Ethylene induced cyanide resistant respiration in orchid petal cells

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Abstract. Respiration of isolated Aranda orchid petal cells increased markedly after cut flowers were treated with ethylene. An increase in respiration was observed 15 to 20 h after treatment which was further enhanced in the presence of oxygen and ethylene. Ethylene induces the development of a cyanide resistant pathway in fully opened orchid flower tissues where the cyanide resistant capacity is negligible. However, there appears a shift back to the cyanide sensitive pathway some time after induction.

1. Introduction

The biochemistry and physiology of cyanide resistant respiration in plants have been extensively reviewed [7, 8, 9, 12, 13]. It is generally believed that cyanide resistant respiration takes place on the inner mitochondrial membrane and constitutes a pathway 'alternative' to the main cyanide sensitive cytochrome pathway. Respiration of roots, leaves and storage organs is more often than not resistant to cyanide [7].

The development of cyanide resistant respiration is influenced by a number of factors, including ethylene and cyanide [4, 10, 15, 16, 17, 18, 19]. However, for the stimulation of respiration by ethylene, the existence of a cyanide resistant electron transport pathway is a prerequisite [15, 16, 17].

The occurrence of a cyanide resistant pathway in flowers other than aroids has been reported in carnations [20, 21], orchid flowers [2] and roses [14]. Interestingly enough, cyanide resistant respiration was demonstrated in young tight buds of carnation and orchid flowers [2, 21]. There was a shift towards cyanide sensitive respiration as the flower developed such that respiration in fully opened flowers was completely cyanide sensitive. With the onset of senescence, the respiration became cyanide resistant again [2].

In our present studies, we investigated the effect of ethylene on the induction of cyanide resistant respiration in fully opened orchid flowers.
2. Materials and methods

Fully opened flowers of *Aranda* Christine 130 (*Arachnis hookeriana × Vanda* Hilo Blue) were harvested. Each cut orchid flower with its base in a plastic vial containing a small amount of water was placed in a specially made chamber. Flowers were subjected to either a continuous short-term or a long-term ethylene (3 ppm) treatment. A continuous open system for treating the orchid flowers with trace amounts of ethylene was essentially the method of Pratt et al. [11]. The volume of air required was provided from a supply of pure compressed air or oxygen which was humidified and metered through a calibrated flowmeter. Ethylene in a reservoir flask was displaced by 20% ammonium sulphate solution. The displaced gas passed through the flow regulator and mixed with incoming air or oxygen. This open system provided a steady and reliable flow of air or oxygen with the desirable level of ethylene. The concentration of ethylene in the air or oxygen stream was regularly monitored using a Hewlett Packard Gas Chromatograph (Model 5890A) equipped with a flame ionization detector and a Porapak R column. Nitrogen (60 psi) was used as the carrier gas at a flow rate of 35 ml min⁻¹ with an oven temperature of 100 °C (isothermal), injector temperature 150 °C and detector temperature 200 °C [22].

Following ethylene treatment, the petal cells were isolated by enzymic digestion and mechanical agitation as described by Jensen, Francki and Zaitlin [5]. Orchid petal tissue was first cut into strips of about 0.1 x 0.15 cm in size and 1 g of the tissue was then vacuum-infiltrated with 15 ml of maceration medium [5]. The tissue was agitated on a reciprocating shaker maintained at 29 °C. The cell suspension was filtered through a nylon sieve (Hydro-Bios, Apparatebau, GmbH, FRG) with a pore size 2 µm smaller than the cell size. Cells retained on the sieve were washed gently with a washing medium and the cells centrifuged at 80 x g for 3 min. The pellet was resuspended in 5 ml of incubation medium [5]. O₂ uptake was measured polarigraphically using a O₂ Hansatech electrode. Treatment of isolated petal cells with potassium cyanide (KCN)(5 mM) and salicylhydroxamic acid (SHAM)(0.25 mM) were similar to those described previously [2]. All determinations were done in triplicate.

3. Results

3.1 Ethylene stimulated respiration

Respiration rates of isolated petal cells in air and in high oxygen concentration (100%) were comparable, with the rate in O₂ being slightly higher.