Corchorus pascuorum: Transmission of Chemically Induced Fruit Formation with Environmental Change

Abstract. *When* Corchorus pascuorum *(Tiliaceae), native to Australia, was grown in the arid region of West Pakistan, it flowered abundantly but produced no fruit. It was induced to form fruit by treatment with 25 percent sucrose solution containing indolebutyric acid (300 parts per million). When plants were raised from the induced fruits, they fruited without any further treatment and retained the ability to produce fruits in two subsequent generations, whereas those raised from the original seeds produced no fruit.*

We raised a population of *Corchorus pascuorum* Domin., a natural tetraploid species from Australia. All the plants in the population grew normally and flowered profusely, but no fruit developed either from open or self pollination.

The stigmatic and stylar regions of a few self-pollinated flowers were dissected and stained. Germination of pollen grains on the stigma was poor, and the growth of a few pollen tubes into the upper region of the style was slight. Twenty-five percent sucrose supported maximum pollen grain germination and pollen tube growth in vitro. Such a solution was then applied to the stigmas of 35 flowers to induce full growth of pollen tubes in a self-pollinated flower. The treatment proved effective insofar as the full pollen tube growth and fertilization were concerned, but the flowers dropped 2 to 5 days after the treatment (Table 1).

Inasmuch as the dropping of flowers in *Corchorus* species crosses can be prevented by the use of indolebutyric acid (300 ppm) (*1*), an aqueous solution of hormone was applied along with 25 percent sucrose on the stigmas of 35 self-pollinated flowers. Thirty flowers developed into fruits with good seeds (*2*). The untreated flowers of the same inflorescence also developed into small fruits, presumably because of translocation of indolebutyric acid. Somewhat similar results were obtained in cantaloupe [cited in (*3*)]. Production of cantaloupe fruit was considerably increased by the application of a mixture of 1 percent indoleacetic acid in lanolin to one lobe of the stigma after pollination.

The following year, 1967, two populations (A and B) were raised simultaneously in pots under identical conditions in natural daylight. Population A, consisting of 15 plants, was grown from the seeds obtained from the fruits induced by sucrose and hormone; population B, consisting of 13 plants, was grown from the original seed stock obtained from Australia. The latter served as control. As expected, control plants flowered but did not fruit. On the other hand, the 15 plants raised from the seeds of induced fruits not only flowered but produced fruits as well (Table 1). One would expect that, in each generation, the flowers would require treatment with sucrose and hormone for fruit formation; but fruiting took place in the progeny without any treatment. Dissected stigmas and styles of 12 pollinated flowers of the progeny producing fruits (population A) showed extensive germination of pollen grains and a large number of pollen tubes in the ovarian chamber; flowers of the control (population B) had none. From the seeds of the population A, which were grown in 1968, only one adult plant was obtained. This plant fruited without any chemical treatment, and the seed size in the fruits was normal.

Further, it was noted that fruit formation took place in crosses, *C. pascuorum* × *C. hirtus* L. (an Argentinian tetraploid species) only when the plants of population A (raised from the seeds of induced fruits) were used as female. When the plants of the control (population B) were used as female, no fruit were set. Thus, the chemical treatment not only restored the ability of plants to form fruits under a different environment but caused the flowers of population A to set fruit when crossed with another species of *Corchorus*.

In *Linum* (*4*), fertilizer-induced changes are transmitted. Height, branching, and vigor induced by the addition of suitable combinations of N, P, and K in the parental generations are passed unchanged for many generations. In peas (*5*), both reduction in vigor and size and seed immaturity induced by constant temperature are transmitted from one generation to another in the same environment which brought about the change. Upon return to fluctuating temperature which represents the natural environment of growth, the loss of vigor was gradually restored in three generations.

The situation in *C. pascuorum* in which no heritable change was induced is not strictly comparable to that in *Linum* and peas. In the former, only the ability of the self-pollinated flower to fruit which remained suppressed under different environmental conditions was restored by chemical treatment, and this restoration maintained itself in subsequent generations.

By way of explanation, the action of genes responsible for fruit formation may have become ineffective under the local climatic conditions which differ from those of Australia. Once these genes were activated (put to commission) under adverse conditions by the chemical treatment, they remained op-

Table 1. Induction of fruiting in self-pollinated flowers of *C. pascuorum* under a different environment and transmission of induced fruiting ability to subsequent generations.

Stock No. type	Source	Mode, treatment of stigma	Plants fruited (No.)	Average No. of seeds per fruit
		1966		
75 Flowers	Australia, original stock	None	None	None
35 Flowers	Original stock	Sucrose	Flowers dropped between 2–5 days	*
35 Flowers	Original stock	Sucrose + IBA	30	20
		1967		
13 Plants	Original stock (pop. B)	None		
15 Plants	Seeds from induced fruits of 1966 (pop. A)	None	15	11
		1968		
1 Plant	Seeds from pop. A of 1967		1	9†
3 Plants	Seeds from induced fruits of 1966		3	10
2 Plants	Original stock	None	None	

* Twelve dissected for study of PT growth. † The rest of the population died as a result of disease.

erative even after their transmission to the next generation. Or the treated plants may use only a portion of the hormone and sucrose for fruit formation and store the rest in their seeds. If this were so, these seeds might germinate with an initial extra reserve of hormone and sucrose and, therefore, would not require any further chemical treatment for fruit formation. Which of these explanations is more acceptable will depend upon the performance of the progeny in subsequent generations. They may retain the ability to produce fruits generation after generation, or they may show progressive reduction in their fruiting ability in proportion to the utilization of hormone and sucrose initially supplied to the parental plants for fruit formation.

A. S. ISLAM
R. MUGHAL
Department of Botany, University of Sind, Jamshoro, West Pakistan

References

1. A. S. Islam, *Sind Univ. Sci. Res. J.* **1**, 71 (1965).
2. R. Mughal and A. S. Islam, *ibid.* **2**, 33 (1966).
3. S. L. Emsweller, in *Plant Regulators in Agriculture*, H. B. Tukey, Ed. (Wiley, New York, 1954), pp. 161–169.
4. H. R. Highkin, *Amer. J. Bot.* **45**, 626 (1958).
5. A. Durrant, *Heredity* **17**, 27 (1962).

29 January 1969

Graft versus Host Inhibition: Fetal Liver and Thymus Cells to Minimize Secondary Disease

Abstract. *Long-lived radiation chimeras were produced in mice differing at the major histocompatibility locus. Survival occurred in lethally irradiated recipients inoculated with allogeneic fetal liver and allogeneic fetal thymus cells in combination. The survival rate was equal or superior to that of mice with transplanted syngeneic fetal, neonatal, or adult hematopoietic cells.*

Clinical application of transplantation of allogeneic bone marrow has been deterred, chiefly because of complications after transplant from infection due to immune inadequacy (*1*) and from graft-versus-host (GVH) disease (*2*). The two problems are inversely related in that attempts to improve the immune status worsen the GVH reaction and efforts to minimize GVH disease tend to increase immune inadequacy.

Shortly after GVH disease was established as a cause of delayed deaths in radiation chimeras transplanted with allogeneic cells, Uphoff reported (*3*) inhibition of secondary disease in the mouse by the use of transplanted fetal liver cells. We have shown that suspensions of fetal liver cells are immunologically incompetent against transplantation antigens (*4*). This finding, and the results reported here, suggest that the delayed deaths which occur in lethally irradiated mice subsequently treated with allogeneic fetal liver cells are probably not due to a graft-versus-host reaction. Others have made the same suggestion based on different data (*5*).

In the case of mice, radiation chimeras often have impaired immune capability (*6*). The immune inadequacy of radiation chimeras with transplanted fetal liver cells may be exaggerated when compared with that of mice with transplanted adult hematopoietic cells. The fetal lymphoid cells, or lymphoid precursors, may not yet have come under the influence of the donor's thymus when the cells were removed for transplantation. In addition, the thymus glands of the host mice are injured by the x-irradiation. Thus, in the radiation chimeras with transplanted fetal liver cells, the precursors of antigen-reactive cells (*7*) may not have received adequate thymic "immunological instruction" before transplantation and may not benefit by thymic influence in the host mice after transplantation.

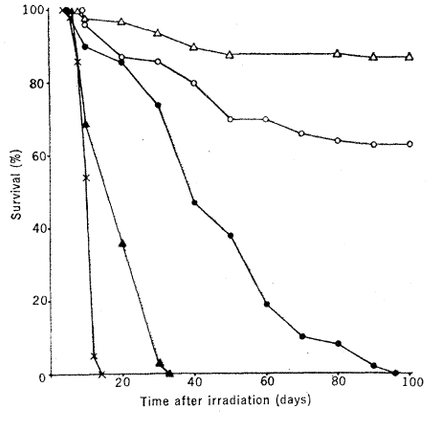

Fig. 1. Survival of CBA/J mice that received 950 r of total body x-irradiation plus 2×10^7 syngeneic or allogeneic (A/J) adult spleen or bone marrow cells. △, Syngeneic adult spleen (52); ○, syngeneic adult bone marrow (51); ▲, allogeneic adult spleen (59); ●, allogeneic adult bone marrow (50); X, radiation controls (150). Numbers in parentheses indicate number of mice in each group.

We used donor and recipient mouse strains which differed at the H-2 locus, the major histocompatibility locus in mice. Host mice were lethally irradiated. Inoculation of these mice with allogeneic fetal liver cells and with fetal thymus cells, in combination, resulted in long-lived chimeras.

Inbred CBA/J (*H-2*^{k}) and A/J (*H-2*^{a}) mice were used. Pregnancies were timed at the Jackson Laboratory, Bar Harbor, Maine. The hosts were CBA mice, 3 to 4 months of age. Liver hematopoietic cells were obtained from fetuses at day 13 and day 18 and from 5-day-old mice. Thymus cells were obtained from fetuses at day 18 and from 5-day-old mice. Donors of adult spleen and bone marrow were 3 to 4 months of age. Cells were collected, processed, counted, and tested for viability (*8*). X-irradiation conditions were: 280 kv at 20 ma; rate in air was approximately 55 r/min; air distance was 82 cm; and 0.7 mm Cu half-value layer. The mice were placed in a perforated, partitioned Plexiglas container on a rotating platform. A single total-body exposure (TBR) of 950 r was given. Then 10^7 or 2×10^7 viable cells were administered intravenously within 4 hours after exposure to radiation. When both liver and thymus cells were given, the suspensions were prepared from different mice, the injections were made separately, and 10^7 cells of each type were administered. Two untreated mice from each irradiated group served as controls. To test chimerism, A-strain skin grafts were used. Chi-square analysis was used to test differences between survival rates.

The results of survival of radiation chimeras that received syngeneic and allogeneic adult spleen and bone marrow are shown in Fig. 1. Whereas 87 percent of mice inoculated with syngeneic adult spleen cells survived 100 days, none of the mice that received allogeneic adult spleen cells survived beyond day 33. Mice that received allogeneic bone marrow cells survived longer than those given allogeneic adult spleen cells; however, all were dead within 96 days. Over the 100-day test period, syngeneic spleen cells provided better survival than syngeneic bone marrow cells ($P < .001$).

Survival curves for mice that received syngeneic or allogeneic fetal liver cells