

Irradiation and Heat Affect Peach Pollen Germination and Fertility

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Abstract. Pollen from the doubled haploid peach [*Prunus persica* (L.) Batsch] 'Hall-D' was irradiated with 0, 290, 530, 820, 1000, 5000, or 9000 Gray (Gy) of gamma radiation, 113 $\mu\text{W}\cdot\text{cm}^{-2}$ of ultraviolet (UV) radiation, or exposed to 100 °C for 2 h. In vitro pollen germination percentages were recorded and pollen was used to pollinate more than 10,000 emasculated or male-sterile peach flowers. Although pollen germination in vitro was stimulated by <1000 Gy of gamma irradiation, seed set following pollination was greatly reduced in all treatments. These results suggest that low levels of irradiation are sufficient to render pollen infertile while still maintaining germination capacity. Such results may be useful for pollination-induced parthenogenetic egg division for the production of maternally derived haploids and for the production of interspecific hybrids.

The effects of ionizing irradiation on pollen germination have been described for many plant species and for many radiation treatments (for review see Brewbaker and Emery, 1962). Generally, in vitro pollen tube germination percentages decrease with increasing gamma-ray doses; however, several studies have indicated that low doses of irradiation can have a stimulatory effect on pollen tube growth in vitro (Klimenko and Zykov, 1976; Michie and Bohm, 1989; Seibold et al., 1979). Pollen from many species can remain viable even when exposed to >5000 Gy of gamma-radiation (Klimenko and Zykov, 1976; Rudolph, 1978; Visser and Oost, 1981; Zhang et al., 1990), although the effects of irradiation on a given species depend not only on total dose, but on dose rate (Speranza et al., 1982), hydration state of irradiated pollen (Visser and Oost, 1981), and ploidy (Klimenko and Zykov, 1976).

Irradiated or heat-treated pollen has been used for various manipulations in plants, including overcoming crossing barriers by the use of mentor pollen (Andreichenko and Grodzinskii, 1991; Shintaku et al., 1988) and effecting gene transfer (Chyi and Sanford, 1985; Engvild, 1985; Pandey, 1983). "Disabled" pollen has also been useful in obtaining maternally-derived haploids resulting from pol-

lination-induced stimulation of egg cell or synergid division (Raquin, 1985; Zhang et al., 1990). Haploid plants are valuable to breeders and geneticists because chromosome doubling results in homozygous lines. Doubled haploid lines of peach would be desirable for the production of uniform, seed-planted F_1 hybrids; for the transfer of genes into homozygous, uniform seed-derived material; and for genetic studies of mutation, pollen competition, and the recovery of recessives. Although haploids have arisen spontaneously in peach (Hesse, 1971; Pratasenja, 1939; Toyama, 1974) and have been doubled to produce fertile, apparently homozygous plants, a reliable method to induce haploidy would have obvious benefits to peach breeders and geneticists. Interspecific hybridization of peach has the potential for introducing traits such as cold hardiness, disease and pest resistance, adaptation to calcareous soils, dwarfing ability, and increased vigor (in rootstocks) from other *Prunus* species (Layne, 1986). The purpose of this study was to assess the effects of various pollen treatments on peach pollen germination in vitro and in vivo, and to determine the consequences of these treatments on fertility, measured as seed production.

Materials and Methods

Pollen was collected in Kearneysville, W.Va., in Apr. 1992 from 'Hall-D', a doubled-haploid developed from a haploid seedling from open-pollinated 'Halford' (Toyama, 1974). Pollen was air-dried at room temperature overnight and then was stored in liquid N until Mar. 1993, when it was subjected to one of the following three treatments: 1) exposure to 290, 530, or 820 Gy (first samples) or 1000, 5000, 9000 Gy (second samples) gamma radiation using a ⁶⁰Co source at a rate of 0.22 Gy·s⁻¹; 2) exposure to 113 $\mu\text{W}\cdot\text{cm}^{-2}$ UV radiation for 12 h; 3) heating for 2 h at 100 °C. Treated and nontreated control pollen was grown in vitro on a medium consisting of 13% sucrose, 1.2% agar (Ultrapure, USB, Cleve-

land) and 1.6 μM boric acid (Werner and Chang, 1981). Drops of medium were placed on microscope slides. About 200 pollen grains were placed on each drop of medium, and slides were placed in a covered petri dish with moistened filter paper to maintain high humidity. Pollen germination percentages were analyzed after 6 h. Three samples were analyzed for each pollen treatment.

Trees were pollinated in Byron, Ga., in Mar. 1993, and in Kearneysville, W.Va., in Apr. 1993. Byron trees 84P512, 81P2467, and 'Hakuto' were emasculated then pollinated the following day. Tree 84P512 was pollinated with nontreated pollen and pollen exposed to 530, 820, or 5000 Gy, UV, or heat. Trees of 81P2467 and 'Hakuto' were pollinated with pollen exposed to 0, 530, 820, or 5000 Gy. In Kearneysville, wooden frames were built over male-sterile 'Hakuto' trees and these frames were covered with parachutes to prevent outcrossing. When flowers opened, they were pollinated with untreated, 530 Gy, 5000 Gy, UV, or heat-treated pollen. The total number of pollinations made for each pollen treatment on each tree was recorded. Fruit that were produced from these pollinations were collected. Seed were extracted, stratified for 90 days, and planted in the greenhouse.

Stigmas from five pollinated flowers from each pollination treatment on the 'Hakuto' pollinated in Kearneysville were collected 6, 12, and 24 h after pollination to observe in vivo pollen tube growth. Stigmas were fixed in FAA, hydrolyzed in NaOH, stained with 0.1% aniline blue according to Gonzalez (1984), and observed using fluorescent microscopy.

Results and Discussion

Analysis of variance (ANOVA) indicated that the effect of pollen treatment on in vitro germination was highly significant ($P < 0.0001$). Examination of differences between each treatment and the control using Dunnett's two-sided test (Steel and Torrie, 1960) indicated that lower levels of irradiation of "Hall-D" pollen increased in vitro germination, whereas higher levels of irradiation (≥ 1000 Gy) were inhibitory (Table 1). After 280, 530, and 820 Gy of irradiation had not reduced in vitro germination in the first set of pollen samples, a second set of pollen samples was irradiated with 1000, 5000, and 9000 Gy. Although both batches of irradiated pollen were treated similarly and the nontreated control pollen from both batches had similar germination rates, differences in physiological state between the two batches may have affected the response to irradiation.

Irradiation by UV light had no significant effect on in vitro germination, while heat treatment had a pronounced inhibitory effect, similar to the higher gamma irradiation levels (Table 1).

Although in vitro germination percentages provide an estimate of pollen viability, a more reliable test of pollen viability is in vivo germination. Pollen from all treatments germinated in vivo, grew through the style, and reached

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the ovule (data not presented). These results indicate that pollen grains from all treatments were capable of growth through the style, regardless of in vitro germination capabilities.

Pollen fertility was evaluated by analyzing seed set. Using individual trees as blocks, we analyzed the effect of six pollen treatments on percent seed set, measured as the number of seeds harvested out of the total number of flowers pollinated. ANOVA indicated that both treatment and block were highly significant ($P \leq 0.005$). Furthermore, all pollen treatments resulted in decreased seed set compared to the control (Table 1, Fig. 1). Thus, although low levels of irradiation have an apparent stimulatory effect on in vitro germination, the ability of such pollen to fertilize the ovule is greatly reduced. Reduced seed set may be caused by radiation-induced chromosomal abnormalities that render the pollen generative nucleus incapable of effecting fertilization. The pollen grain apparently contains intact proteins and other factors necessary for elongation, but cannot form a viable zygote.

A few plants were produced from seed obtained from crosses with treated pollen. These plants were generally weak, with narrow leaves, resembling haploids as described by Toyama (1974). These plants did not survive, and their ploidy level could not be evaluated. Thus, although the production of these plants suggested that the pollen treatments that we used may produce haploids, the production of haploids could not be confirmed.

Viable but infertile pollen has been used for the production of haploids from pollination-induced egg cell development in other crops, including woody perennials, such as poplar (*Populus alba* L., *P. trichocarpa* Hook.) (Kexian and Nagarajan, 1990) and apple [*Malus domestica* Borkh., *M. prunifolia* (Willd.) Borkh.] (Zhang et al., 1990). Viable, unfertile pollen is also useful as mentor or pioneer pollen for interspecific and other incompatible hybridizations (Andreichenko and Grodzinskii, 1991; Shintaku et al., 1988).

We have demonstrated the production of infertile but viable peach pollen. This pollen

had in vitro germination rates similar to and higher than that of nontreated control pollen, but fertility levels were only $\approx 20\%$ of the nontreated control. Irradiated or heat-treated peach pollen may be useful for haploid production or as mentor pollen.

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Table 1. Mean percentage of in vitro pollen tube germination after 6 h of growth at room temperature, and mean percent seed set for indicated pollen treatments.

| Treatment | Mean in vitro germination (%) | Mean seed set (%) |
|---------------------------|-------------------------------|-------------------|
| γ irradiation (Gy) | | |
| 0 | 17.9 | 24.0 |
| 290 | 22.8* | nt ² |
| 530 | 31.7* | 5.6* |
| 820 | 27.0* | 4.0* |
| 1000 | 8.0* | nt |
| 5000 | 2.3* | 3.5* |
| 9000 | 2.8* | nt |
| Other | | |
| UV | 15.4 | 6.7* |
| 100 °C | 6.3* | 6.2* |

²nt = not tested.

*Means different from control by Dunnett's two-sided test, $P \leq 0.05$.

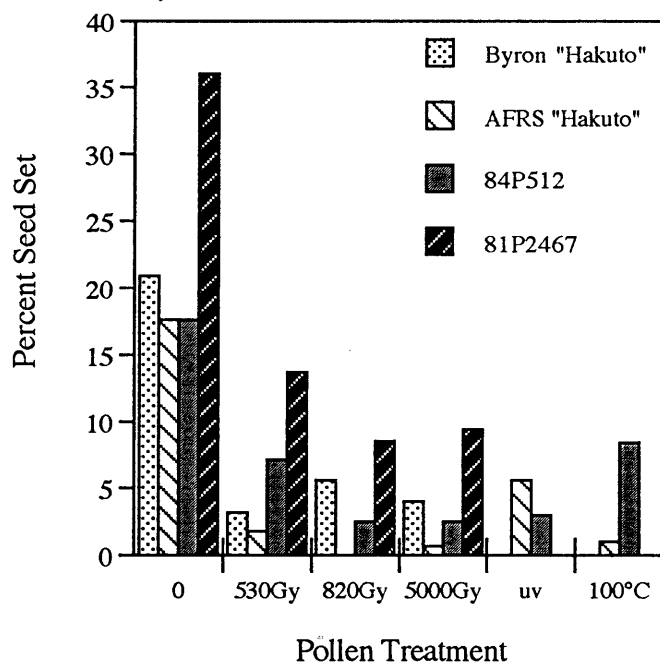


Fig. 1. Percentage of seed set (number of seeds harvested out of total number of flowers pollinated) on four trees pollinated with gamma-irradiated (530, 820, 5000 Gy), UV-irradiated ($113 \mu\text{W}\cdot\text{cm}^{-2}$), and heat-treated (100°C) pollen. Each bar represents data from a single treatment for a single tree, as averaged in Table 1. Total number of flowers pollinated for each tree were: Byron 'Hakuto'—2803; AFRS 'Hakuto'—3362; 84P512—2408; 81P2467—1590.