IS THE SUNFLOWER MITOCHONDRIAL PLASMID PLT ALSO PRESENT IN THE LECLERCQ'S CMS PLANTS ?

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SUMMARY

The 1.45kb circular plasmid has been detected in the mitochondria of several B lines (Leclercq's maintainer lines) by the ethidium bromide technique, but not in the A lines (Leclercq's CMS lines). Total DNA of A and B lines were probed with the P1T plasmid. Hybridization signals at the estimated size of the free plasmid were detected both in A and B lines. We discuss here about several explanations for the presence of the P1T sequence at a different stoechiometry ratio in A line and B line mitochondria.

INTRODUCTION

The presence of the P1T plasmid in the B lines has been established both by ethidium bromide staining technique, Leroy et al. (1985), and by hybridization with the cloned plasmid used as a probe from CANP3 B line, Perez (1987), and from HA89 B line, Crouzillat et al. (1987). Nevertheless some total DNA preparations of the A line gave a sporadic hybridization signal depending upon the samples. So we wonder whether the presence of P1T or any sequence homologous to P1T can be present in total DNA from the A lines.

MATERIALS AND METHODS

Total DNA of the CANP3 A line and B line were prepared according either 1) to Murray and Thompson (1980) from etiolated seedlings or 2) to O'Rogers and Bendisch (1985) from individual seeds. Restriction, electrophoresis, DNA transfer and hybridization protocols were from Maniatis et al. (1982).
Labelled probes of P1T have been prepared according to Hu and Messing (1982) for the large Pst1 EcoRI fragment of P1T CANP3 B and by random primer technique following the seller instructions (Boehringer) for the P1T HA89 B. The two probes did not display differences for the B lines.

RESULTS

Experiment I : total DNA has been prepared from c.a. fifty etiolated seedlings both from the B line and the A line. Ten micrograms of total DNA has been restricted by Eco RI for each sample and electrophorezed on agarose gel. The gel has been transferred onto nitrocellulose membrane.
The DNA from the B line preparation was diluted ten by ten until 10^{-4} with the A line DNA sample. A 10^{-5} dilution has been prepared directly in water.
By probing with the P1T CANP3 B plasmid an hybridization signal was detected at the estimated size of the linearized plasmid in all samples except the 10^{-5} water dilution. The signal intensity in A line preparation was similar to that of the 10^{-3} and 10^{-4} CANP3 B/CANP3 A dilutions.

Experiment II : preparations of total DNA from the A line and the B line were obtained from individual seeds. The unrestricted total DNA amount of each preparation (c.a. 50ug) was loaded onto an agarose gel. The Southern transfer of that gel has been probed with the P1T HA89 B plasmid. A strong hybridization signal has been observed in the B line preparation. A light signal was also present in the A line preparation at the expected size of the plasmid.

DISCUSSION

The free P1T plasmid was detected in the total DNA of CANP3 B line as expected but also in the total DNA of the A line. This result was in contradiction with the idea that the A line mitochondria did not contain the P1T plasmid (Perez et al. 1985, Leroy et al. 1985, Brown et al. 1986, Crouzillat et al. 1987). The copy number of the plasmid is c.a. 1000 times lower in the A line than in the B line.
Consequently we wonder if whether or not the P1T plasmid exists in the A line mitochondria. According to 1) the intensity of the hybridization signal, 2) the fact that free copies of the plasmid were detected in one seed, it is likely that P1T should be present in the A line mitochondria at such a level which ruled out its detection by ethidium bromide fluorescence.
However we could not eliminate the existence of one copy or few copies of P1T integrated in the nuclear genome.
The examination of many A line seeds should permit to verify wether or not all the A line individuals do carry the plasmid.

CONCLUSION

The use of the P1T plasmid as molecular marker of cytoplasms has to be modulated with respect to the sensitivity of the detection methods : fluorescence or hybridization followed by autoradiography. Nevertheless the ratio of P1T between the B line and the A line allowed the characterization of the cytoplasms.

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