Shoot induction and plant regeneration from cotyledon segments of the muskmelon variety "hógolyó"

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Summary: Cotyledonary segments of the casaba type muskmelon variety "Hógolyó" were used to induce organogenesis. Fifty different hormone combinations were applied to enhance the induction of shoot formation on the edge of the segments. The phases of organogenesis were followed with light- and scanning electron microscope. Shoot induction was achieved with high frequency. The shoots were transferred to hormone free media for root induction. The rooted plantlets were planted out to soil.
NAA was feasible and the method can be applied in transformation experiments.

Introduction

In muskmelon (*Cucumis melo* L.) plant regeneration is considered to be a difficult task although in vitro shoot induction has been reported in case of different varieties and from different organs like from cotyledons (*Moreno et al.*, 1985; *Dirks* and *van Buggenum*, 1989; *Niedz et al.*, 1989; *Chee*, 1991), from hypocotyls (*Moreno et al.*, 1985; Kathal *et al.*, 1986), from roots (*Kathal et al.*, 1984), from leaves (*Kathal et al.*, 1988; *Dirks* and *van Buggenum*, 1989). Embriogenesis form cotyledonary segments (*Oridate* and *Oosawa*, 1986; *Tabei et al.*, 1i91; *Grey et al.*, 1993) or protoplasts (*Li et al.*, 1990) has also been reported. We tried to induce organogenesis and plant regeneration from cotyledonary segments of the muskmelon variety 'Hógolyó' (a late casaba type variety) using different hormone combinations and with special attention to the formation of primary structures.

Material and methods

Plant material

Seeds of the muskmelon variety Hógolyó were obtained from the Vegetable Crop Research Institute, Budatétény, Hungary. The seed coat was removed and seeds were sterilised in 10% H_2O_2 for 30 minutes (*Bársony* et al., 1998). After several washing with sterile water, seeds were put on the surface of 8% solidified agar supplemented with 2mg/l Chobalt chloride ($CoCl_2$). Seeds for germination were kept in light cabinet under 18/8h light/dark period at 32 °C. Only three day old green, expanded cotyledons were used in the experiments.

Culture conditions

Cotyledons were cut into four. The edges of the cotyledons were also removed. Explants were kept on half MS media (pH 5.6–5.8) supplemented with different combinations of IAA and BA (Sigma) in light cabinet under 18/8h light/dark period at 25 °C. Number of shoots/explants were counted and data from three independent experiments were analysed with T probe. Root induction media was half MS without hormones. Rooted plantlets were transferred to soil/vermiculite mixture (1:1) and incubated some days under 100% relative humidity at 23 °C. After several days they were transferred to the greenhouse and later to open air.

Documentation

Light and scanning electron microscope (SEM) were used to follow the formation of organogen structures. SEM pictures were made in the Central Laboratory of the University of Horticulture and Food Industry.

Results

Organogenesis

Keeping the cotyledonary segments on the most effective induction medium callus formation and organogen structures emerging on the edge of the cut segments were

observed after 5–10 days. The globular dark green organogen structures seen under light microscope *(Fig. 1a)* had embryo or shoot type forms *(Fig 1b)*. After three weeks these structures were large enough to be recognised clearly as hairy shoots with open stomata structures on their surface *(Fig. 2a and 2b)*.

When the leafy shoots were well formed *(Fig. 3)* they were cut and transferred to root induction media. After 3–6 weeks when roots were well formed rooty plantlets were transferred from the sterile medium to the greenhouse and later to open air.

Induction of organogenesis with high frequency

Cross table (7x7) experiments with different hormone concentrations gave satisfactory combinations for shoot induction. On media with combinations of 2.4 mg/l IAA and 2.5 mg/l BA, high frequency of organogenesis was achieved.

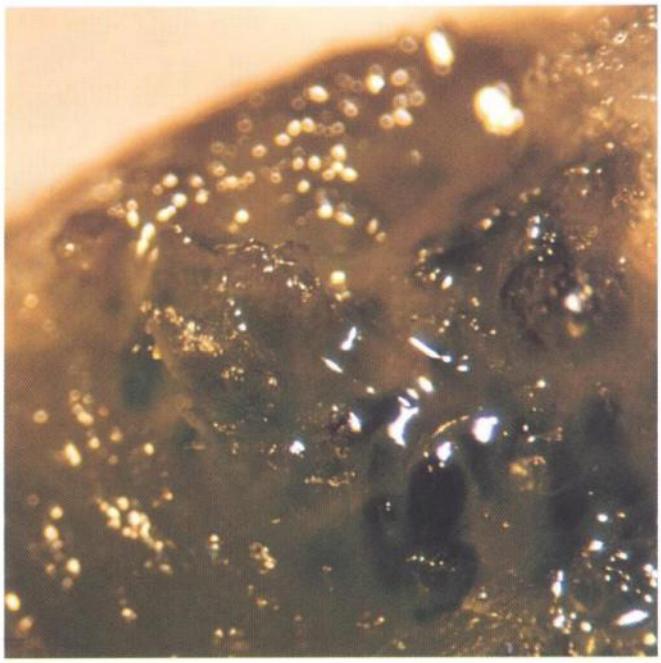

Fig. 1a Light microscope picture of globular organogen structures (with some hair) on the edge of a cotyledonary segment of the muskmelon variety "Hógolyó".

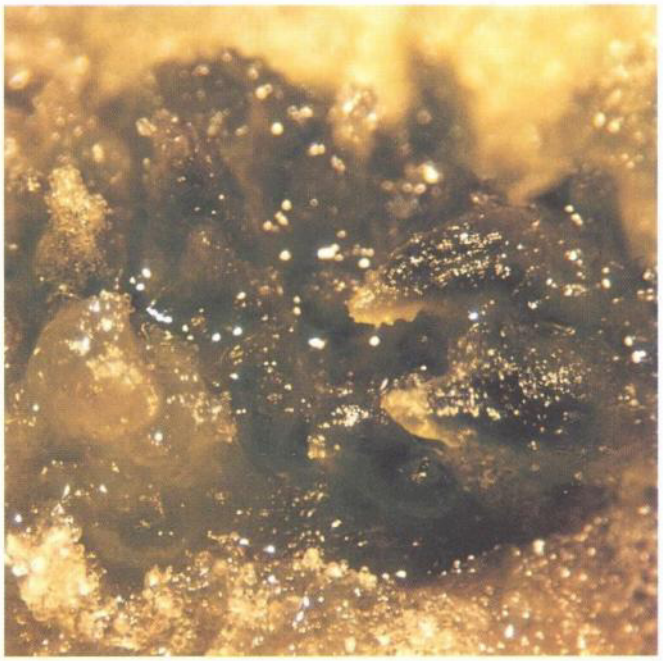

Fig. 2a Shoot formation after three weeks on induction media

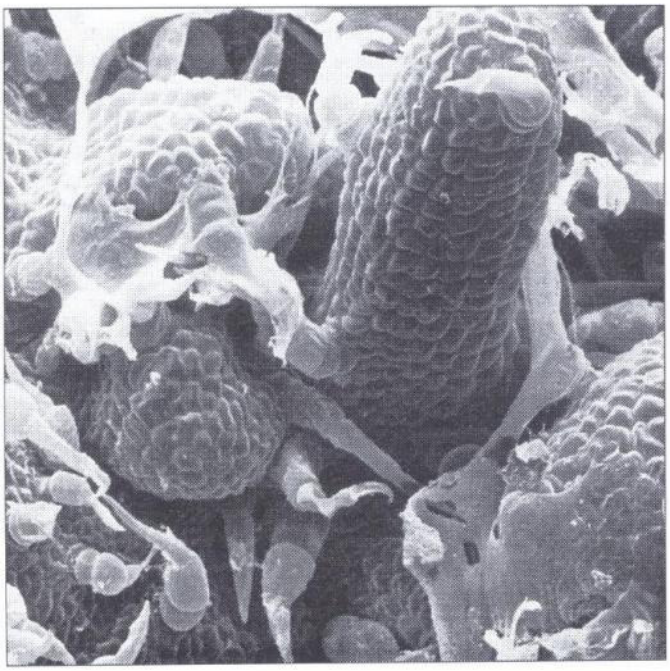

Fig. 1b Birth of early globular structures (SEM, magnification 250 000x)

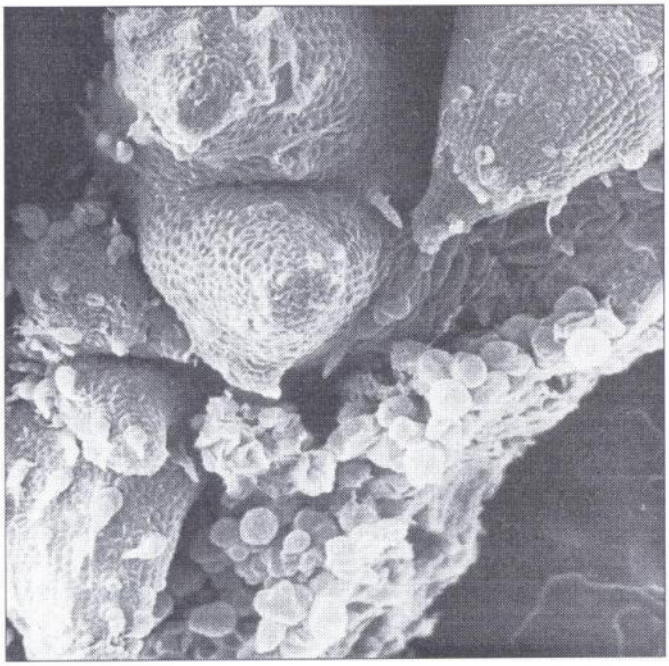

Fig. 2b SEM picture of three weeks old shoot initiatives with hair and stomata on the surface (M: 82 000x)

Some combinations produced less shoots or callus only, while others were not effective at all *(Fig.4)*.

Discussion

The induced organogenesis by direct shoot formation from cotyledonary segments has been reported in case of some muskmelon varieties but Hógolyó which is a casaba type late melon variety. The published induction media did not give satisfactory results with Hógolyó. This was not surprising because organ induction depends on the responsive ability of the genotype and on the applied hormones. In our experiments the effect of 49 hormone combinations on the induction was investigated. The best induction medium produced 4–5 regenerated plantlets from one cotyledonary segment. This is in the average of the published frequencies with other varieties where BA and IAA were used for organ induction (*Moreno et al.*, 1985; *Dirks* and *van Buggenum*, 1989; *Niedz et al.*, 1989; *Chee*, 1991).

In our experiments we cut round the cotyledons before cutting them into four to obtain more wounded surfaces useful for agrobacterial infection. On the right induction medium the callus and organ formation mostly occurred on the cut surfaces. The dark green spherical-elongated structures observed by light- and scannning electron mycroscope (SEM) emerging during the first three weeks proved to be induced shoots. These structures harbour hair on their epidermal surface and later open stomata structures were observed. These differentiated cells and structures are typical in case of leaves and shoots.

Therefore we can assume that the optimised procedure we used for induction of organogenesis from cotyledonary explants of the muskmelon variety 'Hógolyó' can be effective for the purpose of transformation using Agrobacterium.

The site of the induction of the organogenesis is very important although it has not been investigated carefully, as in case of Agrobacterium mediated plant transformation the contact between plant cell and bacteria mostly occur on wounded surfaces. We could observe under light- and electron microscope that the primary structure formation happens mostly on and around the cut surface.

Fig. 3 Leafy shoot formed on the cotyledonary segments 5 weeks after induction

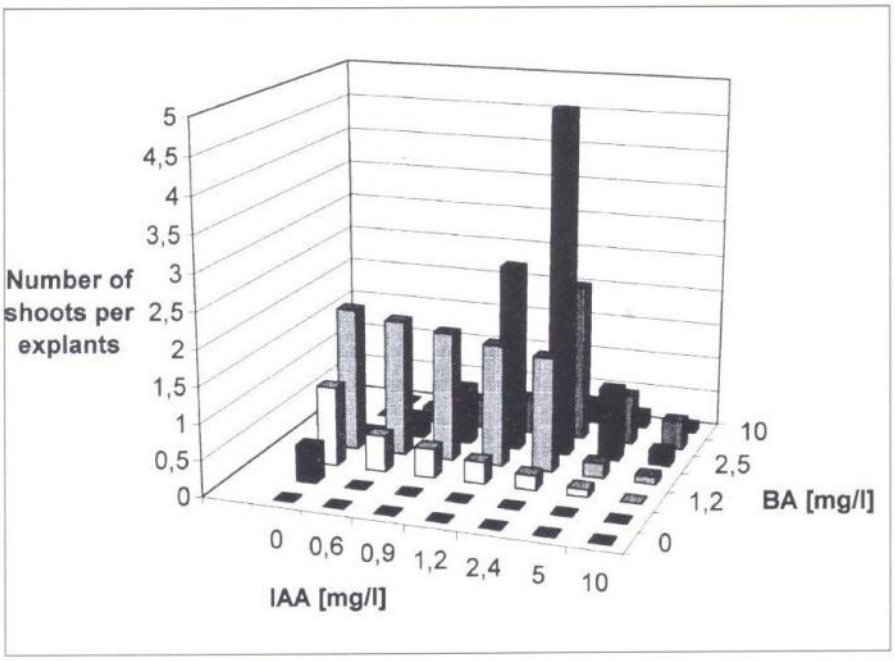

Fig. 4 The frequency of organ (shoot) formation on the cotyledonary segments of the muskmelon variety "Hógolyó". Column height shows the average number of shoots per explant (from three independent experiments) on a particular hormone combination.

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