β-Glucosidase Activity and Scent Production in Some Flowers

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Keywords: non-scented glycoside precursors, β-glucosidase, scent emission

Abstract

In some flowers the aromatic compounds are stored as non-scented glycoside precursors in the vacuoles. The removal of the sugar moiety from these compounds was facilitated by the activity of the β-glucosidases in floral tissue and is correlated with scent production. Crude extracts from different stages of three scented flowers (*Wrightia religiosa, Ervatamia coronaria* and *Gardenia jasminoides*) were assayed for β-glucosidase using p-nitrophenyl-β-D-glucoside (p-NGP) as the substrate. Different kinds of flowers had different level of β-glucosidase activity with the highest level found in *Wrightia religiosa*. The activity of this enzyme was detected in closed buds and was found to increase significantly in open flowers.

INTRODUCTION

Modern ornamentals have been bred, to a large extent, for color, shape or longer shelf life while their scents are of low priority and may have diminished or even lost in the selection and breeding processes. The scent emitted by flowers is generally believed to be a chemical means of communication by which a plant succeeds in attracting specific pollinators (Dudareva and Pichersky, 2000). Reintroducing floral scents will not only make flower beds more aesthetically pleasing but also improve the yield and quality of many crops (Purdue, 2000).

Scents emitted by flowers are typically complex mixtures of low molecular weight compounds (100-250D) that give the flowers their unique, characteristic fragrances (Dudareva, 2001). The scent of some flowers is made up of as few as seven to ten different compounds, as in snapdragon and petunia, or as many as one hundred different compounds as found in orchids (Dudareva et al., 2000). Several thousand compounds from various floral scents have been identified, mostly by steam distillation or head space trapping in combination with GC-MS (Knudsen et al., 1993). Most of the volatile compounds in floral scents are dominated by fatty acid derivatives, terpenoid, phenylpropanoid and benzenoid compounds with some other chemicals, all of which are secondary metabolite (Dudareva et al., 2000).

The mechanism of flower fragrance formation has not been well understood. Aroma and flavor components are found in many parts of the plant as either volatile or bound forms, which are mostly glycosylated (Reuveni et al., 1999). The glycoside precursors of volatile compounds have been isolated from many flowers such as Rosa species (Ackermann et al., 1989), *Gardenia jasminoides* (Watanabe et al., 1993) and *Narcissus papyraceus* (Reuveni et al., 1999). β-Glucosidase activities was found to increase during the flower opening in parallel with the aroma formation (Watanabe, 1993).

In this paper, the β-glucosidase activities from three different species of white flowers, *Wrightia religiosa*, *Ervatamia coronaria* and *Gardenia jasnimoides* were investigated to see if deglycosylation plays a role in the scent production in these flowers.

MATERIAL AND METHODS

Plant Material

Flowers buds and flowers from different stages (closed buds, half open and open flowers) of *Wrightia religiosa*, *Ervatamia coronaria* and *Gardenia jasminoides* were

Proc. WOCMAP III, Vol. 5: Quality, Efficacy, Safety, Processing & Trade in MAPs
Eds. E. Brovelli, S. Chansakaow, D. Farias, T. Hongratanaworakit,
M. Botero Omary, S. Vejabhikul, L.E. Craker and Z.E. Gardner
Acta Hort. 679, ISHS 2005

obtained from the garden of Silpakorn University, Sanamchan palace campus. The collected flower petals were frozen in liquid nitrogen and stored at -80°C until used (Fig. 1).

Preparation of Crude Extract

The crude extract from frozen petal tissue was prepared by the method of Reuveni et al. (1999). The frozen petal tissue was grounded in the extraction buffer containing 5 mM ascorbic acid, 50 mM Mes, 20 mM DTT, 10 mM $MgCl_2$, 4% PVPP, 3 mM PMSF, brought to pH 7.4 with solid Tris. The homogenate was then filtered through a few layer of cheesecloth and centrifuged at 10000 g for 20 minutes. The supernatant or crude extract was assayed for β-glucosidase.

Protein Determination of Crude Extract

The amount of protein in the crude extract from petal tissue was determined by the method of Lowry (Lowry et al., 1951).

β-Glucosidase Assay of the Crude Extract

β-Glucosidase activity in the crude extract from petal tissue was assayed using p-nitrophenyl-β-D-glucopyranoside (pNGP) as a substrate (Reuveni et al., 1999). The crude extract was incubated in 0.1 M acetate buffer pH 5.5 and 0.10 mM pNGP for 10 minutes at 25°C and the reaction was terminated by adding 1 ml of 1.0 M Na_2CO_3.The cleavage of the glycosyl moiety of pNGP was monitored by measuring the release of p-nitrophenolate spectrophotometrically at 400 nm.

RESULTS AND DISCUSSION

β-glucosidase activity was found in the crude extract from all the flowers we have studied. In these flowers the enzyme activities dramatically increased from closed bud and reached a maximum level at open flower stage. The enzyme activity in the closed bud stage of *Ervatamia coronaria* was much lower than the other two flowers. When we considered only *Ervatamia coronaria*, the enzyme activity in the closed bud stage was very low but rose to about 60x in half open and 80x in open flowers when compared to closed bud. In *Gardenia jasminoides* and *Wrightia religiosa*, both of which are strong fragrant flowers, the flowers in all stages had much higher β-glucosidase activities than the less fragrance counterpart *Ervatamia coronaria* (Fig. 2). The scent emission of these two fragrant flowers also increased during the opening stages of the flower.

Improving floral scent is very important not only for floriculture and horticulture industry but also agriculture. In contrast to the emphasis on research into chemical aspects of floral scents, there have been only few studies on the biosynthesis of floral scents, mechanism of scent emission and the molecular biology concerning these processes (Dudareva and Pichersky, 2000). Many investigation have proposed the hypothesis that the floral scent compounds are synthesized elsewhere in the vegetative tissue and then transported to the flower (Dudareva et al., 2000). Several line of evidence support that hypothesis. First, many fruit and floral scent components are often found in both free and glycosylated form, and some of these may have been transported from the vegetative part of the plant (Gunata et al., 1985; Tang et al., 1990). Second, glycosylated compounds are often found in flower buds, which are usually not scented, and later found in flowers (Ackermann et al., 1989; Loughrin et al., 1992; Watanabe et al., 1993). Lastly the Russian group claimed that the transport of glycosylated precursors into flower buds occurs in roses. The increase of β-glucosidase in our tested flower is similar to those in roses (Ackermann et al., 1989) and Narcissus (Reuvini et al., 1999). Reuveni et al. (1999) also demonstrated that the inhibition of β-glucosidase activity decrease scent emission from Narcissus flowers.

CONCLUSIONS

The good correlation between β-glucosidase activity and scent emission in many

flowers and fruits strongly supported the involvement of this enzyme in releasing sugar moiety from nonvolatile glycoside precursors to create volatile compounds. Even though it is not the whole story for the mechanism of flower fragrance formation, the activity of β-glucosidase would be another important factor in scent emission of many flowers.

The molecular biology study will be valuable in providing a more complete picture of the involvement of β-glucosidase in scent production and also to elucidate the whole process of flower scent biosynthesis.

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Figures

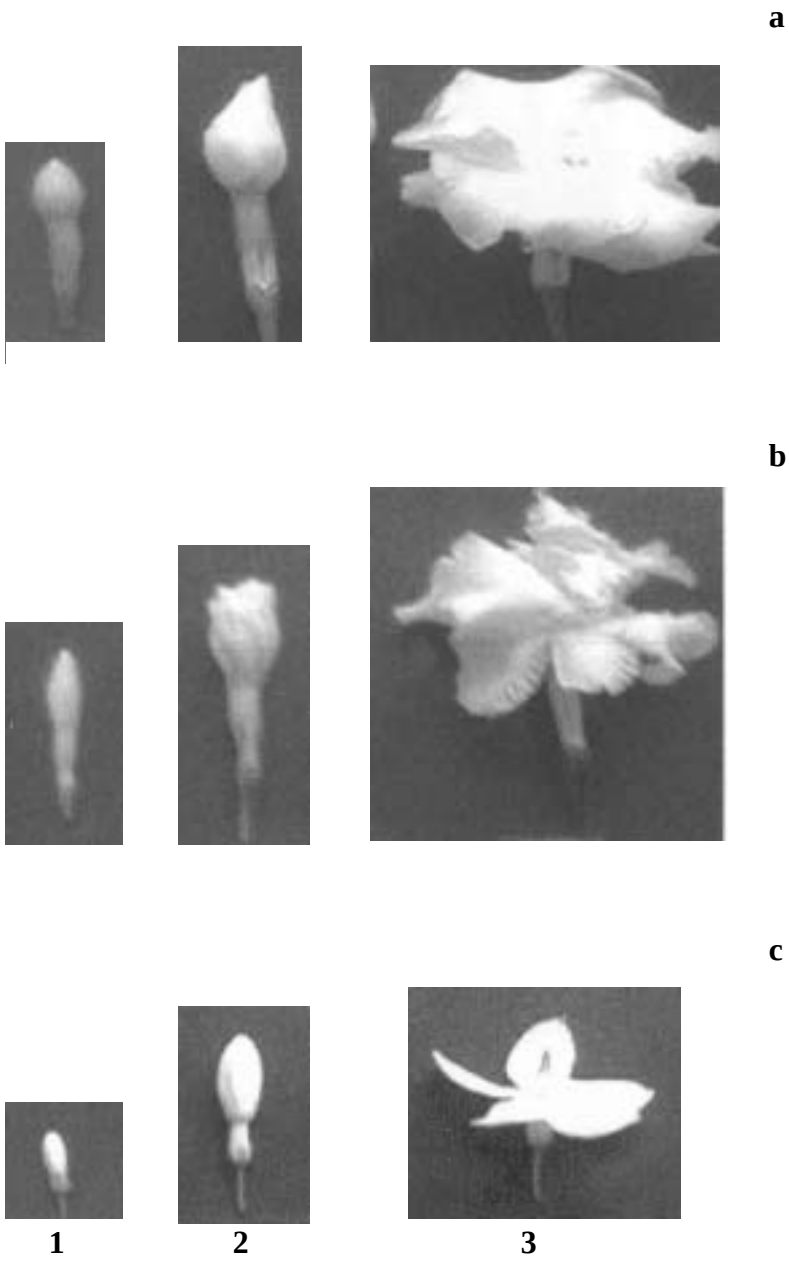

Fig. 1. Different stages of flowers: flower bud (1), half open flower (2) and open flower (3). a) *Ervatamia coronaria*, b) *Gardenia jasminoides*, c) *Wrightia religiosa*.

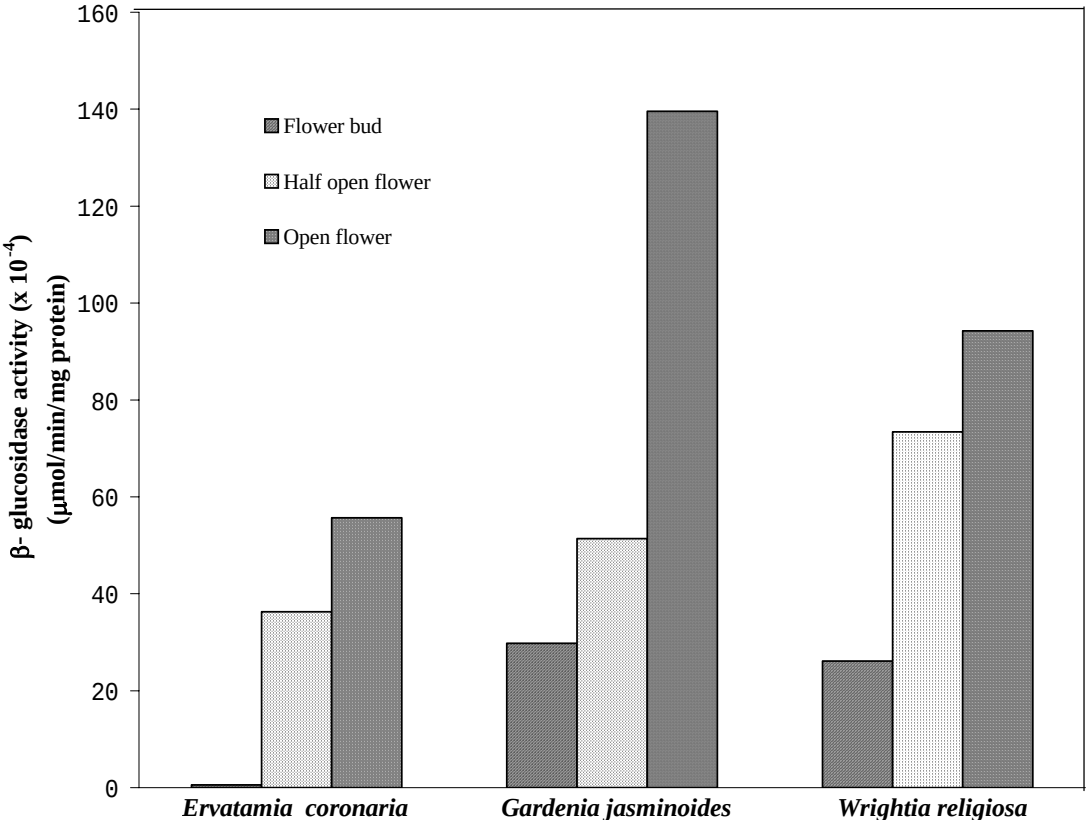

Fig. 2. Distribution of ß-glucosidase activity in different stages of the three flowers.