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EFFECT OF THE ETHYLENE PROMOTER, 1-AMINOCYCLOPROPANE CARBOXYLIC ACID (ACC) AND THE ETHYLENE INHIBITOR, AMINOOXY ACETIC ACID (AOA) ON *IN VITRO* POLLEN TUBE GROWTH IN THE TERRESTRIAL ORCHID, *SPATHOGLOTTIS PLICATA* BLUME.

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Orchid pollinia exhibit a deviant behaviour of pollen tube growth *in vitro* compared to other angiosperms. In the terrestrial orchid species, *Spathoglottis plicata*, the rate of pollen tube elongation increases steadily for a period of 12 h, after which it declines. It is suggested that pollen - borne auxin, indole-3-acetic acid (IAA) induced ethylene synthesis, which stimulated the rate of pollen tube growth *in vitro*. Addition of the ethylene promoter, aminocyclopropane carboxylic acid (ACC) to the pollen germination medium significantly stimulated the rate of pollen tube growth, whereas when the ethylene inhibitor aminoxy acetic acid (AOA) was added, it significantly retarded the rate of pollen tube growth.

Keywords : Aminocyclopropane carboxylic acid; Aminoxy acetic acid; Ethylene; *In vitro* pollen tube growth; *Spathoglottis plicata*.

Introduction

Pollinia or pollen associations demonstrate the same general features of tube development as angiospermous pollen. In Asclepiadaceae, where the pollen grains are enclosed within a pollinial wall, germination is normal but tube growth progressively declines and finally ceases before attaining a fraction of the length attained *in vitro* [1,2]. The behaviour of tetrads and polyads is also not different[3]. It has been reported that the orchid pollinium exhibits a deviant behavior of tube growth *in vitro*[4]. In the terrestrial orchid species, *Spathoglottis plicata,* the rate of tube elongation increases steadily for a period of 12 h after which it declines. It is suggested that pollen - borne auxin, indole-3-acetic acid (IAA) induces the synthesis of ethylene, which stimulates the rate of pollen tube growth *in vitro*.

Herein, the effect of the ethylene promoter, 1- aminocyclopropane carboxylic acid (ACC) and the ethylene inhibitor, aminooxy acetic acid (AOA) on pollinial tube growth in *Spathoglottis plicata* is reported.

The orchid species used in the study was *Spathoglottis plicata* Blume, a common Malaysian terrestrial orchid. It was grown in the Orchidarium of Tropical Botanic Garden and Research Institute, Palode. ACC and AOA were obtained from Sigma Chemical Company, USA. All other chemicals used were of reagent grade.

Pollinia from fresh flowers were dissected out and grown in Brewbaker medium[5] with 10% sucrose and 1mM of the ethylene promoter, ACC or the ethylene inhibitor, AOA. The concentration of the ethylene promoter ACC and ethylene inhibitor AOA used was fixed, based on previous experiments in the laboratory. Appropriate controls were maintained for all experiments. All experiments were conducted at 28 ± 2°C. Emergence of the first few pollen tubes outside the pollinium was taken as the initiation of pollen gerimation. Pollen tube measurements were made at 2 h intervals, after staining the preparation with aniline blue. Pollen tube length values were the mean of 50 measurements of the longest pollen tubes.

In the Brewbaker medium (control), pollinia of *Spathoglottis* germinated only after 5-6 h of incubation and pollen tube growth continued upto 30-36 h, at the end of which the tube attained a length of 600 µm. The rate of tube growth increased

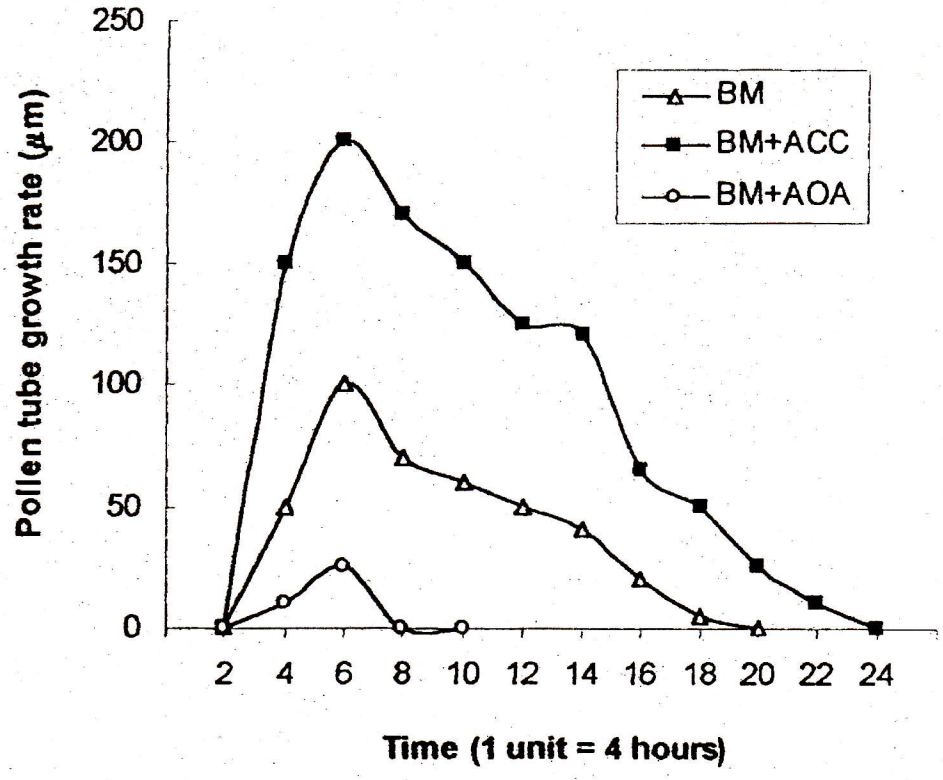

Fig. 1. Effect of ethylene promoter, ACC and ethylene inhibitor, AOA on pollinial tube growth *in vitro* of *Spathoglottis plicata*.

steadily (100 µm). during the first 10-12 h period, then it decreased and became negligible (Fig. 1). Addition of ACC, the ethylene precursor into the medium, stimulated the rate of pollen tube growth. The rate of pollen tube growth increased steadily (200 µm) during the first 2-12 h period, then it decreased and became negligible by 48-50 h, by which time it had attained a length of 800 µm. Addition of AOA, the ethylene inhibitor to the medium significantly inhibited the rate of pollinial tube growth. The rate of pollen tube growth was only 25 µm during the first 2-12 h, then it declined and became negligible by 16h, by which time the tube length reached 100 µm. The pollen tubes appeared stunted, curled, deformed and stub-like, around the pollinium.

The sustained high rates of tube growth for 12 h in orchids can be explained on the basis of growth inhibition - growth promotion system postulated by Thimann[6]. According to him, plants may have a normal growth inhibiting system and normal growth is the result of a balance between this and a growth promoting system. Mcleod[7] extended this concept in relation to pollen development by suggesting that the growth of pollen tube is subject to a natural restraint, which can be released by a variety of factors. In compatible pollinations, the products of pistil-pollen interaction might overcome the inherent inhibition and stimulate the pollen tubes to grow rapidly and reach the ovule. In orchid pollinia, the factor that overcomes the natural restraint of pollen growth *in vitro* may be the pollen - borne auxin, indole-3-

acetic acid (IAA), which promotes the release of tube growth promoting ethylene[4]. Orchid pollinia are rich in IAA[8], with concentrations as high as 100 mg g^{-1}. Latha and Namboodiri[4]. reported that while auxin induced ethylene evolution is responsible for orchid perianth senescence following pollination, the same gas helps to maintain high pollen tube growth rate during the first 12 h following pollination, before the onset of senescence of perianth parts of the flower.

Orchid pollen also contain ACC, the precursor of ethylene, which helps to increase ethylene production by the pollen[9]. It is therefore possible that incorporation of ACC into the culture medium. stimulated the production of ethylene, which led to the observed increase in pollen tube growth rate, during the first 12 h following incubation, in the present study. It has already been reported that IAA in conjunction with ACC resulted in enhanced ethylene production[10]. In similar studies, peach pollen growth *in vitro* was also stimulated by ethrel, a source of ethylene[11]. Almond pollen tube growth was stimulated by calmodulin[12]. On the other hand, when the pollinia were incubated in medium containing the ethylene inhibitor, AOA, suppression of pollen tube growth was observed. This can be explained as due to the inhibition of auxin induced ethylene production by AOA. AOA is known to inhibit enzymes in the ethylene biosynthetic pathway, especially pyridoxine phosphate requiring enzymes, of which ACC synthase is an exameple[13].

Our results support the suggestion that auxin and ethylene play an important role in orchid pollinial tube growth *in vitro*. It is also clear that other factors from pollinium are important and may interact with auxin to regulate pollen tube growth. These endogenous factors may be regulation processes such as wall biogenesis, membrane biosynthesis and respiration needed for pollen tube growth[14].

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