J Plant Growth Regul (1998) 17:81–88

Journal of Plant Growth Regulation
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Distribution of [^{14}C] Dichlorophenoxyacetic Acid in Cultured Zygotic Embryos of *Zea mays* L.

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Received August 18, 1997; accepted February 12, 1998

Abstract. The uptake of 2,4-dichlorophenoxyacetic acid (2,4-D), necessary for the in vitro induction of callus formation and somatic embryogenesis in cultured immature maize embryos, was quantified after culture on nutrient medium with [^{14}C]2,4-D. The identity of the ^{14}C label in the embryos was determined by high performance liquid chromatography (HPLC), and its distribution within embryos was visualized on sections of plastic embedded material. Quantification of the ^{14}C label after a pulse label of 16 h showed a hundredfold accumulation of 2,4-D in the embryos with respect to the initial medium concentration. During tissue processing for in situ detection of ^{14}C, however, up to 70% of the label disappeared because of the embedding process. The best structural preservation was obtained after ethanol-mediated infiltration of Technovit 7100. Water-mediated infiltration of Technovit 7100 gave the highest retention of ^{14}C. HPLC analysis showed that more than 95% of the residual ^{14}C label found in embryos was still 2,4-D. Autoradiography showed that the embryogenic inbred line A188 contained ^{14}C label in distinct regions of the scutellum, coleoptile, and suspensor. The nonembryogenic inbred line A632 contained more label after 16 h of culture in a different distribution compared with A188. Subculture of the embryos for 24 and 72 h and histologic analysis showed that cell proliferation and callus formation were restricted to specific regions of the embryo in both inbred lines. The pattern of 2,4-D distribution did not codistribute with regions of proliferation, indicating that 2,4-D is not the only trigger for proliferation.

Key Words. Maize—2,4-D-Autoradiography—Callus induction—In vitro culture—TIBA

Immature maize embryos excised 11–12 days after pollination form callus when cultured on modified N6 medium (Chu et al. 1975) with 9 μM 2,4-dichlorophenoxyacetic acid (2,4-D) or DICAMBA (Duncan et al. 1985, Emons and de Does 1993, Emons et al. 1993, Green and Phillips 1975, Van Lammeren 1988). Depending on the genotype 2,4-D also induces the formation of somatic embryos, e.g. in the inbred line A188. In contrast, the inbred line A632 is unable to regenerate somatic embryos (Bronsema et al. 1996, 1997, Van Lammeren 1988). In both inbreds, the 1st day of culture is characterized by cytologic changes, such as an increase in the number of organelles, and changes in vacuolation and nuclear morphology. Determination of the incorporation of [^{3}H]thymidine in the scutellum showed that the lines had similar mitotic indexes (Fransz et al. 1990). This early phase is called the *shock response phase,* and aforementioned characteristics also occur in the absence of 2,4-D (Fransz and Schel 1987, 1991). A second phase, the *growth response phase,* starts after day 1, and, depending on the genotype and culture conditions, variations in developmental patterns occur (Fransz and Schel 1987). After 3 days, A188 embryos form a broad meristematic zone in the scutellum and a meristematic zone around the coleorhiza. These meristematic zones give rise to somatic embryos. In A632, the recalcitrant line, a meristematic zone develops only in the coleorhizal region (Fransz and Schel 1987, Fransz et al. 1990). Our aim was to determine whether the uptake and distribution of 2,4-D differed in cultured embryos of A188 and A632 and whether the distribution pattern was related to the zones of cell proliferation. To this end we quantified the uptake of [^{14}C]2,4-D after the first 16 h of culture, and

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; TIBA, triiodobenzoic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HPLC, high performance liquid chromatography; IS, ion suppression.
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we visualized the distribution of ^{14}C within the embryos immediately after labeling and after two periods of chase by autoradiography on sections of Technovit-embedded embryos.

Materials and Methods

Plants and Culture Conditions

Plants of the embryogenic maize inbred line A188 and the nonembryogenic maize inbred line A632 (both kindly provided by Dr. C. E. Green, St. Paul, MN) were grown in a growth chamber with a 16 h/8 h day/night regime at 24/20°C and a light intensity 50 W/m^2. Immature embryos with sizes ranging from 1.5 to 2.0 mm were excised 11–12 days after pollination and cultured in Petri dishes (diameter, 6 cm) with 5 mL of modified N6 medium (Chu et al. 1975) for 16 h in the dark at 25°C (Emons et al. 1993). They had their shoot meristem side in contact with the medium, which contained 8 g/liter agar, 2.3 g/liter L-proline, 200 mg/liter casein hydrolysate, 2% sucrose, pH 5.8, and 9 μM [2-^{14}C]2,4-D (Sigma, St. Louis, MO; specific activity, 12.7 mCi/mmol). Some of the embryos were analyzed for the uptake and distribution of [^{14}C]2,4-D immediately after culture. Other embryos were subcultured for 24 h or 72 h on medium in which the ^{14}C-labeled 2,4-D was replaced by unlabeled 2,4-D. Embryos that were killed after excision by submersion in liquid nitrogen were cultured on [^{14}C]2,4-D-containing medium as a control.

The influence of triiodobenzoic acid (TIBA) on the uptake and distribution of [^{14}C]2,4-D was examined by preculturing embryos on N6 culture medium with 10 μM TIBA for 8 h and then culturing them on [^{14}C]2,4-D-containing medium as described above.

Quantification of ^{14}C in Cultured Embryos

Uptake of [^{14}C]2,4-D and the loss of label during fixation procedures and embedding in Technovit 7100 were determined. After 16 h of culture on labeled medium, samples of five embryos of line A188 were collected, dried on filter paper, weighed, and either (A) processed immediately for scintillation counting (see below); or (B) fixed in a mixture of 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M phosphate buffer for 2 h at room temperature, rinsed in phosphate buffer for 1 h and in Milli-Q H_2O for 30 min, and processed for scintillation counting; or (C) fixed in aldehydes as described above and then processed further in aqueous solutions until the 100% Technovit 7100 embedding step. Procedures B and C were also performed after prefixation in an aqueous solution of 2% 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) for 30 min to bind 2,4-D to structural proteins in the cells (Shi et al. 1993) (procedures D and E). As a control, unfixed embryos were kept in water for 3.5 h and then processed through a graded series until the 100% Technovit step (procedure F). Each sample was collected in 0.5 mL of Milli-Q H_2O and boiled for 30 min. Finally, 4 mL of scintillation fluid (Ready Safe, Beckman, Fullerton, CA) was added, and the samples were measured in a Beckman LS 6000TA scintillation counter for 30 min with a 2.5% error count and background subtraction.

Fixation and Embedding of Cultured Embryos in Technovit 7100

After culture, embryos were removed from the Petri dish and put on filter paper to reduce the film of attached liquid medium. They were prefixed in an aqueous solution of 2% EDC for 30 min, rinsed in 0.1 M phosphate buffer, pH 7.2, for 30 min, and fixed in a mixture of 2% freshly prepared paraformaldehyde and 3% glutaraldehyde in 0.1 M phosphate buffer for 2 h at room temperature. After rinsing in phosphate buffer for 1 h and Milli-Q H_2O for 30 min, embryos were either infiltrated directly with monomer Technovit 7100 by incubation in aqueous mixtures with increasing concentrations of Technovit up to 100%, or embryos were first dehydrated through an ethanol series and then infiltrated with mixtures of ethanol and increasing concentrations of Technovit. Once in 100% Technovit, the samples were put in plastic molds and polymerized for 1 h at room temperature and for 2 h at 40°C.

In Situ Localization of ^{14}C in Sectioned Embryos

Sections of 2 and 4 μM thickness were cut on a Leitz rotation microtome. The median sections of the embryos were mounted on slides by stretching them on drops of Milli-Q H_2O at 40°C and drying them overnight. Slides were dipped in Amersham LM-1 photoemulsion and left for exposure in the dark for 3 weeks at 4°C. The photoemulsion was developed in Kodak D19 (Eastman Kodak, Rochester, NY) for 5 min, rinsed in water, and fixed in Kodak fix for 7 min. Sections were stained with 1% toluidine blue in water, rinsed with water, dried, mounted in DePeX (BDH Laboratory Supplies, Poole, UK), and dried overnight at room temperature. Black and white micrographs were made using bright-field and epipolarization microscopy with a Labophot (Nikon).

Identification of [^{14}C]2,4-D

To identify the nature of the ^{14}C label that caused the autoradiography in the Technovit sections, embryos were first cultured on [^{14}C]2,4-D, prefixed with EDC, fixed with aldehydes, and processed through a graded aqueous series of Technovit. Then four samples of five embryos each were homogenized and extracted overnight in 2 mL of 80% methanol instead of polymerizing the 100% Technovit. The tissue was separated by centrifugation (13,000 $\times g$ for 15 min at room temperature using a MSE microcentaur, UK). The extracts (supernatants), containing the nonbound fraction of 2,4-D in the tissue, were counted for ^{14}C by liquid scintillation counting (Tri-Carb 1500, Packard, Meriden, CO) using Ultima gold (Packard) as scintillator. Data were expressed in dpm. The pellets (remaining tissue) were resuspended in 8 N NaOH. Direct tissue hydrolysis was performed for 3 h at 100°C under water-saturated nitrogen conditions (Bialek and Cohen 1989) to hydrolyze all 2,4-D that was apparently bound by the fixation. After hydrolysis, the extracts were titrated to pH 2 and desalted using a C18 cartridge (Varian). These extracts were submitted to HPLC-IS (Shimadzu LC9A pump with on-line UV detection at 260 nm; Applied Biosystems 757 absorbance detector, 60/39.5/0.5; methanol/H_2O/acetic acid; v/v/v, flow 0.8 mL/min, Alltech Alltima C18 5 μM, 150 mm × 4.6 mm). The retention time for 2,4-D under these conditions was 15 min. Fractions of 0.8 mL were collected, 4 mL of scintillation fluid (Ultima gold) was added, and the ^{14}C content was counted. Concentrations of 2,4-D were calculated by means of the radioactivity at the 2,4-D-specific retention time and the specific radioactivity of [^{14}C]2,4-D (12.7 mCi/mmol). The metabolized 2,4-D was calculated by means of the fraction of radioactivity in the total extract after hydrolysis and desalting before HPLC, subtracted by the amount of radioactivity at the 2,4-D-specific retention time. Recovery losses during HPLC were taken into account using a [^{14}C]2,4-D reference solution.

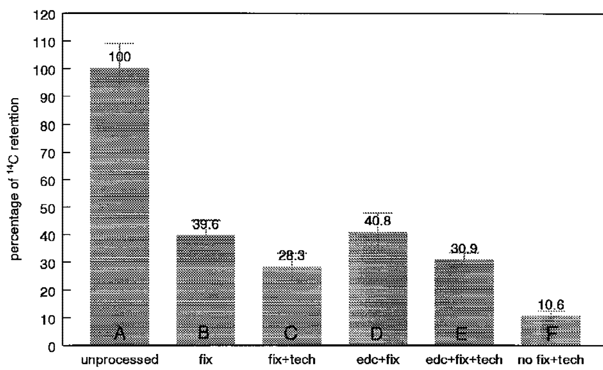

Fig. 1. Influence of prefixation, fixation, and embedding in Technovit 7100 on the retention of 2,4-D in cultured maize embryos of A188 after 16 h of culture with 9 μM [^{14}C]2,4-D. Uptake before fixation is 100%. Data are the means of independent experiments (*n*) with the standard error of the mean. *A,* embryos analyzed immediately after culture without processing ($n = 4$). *B,* after fixation with glutaraldehyde and paraformaldehyde and rinses ($n = 2$). *C,* after fixation with glutaraldehyde and paraformaldehyde and water-mediated embedding in Technovit 7100 ($n = 4$). *D,* after treatment with EDC and fixation with glutaraldehyde and paraformaldehyde and rinses ($n = 2$). *E,* after treatment with EDC and fixation with glutaraldehyde and paraformaldehyde and water-mediated embedding in Technovit 7100 ($n = 4$). *F,* no fixation and water-mediated embedding in Technovit 7100 ($n = 2$).

Results

Retention and Biochemical Analysis of ^{14}C in Embryos Cultured with [^{14}C]2,4-D

The uptake and retention of ^{14}C in unfixed and fixed embryos were quantified by scintillation counting. The filter paper, used to dry the five embryos of each sample, contained 110 dpm of ^{14}C/embryo. Embryos had accumulated an average of 17,668 dpm immediately after culture, which is equivalent to 24,203 dpm/mg or 858 μM 2,4-D. This concentration is 95 times the initial medium concentration (9 μM). About 60% of the label taken up by embryos was rinsed away during the fixation and rinsing (Fig. 1). Further loss of label during the water-mediated infiltration of Technovit was about 10%, but unfixed embryos lost up to 90% of the label during the whole embedding procedure (Fig. 1). EDC did not improve the retention of ^{14}C during fixation and Technovit embedding.

Some embryos were processed until the 100% Technovit step via procedure E, but they were not treated with high temperature for polymerization. Instead they were used to identify the nature of the residual ^{14}C label by methanol extraction and HPLC. A minor part of 3.6 ± 0.9% of the total radioactivity was extracted by methanol. After direct tissue hydrolysis and HPLC, 95.6 ± 0.9% of the radioactivity was detected at the 2,4-D-specific retention time. A minor portion of 0.8 ± 0.1% was not detected at the 2,4-D-specific retention time.

Once embedded in Technovit and sectioned, embryos did not loose ^{14}C label as was determined by analysis of the water in which sections were rinsed for 0.5 h.

In Situ Localization of ^{14}C in Embryos Cultured with [^{14}C]2,4-D

The influence of tissue processing on the retention of ^{14}C and the preservation of the structure was analyzed on median sections of Technovit-embedded embryos. Fig. 2 presents four autoradiographs of A188 embryos obtained after 16 h of culture with 9 μM [^{14}C]2,4-D, fixed without or with EDC, and embedded in Technovit after ethanol- or water-mediated infiltration. Ethanol-mediated Technovit infiltration resulted in good preservation of the structure, but labeling was most abundant after water-mediated Technovit embedding (compare Fig. 2, *A* and *B* with *C* and *D*). Prefixation with EDC resulted in decreased cell preservation; but in the case of water-mediated Technovit infiltration, a slightly increased density of silver grains was observed (compare Fig. 2, *C* and *D*).

The distribution of ^{14}C was studied in median sections of embryos after various periods of culture. The topography of a median section is given in Fig. 3*A*. After 16 h of culture with 9 μM [^{14}C]2,4-D, A188 embryos showed labeling in the basal part of the scutellum, in the top part of the scutellum opposite the shoot meristem, in the coleoptile near the scutellum, and in the lower part of the suspensor (Figs. 3*B;* see Fig. 5). Most label was found in the scutellum opposite the shoot meristem. In the non-embryogenic line A632 label was observed in the top, middle, and basal part of the scutellum except for the procambium strand, in the coleoptile near the scutellum, and in the lower part of the embryo axis away from the scutellum (Fig. 4*A* and 5). Compared with A188, line A632 exhibited more silver grains on sections. In both lines low amounts of label were found in the root and shoot meristems of embryos and in the central region of the embryo axis. Control embryos, frozen in liquid nitrogen before culture, showed no label above the background after 16 h of culture with 9 μM [^{14}C]2,4-D (Fig. 4*D*).

Other embryos were first cultured on [^{14}C]2,4-D medium for 16 h and then subcultured on chase medium with 9 μM unlabeled 2,4-D for another 24 or 72 h to examine changes in the distribution of ^{14}C label during subculture. After a chase of 24 h, fixation, and embedding, A188 embryos showed most label in the scutellum, predominantly in those parts where it was also found without subculture (see Fig. 3, *B* and *C*). The intensity, however, diminished, especially the high amount of label in the top part of the scutellum opposite the shoot meristem. Distinct spots of label appeared in this region. After a chase of 72 h the distribution of label had

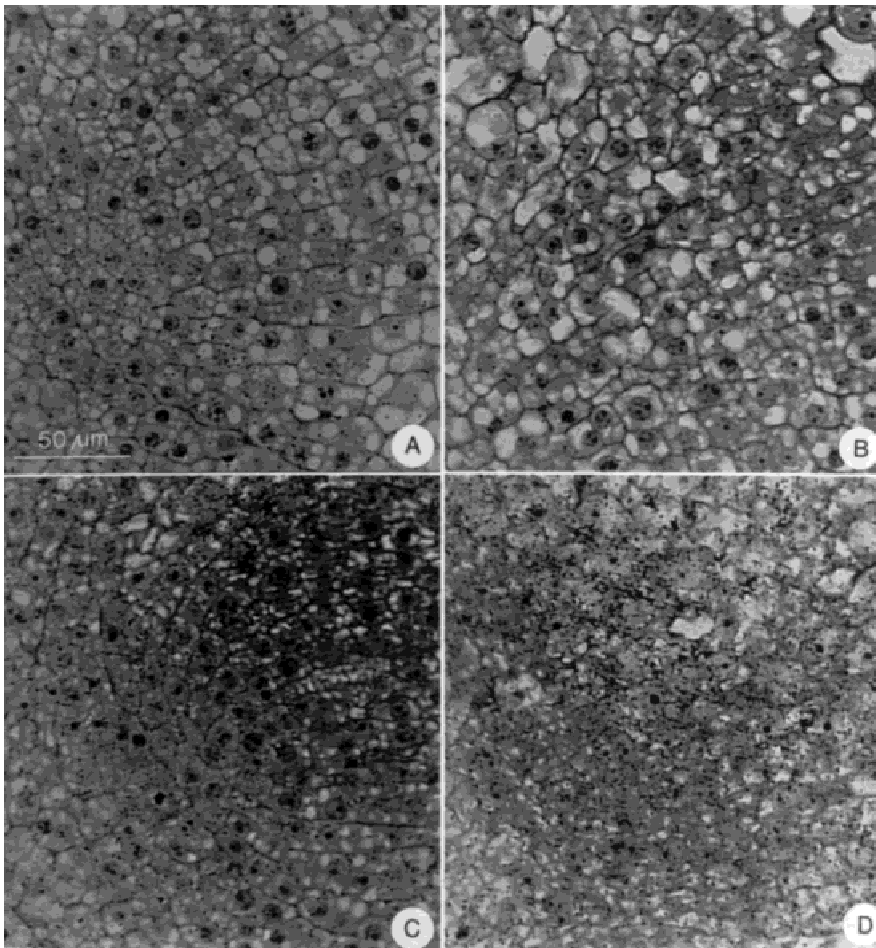

Fig. 2. Autoradiographs of 2-μM-thick median sections of cultured maize embryos showing the structural preservation and ^{14}C labeling of the root zone of A188 embryos, fixed and embedded in various ways after pulse label with 9 μM [^{14}C]2,4-D for 16 h. Embryos were either obtained after ethanol-mediated Technovit infiltration (*A* and *B*) or via a water-mediated Technovit infiltration (*C* and *D*). Embryos in *A* and *C* were not treated with EDC before aldehyde fixation; embryos in *B* and *D* were treated. The best structure preservation was obtained in *A,* but the most label was observed in *D*. The *bar* represents 50 μM for all figures.

changed in A188 embryos. More label was observed in the basal part of the scutellum and in the area around the coleorhiza. Tissues with low concentrations of label had increased in size, especially in the top part of the scutellum (Figs. 3*D* and 5).

When A632 embryos were subcultured on unlabeled medium for 24 h, the general distribution of label had not changed, but the label intensity had decreased, and distinct spots of label appeared in all scutellar parts (Figs. 4*B* and 5). After 72 h of subculture, label was still observed in the basal and top parts of the scutellum. The middle part of the scutellum opposite the coleorhiza had enlarged and showed less silver grains. The embryo axis and coleoptile, which had increased in size, exhibited low concentrations of label (Figs. 4*C* and 5).

In Situ Localization of ^{14}C in Embryos Cultured with [^{14}C]2,4-D after Pretreatment with TIBA

The influence of an 8-h pretreatment with 10 μM TIBA on the uptake of [^{14}C]2,4-D and the distribution of the label in embryos is shown in Fig. 3*E*. Less label is seen but with a similar distribution, indicating a lower uptake of [^{14}C]2,4-D (compare Fig. 3, *B* and *E*). Most label accumulated in a region between the scutellum and coleoptile and less at the base of the scutellum and base of the suspensor. After 24 and 72 h of chase the densities of label were lower than shown in Fig. 3*E* (data not shown). A spot-like accumulation was observed at the adaxial side of the scutellum. The regions with most label exhibited fewest enlargement.

Discussion

Fate of 2,4-D after Uptake by Cultured Embryos

Autoradiography of ^{14}C label on sectioned material cannot distinguish among free, conjugated, or metabolized forms of 2,4-D because all ^{14}C label will cause the formation of silver grains in the dipping film. Quantitative and qualitative analysis of ^{14}C after uptake in cultured embryos of A188 has shown, however, that after a pulse of 16 h with [^{14}C]2,4-D, 73% of the ^{14}C was free 2,4-D, 11% was conjugated 2,4-D, and 16% was metabolized 2,4-D; embryos took up 275 μM 2,4-D (Bronsema et al.

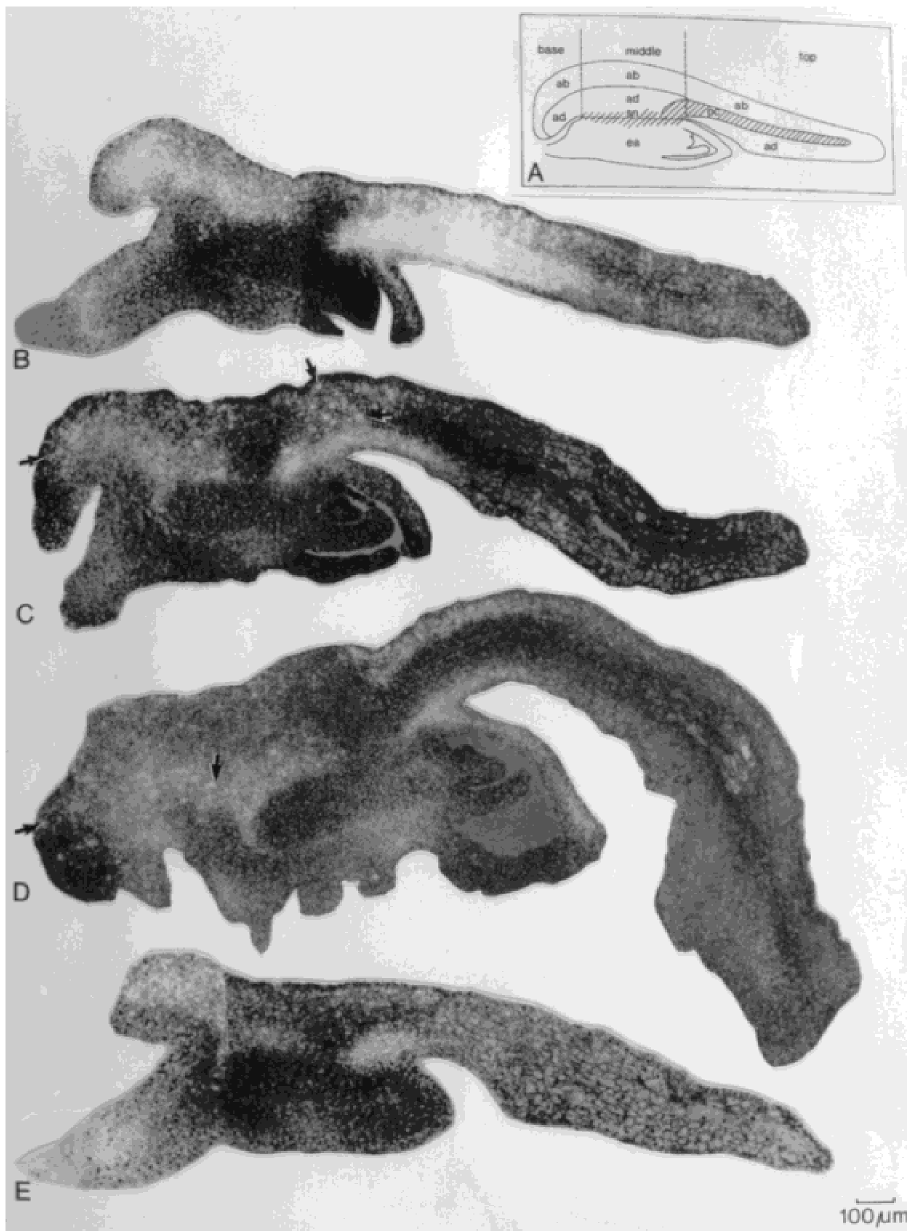

Fig. 3. Median sections of immature maize embryos showing histology (*A*) or morphogenesis and distribution of [^{14}C]2,4-D (*B–E*). *A,* schematic representation showing the various subregions. *ab,* abaxial side; *ad,* adaxial side; *ea,* embryo axis; *pc,* procambium strand; *sn,* scutellar node. (Adapted from Fransz et al. 1990.) *B–E,* light micrographs showing distribution of [^{14}C]2,4-D by autoradiography and epipolarization illumination in 4-µM sections of A188 embryos. The *white* regions and *spots* in the micrographs are caused by illumination of the silver grains by epipolarization and represent the label in the various tissues of the embryo. The *dark* regions represent the tissues of the embryos after staining with toluidine blue. *B,* distribution after 16 h of pulse with 9 µM [^{14}C]2,4-D. Note the accumulation of label in the basal part of the top region of the scutellum and in its basal zone. *C,* distribution after 16 h of pulse with 9 µM [^{14}C]2,4-D and 24 h of chase with 9 µM 2,4-D. *Arrows* indicate spots of silver grains. *D,* distribution after 16 h of pulse with 9 µM [^{14}C]2,4-D and 72 h of chase with 9 µM 2,4-D. *Arrows* indicate spots of silver grains. *E,* distribution after 8-h pretreatment with 10 µM TIBA and a 16-h pulse with 9 µM [^{14}C]2,4-D. Note the reduced autoradiography signal compared with *B.*

1996). For A632, 63.5% of the 2,4-D was free after 16 h of culture, 12.5% conjugated, and 24% metabolized, and embryos took up 402 µM 2,4-D. The present results show an accumulation of 858 µM 2,4-D which is about a hundredfold with respect to the initial medium concentration and about three times as much as found previously (Bronsema et al. 1996). The difference could have been caused by a variation in metabolic activity due to seasonal influences.

Determination of the nature of the ^{14}C present in the plastic-embedded embryos is crucial for the interpretation of the autoradiographs. From the biochemical analysis with HPLC it is concluded that 95% of the ^{14}C analyzed corresponded to free and conjugated 2,4-D which was fixed to the tissue after fixation with EDC and aldehydes, whereas only a minor fraction (0.8%) of the radioactive 2,4-D was metabolized. With A188 up to 70% of the radioactivity was rinsed away during the embedding procedure; however, based on the biochemical analysis directly after the culture mentioned above, we do not expect that the loss is specific, although metabolized 2,4-D might have been rinsed away with preference. We cannot exclude a tissue-specific loss, but the similar distribution in various embryos of one inbred line and the differences in distribution between A188 and A632 rather indicate a genotype-specific distribution.

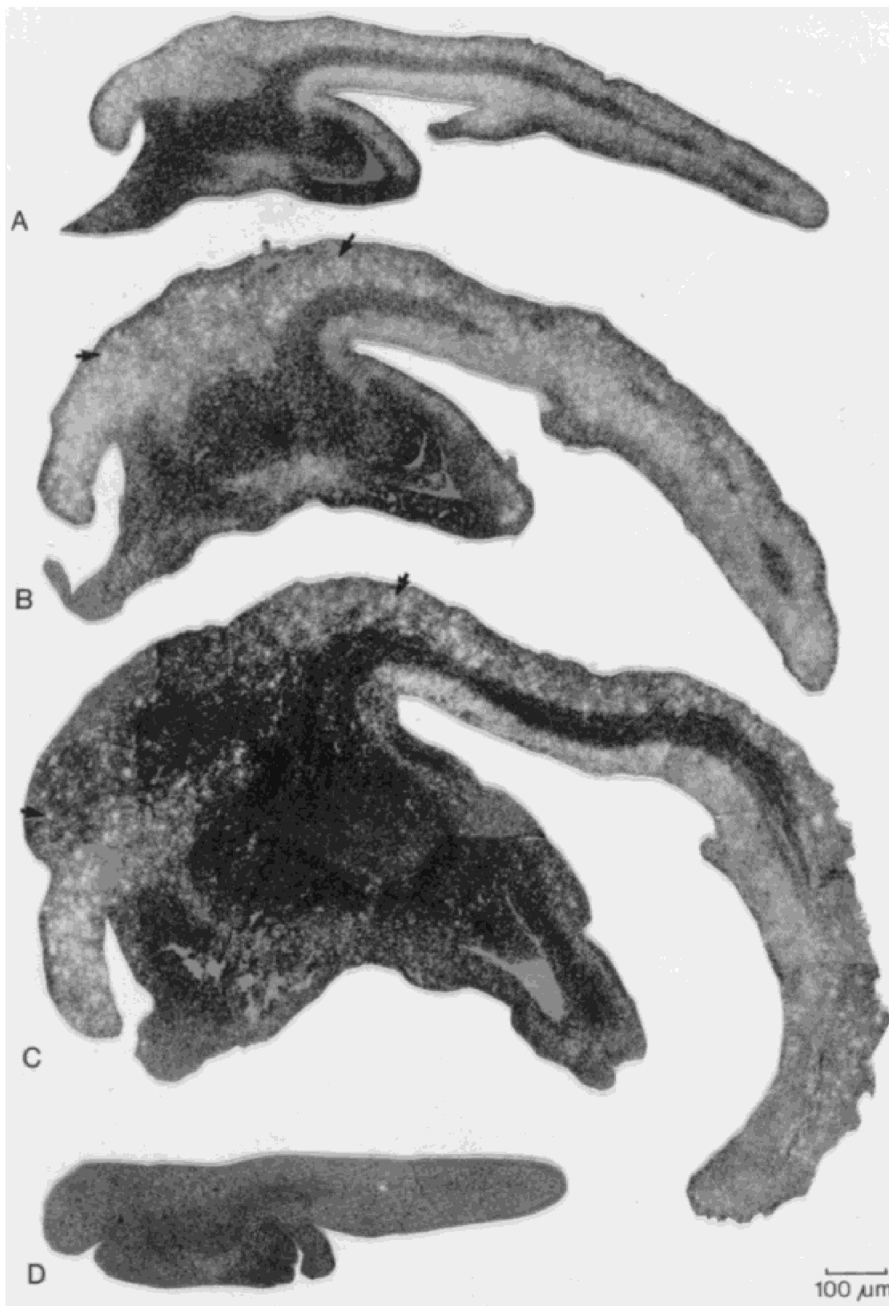

Fig. 4. Distribution of [^{14}C]2,4-D shown by autoradiography and epipolarization illumination in 4-μM median sections of A632 embryos. *A,* distribution after 16 h of pulse with 9 μM [^{14}C]2,4-D. *B,* distribution after 16 h of pulse with 9 μM [^{14}C]2,4-D and 24 h of chase with 9 μM 2,4-D. *C,* distribution after 16 h of pulse with 9 μM [^{14}C]2,4-D and 72 h of chase with 9 μM 2,4-D. *Arrows* indicate spots of label. *D,* distribution of [^{14}C]2,4-D by autoradiography and epipolarization illumination in 4-μM median sections of an A188 embryo submerged in liquid nitrogen after 16-h pulse with 9 μM [^{14}C]2,4-D.

Retention of Accumulated 2,4-D during Embedding Procedures

Processing of unfixed embryos until the 100% Technovit embedding step resulted in the loss of up to 90% of the label. When a combination of EDC pretreatment, fixation, and water-mediated Technovit embedding was performed 30% label remained in the embryos. ^{14}C label was best retained after prefixation with EDC, although the preservation of the cell structure was inferior. Loss of ^{14}C label during fixation, rinsing, and embedding steps was also reported by Coetzee (1985) who fixed, dehydrated, and embedded bean leaves after exposure to $^{14}CO_2$. In the present experiments the loss of ^{14}C during ethanol dehydration was reduced by a protocol in which the monomer Technovit 7100 was dissolved in water. Although only 30% of the label was retained in the tissues of the embryo, the biochemical analysis showed that the formation of silver grains in the dipping film represents the presence of [^{14}C]2,4-D.

Uptake and Transport of 2,4-D in Cultured Embryos

The in situ localization of ^{14}C label showed that although the embryos were cultured with the meristem side on the agar surface, most of the label was found in regions of

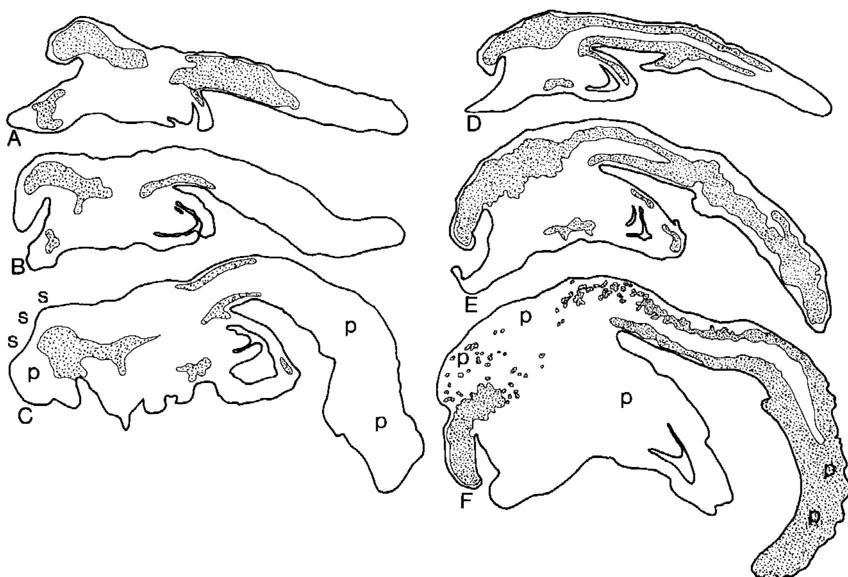

Fig. 5. Schematic representation of the distribution of [^{14}C]2,4-D in median sections of A188 (*A–C*) and A632 (*D–F*) embryos. *A,* distribution after 16 h of pulse with 9 μM [^{14}C]2,4-D. *B,* distribution after 16 h of pulse and 24 h of chase with 9 μM 2,4-D. *C,* distribution after 16 h of pulse and 72 h of chase with 9 μM 2,4-D. *D,* distribution after 16 h of pulse with 9 μM [^{14}C]2,4-D. *E,* distribution after 16 h of pulse and 24 h of chase with 9 μM 2,4-D. *F,* distribution after 16 h of pulse and 72 h of chase with 9 μM 2,4-D. *Dotted* areas represent high concentrations of [^{14}C]2,4-D; areas of proliferation are represented by *p.* Areas where somatic embryos and friable embryogenic callus are formed in A188 are represented by *s.*

the embryo distant from the meristem side, indicating transport of label. We, however, cannot define the transport route for 2,4-D because a 2,4-D-containing water film always surrounded embryos in the culture. The low radioactivity in the adhering film, found by counting the filter paper, however, indicates that the gradient from the agar-containing medium to the embryo might induce the most intensive transport in the contact region between the embryo and nutrient medium.

Transport is partly passive caused by pH trapping and partly active by carrier-mediated efflux of auxin-anions (Jacobs 1983). Bronsema et al. (1996) demonstrated active uptake of 2,4-D in cultured maize embryos by applying pretreatment with the synthetic polar transport inhibitor TIBA (Lomax et al. 1995), which caused a 30% reduction of 2,4-D uptake. Autoradiography now showed that the distribution pattern was maintained after pretreatment with TIBA, but the intensity of labeling was reduced, indicating that active transport was only partly reduced.

Since freeze-killed embryos did not show any labeling because of the destruction of cell membranes, it is concluded that pH trapping also causes a significant contribution to the uptake of 2,4-D in living embryos.

Distribution of ^{14}C Label in Embedded and Sectioned Embryos

Random labeling of 2,4-D in embryonal tissues was not observed in any of the embedding protocols used. Procambium strands were only weakly labeled, indicating that the transport of 2,4-D might either be cell to cell transport, not mediated by the cells of the procambial strands, or that the procambial strands do not accumulate 2,4-D in a way seen in the parenchyma cells of the embryo.

Comparing the two lines for their distribution of label, the more intensive labeling found in A632 is in agreement with data of the quantitative analysis (Bronsema et al. 1996). Differences in distribution are presented schematically in Fig. 5 together with the sites of proliferation and callus formation. Spots of intensive label appeared in both lines. These spots were found in cells that, however, did not differ in morphology from cells surrounding the spots. Cells that formed callus were never found in regions with dense labeling, and regions with a high label intensity did not proliferate. In A632, however, the top part of the scutellum enlarged despite the presence of label. Thus, the pattern of 2,4-D distribution did not codistribute with proliferation.

Cell proliferation in cultured gramineous embryos is generally initiated first in the nodal and basal regions of the scutellum. Both regions are in close proximity to the root meristem and the procambium of the embryo axis (Vasil et al. 1985). It has been suggested that this may be related to the high levels of plant growth regulators presumed to be present in root and procambium (Vasil and Vasil 1982). With our autoradiographic localization we indeed observed high levels of label in the nodal and basal regions of the scutellum but not in the root meristem and the procambium.

The suggestion that the uptake of 2,4-D by embryos cultured in vitro is probably channeled more effectively through the embryo suspensor, and the coleorhiza (Vasil et al. 1985) could not be confirmed by our results because the highest amount of label observed in A188 was found in the scutellum near the coleoptile, distant from the suspensor.

In all, the detection of 2,4-D by autoradiography vi-

sualizes the distribution of the growth regulator over the various tissues of the zygotic embryo. The regulator is taken up rapidly from the medium by passive and active processes and distributed throughout the embryo. The patterns of 2,4-D distribution did not codistribute with regions of proliferation. On the contrary, the regions of high accumulation of label always showed reduced enlargement and proliferation. These observations indicate that the presence of 2,4-D in a certain tissue itself is not the only prerequisite for triggering cell division and callus formation.

Acknowledgments. We thank Prof. Dr. M. T. M. Willemse and Prof. Dr. H. A. van Onckelen for discussion; Dr. A. K. Pawlowski of the Department of Molecular Biology, Wageningen Agricultural University, for assistance with autoradiography; and S. Massalt, A. Haasdijk, and P. van Snippenburg for photography and artwork. This work was supported by the Dutch Organisation for Scientific Research.

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