THE RELATIONSHIP BETWEEN THE ENDOGENOUS ABSCISIC ACID CONTENT OF ANTHERS AND IN VITRO ANDROGENESIS IN PEPPERS (*Capsicum annuum* L.)

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Abstract

The effects of cold pretreatments on pepper (*Capsicum annuum* L.) buds to the relationship between the endogenous abscisic acid (ABA) content of anthers and *in vitro* androgenesis were investigated. Cold shock treatments at +4°C at periods of 48 and 96 hours were used. The anthers were cultured in the MS medium with 4 mg/l NAA and 1 mg/l BA. Activated charcoal was added to the medium at a concentration of 0.25%. The cultures were incubated at +29°C under conditions of continuous illumination. The ABA analyses were made at different stages, (1) after the buds were separated from the plants (control), (2) after the cold treatments, and (3) at the 50th day of the incubation. The ABA amount of the anthers that had cold treatment always remained lower than the control anthers. However, the most embryo formation was obtained from the control anthers not subjected to cold treatments (12.5%). These results indicated that the ABA content in anthers is not the only factor for obtaining embryos from the anther culture of peppers.

1. Introduction

Anther culture is a technique for the production of double haploids in cultivated plants. This method also has a practical application in the acceleration of the breeding program. However, the yield has remained extremely low in many cases. The method can only become useful for applied agricultural projects when the haploids are produced in large numbers. Consequently, many efforts are being made to increase the yield of anther cultures. It has been shown that the addition of certain amino acids (Nitsch, 1974), AgNO₃ (Paksoy et al., 1995), and activated charcoal (Anagnostakis, 1974; Tipirdamaz and Ellialtoğlu, 1998) to the culture medium and pretreatment of whole buds or anthers (Morrison et al., 1986) can increase embryo production.

Abscisic acid (ABA) is a hormone which also displays an inhibitory effect on the tissue culture. In the anther culture studies made with different plant types, it has been claimed that the ABA found in the anthers could prevent embryogenesis and this negative effect could be removed with the addition of activated charcoal to the culture medium (Johansson, 1983) and by applying cold shock treatments to the buds (Johansson and Eriksson, 1977; Johansson et al., 1982).

In this paper the effects of the cold shock treatment of buds on the endogenous ABA content of anthers and on pollen embryogenesis were investigated in peppers.
2. Materials and methods

*Capsicum annuum* L. variety Malatya were used in the present study. Donor plants were grown under field conditions. Flower buds 5-7 mm in size were sampled from each plant when the petals were just visible from the sepals. The anthers had uninucleate microspores at this stage. The stages of the microspores were determined by comparative studies between the bud size and microspore development (Özkum, 2000).

Cut flower buds were placed in a flask and stored in a refrigerator at +4°C for 48 and 96 hours for the cold pretreatments. The control buds were not subjected to cold conditions. Before isolating the anthers, the buds were sterilized with a 20% commercial bleach for 15 minutes and then washed 3 times in sterile distilled water. The anthers were placed in an MS medium (Murashige and Skoog, 1962) containing 30 g/l saccharose, 0.8% agar, with 0.25% activated charcoal and 4 mg/l NAA and 1 mg/l BA. The anthers were placed in petri dishes and kept continuously illuminated at +29°C for 3 months. The anthers were used at two different stages for the ABA analyses: at the beginning of the culture and at the 50th day of the culture taken from a control sample without cold treatment and from the anthers cold treated at +4°C for 48 and 96 hours. The ABA was analyzed as its methyl ester (ABA-Me) with the method used by Johansson et al. (1982) and Ellialtioğlu and Tipirdamaz (1998).

3. Results

The effects of cold shock treatments on the ABA contents of anthers as well as the relationship between the endogenous ABA contents of anthers and pollen embryogenesis were investigated. The ABA amounts in the anthers of buds of *C. annuum* L. that had been cold treated at +4°C for 48 and 96 hours and the control anthers were chemically analyzed. These analyses showed that the control anthers (without cold treatment) contained 2.90 ng/g ABA at the beginning of the experiment. The ABA amounts of the anthers cold treated at +4°C for 48 and 96 hours were 2.58 and 2.04 ng/g, respectively.

The ABA amounts in the anthers were also analyzed at the 50th day of the culture. The ABA contents of the anthers were reduced to 2.56 ng/g in the control group and to 2.20 and 1.81 ng/g in the cold treated anthers for 48 and 96 hours, respectively, as shown in Table 1. Cold treatments reduced the ABA contents of the anthers in both sample times for the analyses; at the beginning of the culture and at the 50th day of the culture. The differences between the 48 hour and 96 hour treatments were not statistically significant. The decreases in the ABA values in the cold treatments compared with the control values, both at the beginning of the culture and at the 50th day of the culture, were statistically insignificant for the cold treatments at +4°C for 48 hours, but were statistically significant for the cold treatments at +4°C for 96 hours (Table 1).

A considerably higher number of embryos (12.5%) were produced in the control cultures which were cultivated. On the other hand, 1-2% embryos were obtained from the cold treated anthers.
4. Discussion

It has long been known that the number of embryos produced by anther cultures could be increased if the pollen is exposed to some kind of shock treatment before cultivation. The most common method has been a cold treatment of the buds before the anthers are placed in the medium (Sunderland and Roberts, 1979; Morrison et al., 1986). The mechanism of this effect has not yet been clarified. Our results showed that in general the cold treatments reduce the ABA content of the anthers. These decreases in the ABA content were found to be insignificant except for the cold treated anthers at +4°C for 96 hours at both stages; at the beginning of the culture and at the 50th day of the culture. These results display a similarity with the other research results which determined that the cold treatment applications make a decreasing effect on the ABA contents of the anthers (Johansson et al., 1982; Tipirdamaz and Ellialtoğlu, 1998).

The ABA amounts at the 50th day of the culture were determined to be much lower than the ABA values at the beginning of the culture. These results are probably due to the loss of vigor in the anthers and inhibition of the ABA as well as in the other metabolites (Johansson et al., 1982).

In spite of the effects of cold shock treatment, a number of pollen grains are forced to divert from the normal path of development and start developing into embryos (Bajaj et al., 1977). In our study, we were able to obtain embryos only from the control groups. However, the cold pretreatments did not increase the embryo formation in the medium to which activated charcoal was added. These results reveal that cold treatment to the buds and the addition of activated charcoal to the culture medium creates a negative effect in pepper anther cultures. It is thought that the existence of ABA in the control anthers is not at a level which would prevent embryogenesis. It has been suggested in the same manner by Johansson et al. (1982), Tipirdamaz and Ellialtoğlu (1998) that the preventive effect of ABA on pollen embryogenesis only appears when the ABA is above a specific level of concentration. In conclusion, it has been determined that there are other factors apart from the ABA which are influencing embryogenesis in pepper anther cultures.

Acknowledgement

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References


Table 1. The ABA contents (ng/g fresh weight) of anther samples of C. annuum L. cv. Malatya taken at different stages from time cold treated anthers.

<table>
<thead>
<tr>
<th>Time of sample taken</th>
<th>Control</th>
<th>48 hours</th>
<th>96 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning of the culture</td>
<td>a* 2.90±0.18 A**</td>
<td>a 2.58±0.06 AB</td>
<td>a 2.04±0.19 B</td>
</tr>
<tr>
<td>50th day of the culture</td>
<td>a 2.56±0.30 A</td>
<td>a 2.20±0.04 AB</td>
<td>a 1.81±0.28 B</td>
</tr>
</tbody>
</table>

* The same letters in each column do not differ significantly at the 1% level.
** The same letters in each row do not differ significantly at the 1% level.