Tumor Formation in Interspecific Hybrids of Lilium

Abstract. Tumors were formed on germinating seeds of a hybrid of *Lilium speciosum* "Album" × *L. auratum*. Ferulic acid and the glucose ester were isolated from both pericarp and seeds. Tumors were induced on excised embryos only when the embryos were grown on nutrient agar containing ferulic acid. Tumors may result from the limited amount of glycosylation and from detoxification of ferulic acid.

Tumors and galls occur in several genera of plants (*1*). These abnormal growths may result from some exogenous or endogenous inducing agent which can incite the functioning of a genetic mechanism controlling abnormal physiological responses within the plant. Genetic tumors on interspecific hybrids of *Nicotiana* have been investigated by Kehr and Smith (*2*).

In the lily breeding program at Beltsville, Maryland, attempts have been made to obtain interspecific hybrids between *Lilium speciosum* Thunb. "Album," *L. speciosum* Thunb. "Rubrum," and *L. auratum* Lindl. A clone of Album and one of Rubrum were used as maternal parents in all crosses, and the pollen was from plants comprising a mixed group of *L. auratum*. In a previous paper it was reported that a large number of seeds were obtained but that very few germinated and produced mature plants (*3*). Large numbers of hybrids were obtained by excising embryos and culturing them on artificial media. When mature seeds were cultured, growth was initiated and 7.6 percent developed tumors (Fig. 1*A*) and died. A few others developed to various seedling stages, but none survived. By far the largest number of embryos failed to survive within the seeds.

To determine the cause for these abnormalities, the possibility of the presence of inhibitors was considered. Only seeds obtained by crossing Album and *L. auratum* were used, and they were surface sterilized in $Ca(OCl)_2$ and placed in running water for 10 to 12 hours. When cultured these seeds grew normally. Similarly sterilized seeds were soaked in water for 72 hours, and the water extract was examined for known inhibitors. The growth of wheat coleoptiles was inhibited by the water extract. Ferulic acid was isolated from the water extract and identified by spectrophotometric and chromatographic methods (*4*). Other closely related phenolic componds found were sinapic and *p*-coumaric acids. When ferulic acid was incorporated in a medium on which excised embryos were cultured, growth was completely inhibited at a concentration of $5 \times 10^{-4}M$, and the same results were obtained with sinapic and *p*-coumaric acids at a concentration of $10^{-3}M$. Tumors similar to those obtained previously from cultured, unwashed seeds (*1*) were produced by 7.2 percent of excised embryos cultured on a medium containing $5 \times 10^{-5}M$ ferulic acid (Fig. 1*B*).

The incidence of tumor formation was of the same order whether tumors were produced on cultured unwashed seeds or on excised embryos cultured in the presence of ferulic acid. Tumors were also observed on embryos on culture media containing *p*-coumaric or sinapic acids, but at a lower frequency than when ferulic acid was added to the media. Tumors were not observed on the several thousand embryos cultured on a medium lacking the cinnamic acids.

The amount of ferulic acid in seeds as well as in pericarps was determined (*5*). The seeds of Album × *L. auratum* contained 11 μg of ferulic acid per gram wet weight, whereas those of Album × Rubrum (an intraspecific hybrid that germinates readily) contained only 1 μg. Ferulic acid was also present as a glucose ester. When the glucose ester was expressed as the free acid, seeds of Album × *L. auratum* contained 8 μg per gram wet weight as compared to 18 μg for seeds of Album × Rubrum. In pericarps of both these hybrids ferulic acid was present primarily as the glucose ester but with trace amounts of the free acid.

Approximately 60 percent of the total ferulic acid is present as the free acid in seeds of the interspecific hybrid Album × *L. auratum*, whereas only 6 percent is present as the free acid in seeds of the intraspecific hybrid Album × Rubrum. The toxic effects of some phenols can be readily reduced by glycosylation of one or more hydroxyl groups, and one valid function of glycoside formation would appear to be the detoxification of compounds that are harmful (*6*). Harborne and Corner have shown that

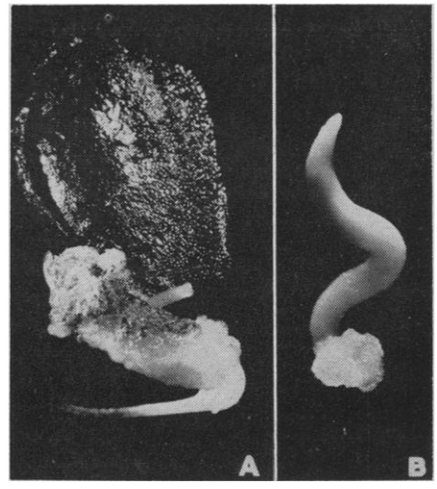

Fig. 1. *A*, Tumor on enlarged cotyledon of a germinating seed of *Lilium speciosum* "Album" × *L. auratum* grown on agar medium. *B*, Tumor on excised embryo grown on agar medium containing ferulic acid ($5 \times 10^{-5}M$).

when ferulic acid was supplied to plants it was readily converted to glucose esters (*7*). A possible explanation for the failure of seeds of Album × *L. auratum* to produce normal seedlings might be found in the relatively high concentrations of ferulic acid they contain. The mechanism for the esterification of ferulic acid is probably limited in the interspecific hybrid Album × *L. auratum*, the hybrid in which a few tumors are formed and in which development of seedlings is almost completely inhibited. Esterification of ferulic acid is probably not affected in the intraspecific hybrid Album × Rubrum, where normal seedlings are produced.

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