THE MITOCHONDRIAL PLASMID OF SUNFLOWER : ITS USE TO ESTABLISH RELATIONSHIPS FOR THE HELIANTHUS PETIOLARIS SPECIES

C. Perez and A. Bervillé
INRA, Station d'Amélioration des Plantes, Laboratoire de Génétique et Mutagenèse, BV 1540, 21034 Dijon Cédex, France.

SUMMARY

The circular plasmid called P1T found in the mitochondria of the sunflower has been used as a probe on sunflower lines used by breeders and on wild forms or wild form derived CMS. P1T cross-hybridized other various size plasmids but not all of the H. petiolaris species. These results allowed us to differentiate subspecies rapidly and efficiently.

INTRODUCTION

Cytoplasmic male-sterility was first induced from the H. petiolaris No 6392 ecotype, which has been lost, Leclercq (1969). In the mitochondria of the maintainer plants one 1.45kb plasmid has been detected, Leroy et al. (1985), Perez et al. (1986), Brown et al. (1986). Compared to the maintainer plants, the Leclercq's CMS plants CMS did not carry the plasmid in their mitochondria. The plasmid was recognized as a circular molecule present under two forms either open circle (OC) or supercoiled (CCC) in the mt DNA preparations.
The plasmid named P1T was cloned and sequenced, Perez (1987). We used it as a probe on various ecotypes from the INRA collection from Montpellier. The subspecies of H. petiolaris we studied displayed different plasmid set profils for the unrestricted mitochondrial DNA : some of the plasmid bands cross-hybridized with P1T.

MATERIAL AND METHODS

The plants were grown in a green house ; the leaves were picked up on three month old plants. Mitochondrial DNA was obtained as described by Perez et al. (1988).
Restriction of the mt DNA, agarose gel electrophoresis and hybridization were done according to Maniatis et al. (1982). The P1T plasmid was cloned at the EcoRI site of the plasmid vector Pembl8 (-) in 71/18 E. Coli strain. DNA insert was purified from vector after Eco RI digestion and then labelled with ^{32}P ATP by nick-translation according to Maniatis et al. (1982).
The following ecotypes were studied (Serieys nomenclature) :

91 : H. petiolaris
199 : H. petiolaris petiolaris
200 : H. petiolaris fallax
674 : H. petiolaris fallax.

The A and B lines HA89 and CANP3 were used as controls, other genotypes lines are indicated in table 1.
The hybridization of the P1T plasmid with DNA fixed onto membrane filters allow to identify specifically if any DNA sequences homologous to that of P1T that are present.

RESULTS

The direct observation of unrestricted mitochondrial DNA stained by ethidium bromide was made for the ecotypes 91, 199, 200, 674 and several B lines and A lines of sunflower.
Data are summarized in table 1. The position of the band on the gel is used to named the band by the molecular weight of a linear fragment which should migrate at this position, i.e., apparent molecular weight (AMW).
According only to the bands revealed on the agarose gel we compared their positions to the ones of the OC and CCC forms of P1T of 1.6kb AMW and 1.0kb AMW respectively.

Table 1

Low molecular weight DNA in the mitochondria of several lines and genotypes of sunflower

	Apparent molecular weight for the plasmid bands in kb
Helianthus annuus	
CANP3 B	1.8 ; 1.6 ; 1.2 ; 1.0
HA89 B	1.6 ; 1.0
CCM60 B, CC40 B, CA4 B, 2603 B	1.6 ; 1.0
CANP3 A	no band
HA89 A, CVH11 A, CIC 61 A, CC60 A, CC40 A, CA4 A, RHA274	no band
Helianthus petiolaris group	
No 91	no band
No 199	1.6 ; 1.0
No 200	2.3 ; 1.6 ; 1.4 ; 1.0 ; 0.8 ; 0.7 ; 0.5
No 674	2.8 ; 1.6 ; 1.4 ; 1.0 ; 0.8 ; 0.7 ; 0.5

Underlined AMW correspond to minor Low molecular weight DNA.

So the main observations are :
- B lines displayed 2 bands except CANP3 B line which showed two extra bands of 1.2kb and 1.8kb AMW.
- A lines did not display any band when mt DNA is loaded in the agarose wells

in equivalent amount for A and B lines.
- The H. petiolaris ecotypes displayed 3 specific profils : one (91) displayed no band, one (199) looked like B lines, the other (200, 674) was quite different with more than two bands.
The mt DNA of some lines and ecotypes were blotted onto nitrocellulose filters (Southern transfer) and were hybridized with ^{32}P labelled P1T probe.
The hybridization signals we observed are summarized in the table 2.

Table 2

Recapitulation of hybridization signals (+) with P1T used as a probe on Southern transfer of unrestricted mt DNA

H. annuus					
	1.00	1.6	1.2	1.8	
CANP3 B	+	+	+	+	
HA89 B	1.00	1.6			
	+	+			
CANP3 A	No signal				
HA89 A	No signal				
H. petiolaris fallax					
No 200	2.3	2.0	1.4	1.0	0.8
	+		+		
No 674	2.3	2.0	1.4	1.0	0.8
	+		+		

DISCUSSION

In the B lines two hybridization signals of 1.6kb and 1.0kb AMW were interpreted as the open circular form and supercoiled form respectively of a circular 1.45kb plasmid. That plasmid has not been detected in the mitochondria of A lines. Moreover in the CANP3-B mitochondria the 2 hybridization signals at the 1.8kb and 1.2kb AMW should correspond to a second plasmid with closely related sequences to P1T.
In the ecotypes 200 and 674 of H. petiolaris fallax species 2 bands of 2.3kb AMW and 1.4kb AMW shown cross hybridization with the P1T probe. The 2.3kb AMW band corresponded to a open circular form of a circular 2.150bp plasmid, as observed by electron microscopy. The 1.4kb AMW band should correspond to the AMW of the supercoiled form of the molecule.
Since H. petiolaris fallax wild form 200 and CMS derived plants (ST645 x RHA645) displayed the same plasmid set we conclude that the mitochondria are maternally inherited through the interspecific cross.
Consequently the results indicate that since the A lines did not display the P1T plasmid the mother plant used by Leclercq (1969) in the cross by sunflower did not carry it. Such a plant does exist in H. petiolaris since ecotype 91 corresponds to that situation.
The plasmids cross-hybridizing with P1T probably have a common origin.

CONCLUSION

The use of the sunflower mitochondrial plasmid as molecular probe allows to follow cytoplasmic markers through the interspecific crosses. Furthermore the cytoplasms of the wild form can be differentiated directly without cross leading to rapid and accurate traits to establish the relationships between the subspecies. This work ought to be extended to more origins of H. petiolaris species.

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