Technical Papers

Anomalous Structure in the Hypocotyl of Soybean Following Treatment with 2,4-Dichlorophenoxyacetic Acid

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The effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on the histological responses of certain plants have been described by various investigators. However, little response has been found in the pith cells of the hypocotyl region (*1*). This paper deals with the effects of 2,4-D in producing anomalous structures in the pith of the hypocotyl region of soybean seedlings (Monroe variety).

The seeds, after having been soaked for 24 hr in tap water, were transferred to moist blotting paper in Petri dishes for another 24-hr period. The seedlings were then immersed for 10 min in an aqueous solution of 2,4-D (500 mg/lit), after which they were cultured on moist blotting paper in Petri dishes and washed daily. The chloroplasts disappeared in the cortical parenchyma cells of the treated hypocotyl. The latter became yellowish and stunted but considerably enlarged in diameter. The four-winged broad shoulders developed throughout the entire region, owing primarily to the proliferation of the pericycle.

The pith cells of the hypocotyl region showed a very interesting response to 2,4-D. After 5 days of treatment, they started meristematic activity, the activity being greatest at the upper end of the hypocotyl. Only a few cells next to the primary xylem at the lower end of the hypocotyl divided at this stage. After 10 days of treatment, almost all the cells in the pith at any level within the hypocotyl had become meristematic. The pith cells proliferated and differentiated most rapidly directly beneath the cotyledons.

At the periphery, the active cell division resulted in the formation of a continuous, circular cambium layer from which vascular elements differentiated. A few derivatives centripetal to this cambium layer differentiated into tracheids and other vascular elements (Fig. 1, left). At a lower level, the cambium layer was irregular and curved along a random path so that it did not present a circular pattern in cross section. At the root end of the hypocotyl, dividing cells were observed in the pith, but no cambium layer was formed (Fig. 1, right). Pitted tracheids were present among the primary xylem elements and also in the vascular strands of the pith.

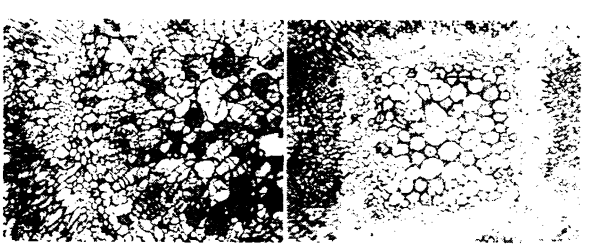

Fig. 1. Transverse sections of the hypocotyl region of soybean seedling after 10 days of treatment with 2,4-D. (Left) Transverse section at the upper end of the hypocotyl showing cambium and vascular strands in the pith. (Right) Transverse section at the root end of the hypocotyl showing the dividing cells in the pith. (×46)

It is obvious that one of the striking responses of the soybean hypocotyl to 2,4-D is the tendency of cells to become highly meristematic; another response is the development of vascular strands in the pith. These responses show that differentiated cells retain their totipotential capacities following treatment. The 2,4-D apparently disrupts the orderly metabolic and physiological processes and, thus, leads to nonpolarized cell division and abnormal growth patterns.

References and Notes

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Genetic and Nongenetic Effects of Radiations in *Neurospora*

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The mechanism of radiation damage in biological systems eludes detailed description. It is concluded that, on the one hand, the lethal effects of x-rays are mediated entirely through the nuclei in *Neurospora* conidia (*1*), whereas, on the other hand, in barley (*2*) and *Habrobracon* (*3*), genetic (nuclear) damage alone cannot account for all the lethal effects of x-rays (*4*).

This paper (*5*) describes genetic and nongenetic effects of radiations in *N. crassa*, strain 74A-3b. The criterions used to detect radiation effects included genetic mutation, inactivation, and a "stimulatory" effect. The last criterion was measured as an increase in the number of conidia that germinated and as an accelerated growth rate following irradiation.

The mutation rate of one glutamic acid locus has been studied (*6*). The mutants were recovered according to the filtration and selective-plating technique (*7*). The observed mutation rate, expressed as mutants per 10,000 conidia, increased with x-ray dosage as follows: no dose, 0.85; 250 r, 0.93; 1000 r, 1.12; 2000 r, 1.03; 3000 r, 1.50; 7000 r, 3.05; and 10,000 r, 3.72 (Fig. 1).

Inactivation and the increase in the number of conidia that germinated following irradiation were measured concomitantly. Survival values of marcroconidial populations were determined following expo-

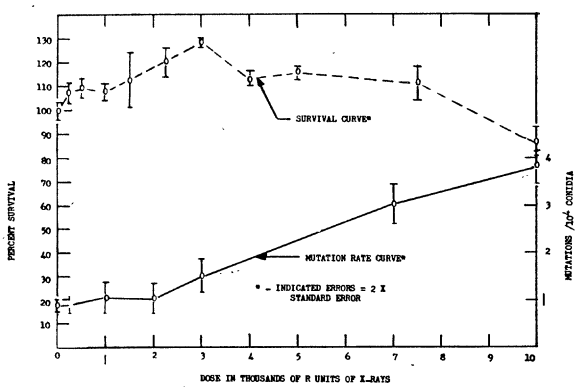

Fig. 1. Relationship of survival and mutation rate to dosage.

sures to relatively low dosages of x-rays; population sizes were determined by hemocytometer counts; and viability was determined by plating appropriately diluted aliquots of conidia onto minimal agar supplemented with glutamic acid (*8*). The number of conidia that germinated following irradiation increased with dosage up to 3000 r, at which dose between 20 and 25 percent more conidia germinated than in the untreated populations (Fig. 1). It is also evident from this figure that more conidia germinated following treatments of 250 to 7000 r than germinated without irradiation.

The second measurement of "stimulation" was that of a radiation-induced growth-rate alteration. Following irradiation, the conidia produced mycelia, in minimal medium (solution), that became perceptible to the naked eye at varying time intervals. By removing the developing mycelia (*7*), the duration of germination was measured. Unirradiated macroconidia produced mycelia in amounts sufficient to be perceptible in 15 to 20 hr at 24°C. The duration of germination was 24 to 36 hr. Conidia subjected to doses of x-rays or gamma rays between 500 and 50,000 r produced visible hyphae in 8 to 10 hr; the duration of incubation was 24 to 30 hr. Conidia treated with ultraviolet light or with thermal neutrons of varying doses were, on the contrary, retarded in growth. Perceptible growth was not detected until after 24 hr of incubation; and it continued at a rather constant rate for approximately 48 hr. There was a rather extended dose-independent range with all radiations. Since conidia one generation removed from the conidia treated with x-rays, thermal neutrons, and ultraviolet light and from untreated conidia germinated at the same rate, it was concluded that the stimulation was nongenetic.

It is evident from these data that x-rays effect changes in both genetic and nongenetic components of *Neurospora* conidia. X-rays affect the growth rate and the increased conidial germination similarly; but, in the case of the growth rate, either the biological component responsible is affected differently by the different types of radiation or different components are affected.

The relationship between mutation rate and conidial survival is more complex. The survival curve appears to be a composite of at least two independent events: (i) conidial "activation" or stimulation and (ii) inactivation. Therefore, it is difficult to determine the extent of either event, since a given survival value represents a combination of both. *A priori,* one would expect inactivation to be insignificant at low dosages, at which a rather large increment of stimulation occurs. At higher dosages, inactivation, no doubt, becomes more significant, but the increment of stimulation becomes difficult to measure. Since the survival curve is a composite, it becomes difficult to establish the correlation between either component and the mutation process. According to their relationship with x-ray dosage, stimulation is independent of genetic mutation; but, before the relationship between inactivation and mutation is established, it will be necessary to separate, experimentally, the two components that combine to produce a given survival curve.

The conidial "activation" caused by x-rays imposes certain difficulties to the interpretation of killing curves in *Neurospora.* Until the separate components are isolated, it would seem risky to attempt to separate nuclear from nonnuclear x-ray effects solely on the basis of existing killing curves.

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Liquid Sulfur Dioxide as a Solvent for Proteins and the Infrared Spectrum of Proteins in Solution

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Pure liquid sulfur dioxide (boiling point, −10.02°C) has previously been observed to have little or no solvent properties for proteins (*1*). In the case of water, it is well known that many proteins are insoluble in pure water but dissolve readily on the addition of small amounts of neutral salts (*2*). It has now been found (*3*) that a similar salting-in effect occurs with liquid sulfur dioxide, and that proteins are soluble in sulfur dioxide-neutral salt solutions.

Alkali metal (and ammonium) iodides and thio-