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MOLECULAR DIVERSITY OF CMS SOURCES AND FERTILITY RESTORATION IN THE GENUS *Helianthus*

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SUMMARY

Molecular characterization of 28 CMS sources and the fertile cytoplasm of *H. annuus* in sunflower demonstrated that cytoplasms different by their origin show a considerable similarity that cannot be expected from their pedigree. Cluster analysis using the UPGMA method allowed differentiating 10 mitochondrial (mt) types based on RFLP data. Characteristic proteins specific for one or more CMS sources could be identified which correspond to the mt types. In addition, the data obtained on fertility restoration for a subset of nine new CMS sources support the classification of the cytoplasms made on mtDNA level as demonstrated in the UPGMA dendrogram.

A map-based cloning approach is followed for the isolation of the restorer gene *Rf1*, which restores pollen fertility in hybrids based on PET1. Markers closely linked to the restorer gene could be identified using AFLP and RAPD technologies. A bacterial artificial chromosome (BAC) library was constructed using the sunflower restorer line RHA325 and pBeloBAC11 as vector. The first sunflower BAC library corresponds to 1.7 haploid genome equivalents (104,736 clones, average insert size approx. 50 kb). Use of STS markers, which had been developed from RAPD markers, allowed to identify three BAC clones by colony hybridization against high density filters. DNA fingerprinting using *Hind*III allowed to build a contig for the restorer locus *Rf1*.

Key words: CMS, fertility restoration, hybrid breeding, mtDNA, similarity matrix, sunflower

INTRODUCTION

Development of commercial sunflower hybrids based on new sources of cytoplasmic male sterility (CMS) is of special interest to reduce the potential risk of vul-

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nerability to pathogens and to increase genetic diversity. In sunflower, more than 60 CMS sources have been described until now (Serieys, 1999) occurring spontaneously or after intra- and interspecific hybridization as well as due to mutagenesis. This large number of CMS sources allows now for more general conclusions to be made about CMS mechanisms in the genus *Helianthus*.

In sunflower, commercial hybrid breeding is based on a single CMS source, the PET1 or French cytoplasm, which was derived from an interspecific cross between *H. petiolaris* and *H. annuus* (Leclercq, 1969). This was the first CMS source in sunflower that has been thoroughly studied (Horn *et al.*, 1991; Köhler *et al.*, 1991; Laver *et al.*, 1991). An inversion of 11 kb and an insertion of 5 kb led to the new open reading frame *orfH522* which is cotranscribed with the *atpA* gene in the male sterile lines. *OrfH522* encodes a 16-kDa protein which is anther-specific reduced in fertility restored hybrids (Moneger *et al.*, 1994).

To facilitate selection of new CMS sources for hybrid breeding, 28 cytoplasms were characterized by organization of the mitochondrial DNA, expression of new mitochondrially encoded proteins and fertility restoration. In addition, markers were identified for the restorer gene *Rf1*, which leads to fertility restoration in hybrids based on the PET1 cytoplasm, to perform marker-assisted selection and to isolate the restorer gene for functional studies. As large insert DNA libraries represent an essential tool for map-based cloning approaches a sunflower bacterial artificial (BAC) library was constructed.

MATERIALS AND METHODS

Plant material

Apart from the fertile cytoplasm of *H. annuus*, 28 CMS sources different by their origin were investigated. The origin of these sources, which were kindly provided by Dr. Hervé Serieys (Montpellier, INRA), is given in Table 1. Restorer lines used for testcrosses with a subset of nine new CMS sources are described in Horn and Friedt (1997). Forty-two plants for each testcross were evaluated in the years 1995 and 1996 in field trials at Gross-Gerau near Frankfurt/Main (Germany).

The F_2 population used for mapping the restorer gene *Rf1*, which restores pollen fertility in hybrids based on PET1 cytoplasm, was developed from the cross RHA325 x HA342. F_2 and F_2BC_1 were evaluated for male sterility and fertility in trials at the field station in Gross-Gerau.

Molecular markers

Isolation of mitochondria for preparation of mtDNA and *in organello* translation were performed according to Horn *et al.* (1991, 1996). Performance of Southern hybridization and probes were described in Horn (2001).

Table 1: Overview of the investigated CMS sources (References are cited in Serieys, 1996)

Name	FAO- Code	Origin	Reference
1. Spontaneously occurring CMS sources			
H. annuus 367	ANN1	*H. annuus*	Serieys and Vincourt,1987
H. annuus 517	ANN2	*H. annuus*	Serieys and Vincourt, 1987
H. annuus 519	ANN3	*H. annuus*	Serieys and Vincourt, 1987
H. annuus 521	ANN4	*H. annuus*	Serieys and Vincourt, 1987
NS-ANN-81	ANN5	*H. annuus*	Škoriæ, 1988
AN-67	ANN10	*H. annuus*	Christov, 1992
2. Intraspecific crosses			
Kouban	ANL1	*H. annuus* ssp. *lenticularis*	Anashchenko *et al.*, 1974
Indiana1	ANL2	*H. annuus* ssp. *lenticularis*	Heiser, 1982
Fundulea 1	ANT1	*H. annuus* ssp. *texanus*	Vrânceanu *et al.*, 1986
3. Interspecific crosses			
Anomalus	ANO1	*H. anomalus*	Serieys and Vincourt, 1987
Argophyllus	ARG1	*H. argophyllus*	Christov, 1990
Argophyllus	ARG2	*H. argophyllus*	Christov, 1990
Argophyllus	ARG3	*H. argophyllus*	Christov, 1992
Bolanderi	BOL1	*H. bolanderi*	Serieys and Vincourt, 1987
Exilis	EXI1	*H. exilis*	Serieys and Vincourt, 1987
Exilis	EXI2	*H. exilis*	Serieys, 1991
CMG2	GIG1	*H. giganteus*	Whelan, 1981
CMG3	MAX1	*H. maximiliani*	Whelan and Dedio, 1980
Neglectus	NEG1	*H. neglectus*	Serieys and Vincourt, 1987
Fallax	PEF1	*H. petiolaris* ssp. *fallax*	Serieys and Vincourt, 1987
PET/PET	PEP1	*H. petiolaris* ssp. *petiolaris*	Serieys and Vincourt, 1987
Petiolaris	PET1	*H. petiolaris*	Leclercq, 1969
CMG1	PET2	*H. petiolaris*	Whelan and Dedio, 1980
PHIR 27	PRH1	*H. praecox* ssp. *hirsutus*	Christov, 1993
PRUN 29	PRR1	*H. praecox* ssp. *runyonii*	Christov, 1993
Vulpe	RIG1	*H. rigidus*	Vulpe, 1972
4. Induced by mutagenesis			
HEMUS	MUT1	Irradiation of 'Hemus'	Christov, 1993
PEREDOVIK	MUT2	Sonification 'Peredovik'	Christov, 1993

AFLP-analyses were made according to Vos *et al.* (1995). Two bulks of homozygous fertility restored and male sterile F_2 plants were used for bulked segregant analyses (Michelmore *et al.*, 1991). RAPD amplifications were performed according to Sobral and Honeycutt (1993). A total of 1,200 arbitrary decamer primers (Operon Technologies, kits A to Z and AA to AI) was analyzed for the ability to produce polymorphic bands between the bulks. Polymorphisms were mapped in the F_2 population.

Analysis of Southern hybridization patterns

Genetic similarities (Sn) were estimated according to the formula developed by Nei and Li (1979): $Sn_{xy}=2n_{xy}/n_x+n_y$, (n_{xy} represents the number of bands shared by genotypes X and Y; n_x and n_y are the total number of bands shown by genotypes X and Y, respectively). Applying the program NTSYS-pc (Rohlf, 1993), cluster analyses based on the matrix of genetic similarity were performed using the unweighted pair group method of arithmetic means (UPGMA). The results of the cluster analyses are shown as a dendrogram.

BAC library

A BAC library was constructed using the vector pBeloBAC11 and the restorer line RHA325. High molecular weight (HMW) DNA was isolated from nuclei of young leaves according to Ganal (1996) and partially digested with *HindIII* for cloning (Woo *et al.*, 1994). Size selection was performed by pulse-field gel electrophoresis. DH10B cells were transformed by electroporation. BAC clones were picked into 384-well microtiter plates and stored at -80°C after adding glycerol to the cells to a final concentration of 10%. High-density filters were prepared for hybridization according to standard procedures including alkaline denaturation, neutralization and fixation of the DNA on the membranes by air-drying (Woo *et al.*, 1994).

RESULTS AND DISCUSSION

Characterization of the mitochondrial DNA of CMS sources

The organization of mtDNA was investigated for 28 sources of cytoplasmic male sterility (CMS) and a fertile line (normal cytoplasm) of *Helianthus annuus* by Southern hybridizations. Besides nine known mitochondrial genes (*atp6*, *atp9*, *cob*, *coxI*, *coxII*, *coxIII*, *18S*, *5S* and *nd5*) three probes for the open reading frames in the rearranged area of PET1, *orfH522*, *orfH708* and *orfH873*, were used (Horn and Friedt, 1999; Horn, 2001). Genetic similarities of the investigated cytoplasms estimated according to Nei and Li (1979) varied between 0.3 and 1 (Horn, 2001). Cluster analyses using the UPGMA method allowed the distinction of 10 mitochondrial (mt) types among the 29 investigated cytoplasms (Figure 1). Most mitochondrial types comprise two or more CMS sources, which could not be further separated, like the PET1-like cytoplasms (with exception of ANO1 and PRR1), or ANN1/ANN2/ANN3, ANN4/ANN5, ARG3/RIG1, BOL1/EXI1/PEF1/PEP1 and GIG1/PET2. ANL1, ANL2 and the fertile cytoplasms are also regarded as one mitochondrial type.

The hybridization patterns obtained for the different CMS sources and the fertile cytoplasm are summarized in Tables 2 and 3. Unique banding patterns were only observed for ANT1 (*atp6*), MAX1 (*atp6*, *orfH522* and *orfH708*) and PRR1 (*coxII*). However, four of the mitochondrial types showed unique hybridization sig-

nals: ANN4/ANN5 had characteristic bands for *atp6* and *orfH708*, PEF1/PEP1/EXI1/BOL1 for *atp6* and *coxII* and PET2/GIG1 for *atp9*. The PET1-like cytoplasms all shared the same patterns for *orfH522*, *orfH708* and *cob* (except ANO1).

Table 2: Restriction patterns of new CMS cytoplasms which occurred spontaneously, were induced by mutagenesis in *H. annuus* or were derived from intraspecific crosses. MtDNA was digested with *Bgl*II and hybridized against *atp6*, *atp9*, *coxI*, *coxII*, *coxIII*, *orfH522*, *orfH708*, *orfH873* and *18S*, *5S rRNA* and *nd5*

Probe	HA89	ANN1	ANN2	ANN3	ANN4	ANN5	ANN10	MUT1	MUT2	ANL1	ANL2	ANT1
atp6 [1]	a	b	b	b	c	c	a	a	a	a	a	d
atp9 [2]	a	b	b	b	a	a	a	a	a	a	a	b
coxI [3]	a	b	b	b	a	a	a	a	a	a	a	b
coxII [4]	a	a	a	a	a	a	a	a	a	a	a	a
coxIII [5]	a	b	b	b	c	c	d	d	d	c	a	b
orfH522 [6]	-	-	-	-	-	-	a	a	a	-	-	-
orfH708 [7]	a	-	-	-	c	c	b	b	b	a	a	-
orfH873 [8]	a	b	b	b	a	a	b	b	b	a	a	a
18S, 5S and *nd5* [9]	a	b	b	b	a	a	a	a	a	a	a	b

[1]a: 5.9 kb, b: 9.3 kb, c: 9.3 kb and 6.2 kb, d: 8.1 kb, e: 9.3 kb and 5.9 kb, f: 5.0 kb, g: 9.3 kb and 3.0 kb
[2]a: 10.3 kb, b: 11.1 kb and 7.3 kb, c:10.3 kb and 4.7 kb
[3]a: 10.7 kb, b: 11.5 kb, c: 10.1 kb
[4]a: 5.1 kb and 2.4 kb, b: 7.8 kb and 2.4 kb, c: 5.1 kb, 4.0 kb and 2.4 kb
[5]a: 8.7 kb and 6.6 kb, b: 5.3 kb, c: 6.6 kb, d: 8.7 kb
[6]a: 4.9 kb, b: 10.2 kb
[7]a: 6.2 kb and 3.4 kb, b: 1.2 kb, c: 1.4 kb, d: 3.4 kb, e: 2.4 kb
[8]a: 8.5 kb, b: 8.9 kb, c: 9.9 kb
[9]a: 10.7 kb and 8.3 kb, b: 11.5 kb and 8.3 kb

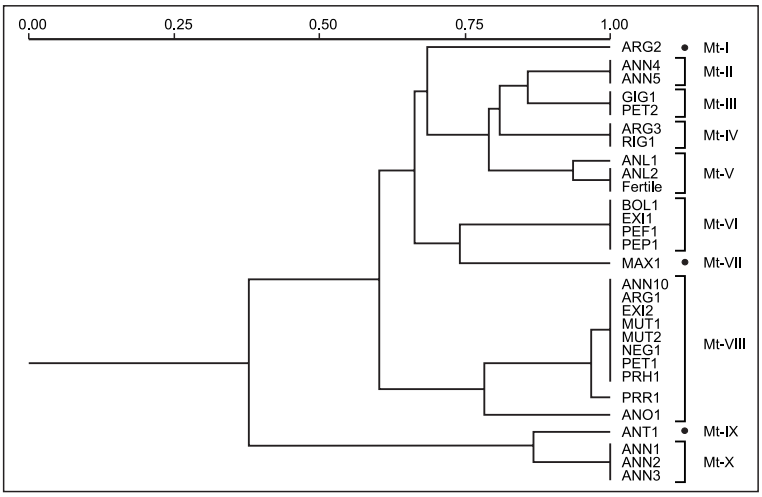

Figure 1: UPGMA dendrogram of 28 CMS sources in the genus Helianthus and the fertile (normal) cytoplasm of H. annuus based on RFLP data

Investigating 15 CMS sources and the fertile cytoplasm in sunflower using 12 mitochondrial probes, Crouzillat *et al.* (1991, 1994) could differentiate 13 cytoplasms but also were unable to distinguish the groups ANN1/ANN2/ANN3 and

PEF1/PEP1. However, 11 of 12 probes detected polymorphisms comparing ANN1/ ANN2/ANN3 with ANN4. Using different restriction enzymes, PET2 and GIG1 were polymorphic in hybridizations with the mitochondrial probes *coxII*, *atpA* and *atp9* (Crouzillat *et al.*, 1991) indicating that these cytoplasms have a very similar organization but are not identical.

Table 3: Southern analyses of CMS sources which originate from different interspecific crosses. MtDNA was digested with *Bgl*II and hybridized against *atp6*, *atp9*, *coxI*, *coxII*, *coxIII*, *orfH522*, *orfH708*, *orfH873* and *18S*, *5S rRNA* and *nd5*. Fragment sizes for the different patterns are given in Table 2

Probe	PET1	PET2	PEF1	PEP1	EXI1	EXI2	ARG1	ARG2	ARG3	PRH1	PRR1	ANO1	BOL1	NEG1	GIG1	MAX1	RIG1
atp6 [1]	a	e	f	f	f	a	a	b	a	a	a	a	f	a	e	g	a
atp9 [2]	a	c	a	a	a	a	a	a	a	a	a	a	a	a	c	a	a
coxI [3]	a	a	a	a	a	a	a	c	c	a	a	a	a	a	a	a	c
coxII [4]	a	a	b	b	b	a	a	a	a	a	c	a	a	a	a	a	a
coxIII [5]	d	c	c	c	c	d	d	b	c	d	d	c	c	d	c	c	c
orfH522 [6]	a	-	-	-	-	a	a	-	-	a	a	a	-	a	-	b	-
orfH708 [7]	b	-	-	-	-	b	b	d	-	b	b	b	-	b	-	e	-
orfH873 [8]	b	a	c	c	c	b	b	a	a	b	b	c	c	b	a	c	a
18S, 5S and *nd5* [9]	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a

The clustering of CMS sources in the UPGMA dendrogram does not correspond to the origin of the CMS sources (Serieys 1996, 1999) or the phylogeny of the species in the genus *Helianthus* (Rieseberg, 1991). Crouzillat *et al.* (1994) also came to the conclusion that no correlation between the original species and mtDNA-type could be observed.

***In organello* translation products of CMS sources**

Comparing the mitochondrially encoded proteins of the 28 CMS sources in sunflower, nine additional cytoplasms could be identified as having the same CMS mechanism as PET1 (Horn *et al.*, 1996). This was surprising as these PET1-like cytoplasms had different origins (Table 1). According to Serieys (1996), these cytoplasmic male sterile germplasms had been produced by either different interspecific crosses involving *H. argophyllus* (ARG1), *H. neglectus* (NEG1), *H. exilis* (EXI2), *H. anomalus* (ANO1), and two subspecies of *H. praecox* (PRR1, PRH1), or by mutagenesis of two maintainer lines for the PET1 cytoplasm (MUT1 and MUT2). In addition, one of the CMS types that arose spontaneously (ANN10) expressed the 16-kDa protein. All these PET1-like cytoplasms showed the same organization at the *atpA* locus (Horn and Friedt, 1999). Apart from these PET1-like germplasms, other groups of CMS cytoplasms could be identified which expressed new proteins (Horn and Friedt, 1999). ARG3 and RIG1 showed an additional 16.9-kDa protein but missed a 17.5-kDa protein common to the other cytoplasms. ANN1 and ANN3 expressed three specific proteins of 34.0, 16.9 and 16.3 kDa in common. A protein

of 12.4 kDa was unique for PET2 and GIG1. So far, ANT1 is the only CMS source that shows a unique pattern of three additional proteins of 19.7, 17.8 and 13.4 kDa. Table 4 gives an overview of the identified mitochondrially encoded proteins in the 28 CMS sources.

Table 4: Overview of mitochondrially encoded proteins specific for CMS sources in the genus *Helianthus*

CMS source	Origin	Mt-type	Characteristic protein
PET2/ GIG1	*H. petiolaris*, *H. giganteus*	Mt-III	+12.4 kDa
ARG3/ RIG1	*H. argophyllus*, *H. rigidus*	Mt-IV	+16.9 kDa, - 17.5 kDa
ANN10/ ANO1/ ARG1/ EXI2/ MUT1/ MUT2/ NEG1/ PET1/ PRR1/ PRH1	*H. annuus*, *H. anomalus*, *H. argophyllus*, *H. exilis*, both mutagenesis induced, *H. neglectus*, *H. petiolaris*, *H. praecox* ssp. *hirsutus*, *H. praecox* ssp. *runyonii*	Mt-VIII	+ 16.0 kDa
ANT1	*H. annuus* ssp. *texanus*	Mt-IX	+ 19.7 kDa, + 17.8 kDa, + 13.4 kDa
ANN1/ ANN3	*H. annuus, H. annuus*	Mt-X	+ 34.0 kDa, + 16.9 kDa, + 16.3 kDa

Fertility restoration of selected CMS sources

From 263 testcrosses involving nine new CMS sources, i.e. ANL1, ANL2, MAX1, PEF1, PET2, ANN1, ANN2, ANN3, and ANN4, five restorer lines of PET1 were selected as potential restorers for PEF1, PET2 and ANN4. Testcrosses of all nine cytoplasms with these five lines were evaluated in two years at the field station Gross-Gerau to study if these lines can be classified as stable restorers and allow a differentiation between the CMS sources (Table 5). The five lines used in the testcrosses allowed to differentiate between the cytoplasms PET1, MAX1, PEF1, PET2, and ANN4 as well as from ANN1/ANN2/ANN3, which were not restored by any of the five lines, indicating that these sources possess different CMS mechanisms. ANL1 and ANL2 cannot be distinguished from each other, but can be differentiated from MAX1, PEF1, PET2, and the four spontaneously occurring CMS sources. The data obtained on fertility restoration support the classification of the CMS sources made at the mtDNA level as demonstrated in the UPGMA dendrogram (Figure 1).

Test hybrids based on ANL1, ANL2, MAX1, PEF1, PET2, and ANN4, showed good agronomic performance regarding plant height, days to flowering, maturity and oil content (Horn and Friedt, 1997). Segregation analyses of the F_2 populations indicate that a single dominant restorer gene was sufficient to restore pollen production of hybrids based on ANL2, PEF1 and PET2 (Horn and Friedt, 1997). For restoration of ANN4, two dominant complementary genes are required. Conversely,

two dominant genes which each on its own allow to produce fertile plants are involved in the restoration of fertility in the investigated crosses of ANL1 and MAX1.

Table 5: Pattern of male fertility restoration and male sterility maintenance in F_1 hybrids of crosses using nine different CMS sources and five restorer lines of cytoplasm PET1 as pollinators. The percentage of male fertile plants in the F_1 generation is given in brackets as the average of the years 1995 and 1996. M=maintainer, R=restorer, M/R=partial restorer (intermediate or segregating phenotype)

CMS source	Pollinator (male parent)									
	NS		IH		RO		LC		FU	
ANL1	M/R	(97.5)	M/R	(71.0)	R	(100)	M/R	(49.5)	M/R	(96.5)
ANL2	M/R	(97.5)	M/R	(98.5)	R	(100)	M/R	(99)	M/R	(99)
MAX1	M/R	(84)	R	(100)	M/R	(93)	M/R	(100)	M/R	(100)
PEF1	R	(100)	M	(0)	M	(0)	R	(100)	M	(0)
PET2	M	(5)	R	(100)	M	(0)	M	(0)	M	(0)
ANN1	M	(0)	M	(0)	M	(0)	M	(0)	M	(0)
ANN2	M	(0)	M	(1.5)	M	(0)	M	(0)	M	(0)
ANN3	M	(0)	M	(0)	M	(0)	M	(0)	M	(0)
ANN4	M	(0)	M	(2.5)	R	(100)	M	(0)	M/R	(100)

Mapping of the restorer gene *Rf*1 for fertility restoration of PET1

Segregation analysis using the F_2-population RHA325 x HA342 demonstrated that one restorer gene *Rf1* is sufficient to restore fertility of the PET1 cytoplasm in this cross. Analyses using 1,200 RAPD primers and 712 AFLP primer combinations were performed to identify markers closely linked with the restorer gene (Horn *et al.*, 1998). Three RAPD markers were successfully converted into STS markers (STSY10-750, STSHP4-426, and STSW03-280) and are now available for marker-assisted selection in backcross programs. In addition, these markers were used to screen the BAC library in order to develop a contig around the restorer gene *Rf1*.

BAC library

The bacterial artificial chromosome (BAC) library was constructed using the sunflower restorer line RHA325 and pBeloBAC11 as vector. Preparation of high-molecular-weight DNA from nuclei using leaf material from two-week-old seedlings was successfully optimized. This first BAC library for sunflower comprises 104,736 clones. The average insert size was approximately 50 kb, with an insert size range of 20-270 kb. The sunflower BAC library corresponds to 1.7 haploid genome equivalents. The whole BAC library was spotted in duplicate on four high-density filters, each carrying 55,296 clones. Content of organellar DNA as estimated by colony hybridization against the mitochondrial probe *coxII* and the chloroplast probe *rbcL* proved to be lower than 0.03% and 0.1%, respectively.

Colony hybridization against high density BAC filters using the marker STSHP4-426 allowed to identify three positive BAC clones. DNA fingerprinting

using *Hind*III as restriction enzyme demonstrated that two clones of 74 kb were identical and the third clone of 23.5 kb was overlapping with these two.

CONCLUSIONS

In the genus *Helianthus*, the characterization of the 28 CMS sources in comparison to the fertile cytoplasm of *H. annuus* demonstrated that cytoplasms derived from a different origin could nevertheless have the same CMS mechanism. Therefore, the development of commercial hybrids based on new CMS sources requires a careful characterization of the cytoplasms in advance, to avoid using the same type of CMS mechanism again. Certain organization of the mitochondrial DNA seems to occur preferentially in the genus *Helianthus* independently of the species origin. It could be demonstrated that CMS sources such as PET2 and PEF1 are different from PET1 in their mtDNA organization and the CMS mechanism. Therefore, these cytoplasms represent interesting candidates for the development of new hybrid breeding systems based on new CMS mechanisms.

To facilitate genome analysis and map-based cloning of agronomically important genes in sunflower, a bacterial artificial chromosome (BAC) library was constructed using the sunflower restorer line RHA325 and pBeloBAC11 as vector. Markers closely linked to the restorer gene *Rf1* were identified using AFLP and RADP technology which facilitate selection in backcross programs and allow to proceed in map-based cloning of the *Rf1* gene.

For genome research, sunflower has started to be considered a model species among the *Asteraceae* which represents the largest of all plant families with over 20,000 species (Cronquist, 1988) and comprises a considerable number of economically important crops.

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DIVERSIDAD MOLECULAR DE FUENTES CMS Y REASTAURACION DE LA FERTILIDAD EN EL GENERO *Helianthus*

RESUMEN

La caracterizacion molecular de 28 fuentes CMS y el citoplasma fertil de *H. annuus* mostro que los citoplasmas de origen diferente muestraban la similitud considerable que no tendria que esperarse a base de su origen. El analisis del agrupamiento, por la utilizacion del UPGMA y de los datos obtenidos para RFLP, facilito de constatar la existencia de 10 tipos mitohondriales (mt). Fueron identificadas las proteinas caracteristicas siendo especificas para una o varias fuentes CMS, que convenian a los tipos mt encontrados. Ademas, loa datos de restauracion de la fertilidad fueron obtenidos para el sub-grupo, que cinsistia en nueve nuevas fuentes CMS, confirman la exactitud de clasificacion de citoplasmas hechos a base del nivel de mtDNK constatado, como lo indica el dendrograma de UPGMA.

La accion de clonar a base de la creacion de mapa precedente era utilizado para el aislamiento de los genes de restauracion *Rf1*, que restaura la fertilidad de polen en los hibridos basados en PET1.

Los marcadores estrechamente ligados con el gen restaurante fueron identificados por las tecnologias AFLP y RAPD. La biblioteca del cromosoma bacterial artificial (BAC) ha sido construida por la utilizacion de la lineas restaurantes del girasol RHA325 y pBeloBAC11 como vector. Esta primera biblioteca BAC del girasol corresponde al valor de 1,7 equivalente del genoma haploide (104,736 clones, la grandeza media del insert cerca de 50 kb). El uso de marcadors STS, desarrollados de los marcadores RAPD facilito de identificar tres clones BAC por la hibridizacion de la colonia a traves de filtros de grande densidad. Por el metodo del fingerprinting y la utilizacion de *Hind*III fue hecho el contig para el locus restaurante *Rf1*.

DIVERSITÉ MOLÉCULAIRE DES SOURCES CMS ET RÉTABLISSEMENT DE LA FERTILITÉ DANS LE GENRE *Helianthus*

RÉSUMÉ

La caractérisation moléculaire de 28 sources CMS et le cytoplasme fertile de l'*H. annuus* ont démontré que des cytoplasmes d'origines différentes font preuve d'une similarité importante que leur origine ne pouvait pas laisser prévoir. L'analyse de groupement, au moyen de la méthode UPGMA et les données obtenues pour RFLP, a permis la différenciation de 10 types de mitochondries (mt). Des protéines caractéristiques à une ou plusieurs sources CMS qui correspondent aux types mt ont été identifiées. De plus, les données obtenues sur le rétablissement de la fertilité pour un sous-groupe de neuf nouvelles

sources CMS confirment la classification des cytoplasmes faite d'après le niveau mtADN, comme le démontre le dendrogramme UPGMA.

Un clonage fait d'après la carte précédente a été utilisé pour isoler le gène de restauration *Rf1*, qui rétablit la fertilité du pollen chez les hybrides basées sur PET1. Des marqueurs étroitement liés au gène restaurateur ont été identifiés à l'aide des technologies AFLP et RAPD. Une bibliothèque de chromosomes bactériens artificiels (BAC) a été construite au moyen de la ligne restauratrice du tournesol RHA325 et pBeloBAC11 comme vecteur. Cette première bibliothèque BAC correspond chez le tournesol à la valeur de 1,7 équivalents de génomes haploïdes (104,736 clones, dimension moyenne approximative 50 kb). L'utilisation de marqueurs STS, développés à partir de marqueurs RAPD, a permis d'identifier trois clones BAC par l'hybridation de colonies à travers des filtres à haute densité. Un contig pour le restaurateur locus *Rf1* a été construit par la méthode "fingerprinting" AND et au moyen de *HindIII*.