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SHORT COMMUNICATION

A MODIFIED METHOD FOR EXTRACTION AND IDENTIFICATION OF INDOLE-3-ACETIC ACID (IAA), GIBBERELLIC ACID (GA_3), ABSCISIC ACID (ABA) AND ZEATIN PRODUCED BY *PHANEROCHAETE CHRYSOSPORIUM* ME446

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Summary. Besides higher plants, there is evidence that fungi synthesize IAA, GA_3, ABA and zeatin as well. According to our findings, *Phanerochaete chrysosporium* ME446 synthesized the growth substances IAA, GA_3, ABA and zeatin as primary and secondary metabolites. Recovery of IAA, GA_3, ABA and zeatin were respectively 55.5±10%, 74.6±8%, 51.6±10% and 56.63±6%. We have demonstrated that one can use the same extraction method to determine the different growth substances at the same time, thus this method will be primary and the only method that is applied in this research area.

Introduction

Due to the recent discovery of plant growth regulators, it has been possible to control plant growth and many activities concerned with growth. Besides high plants, there are evidences that fungi synthesize IAA, GA_3, ABA and zeatin as well. For example, many researchers established that IAA is produced by *Dipodascopsis uninuc-*

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leata, GA_3 is produced by *Gibberella fujikuroi* (*Fusarium moniliforme*), ABA is produced by *Cercospora rosicola*, cytokinin is produced by *Fusarium moniliforme* and *F. colmonum* (Elwy, 1989; Rachiev et al., 1993).

Several methods for extracting plant hormones are described in different references. However, most of these methods were developed for use with specific hormones.

The aim of this study was to prove that indole-3-acetic acid (IAA), gibberellic acid (GA_3), abscisic acid (ABA) and zeatin can be obtained by the same extraction method from *P. chrysosporium* ME446. In this respect, a procedure for extracting and identifying plant growth substances (IAA, GA_3, ABA and zeatin) was developed through modification of reference methods. The procedure is particularly useful for the study more than one hormone simultaneously in a given sample even if the sample supply is limited.

Materials and Methods

Extraction, purification and quantitative determination of free and bound IAA, GA_3, ABA and zeatin in both the culture medium and the mycelium of *P. chrysosporium* ME446 were conducted with little modifications in the methods of Prakash and Prathapasenan (1990), Zieslin and Geller (1983), Ames et al. (1979), Beardsell and Cohen (1975) and Van Staden and Nicholson (1989). The extraction and purification procedures are shown on Fig. 1. In the testing procedure of recovery, authentic IAA, GA_3, ABA and zeatin standards were used and each was 1 mg with 100 ml of methanol:chloroform:2 N ammonium hydroxid (12:5:3 v/v/v). IAA, GA_3, ABA and zeatin loses during the extraction and purification steps were determined by passing known amounts of standard synthetic IAA, GA_3, ABA and zeatin samples through the Fig. 1 procedure.

Spectrophotometric techniques were applied to determine the amounts of IAA, GA_3, ABA and zeatin.

Average recovery of IAA, GA_3, ABA and zeatin are displayed in percentage which is calculated from the standard curve.

Results and Discussion

The maximum amounts of IAA, GA_3, ABA and zeatin in both the culture medium and the mycelium of *P. chrysosporium* ME446 were given in Fig. 2.

Average recovery of the authors' method is shown in % on Fig. 3.

Some researchers reported the recovery of IAA, GA_3, ABA and zeatin by their own methods. For example, Lin et al. (1986) reported that recoveries of ABA was ranged from 35 to 77% (average 62%) for leaf samples. Boyer and Zeevaart (1982)

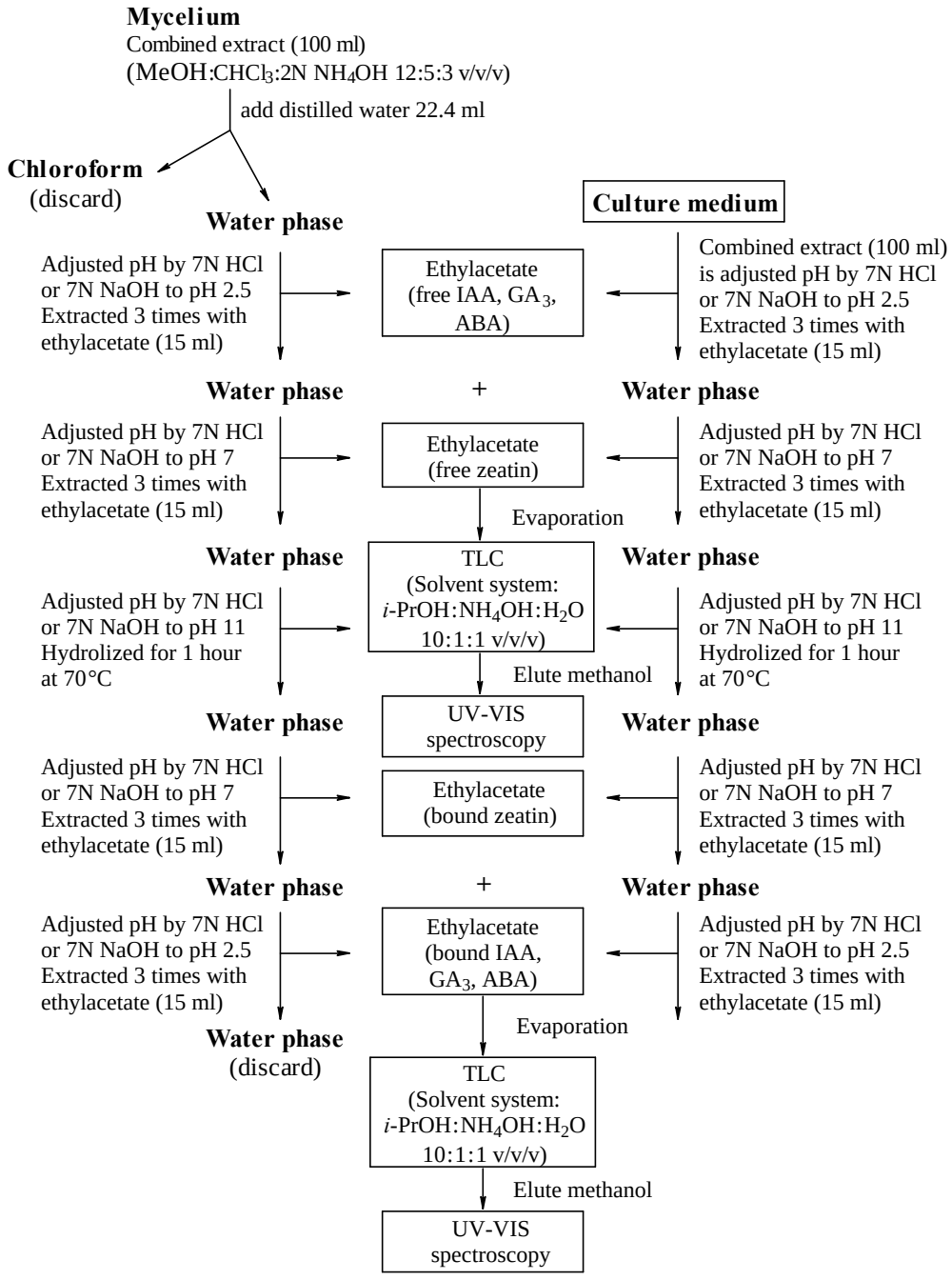

Fig. 1. Flow diagram outlining the extracts used in purification of IAA, GA_3, ABA and zeatin for UV-spectrophotometer

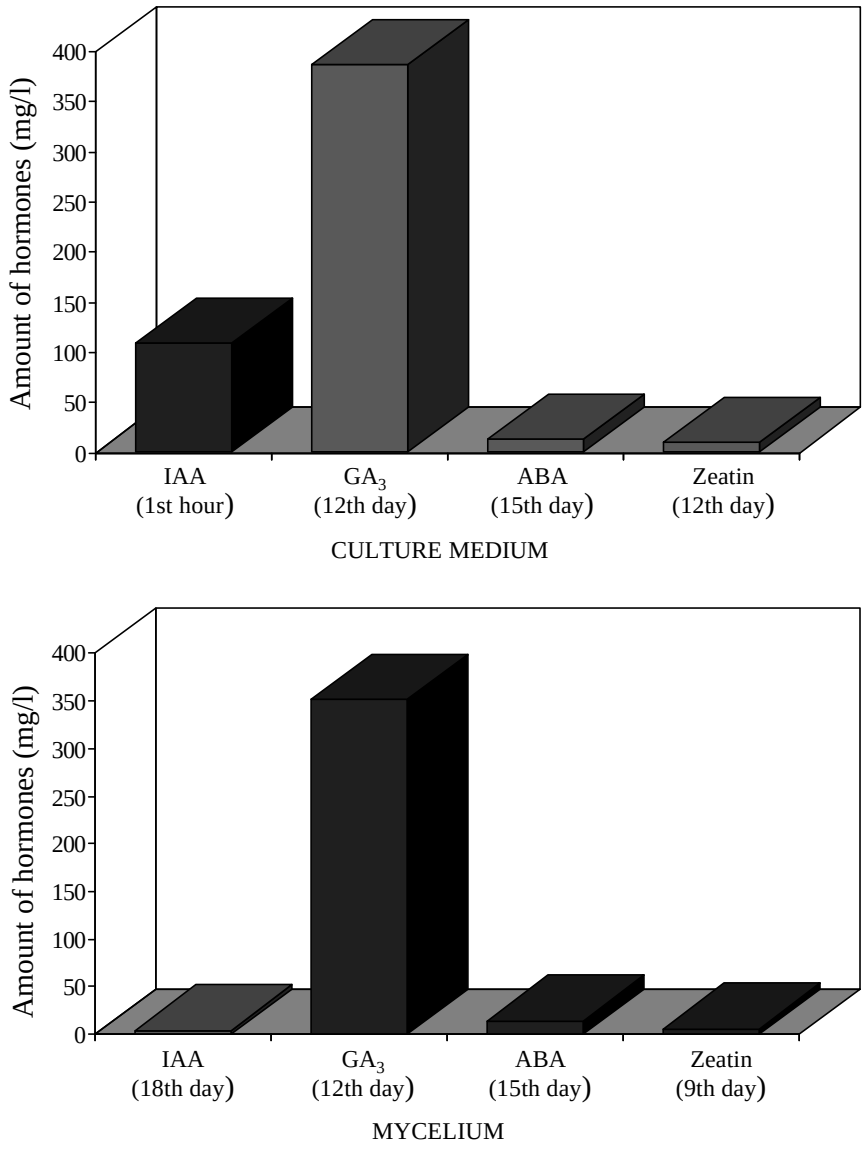

Fig. **2**. The maximum amounts of IAA, GA_3, ABA and zeatin in both culture medium and mycelium of *P. chrysosporium* ME446

reported that recovery of ABA was between 52% and 62% for leaf samples. Goldschmidt and Galily (1974) reported that recovery of GA_3 was 63 to 69%. However, recovery of GA_3 was reported 56–86% (Mettrie et al., 1988). For zeatin, between 20 and 40% recovery was found in the various extracts (Hansen et al., 1988). Moreover, recovery of zeatin was reported 60% (Dekhuijzen and Gevers, 1975). Average

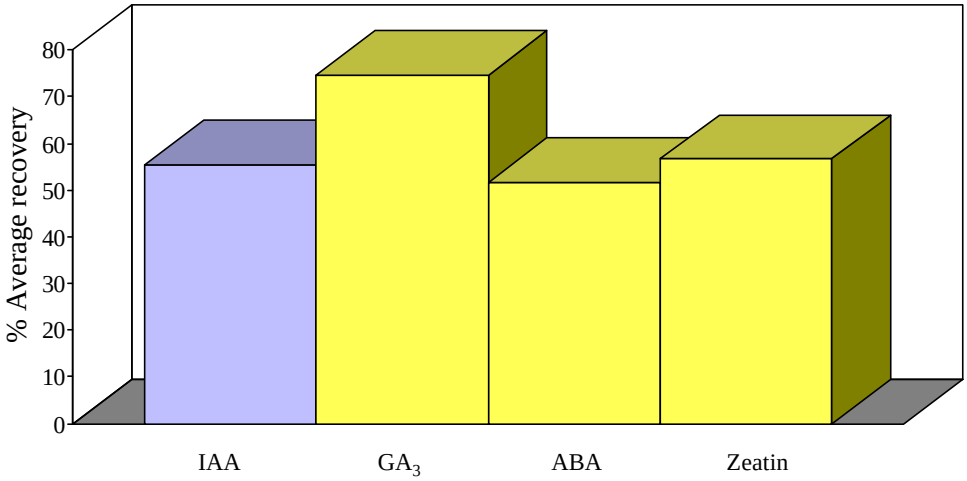

Fig. 3. Average recovery of IAA, GA_3, ABA and zeatin standards

recovery of IAA was reported between 34 and 39% (Lieberman and Knegt, 1977). Prakash and Prathapasenan (1990) reported that the yield of IAA from samples was 59±4.1.

Some of the researchers have reported that the recovery of plant hormones is ultimately dependent on the extraction procedure (Hemberg and Tillberg, 1980).

According to our findings, *P. chrysosporium* ME446 has synthesized the growth substances (IAA, GA_3, ABA and zeatin) as primary and secondary metabolite. As a result, we have demonstrated that one can use the same extraction method for determining the different growth substances at once. This is the first and the only method that is applied in this research area.

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