IN VITRO ORGANOGENETIC POTENTIAL OF COTYLEDON AND LEAF EXPLANTS OF *CAPSICUM ANNUUM* L., CV. BRYZA

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(Received: April 15, 1994. Accepted: September 30, 1994)

ABSTRACT

Regeneration capabilities of *Capsicum annuum* cotyledons and leaves were compared. Adventitious buds, leaf like structures and callus formation occurred on both types of explants, roots on cotyledons only. Murashige and Skoog basal medium containing 1.0 mg dm^{-3} indole-3-acetic acid (IAA) and 1.0; 5.0; 10.0; 15.0 mg dm^{-3} 6-benzylaminopurine (BAP) were used. Adventitious buds were induced more frequently on cotyledons than on leaves. The middle part of cotyledon explants and the distal part of the leaf explants seem to be the best.

KEY WORDS: *Capsicum annuum* L., adventitious bud, root, callus formation.

INTRODUCTION

Regeneration of pepper plants through in vitro culture was obtained from shoot apices, cotyledons and hypocotyls of seedlings, leaves, anthers, immature embryos as well as from protoplasts (Fari 1986, Agrawal et al. 1989, Harini and Lakshmi Sita 1993). Applying various pepper explants, it was shown that auxins are indispensable for callus and root induction, and cytokinins for adventitious bud induction (Gunay and Rao 1978). Apart from growth regulators, the conditions of incubation, particularly the length of the photoperiod and temperature, have the essential effect on the regulation of organogenesis in pepper (Phillips and Hubstenberger 1985). Also endogenic factors such as genotyp (Ochoa-Alejo and Ireta-Moreno 1990), as well as the type of explant (Fari 1986) have an important effect on this process. It has been reported recently that there is a possibility of plant regeneration from immature embryos of pepper via somatic embryogenesis (Harini and Lakshmi Sita 1993). Using Polish varieties of pepper, whole plant regeneration was also obtained and morphogenetic differences, depending on the genotype, were shown (Rogozińska and Drozdowska 1989, Rogozińska and Tobolewska 1992).

The aim of this paper was to determine the morphogenetic potential in the individual segments of cotyledons and leaves of pepper cv. Bryza. The efficient regeneration system of pepper can be used in genetic transformations.

MATERIAL AND METHODS

Seeds of *C. annuum* L., cv. Bryza obtained from PlantiCo in Świętosławie were surface sterilized in 4% sodium hypochlorite for 7 min, then rinsed 3 times in sterile water and put on diluted (1:1) mineral MS medium (1962), solidified with agar (0.8%). From 4-week old pepper seedlings, leaves and cotyledons were isolated, which then were cut into 3 parts

and thus following kinds of explants were obtained: 1-distal part, 2-middle part, 3-proximal part (a petiole with a small fragment of blade) (Fig. 1). The average length of cotyledon explant was about 14 mm, and the leaf 7 mm. The explants were placed on the medium horizontally, with the abaxial side of the leaf or cotyledon facing the medium. MS medium with constant level of indole-3-acetic acid (IAA) (1.0 mg dm^{-3}) in combination with 6-benzylaminopurine (BAP) (1.0; 5.0; 10.0; 15.0 mg dm^{-3}) were used. Seed germination, seedling growth and explant differentiation occurred in 16 h photoperiod with light intensity about 3000 lx and temperature $25 \pm 2^\circ\text{C}$. The experiment was repeated twice, and with each combination of growth regulators 16 explants were incubated. After 4 weeks the morphogenetic potential of the explants was determined.

RESULTS AND DISCUSSION

During the first 7 days of the culture, the explants enlarged and elongated. The presence of growth regulators in the medium was indispensable for adventitious bud formation; they were visible macroscopically after about 14 days from the culture initiation. Depending on the presence of growth regulators in the medium, the bud formation occurred directly on the surface of the explants and on the cut surfaces or indirectly through callus.

Comparing the regeneration capabilities of leaf and cotyledon explants, it was shown that the cotyledons are better material to obtain the entire morphogenetic response.

On each from the analyzed cotyledon explants, callus as well as adventitious bud and root formation occurred. On the other hand, on the individual leaf segments only adventitious bud and callus appeared. Also the number of explants, on which buds' induction occurred was higher in the case of cotyledon than leaf (Fig. 2). Agrawal et al. (1989) reported similar results, obtaining adventitious bud formation twice frequently on cotyledons than leaves. However in *Cucumis*

Fig. 1. Types of cotyledon (C) and leaf (L) explants excised from four-week-old seedlings of pepper cv. Bryza

C₁, L₁ – distal parts
C₂, L₂ – middle parts
C₃, L₃ – proximal parts

melo L., the highest percentage of explant differentiating buds was obtained from the cotyledons of mature seeds, much smaller from young leaves and the smallest from seedling cotyledons (Tabei et al. 1991).

Comparing the morphogenetic capabilities of the 3 tested types of cotyledon explants, it was shown, that bud formation

occurred on larger number of middle segments than on the distal and proximal ones (Fig. 2). The number of buds falling on one cotyledon segment amounted from a few to about 30, and most of them were formed on the distal part. The bud number depended also on the combination of the tested growth regulators (Fig. 3).

Fig. 2. Adventitious bud formation on cotyledon and leaf explants of pepper cv. Bryza in the presence of IAA/BAP after 4 weeks of culture 1 – distal part; 2 – middle part; 3 – proximal part.

the morphogenetic potential. Adventitious buds appeared most frequently in the blade-petiole part (85%), next on leaf petiole (15%).

Comparing the response of the individual cotyledon and leaf segments of pepper cv. Bryza on the increasing cytokinin concentration in the medium some analogies were found (Fig. 2). With increasing BAP concentration in the medium, bud differentiation occurred on more and more number of explants of the distal part of cotyledon and leaf. On the segments of middle part however, this relationship was less regular and similarly on proximal segments. This can be caused, as suggested by Fari (1986), by the presence of endogenic cytokinins. Differential morphogenetic response of various hypocotyl segments of pepper on the level of endogenic growth regulators was shown earlier by Fari and Czako (1981). The segments placed higher in the seedling formed mainly shoot buds and those placed lower only roots and callus. The authors explain this fact by different level of endogenic auxin in the hypocotyl segments.

The higher morphogenetic potential of cotyledons than leaves results from their different physiological function. In the regeneration process the cotyledons are richer source of nutritious substances than the leaves.

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ZDOLNOŚCI ORGANOGENETYCZNE LIŚCIENI I LIŚCI SIEWEK *CAPCISUM ANNUUM* L., CV. BRYZA W KULTURACH IN VITRO

STRESZCZENIE

Porównano zdolności regeneracyjne liścieni i liści siewek *Capsicum annuum* L.. Pąki przybyszowe, struktury liściopodobne oraz kalus tworzyły się na obu typach eksplantatów, korzenie tylko na liścieniach. Stosowano pożywkę MS zawierającą IAA (1.0 mg dm^{-3}) i BAP ($1.0; 5.0; 10.0; 15.0 \text{ mg dm}^{-3}$). Pąki przybyszowe tworzyły się liczniej na eksplantatach liścieniowych. Z przebadanych typów eksplantatów liścienia, najlepszą pod względem częstotliwości różnicowania pąków przybyszowych okazała się część środkowa, natomiast z eksplantatów liścia, część dystalna.

SŁOWA KLUCZOWE: *Capsicum annuum* L., formowanie pąków przybyszowych, korzeni i kalusa.