In vitro regeneration from cotyledons of watermelon

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Summary: Cotyledonary segments of five different genotypes of watermelon were used to induce organogenesis. Five different hormone combinations were applied to enhance the induction of shoot formation on the surface of the segments. The phases of organogenesis were followed with light and scanning electron microscope. Shoots were obtained after four weeks, then the shoots were transferred to hormone free medium for root induction.

This method of regeneration can be applied in transformation experiments. GUS histochemical assay was made to check the expected success of using Agrobacterium for the transformation.

Introduction

Various factors and conditions involved in plant tissue and cell culture techniques in the Cucurbitaceae have been investigated. Cotyledonary explants of mature seeds and young seedlings possess high ability for shoot organogenesis and embryogenesis. Regeneration systems from protoplasts of melon have also been developed *(Tabei,* 1997). *Islam* et al. (1995) used mature zygotic embryos of watermelon for plant regeneration. High frequency of bud induction was obtained from cotyledons on MS medium containing BA and IAA *(Hao Li Xin* et al., 1998; *Islam* et al., 1995; *Wang Chun Xia* et al., 1997; *Tang Shao Hu* et al., 1994; *Compton* et al., 1993; *Dong J. Z.* et al., 1991). Immature cotyledons were used as inocula by *Zhang XP* et al. (1994) and by *Compton* et al. (1993). *Szalai* (1995) used MS medium supplemented with 2,4–D for callus induction.

Our aim was to work out an effective system for regeneration of watermelon as a base of the transformation. We examined the regenerating capability of different genotypes of watermelon and the possibility of the *Agrobacterium* mediated transformation.

Material and methods

Plant material

Five genotypes of watermelon: Favorit F1, Szigetcsépi 51 F1, AS, Zebra and *Citrullus lanatus var. Citroides* /a wild type of watermelon/ were used in the experiments. The first step was removing the coats of the seeds. The helled seeds were sterilised with 20% H_2O_2 for 5 minutes and were rinsed 3 times for five minutes with sterile distilled water. Then the seeds were put on MS medium *(Murashige & Skoog,* 1962) without hormones, solidified with 2 g/l Phytagel (Sigma), supplemented with 30 g/l sucrose and pH 5.8. Seeds for germination were incubated in thermostatic box at 32 °C for 2 days. Then the germinated seeds were transferred to the tissue culture room. They were kept at 25 °C and 16h photoperiod provided by cool white fluorescent lamps. Approximately 3 days old, green, expanded cotyledons were used in the experiments.

Culture conditions

After germination cotyledons were cut as explants, when became half-open. Cotyledons were cut into four parts. The edges of the cotyledons were also removed. Explants were put on MS medium complemented with different hormone combinations *(Table 1.).* Cotyledonary segments were cultured on the various mediums in light cabinet under 18/8h light/dark period at 25 °C. After four weeks adventitious shoots were transferred to hormone free MS medium for root induction.

Table 1 The content of hormones [mg/l] in the different media and pH

	IAA	BA	ABA	NAA	2.4–D	pH
1	0.9	0.6	0.24	–	–	5.3
2	1.8	1.2	0.48	–	–	5.3
3	–	–	–	–	0.2	5.3
4	–	–	–	–	0.4	5.3
5	–	1.2	–	0.1	–	5.7

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 Szent István University, Faculty of Horticultural Science.

Agrobacterium

For the transformation we used EHA105, a disarmed strain of *Agrobacterium tumefaciens*.

The pRGG plasmid carried the nopaline synthase gene (NOS) as a selectable marker for kanamycin resistance, and the GUS gene (*uidA*) as reporter –coded β–Glucuronidase–, driven by the 35S promoter of CaMV. *Agrobacterium tumefaciens* was grown and maintained on AB medium.

Co-cultivation and GUS histochemical assay

Three–day–old cotyledons were excised, cut into four segments and the edges were also removed. This step is important, because *Agrobacterium* can infect only the wounded cells. We made a suspension of 1 ml liquid AB medium and 1 tendril amount of *Agrobacterium tumefaciens*. 10 µl of the suspension was added to each freshly cut segment and 3–4 mm groups of callus cells. Cocultivation was at 26 °C for 2 days. After the cocultivation we could check the success of the transformation by GUS–test. The mechanism of GUS–test is based on a colour reaction. GUS gene expression was confirmed by adding 5–bromo–4–cloro–3–indolyl glucuronide (X–Gluc) to the cells for staining transformed cells to blue colour.

Results

Regeneration

Disinfection of seeds with 20% H_2O_2 for 5 minutes exhibited 100% normal seedlings without infection. In addition to sterilisation procedure didn't increase the time of germination, and we got seeds with higher turgor by rinsing with sterile distilled water.

By the fourth week high frequency of direct organogenesis *(Fig. 1 and 2)* and a little callus formation were established on *medium No. 1*. On *medium No. 2*, emerging organogen structures were observed in 7–8 weeks. The hormone concentration of the *No. 3* and *No. 4 media* causes callus induction *(Fig. 3)*. Plant regeneration from callus wasn't successful. After the first week cotyledonary segments expanded quickly on *medium No. 5*. Later some callus cells developed and few adventitious shoots were observed.

After 4 weeks culture the organogenie structures were large enough to transfer them onto MS medium without hormones for rooting. In 3-5 weeks root differentiation was observed. Finally we obtained entire plants with leaves and roots.

GUS test

As a result we obtained GUS–posive callus *(Fig. 4.)* and about 1mm edges of the cotyledonary segments showed a positive GUS test too.

Discussion

We have developed a protocol for regeneration of watermelon from cotyledonary segments. Inducing organogen structures on MS medium supplemented with 0.9 mg/l IAA, 0.6 mg/l BA and 0.24 mg/l ABA showed the best results. On the other hand the regeneration capability of the watermelon was determined by the genotype. *Citrullus lanatus var. Citroides*, the wild type of watermelon was vigorous and developed shoots from all of the segments. The regeneration capability was over 70% of this genotype.

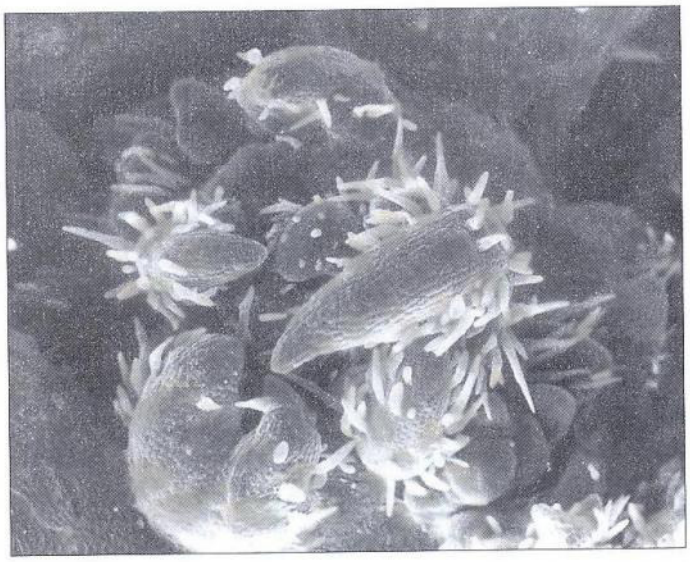

Figure 1 Scanning electron microscopic (SEM) picture of organogen structures emerging from cotyledonary segments of *Citrullus lanatus* var. *Citroides* after four weeks on *medium No. 1* Magnification 60x.

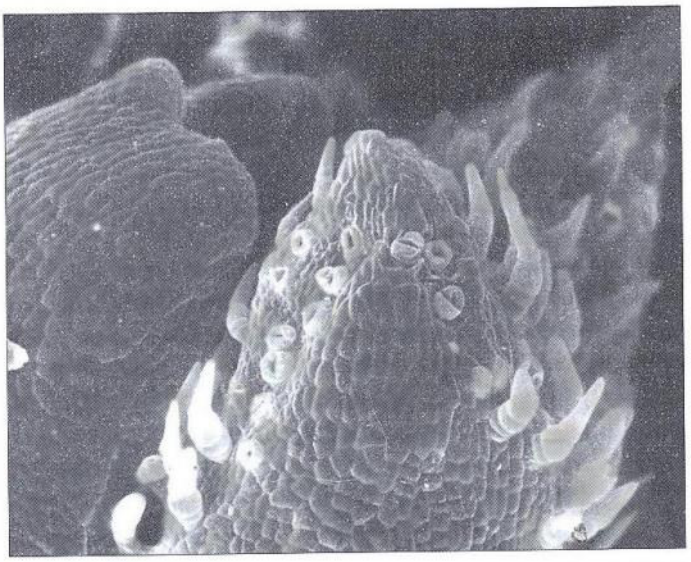

Figure 2 Trichomes and stomata on organogen structures. SEM picture, magnification 150x.

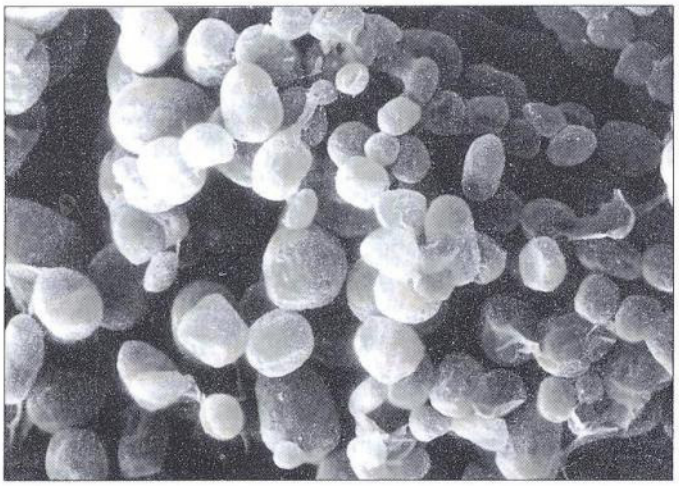

Figure 3 SEM pictures of callus cells developed on cotyledonary segments on *No. 3 medium*. Magnification 100x.

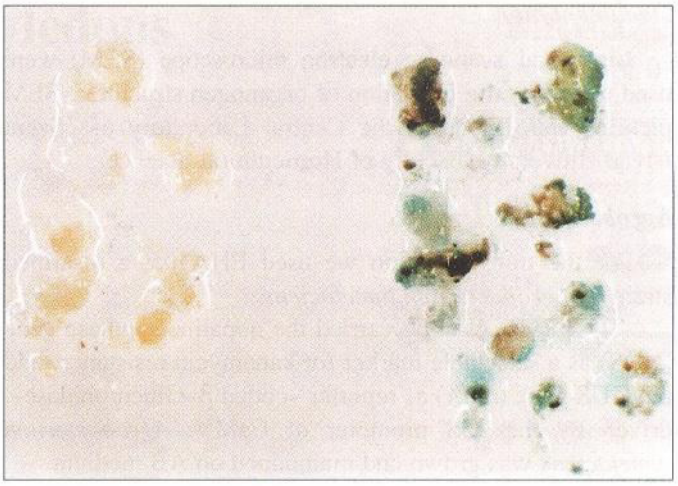

Figure 4 GUS positive callus (left) after *Agrobacterium* mediated transformation and control callus (right).

According to our experiment it is possible to introduce genes into watermelon by *Agrobacterium* and gene expression can be detected using GUS gene as a reporter. The method can be used in the future for transformation of watermelon.

References

Hao, L. X. & Wang, H. M. (1998): A study on building up the regeneration system of watermelon. Acta Agriculturalae Boreali Sinica. 13:3, 112–115; 3 ref.

Compton, M. E., & Gray, D.J. (1993): Shoot organogenesis and plant regeneration from cotyledons of diploid, triploid and tetraploid watermelon. Journal of the American Society for Horticultural Science.118:1, 151–157; 39 ref.

Compton, M. E. & Gray, D. J. (1993): Somatic embryogenesis and plant regeneration from immature cotyledons of watermelon. Plant Cell Reports.12:2, 61–65; 28 ref.

Wang, C. X., Jian, Z. Y., Liu, Y. and Zou, Q. (1997): Genetic transformation and plant regeneration of watermelon using Agrobacterium tumefaciens. Acta Botanica Sinica. 39:5, 445–450, 21 ref.

Islam, R., Ahad, A., Reza, M. A., Mamun, A. N. K. & Joarder, O.I. (1997): in vitro plant regeneration from zygotic embryos of Citrullus lanatus Thunb. Pakistan Journal of Scientific and Industrial Research. 38:11–12, 445–447; 11 ref.

Tabei, Y. (1997): Study on breeding of Cucurbitaceae using biotechnology. Bulletin of the National Institute of Agrobiological Resources. No. 11, 1–107; 158 ref.

Szalai J. (1995): The micropropagation of water-melon. Horticultural Science. 27:3–4, 111–113; 4 pl.; 8 ref.

Tang, S. H., Liao, Y. F. & Xu, R. C. (1994): Medium selection and in vitro culture of seedless watermelon (Citrullus vulgaris Schrad.). Journal of Southwest Agricultural University. 16:6, 540–542; 13 ref.

Dong, J. Z. & Jia, S. R. (1991): High efficiency plant regeneration from cotyledons of watermelon (Citrullus vulgaris Schrad.). Plant Cell Reports. 9:10, 559–562, 15 ref.

Zhang, X. P., Rhodes, B. B. & Alderberg, J. W. (1994): Shoot regeneration from immature cotyledons of watermelon. Report Cucurbit Genetics Cooperative. No. 17, 111–115; 10 ref.