Somatic Embryogenesis in *Miscanthus*

Karen K. Petersen, Inger B. Holme, and Mona Lillelund Hansen
Department of Ornamentals, Research Centre Aarslev, Danish Institute of Plant and Soil Science, Kirstinebjergvej 10, DK-5792 Aarslev

Shoot apices and leaf explants of *Miscanthus* shoot cultures were grown on Murashige and Skoog medium supplemented with 1.36, 13.6, or 136 µM of the auxin 2,4-dichlorophenoxyacetic acid (2,4-D). The explants were exposed to 2,4-D for 1, 2, 4, or 8 weeks and then transferred to 2,4-D free MS medium. A higher percentage of shoot apices produced callus and embryogenic callus than leaf explants. Shoot apices were less sensitive to 2,4-D than leaf explants. Transfer to growth-regulator-free medium after short exposure to 2,4-D caused some of the callus to die. High concentrations of 2,4-D suppressed the development of roots until transfer to growth-regulator-free medium.

INTRODUCTION

Somatic embryogenesis is a potential method for mass propagation of cultivars like the triploid *Miscanthus* ×*ogiformis* Honda 'Giganteus' where propagation is restricted to vegetative propagation methods. The different steps involved in the development of a somatic embryo propagation system are: (1) Induction and selection of a highly embryogenic callus type; (2) Maintenance and proliferation of embryogenic callus either on solid or in liquid medium—when fast-growing suspensions are established they can be transferred to bioreactors where large quantities of cell aggregates can be handled in an controlled environment; (3) Induction and maturation of somatic embryos; (4) Control of germination; and (5) Encapsulation of somatic embryos and germination of artificial seed in the field.

Often several different callus types are produced during callus induction which will differ in their capacity to form somatic embryos, shoots, and roots. For establishment of a reliable and efficient somatic-embryogenesis propagation system it is crucial to induce and select fast-growing highly embryogenic callus. It is also important to control the production of root-forming callus types as they can outgrow the embryogenic callus types (Morrish et al., 1987).

In *Miscanthus* three different callus types—an embryogenic, a non-embryogenic, and a root-forming—were formed when explants were cultured on medium with 4.5 to 31.7 μM of 2,4-dichlorophenoxyacetic acid (2,4-D) (Holme and Petersen, 1996). It was observed that 2,4-D did have some impact on the distribution between callus types but only relatively small differences were found. It would be of great value for somatic embryogenesis in *Miscanthus* if the formation of embryogenic callus could be further increased and the production of root-forming callus decreased.

In order to further specify the effects of 2,4-D on the callus induction percentage and the subsequent differentiation of callus into embryogenic- and root-forming types, a broader 2,4-D range (1.36 to 136 μM) was investigated. Furthermore, the explant exposure time to the different 2,4-D concentrations before transfer to medium without growth regulators was tested.

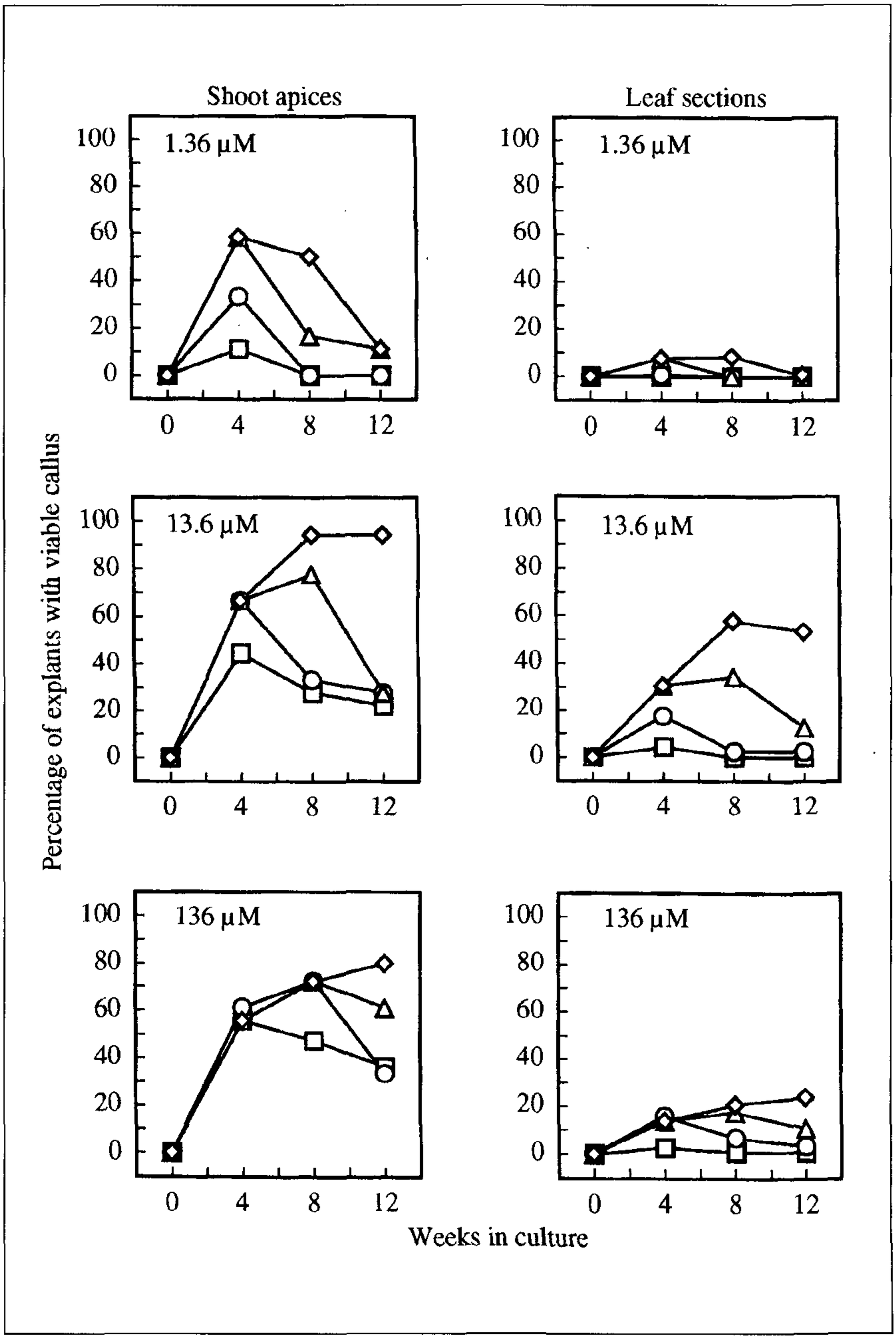

Figure 1. Percentage of explants from *Miscanthus* ×*ogiformis* 'Giganteus' with viable callus as influenced by explant type, 2,4-D concentration, and exposure time recorded after different periods in culture. The exposure times to 2,4-D were □ = 1 week, ○ = 2 weeks, Δ = 4 weeks or ◇ = 8 weeks.

MATERIALS AND METHODS

Plant material of *M.* ×*ogiformis* 'Giganteus' was obtained from in vitro shoot cultures propagated according to Nielsen et al. (1993) with 22.2 μM benzyladenine (BA) and 1.3 μM α-naphthaleneacetic acid (NAA). Four weeks after subculture shoot apices and 2-mm sections from the most basal part of the three youngest leaves were dissected. The callus induction medium was MS basal medium (Murashige and Skoog, 1962) containing 30 g liter^{-1} sucrose, 500 mg liter^{-1} casein hydrolysate, 300 mg liter^{-1} L-glutamine, 2 g liter^{-1} gelrite, 750 mg liter^{-1} $MgCl_2$ $6H_20$, and 0, 1.36, 13.6, or 136 μM of 2,4-D.

Media pH were adjusted to 5.5 prior to autoclaving. Explants were transferred from 2,4-D-containing medium to the same medium without growth regulators (0-medium) after 1, 2, 4, or 8 weeks. Explants were incubated in darkness at 27C and subcultured at l-week intervals for the first 2 weeks and thereafter at 2-week intervals. The percentage of explants with viable callus, viable embryogenic callus, and roots were recorded 4, 8, and 12 weeks after culture initiation.

RESULTS AND DISCUSSION

Percentage of Explants With Viable Callus. No callus was induced on medium without growth regulators. Overall shoot apices produced more callus than leaf explants and were less sensitive to high and low concentrations of 2,4-D (Fig. 1). It is common to find different response patterns between different types of explants dependent on auxin concentration (Conger et al., 1982; Henke et al., 1978). In the present investigation the shorter the period of exposure to 2,4-D the less callus was induced especially on leaf explants. After transfer to 0-medium part of the callus died and the lower the 2,4-D concentration the faster the callus died. Comparison of the callus induction (Fig. 1) and the percentage of explants with viable embryogenic callus (Fig. 2) shows that it is primarily non-embryogenic callus types that die.

Percentage of Explants With Viable Embryogenic Callus. A much higher percentage of embryogenic callus was produced on shoot apices as compared to leaf explants. Shoot apices produced the highest percentages of embryogenic callus when exposed to 13.6 μM 2,4-D for 4 or 8 weeks or to 136 μM 2,4-D for 1 week. Exposure for 1 or 2 weeks to 1.36 μM 2,4-D did not induce embryogenic callus and the other treatments were either suboptimal or superoptimal for embryogenic callus production on shoot apices. The production of embryogenic callus on leaf explants compared to shoot apices is restricted to a more narrow exposure time and 2,4-D concentration range. Differences in the optimal auxin concentration for embryogenic callus formation differs between species and explant types, and the optimal concentration range is often found to be more narrow than for callus induction (Pareddy and Petolino, 1990; Thomas and Scott, 1985).

Percentage of Explants With Roots. The higher the 2,4-D concentration the more suppressed was the development of roots (Fig. 3) when explants were still exposed to auxin, and no roots developed on 136 μM 2,4-D until transfer to 0-medium. Likewise, in suspension cultures of *Dactylis glomerata* (orchardgrass) root development was reduced with increasing concentrations of the auxin dicamba up to 60 μM (Gray and Conger, 1985). In *Sorghum bicolor* 0.09 μM 2,4-D resulted in root development on all calli whereas 2.7 μM 2,4-D reduced the development of roots to 4% of the calli (Wernicke and Brettell, 1982). In *Miscanthus* it was not possible to

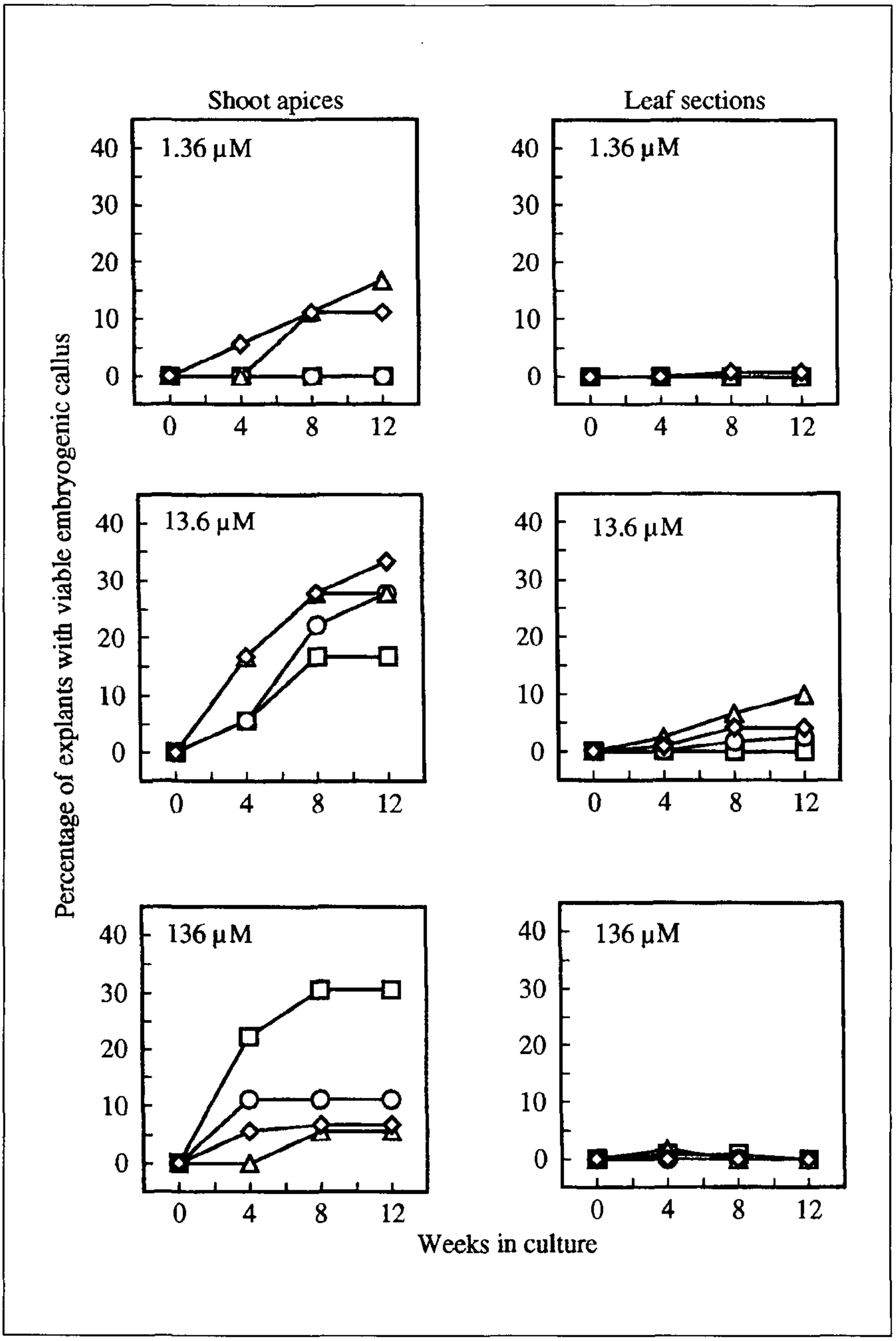

Figure 2. Percentage of explants from *Miscanthus* ×*ogiformis* 'Giganteus' with viable embryogenic callus as influenced by explant type, 2,4-D concentration, and exposure time recorded after different periods in culture. The exposure times to 2,4-D were □ = 1 week, ○ = 2 weeks, Δ = 4 weeks, or ◊ = 8 weeks.

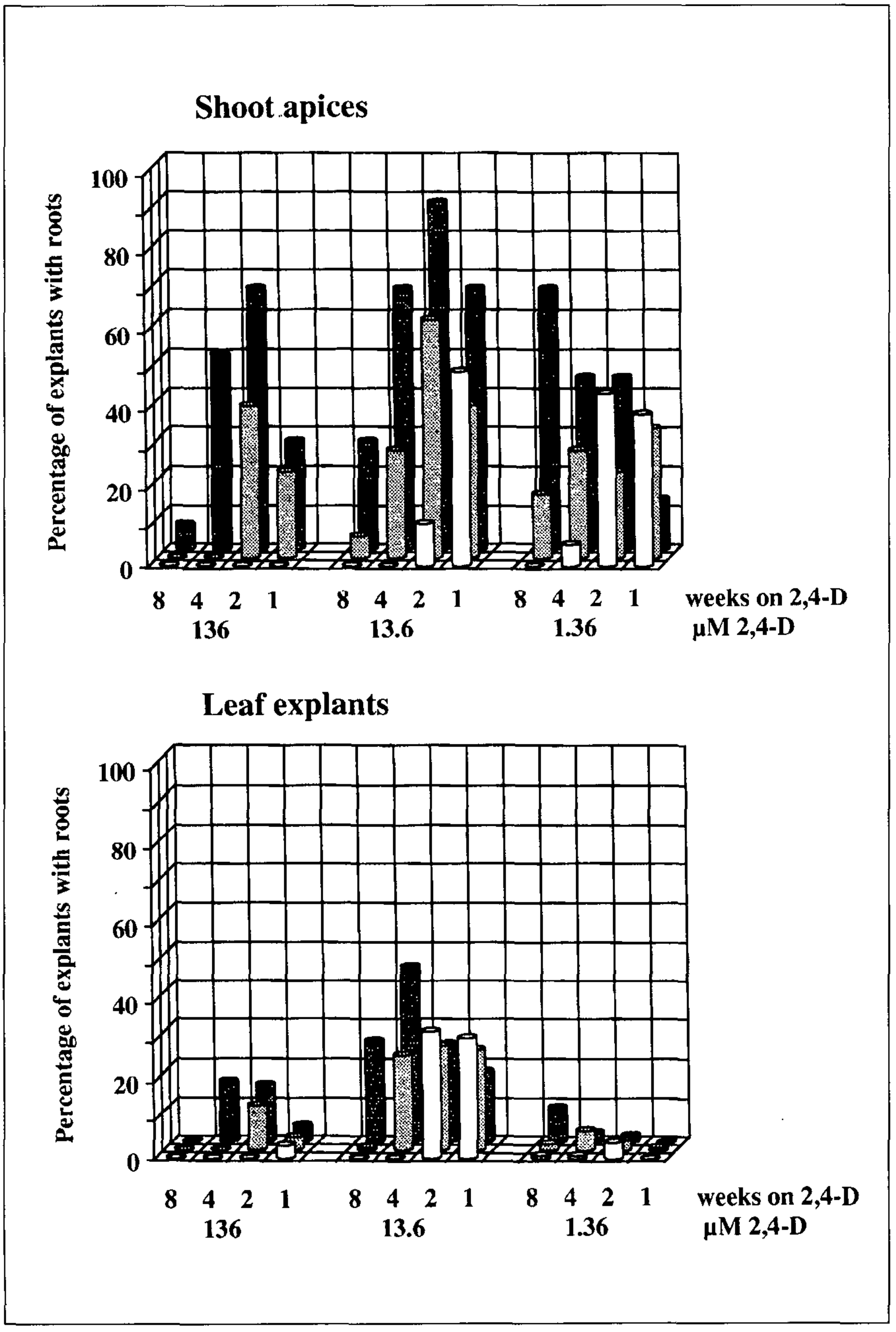

Figure 3. Percentage of explants from *Miscanthus* ×*ogiformis* 'Giganteus' with roots as influenced by explant type, 2,4-D concentration, and exposure time recorded after different periods in culture. □ = 4 weeks, ◻ = 8 weeks, or ■ = 12 weeks in culture.

inhibit the formation of the root-forming callus type with high concentrations of 2,4-D, only to suppress the development of roots. Careful selection is therefore decisive to obtain highly embryogenic cultures.

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