Gene Flow in Medicago Through Somatic Hybridization

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GENE FLOW IN MEDICAGO THROUGH SOMATIC HYBRIDIZATION

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ABSTRACT
The objective of this research was the characterization at both molecular and phenotypic level of three somatic hybrid plants obtained by electrofusion of protoplasts of Medicago sativa with those of M.coerulea, M.falcata and M.arborea, three Medicago species in a different relation with alfalfa. Different kinds of rearrangements including the amplification of new spacer-length variants were detected at rDNA loci in the somatic hybrids. Analysis of field performances confirmed the suitability of these plants for breeding purposes.

KEYWORDS
Medicago sativa, somatic hybridization, rDNA, genomic rearrangements, field performance

INTRODUCTION
Alfalfa, (Medicago sativa, 2n=4x=32), is the most important forage crop in temperate environments and it allows a large forage production by cutting plants several times a year. There are several aspects such as resistance to diseases and pest attacks and adaptation potential to severe environmental conditions which need to be improved. Since germplasm source of alfalfa is restricted to a few species belonging to the M.sativa coerulea falcata complex, there is a need to exploit somatic hybridization as a tool to overcome the sexual barriers that hamper the use of wild Medicago species as donors of the traits above mentioned. Here are reported the obtainment and characterization of three somatic hybrid plants which combined the genome of alfalfa with that of the two diploid species namely M.coerulea and M.falcata and the distantly related tetraploid species M.arborea which is incapable of sexual hybridization with M.sativa. The fate of rDNA gene family in the somatic hybrids was studied with the aid of RFLPs (Restriction Fragment Length Polymorphisms) and FISH (Fluorescent In Situ Hybridization). Some agronomic traits were evaluated in field conditions to monitor the rearrangements and interchanges of the parental genomes in the somatic hybrids at phenotypical level.

MATERIALS AND METHODS
Parental lines M.sativa L.(4x cv. Rangelander 15) M.coerulea and M.falcata were propagated by cuttings, M.arborea lines came from seeds harvested on a protoplast-derived plant. Callus protoplasts of the three Medicago species were treated with fluoresceine isothiocianate and fused with mesophyll protoplasts of M.sativa. Heterokaryons were recognized by virtue of their dual color under fluorescent light: yellow-green due to fluoresceine and red to the chlorophyll. Electrofusion conditions, rDNA analyses and field evaluation of hybrid plants are reported elsewhere (Pupilli et al., 1992;, Pupilli et al., 1995; Cluster et al., 1996; Nenz et al., 1996).

RESULTS AND DISCUSSION
rDNA analysis. RFLP of rDNA spacers and FISH of an 18S-gene probe to mitotic chromosomes were used to compare parental and hybrid plants. The M.sativa-coerulea hybrids (S+C) retained all the parental nucleolar-organizing regions (NORs) and RFLPs representing a complete integration of parental genomes. The M.sativa-arborea hybrids (S+A) retained five of six parental NORs and lost half of the arborea-specific RFLPs, most probably as a consequence of the loss of one arborea NOR chromosome. Dramatic molecular alterations occurred in the M.sativa-falcata hybrids (S+F) where five of six parental NORs were retained and new rDNA RFLPs involving increased numbers of a 340-bp subrepeating element were created and amplified differentially among somaclonal-variant plants.

Cytological analysis. Chromosome counts showed the sum of parental complements (32+16=48) for S+C while seven and fifteen chromosomes were missing in S+A and S+F, respectively. Both S+C and S+F were fertile while S+A was sterile.

Analysis of agronomic traits. Both S+A and S+C showed a plant habit intermediate between the completely erect of M.sativa, the prostrate of M.coerulea and the tree-like of M arborea. S+C exhibited heterosis for some traits such as the main stem diameter and the leaf dimensions (Table 1). S+A showed an average number of stems per plant intermediate between the parents and stems arose from a structure similar to a crown. These last hybrids showed a rather vigorous aspect and the reduced number of stems per plant, with respect to M.sativa, was in part counterbalanced by a higher leafiness so that the apparent total green matter did not differ significantly from that of M.sativa (Table 2). The hybrids S+F were the most similar to the cultivated alfalfa. The formidable stresses created by tissue culture could account for the intensive genome rearragements detected as loss and/or gain of restriction sites at rDNA loci and chromosome loss. Nevertheless such rearragements did not produce dramatic disturbances at phenotypic level with the exception of the sterility of S+A. However these last hybrids demonstrated the feasibility of somatic hybridization for transferring phenotypic features such as the reduced number of stomata per leaf, a trait presumably correlated with drought resistance, from a wild species to cultivated germplasm. Moreover, the hexaploid level of the S+C plants, could reduce the decline in vigour due to self fertilization and such hybrids could be directly used in breeding programs.

REFERENCES
Cluster, P.D., O. Calderini, F. Pupilli, F. Crea, F. Damiani and S. Arcioni. 1996. The fate of ribosomal genes in three interspecific somatic hybrids of Medicago sativa: three different outcomes including the rapid amplification of new spacer-length variants. Theor. Appl. Genet. (in press).

Nenz, E., F. Pupilli, F. Damiani and S. Arcioni. 1996. Somatic hybrid plants between the forage legumes Medicago sativa L. and Medicago arborea L. Theor. Appl. Genet. (in press).

Pupilli, F., G.M. Scarpa, F. Damiani and S. Arcioni. 1992. Production of interspecific somatic hybrid plants in the genus Medicago through protoplast fusion. Theor. Appl. Genet. **84**: 792-797.

Pupilli, F., S. Businelli, M.E. Caceres, F. Damiani and S. Arcioni. 1995. Molecular, cytological and morpho-agronomical characterization of hexaploid somatic hybrids in Medicago. Theor. Appl. Genet. **90**: 347-355.

Table 1
Field evaluation of agronomic traits of the somatic hybrids S+C and their parents

TRAIT	MEAN	SE	MIN	MAX	MS[1]	MC[2]
Main stem diameter (mm)	2.24	+0.039	1.81	2.65	2.10**	2.15*
Blooming time	16.98	+0.627	10.14	25.00	16.33ns	19.87**
Node number	12.12	+0.122	10.85	13.50	14**	13**
Leaflet width	1.17	+0.019	0.96	1.38	1.02**	0.57**
Leaflet length	2.35	+0.037	1.93	2.76	2.03**	1.70**
Leaflet weight	0.16	+0.004	0.10	0.19	0.12**	0.14**
Stem length	56.04	+1.10	44.59	67.50	55.77ns	60.96**
Stem number	18.91	+0.941	9.93	23.72	24.67**	25.62**
Stem weight/ plant	33.21	+1.191	22.70	37.53	34.39ns	23.21**
Leaf weight/ plant	34.41	+2.271	11.18	36.36	35.72ns	19.94**

[1] MS=*M.sativa*
[2] MC=*M.coerulea*
* values not significantly different for P-0.05
** values not significantly different for P-0.01

Table 2
Field evaluation of agronomic traits of the somatic hybrids S+A and their parent

TRAIT	MS	S+A	MA
Number of stems	19a	6b	2b
Leaf mass (score 1 to 5)	1.7a	4.9a	3a
Total green matter (score 1 to 9)	5b	4.2b	9a
Stem diameter (mm)	2.1b	2.1b	5.1a
[1]Leaf number (upper 15 cm)	17b	30.9ab	17b
[2]Leaf number (median node)	4.7a	5.9a	10.7a
[2]Leaf weight (g)	0.6a	0.79a	0.96a
[3]Pubescence (number/mm2)	22b	16.8c	39.6a
[3]Stomata (number/mm2)	94.7a	35.4b	23.1c
[3]Stomata length (µm)	24.4a	31.5a	23.1a
[3]Stomata width (µm)	15.8b	15.8b	19.5a

[1]Traits recorded on the longest stem.
[2]Traits referred to the number and weight of trifoliate leaves of the median internode of the longest stem.
[3]Traits recorded on the median leaflet of the largest trifoliate leaf on the position mentioned above.
MS=M.sativa
MA=M.arborea
Values followed by the same letter did not differ for P-0.05