The leaves and fruits of this plant are mentioned as purgatives[3]. Leaves have shown positive Borntraéger reaction[2] and contain rhein derivatives[4]. In view of *C. podocarpa* being considered a possible substitute to *C. senna*[5], it was deemed of interest to reexamine the leaves of *C. podocarpa* for its anthraquinone compounds.

Experimental

Procedures for extraction of free and combined anthraquinones from leaves of *C. podocarpa* were similar to *Cassia siamea*[6]. Extracts were examined by thin layer chromatographic processes and separated components were isolated by preparative thin layer chromatography[7]. Chrysophanol, emodin and rhein were detected as free anthraquinones but rhein alone was present in glycosidic form.

Free and combined anthraquinones of leaves were estimated as the equivalent of rhein by a colorimetric method[7]. The maximum optical density for rhein in 5% w/v NaOH + 2% ammonia solution was at 505 nm. A linear relationship was obtained between concentration and optical density up to a concentration of 1 mg/100 ml.

The estimated content of fsee anthraquinones was 0·65% w/w and of combined anthraquinone (rhein glycoside) 1% w/w, giving a total of 1·65% w/w in the dry powder. Apparently, rhein emerges as the main component of the leaves and is largely present in the glycosidic form. It would seem that rhein glycoside is probably responsible for the purgative properties of leaves of *C. podocarpa*. Although in comparison with *C. senna*, the estimated anthraquinone content in leaves of *C. podocarpa* is 3–4 times lower, nevertheless *C. podocarpa* promises a plausible alternative to *C. senna*.

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January 6, 1978

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ANTIGENIC AND NODULATION PROPERTIES OF *RHIZOBIUM TRIFOLII* TRANSFORMANTS

ISOLATION of *Rhizobium trifolii* nif+ transformants were described earlier[1]. The five transformants, B_1, B_3, B_5, A_4 and A_{10} derived from the parents *R. trifolii* RT/3 (Recipient) and *Azotobacter chroococcum* B_3 (Donor) were capable of growing in nitrogen free Ashby's mannitol broth. The present report confirms the antigenic homology of the transformants with that of the recipient RT/3, cultured under identical conditions. Nodulation tests on the host *Trifolium alexandrinum* variety tetraploid C further confirmed that the transformants were derived from the recipient *R. trifolii* RT/3 and maintained symbiotic properties.

For preparation of whole cell antigen, *R. trifolii* RT/3 and five transformants were grown on yeast extract mannitol agar (YEMA) slopes[2] and the donor *A. chroococcum* B_3 on Ashby's mannitol agar slopes[3]. The YEMA slopes were preferred to Ashby's medium to avoid antigenic variations brought about by differences in medium components. Rabbit antisera were prepared for the whole cell antigen of RT/3 (Recipient) and B_3 (Donor) strains and precipitin reactions were carried out in immuno-diffusion plates. Procedures adopted for the preparation of antigen, antisera, immunodiffusion plates and for interpretation of precipitin reactions were the same as described by Vincent[2].

Nodulation was tested on *T. alexandrinum* variety tetraploid C under sterilized soil conditions in pots. The moisture in the soil was kept at 1/3 of the water holding capacity and sterilized for 3 consecutive days for four hours a day. Surface sterilized seeds inoculated with the parent as well as the transformant cultures were sown at the rate of 7 seeds per pot in quadruplicate series along with an uninoculated control of the same seed density. Plants were allowed to grow through a punched paper-cover tightly fitted to the pots to avoid external contamination.

The recipient strain RT/3 and the five transformants B_1, B_3, B_5, A_4, and A_{10} formed identical precipitin bands in immuno-diffusion plates with antiserum of RT/3 (Fig. 1A). One somatic (*a*) and one internal (*b*) band were formed with all the antigens. No variation was observed with regard to the position and joining of ends of both the precipitin bands with all transformants, confirming that antigenically the transformants were identical with the recipient parent RT/3. The whole cell antigen of the transformants prepared by growing them on YEMA as well as on Ashby's medium was also tested with B_3 antiserum

(Fig. 1 B and 1 C). While the donor B_3 formed one somatic (*a*) and two internal antigen (*b*, *c*) bands with its antiserum, the transformants did not show any cross reaction when the antigens of these were prepared on YEMA (Fig. 1 B). Only B_1 and B_3 formed cross reacting heterologous '*b*' band with B_3 antisera when cultured on Ashby's medium (Fig. 1 C). Such non-specific bands formed due to antigens or haptens of intracelluar origin would be expected in a drastically changed medium. But the absence of somatic bands with the B_3 antiserum excludes the possibility of these strains being derived from *Azotobacter*.

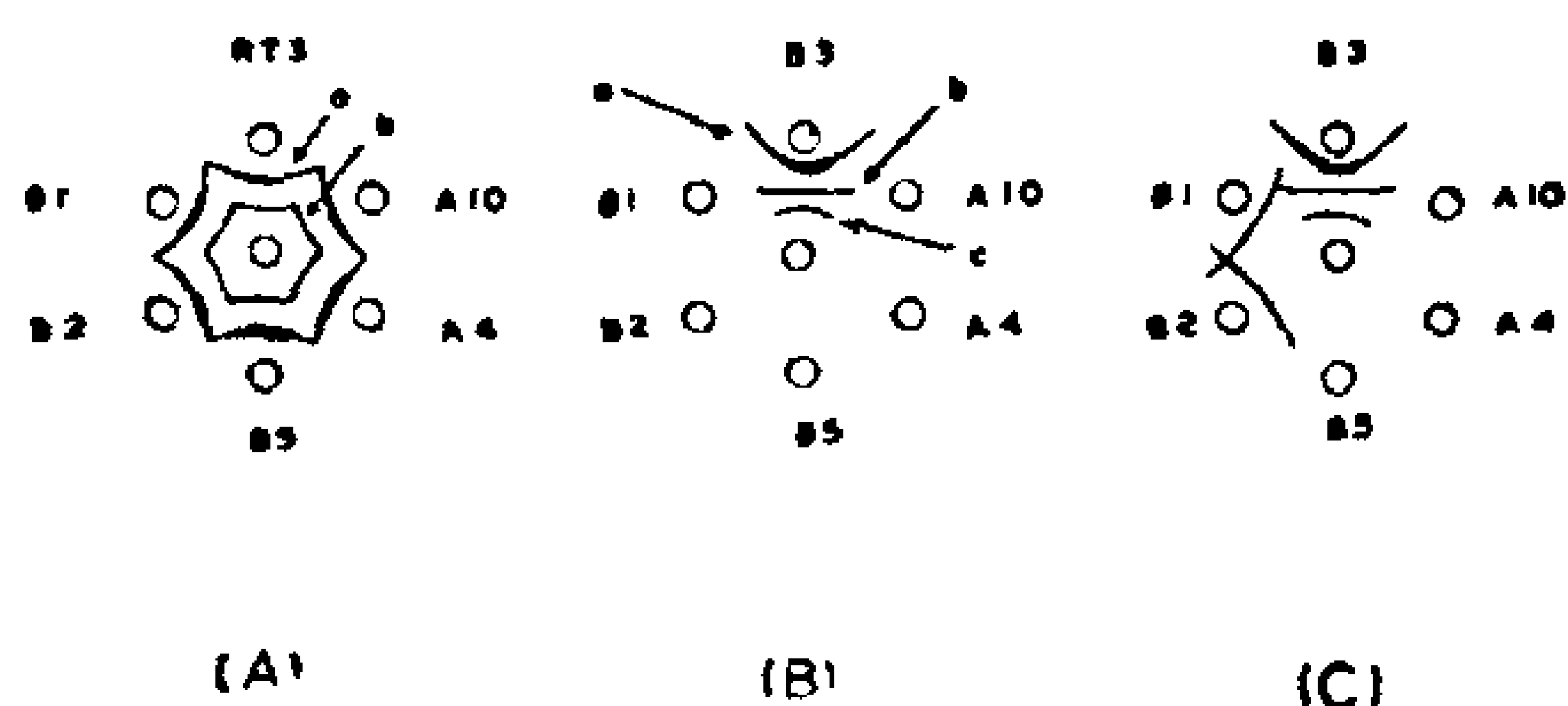

FIG. 1. Precipitin bands formed in agar diffusion against antisera of *R. trifolii* RT/3 recipient (A), and *A. chroococcum* B_3 donor (B and C). Transformantsl B_1, B_2, B_5, A_4 and A_{10} were grown on yeast extract mannitol agar slopes. When transformants were shifted to Ashby's medium, changes in antigenic properties of transformants B_1 and B_2 were observed (*c*) in all the three hexogones.

Nodules were not observed in uninoculated control plants and in those inoculated with *A. chroococcum* B_3, while the recipient strain RT/3 and other transformants formed nodules on *T. alexandrinum* (Table I). The mean number of nodules varied from 9 to 14 and the variation was within statistical limits.

TABLE I

Nodulation of Trifolium alexandrinum (*variety tetra ploid C*) *by* A. chroococcun, R. trifolii *and transformants under pot culture conditions* (*values average of* 28 *plants*)

Strains	No. of nodules/plant
Control	0
A. chroococcum B_3 (Donor)	0
R. trifolii RT/3 (Recipient)	$11 \pm 0 \cdot 33$
Transformants	
B_1	$14 \pm 0 \cdot 99$
B_2	$11 \pm 0 \cdot 69$
B_5	$9 \pm 0 \cdot 62$
A_4	$11 \pm 0 \cdot 97$
A_{10}	$13 \pm 0 \cdot 6$

We are thankful to Dr. N. S. Subba Rao for providing all the facilities.

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REGENERATION OF HYBRID TOMATO PLANTS FROM LEAF CALLUS

THE use of tissue culture method for rapid propagation[1] and protoplast fusion[2] is already described in literature. Tomato plants (*Lycopersicon esculentum*) have been regenerated from leafcallus by Kartha *et al.*[3]. A hybrid tomato TH (Pol × Pusa) has been developed in this laboratory[4] by artificial pollination between Pusa variety (red colored fruits bearing) and Pol variety (high β carotene containing fruits). This hybrid TH has lycopene and high β carotene containing fruits which are nutritious and easily marketable. The present work describes the differentiation of leaf callus of Pol and TH varieties of tomato into full plants. The work was taken up with the objectives of (1) rapid propagation of hybrid and (2) for the study of protoplasts fusions.

Standard methods of White[5] were used for the cultivation of leaves gathered from 4–5 weeks old plants, and plants which were flowering. The sterile leaves were cut into small pieces and put into different media for callus development and differentiation.

The basal medium contained Murashige and Skoof's[6] major and minor salts, vitamins of B 5 medium[7] with 3% sucrose as the carbohydrate source. Agar (1%) was used to solidify the medium. PH was adjusted to 6 before autoclaving and standard techniques were used for autoclaving and culturing. Cultures were incubated at 26° ± 1° C with a light intensity of 1000 Lux and relative humidity of 50–60%. Controls were maintained in all the experiments.. Each experiment had a minimum of 10 replicates and all experiments were conducted twice atleast.

For the Pol leaf explants 12 different media were tried using basal medium with different hormones at various concentrations. It was observed that of the various cytokinins used, benzyladenine and zeatin