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CENTRO DE INVESTIGACION CIENTIFICA DE YUCATAN, A.C.

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December 10, 1999

Re: C/2236-2

Ingrid Lindhe

Assistant, Crop Sciences Area

International Foundation for Science

Please find enclosed the final report of the project "AUXIN SIGNAL TRANSDUCTION MECHANISMS IN *Catharanthus roseus* ROOTS TRANSFORMED WITH *Agrobacterium rhizogenes*.This project was done at:

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Centro de Investigación Científica de Yucatán, A. C.
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México

The IFS financial support started on January 1, 1997 and finished december 10, 1999. Attached to this letter is a brief summary of the report and the manuscripts resulted from the IFS support, as well as the completion form.

Please do not hesitate in contact me if I can be of any further help.

Yours sincerely,

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PROJECT TITLE: Auxin signal transduction mechanims in *Catharanthus roseus* roots transformed with *Agrobacterium rhizogenes*

Grantee No. C/2236-2F

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Report from the period between November 1998 and December 1999

The present project seeks to uncover novel mechanisms of Phospholipase C (PLC) regulation, by growth factors, in plants. Although PLC enzymes in animals and plants catalyze the same basic reaction, the plant system offer a novel biological system in which a differentiated tissue can be routinely generated in the laboratory from undifferentiated cell. Using a s a model *Catharanthus roseus* roots, transformed with *Agrobacterium rhizogenes*, comparative studies at both, the biological an enzymological level, are proposed to be useful to understand more completely the role of PLCs in nature and signal transduction relevant to growth.

We have previously reported that *Catharanthus roseus* transformed roots contain at least two phosphatidylinositol 4,5-bisphosphate-phospholipase C (PLC) activities, one soluble and one membrane associated. In this report, the effect of neomycin and several divalent cations was analyzed. Neomycin, inhibited PLC in a concentration-dependent fashion, the IC_{50} (100 mM) was the same for the inhibition of the soluble and the membrane associated PLC activity. The effect of different divalent cations such as Ni^{2+}, Cu^{2+} and Zn^{2+} was studied as well. For the soluble activity, the inhibition due to the three cations was very similar (IC_{50}s between 0.2 and 0.3 mM). For the membrane associated PLC activity, Cu^{2+} was the most potent inhibitor (IC_{50} 3.6 mM), then Ni^{2+} and then Zn^{2+}. The effect of macerozyme on indole alkaloid, coumarine accumulation, and the induction of tryptophane decarboxylase (TDC) and phenylalanine ammonia-lyase (PAL) activities and their relationship with PLC activity on *C. roseus* hairy roots was also investigated. Increasing concentrations of macerozyme induced an increase in indole alkaloid and coumarine accumulation. There was a 10-fold increase in TDC and 4-fold in PAL activities in hairy roots treated with 1 % of macerozyme. The effect of macerozyme on PLC activity depends on the concentration used. In a dose-response experiment PLC activity decreased 38 % with 0.5 % of macerozyme and increased 40 % with 1 % of macerozyme when compared to control. The addition of neomycin, inhibits

the increment in alkaloid production induced by macerozyme. The properties of PLC partially purified from *C. roseus* transformed roots were analyzed using substrate lipids dispersed in phospholipid vesicles, phospholipid-detergent mixed micelles and phospholipid monolayers spread at an air-water interface. Using ^{33}P-phosphatidylinositol 4,5-bisphosphate (PIP_2) of high specific radioactivity, PLC activity was monitored directly by measuring the loss of radioactivity from monolayers as a result of the release of inositol phosphate and its subsequent dissolution on quenching in the subphase. PLC activity was markedly affected by the surface pressure of the monolayer, with reduced activity at extremes of initial pressure. The optimum surface pressure for PIP_2 hydrolysis was 20mN/m. Depletion of PLC from solution by incubation with sucrose-loaded PIP_2 vesicles followed by ultracentrifugation demonstrated stable attachment of PLC to the vesicles. A mixed micellar system was established to assay PLC activity using deoxycholate. Kinetic analyses were performed to test whether PLC activity was dependent on both bulk PIP_2 concentration and surface concentration in the micelles.

PUBLICATIONS OF THE PROYECT SUPOORTED BY IFS.

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