



In situ RT-PCR in Bixa orellana

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Abstract

An in situ RT-PCR assay was carried out in the tissues where apocarotenoid production was observed, with the aim of identifying the location of the BoCCD mRNA.

Materials

MATERIALS

☒ DNase (DNase I Amp Grade Deoxyribonuclease I Amplification Grade) **Catalog #Cat. No. 18068-015**

☒ SuperScript™ III One-Step RT-PCR System **Invitrogen - Thermo Fisher Catalog #Cat. No. 12574026**

☒ DIG-dUTP **Catalog #Cat. No. 11573179910**

☒ Anti-Dig-AP **Catalog #Cat. No. 11093 274910**

☒ DIG Wash and Block Buffer Set **Catalog #Cat. No. 1158576200**

Thick slices of the seeds

histo-clear

DEPC water

PBS (phosphate-buffered saline)

Axioplan, Zeiss

- 1 In situ RT-PCR was performed to identify the specific cells expressing the BoCCD1-1, BoCCD4-1 and BoCCD4-3 genes. Thick slices of the seeds (50 micrometers) were washed with histo-clear twice for  00:10:00 , deparaffinized and hydrated in a series of alcohol concentrations (100, 95, 85, 70, 50, 30, 10 %) for  00:00:30 and finally washed with DEPC water. This procedure was followed by washing with 1× PBS (phosphate-buffered saline) for  00:02:00 and treated with protease (1 mg/ml) for  00:20:00 at  37 °C 37 °C. The enzyme was then neutralized with 1× PBS + glycine (0.2 %) for  00:02:00 2 min and washed again with 1× PBS for  00:02:00 2 min .

- 2 Subsequently, the tissue was treated with DNase (DNase I Amp Grade, Deoxyribonuclease I Amplification Grade. Invitrogen, Cat. No. 18068-015). Next, in situ RT-PCR was performed using the whole tissue with SuperScript™ III One-Step RT-PCR System, (Invitrogen Cat. No. 12574026) in a reaction consisting of  25 µL of 2× reaction mix buffer, DIG-dUTP (Roche, Cat. No. 11573179910) and Anti-Dig-AP (Roche, Cat. No. 11093 274910). Immediately after, the tissue was treated with DIG Wash and Block Buffer Set (Roche, Cat. No. 1158576200) and observed using an Axioplan, Zeiss (10×, 20×, 100×).