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Short Communication

Actions of Gibberellic and Abscisic Acids on Lettuce Seed Germination Without Actions on Nuclear DNA Synthesis[1]

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Promotion and inhibition of DNA synthesis have been suggested as important causal events in the growth-promoting action of GA[2] and the growth-retarding action of ABA, respectively (11, 12, 15). We here report typical effects of GA and ABA during lettuce seed germination after seed irradiation that prevents detectable DNA synthesis as determined by 2 criteria: Feulgen microspectrophotometry and ^{3}H-thymidine incorporation into nuclear DNA.

Air-dry seeds of lettuce, *Lactuca sativa* 'New York,' were given 1.3 Mrad ^{60}Co gamma-rays and then sown in Petri dishes containing distilled water or solutions of GA ("Gibrel," Merck) or ABA (Shell Development Company) as indicated. Whereas lower radiation doses are sufficient to prevent mitosis during germination (7), the 1.3 Mrad dose was necessary to inhibit nuclear DNA synthesis, as will be shown in this report. In all cases the pH was 5.7, and the dishes were exposed to continuous illumination with 900 μw/cm^2 white light (Sylvania Gro-Lux) at 20°. After 5 days, germination of these irradiated seeds (as the percentage of total number of seeds sown) was 0 % in water controls, 84 % in 5×10^{-4} M GA, and 1.5 % in 5×10^{-4} M GA + 100 mg/l ABA. Thus germination of these seeds was very sensitive to GA and ABA.

By Feulgen microspectrophotometry the relative amount of DNA per nucleus was measured for individual cells from (a) the apical 0.5 mm of the radicle of unirradiated embryos before sowing, and (b) the protruded 1.1 mm of 5-day-old root tips from the irradiated seeds that had germinated in 5×10^{-4} M GA. After histologic examination we chose the 1.1-mm length in (b) because it contains the same number of cells as the 0.5-mm length in (a). Because there was no mitosis, any DNA synthesis during the 5 days after sowing of the irradiated seeds in GA would have shown up as increases in DNA contents in (b) compared to (a). Each slide contained a squash of either the 0.5-mm apical portion of the radicle from an unsown seed (a) or the protruded 1.1-mm root tip from an irradiated seed after 5 days in GA (b). Each slide also contained a squash of a normal root tip (1–5 days old) comprised of cells in all stages of mitosis; the telophase nuclei were used to determine the 2C nuclear DNA level, and the prophase and metaphase nuclei were used to determine the 4C nuclear DNA level. In this way the DNA content of individual nuclei in unsown seeds (a) and in roots from irradiated seeds germinated in GA (b) could be directly measured relative to the normal 2C and 4C nuclear DNA levels on the same slides.

The radicle and root tips were fixed overnight in Carnoy's fixative (3), then washed in 3 changes of 45 % acetic acid, and then squashed in 45 % acetic acid on slides 0.99 mm or less in thickness. Immediately before staining, the squashes were postfixed in 3 parts absolute ethyl alcohol: 1 part glacial acetic acid for one-half hr. After thorough draining and drying, the slides were heated to 60° and then immersed in 1 N HCl at 60° for 12 min (optimum hydrolysis time). The squashes were then stained for 1 hr in freshly prepared Schiff's leuco-basic fuchsin. After three 10-min washes in "sulphur dioxide water" (3), the slides were washed 5 min in running water and air-dried. Just before use, the squashes were mounted with Cargille's oil of refractive index 1.54. Total absorbancy integrated over the area of the nucleus was determined with a scanning microspectrophotometer (Zeiss Model UMSP-I). Because the high intensity of stain produced high absorbancies at the 565 nm maximum, off-peak measurements were made at 520 nm, thereby giving point absorbancies mostly between 0.3 and 0.5. The reliability of such off-peak measurements has been established by Fand and Spencer (4).

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[2] Abbreviations: ABA, abscisic acid (dormin, abscisin II); GA, gibberellic acid; FUdR, 5-fluorodeoxyuridine. In microspectrophotometry the 2C amount of DNA per nucleus represents the DNA content of a diploid, telophase nucleus or of a diploid, interphase nucleus before onset of DNA synthesis; the 4C amount of DNA per nucleus represents the DNA content of a diploid prophase, or diploid metaphase nucleus or of a diploid interphase nucleus after completion of DNA synthesis.

The results of the microspectrophotometry are shown in Fig. 1. All the values for nuclei in the unsown embryo and in the rootlet of seedlings from 1.3 Mrad-irradiated, GA-treated seeds came from interphase nuclei, since mitotic stages are absent from these plants. All nuclei examined in unsown embryos had a 2C DNA content. This finding is similar to that of Brunori and D'Amato (1), who found only 2C nuclei in 14 of 15 unsown lettuce embryos; the fifteenth had some 4C nuclei. Feinbrun and Klein (5) found that in "Grand Rapids" lettuce seed germinated on ^{3}H-thymidine, all nuclei were labeled with the exception of a few epidermal nuclei; therefore, it may be inferred that the vast majority of nuclei in their lettuce seed also had a 2C DNA content.

The DNA level was 2C or less for all except 4 nuclei from the rootlet of seedlings from the irradiated, GA-treated seeds. This observation indicates that DNA synthesis was absent or negligible in the elongating portion of these seedlings during the first 5 days after sowing. The four 4C nuclei in the root tips from the seeds irradiated and then germinated in GA may actually have been initially present before irradiation and germination, because 4 of 300 (Fig. 1b) *versus* 0 of 300 (Fig. 1a) is not a statistically significant difference ($\chi^2 = 2.3$; $p = 0.13$). Brunori and D'Amato (1) and Feinbrun and Klein (5) also suggest the presence of a few 4C nuclei in unsown lettuce embryos. Even if these 4 nuclei did undergo DNA doubling during germination of the irradiated seeds in the GA solution, the amount of DNA synthesis is still rather small per embryo; moreover, some of these germinated seeds, all of which required GA for germination, are still entirely 2C and therefore could not have undergone any doubling of DNA in their cells. We do not imply that either 1.3 Mrad or GA have no effect on DNA itself; in fact the gamma-rays do seem to cause degradation of DNA, as shown by the nuclei containing less than the 2C level in Fig. 1b. Our conclusions pertain only to the absence of significant nuclear DNA synthesis.

Autoradiography was also used to verify absence of nuclear DNA synthesis in both seeds and seedlings in 5×10^{-4} M GA. Irradiated seeds and the seedlings in 5×10^{-4} M GA, when 0, 1, 2, 3, 4, 5, 6, and 7 days old, were exposed for 24 hr to a solution also containing 0.006 mg/ml of ^{3}H-thymidine (50 μc/ml; specific radioactivity 2.0 c/mmole). Those seeds not yet germinated were cut through the widest part of the cotyledons to assure that the ^{3}H-thymidine reached the radicle. Unirradiated seeds and seedlings of the same ages were also exposed for the same time to ^{3}H-thymidine solutions of the same concentration. Fixation was overnight in Carnoy's fixative (3). The radicles and root tips were then stained by means of the Feulgen reaction, squashed on slides, coated with Kodak NTB 3 liquid emulsion, and stored in darkness at 4°. After 7 days the slides were developed. By this criterion, also, no nuclear DNA synthesis occurred in seedlings from the irradiated, GA-treated seeds, because grains over nuclei were absent or negligible (*i.e.*, no greater in number than over nearby cytoplasm). By contrast, unirradiated seeds and seedlings of the same ages showed heavy labeling of nuclei (Fig. 2). The absence of ^{3}H-thymidine incorporation into nuclei suggests that, within the time period studied, the apparent radiation-induced degradation of nuclear DNA (*cf.* figs 1a and 1b) is not followed by "repair synthesis" of DNA (10).

Since by the 2 different criteria there was little or no nuclear DNA synthesis during germination of the irradiated seeds in GA, the promotion of germination by GA here is unrelated to any effect on nuclear DNA synthesis. In these seeds germination involves and is measured by expansion of the very same cells shown here to lack DNA synthesis. This

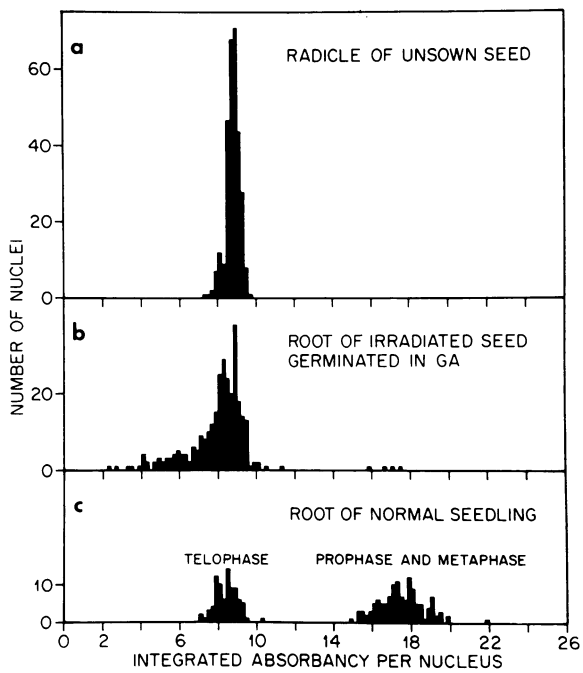

FIG. 1. Frequency distributions of DNA contents of individual nuclei from (a) radicle of unsown seed, (b) root of irradiated seed germinated in GA, and (c) root of normal seedling. (a) Three hundred nuclei were measured. Fifty are from each of 6 radicle tips; only interphase nuclei were present. (b) Three hundred nuclei were measured; 50 are from each of six 5-day-old tips; only interphase nuclei were present. Four of the nuclei, from 4 different tips, have the 4C level of DNA. Those nuclei having less than the 2C level of DNA, presumably reflecting destruction of DNA by the irradiation, were distributed among all 6 root tips examined. (c) The telophase nuclei indicate the normal 2C level of DNA; prophase and metaphase nuclei indicate the normal 4C level. Presumably the 2C and 4C levels of DNA are represented by approximately 8.5 and 17 absorbancy units, respectively.

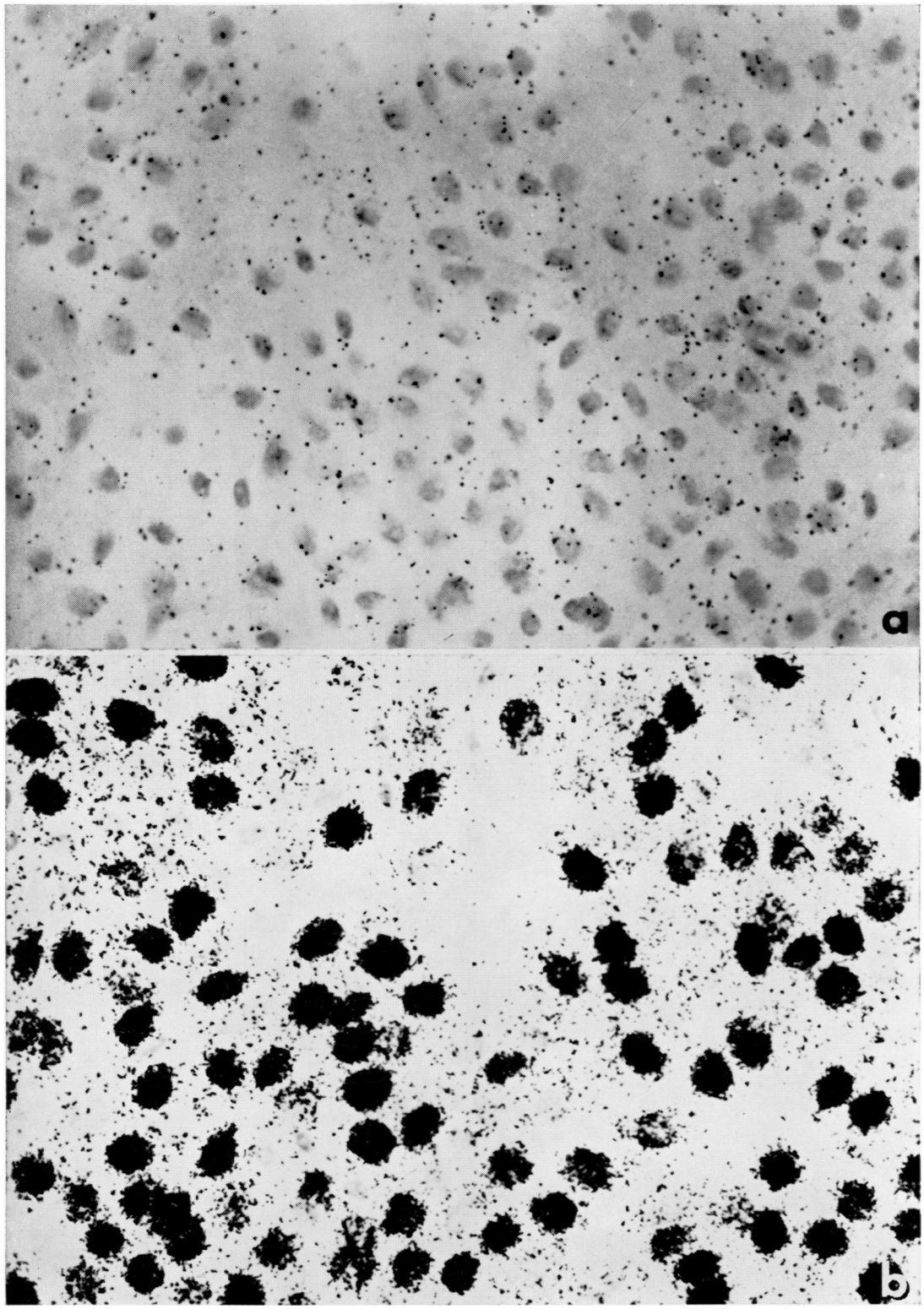

FIG. 2. Autoradiographs of roots after exposure to ^{3}H-thymidine for 24 hr: (a) irradiated seed germinated in GA and (b) normal seedling. In approximately one-half million nuclei of (a) examined, not one gave evidence of either DNA synthesis or mitosis, whereas nuclei of (b) are heavily labeled. These roots were exposed to ^{3}H-thymidine between days 5 and 6, when they were fixed. Similar results were found for all other ages studied (see text).

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expansion is similarly stimulated and inhibited by GA and ABA, respectively, in irradiated lettuce seeds cut so as to remove mechanical restraint of the endosperm (unpublished results). The prevention of germination by ABA in the presence of GA cannot be attributed to prevention of nuclear DNA synthesis, since there was none to inhibit. Although we have no evidence for significant cytoplasmic DNA synthesis, our data cannot rule out the possibility of cytoplasmic DNA synthesis as clearly as nuclear DNA synthesis. Since all or nearly all the nuclei in the lettuce embryo are initially at the 2C DNA level, the normal mitotic divisions during germination of unirradiated seeds must involve DNA doublings. From the very great burst in mitotic frequency that typically accompanies the onset of normal germination (7) there is little question that, with nonirradiated seeds, GA and ABA would have exerted their expected effects on nuclear DNA synthesis associated with these mitoses. The results in this present communication show that such effects on nuclear DNA synthesis are not necessary for the actions of these chemicals on lettuce seed germination.

From experiments with ^{3}H-thymidine similar to those reported in Fig. 2, we find no detectable nuclear DNA synthesis in the aleurone of Himalaya barley endosperm halves treated with GA. Since amylase formation in this system is stimulated by GA and since this stimulation is counteracted by ABA (2), we infer that the actions of both GA and ABA on amylase synthesis in barley aleurone also proceed without actions on nuclear DNA synthesis. [There are other examples of hormone action without concurrent mitosis (8, 13). One should not assume, however, that absence of mitosis implies absence of DNA synthesis. The widespread occurrence of 4C and 8C nuclei among plant cells that have ceased dividing indicates the frequent occurrence of nuclear DNA synthesis apart from the normal mitotic cycle (14). Even after large doses of radiation one cannot assume absence of nuclear DNA synthesis solely from radiation-induced mitotic inhibition (6).]

The foregoing discussion pertains to instances of GA action in systems lacking nuclear DNA synthesis. Since in most systems, including even nondividing ones, nuclear DNA synthesis does occur, we might ask the following question: When DNA synthesis does occur, to what extent does it contribute to GA sensitivity? In earlier papers we discussed the "relative GA effect," defined as

$$\frac{\text{increase in length (\%) with GA treatment}}{\text{increase in length (\%) of water controls}},$$

as a valid expression of GA sensitivity (9, 13). The relative GA effect, in contrast to absolute differences in elongation, is not reduced by treatments that presumably greatly reduce DNA synthesis in 2 different systems: (a) leaf growth in wheat seedlings from grain that had been exposed to gamma rays *versus* normal, unirradiated plants [see Fig. 1 of (8) and Fig. 2 of (9)], and (b) lentil epicotyl elongation in the presence of FUdR *versus* controls not treated with FUdR [see table I of (12) or table VI of (11)]. Therefore, when DNA synthesis occurs, it does not seem to contribute to sensitivity to GA.

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