Facultative Apoximis, Spontaneous Polyploidization and Inbreeding in *Citrus volkameriana* Seedlings

P. Ollitrault
CIRAD-FLHOR, BP 5035
34032 Montpellier cedex 01
France

C. Jacquemond
SRA San Giuliano
INRA/CIRAD-FLHOR
20230 San Nicolao
Corsica

Fruits, vol. 49, n°5-6
p. 398-400 (English)
p. 479-480 (French)

The genetic status of 135 seedlings of Citrus volkameriana *and relationships with plant vigor were analysed.*

introduction

Citrus rootstocks are usually propagated by planting polyembryonic seeds that contain one zygotic embryo and several somatic embryos arising from the nucellus. Competition between these different embryos results in partial apomixis. The apomixis rate is dependent on the genotype and environmental conditions (Khan and Roose, 1988). Moreover, there can be a low percentage of polyploids due to fertilization of non-reduced ovules or spontaneous polyploidization of nucellar tissues (Esen and Soost, 1977; Iwamasa and Nito, 1989).

Citrus volkameriana is a very vigorous and highly heterozygous rootstock, as shown by isozyme analysis (Ollitrault *et al.*, 1992). It was therefore predicted that zygotic seedlings would show very marked diversity. However, *C. volkameriana* seedling populations are generally bimodal, i.e. a highly vigorous true-to-type population and, conversely, a very weak population.

In the present paper, we have analysed the genetic status (somatic or zygotic origin, and ploidy level) of *C. volkameriana* seedlings and relationships with plant vigor.

material and methods

The genetic origins of 135 *Citrus volkameriana* greenhouse-grown seedlings at the SRA San Giuliano station were analysed.

Isozyme polymorphism was used to differentiate zygotic and nucellar seedlings. Five heterozygous loci were analysed in *C. volkameriana* using four enzymatic systems (malate dehydrogenase, MDH; isocitrate dehydrogenase, IDH; phosphoglucose isomerase, PGI; aspartate amino transferase, AAT), as described by Ollitrault *et al.* (1992). Four of them are unlinked (Luro *et al.*, 1995 and our unpublished data), so more than 94% of the zygotic seedlings could be detected.

The ploidy level of each seedling was evaluated by flow cytometry, as described by Ollitrault and Michaux-Ferriere (1992). Samples were analysed at the INSERM Unit 291 laboratory in Montpellier under the supervision of C. Duperray.

Vegetative seedling vigor was estimated 1 year after planting according to three characters: seedling height, number of internodes and diameter of the second internode. A synthetic index of vigor was established from these characters by principal component analysis.

results

Twenty-nine zygotic plants were identified by isozyme analysis (Photo 1), while flow cytometry analysis revealed two tetraploid plants (Fig. 1). These two plants, which had enzymatic patterns identical to *C. volkameriana*, certainly arose through spontaneous tetraploidization of somatic cells. The enzymatic patterns of the zygotic seedlings suggested that they mainly

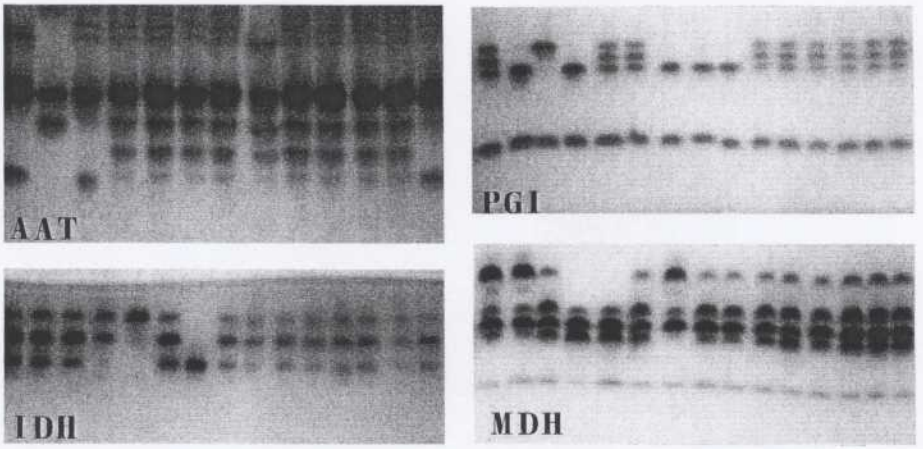

Photo 1
C. Volkameriana *(last sample) exhibits five heterozygous loci for the 4 analysed enzyme systems. From the combination of these systems it appears that samples n°1, 2, 3, 4, 5, 6, 7, 8, 9, 13 and 14 are zygotic seedlings. The others samples are considered as nucellars with a 3% risk (if the five heterozygous loci are unlinked).*

arose *via* self pollination. The enzymatic patterns and flow cytometry histograms of the 104 other plants were identical to *C. volkameriana*; they were thus considered to be somatic diploids of nucellar origin.

The three morphological characters studied were very highly correlated, and the vigor index established from the first axis in the principal component analysis represented more than 90% of the total variance. This vigor index was used to establish the vigor distribution between the three genetic groups (Fig. 2). The vegetative vigor levels of most of the zygotic seedlings (23/29) were substantially lower than those of plantlets of nucellar origin and, similarly, tetraploid plantlets showed very depressed development (Table 1).

discussion

The two main vigor classes in *C. volkameriana* seedlings could be associated with different genetic origins. The weak plants were diploid zygotic or somatic tetraploids, while the vigorous plants were mainly nucellar diploids.

Inbreeding was previously reported for other polyembryonic *Citrus* and *Poncirus* species (KHAN and ROOSE, 1988; SOOST and CAMERON, 1975). Apomixis certainly caused deleterious recessive mutations with respect to the heterozygous status, as noted in self-fertilized zygotic seedlings. The high proportion of depressed *C. volkameriana* seedlings suggested the

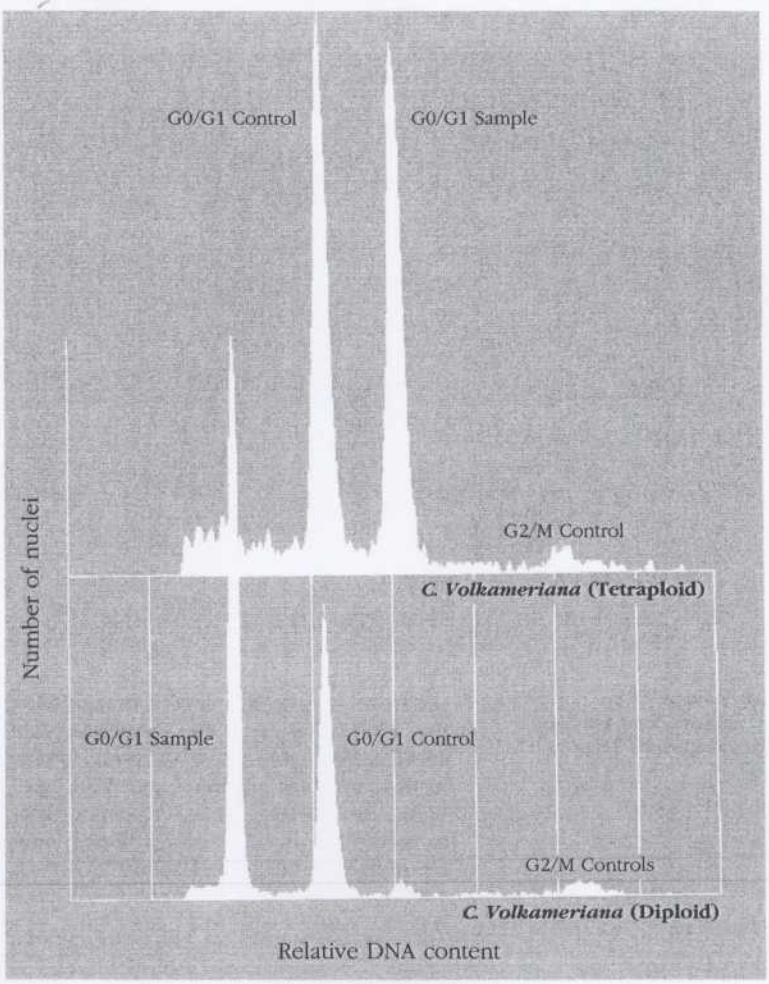

Figure 1
Flow cytometry analysis of ploidy levels in C. volkameriana *seedlings. Tahiti lime (triploid) was used as internal control.*

Table 1
Numbers and characteristics of nucellar, zygotic and tetraploid seedlings.

	Number of plants	%	DI Mean	DI CV	NI Mean	NI CV	H Mean	H CV
Nucellars	104	77.0	11.6	0.13	72.5	0.11	140	0.17
Zygotics	29	21.5	6.6	0.36	22.7	0.59	51	0.89
Tetraploids	2	1.5	5.6	0.20	24.5	0.10	30	0.04

DI: Diameter of the second internode (mm)
NI: Number of internodes
H: Hight of the seedling (cm)

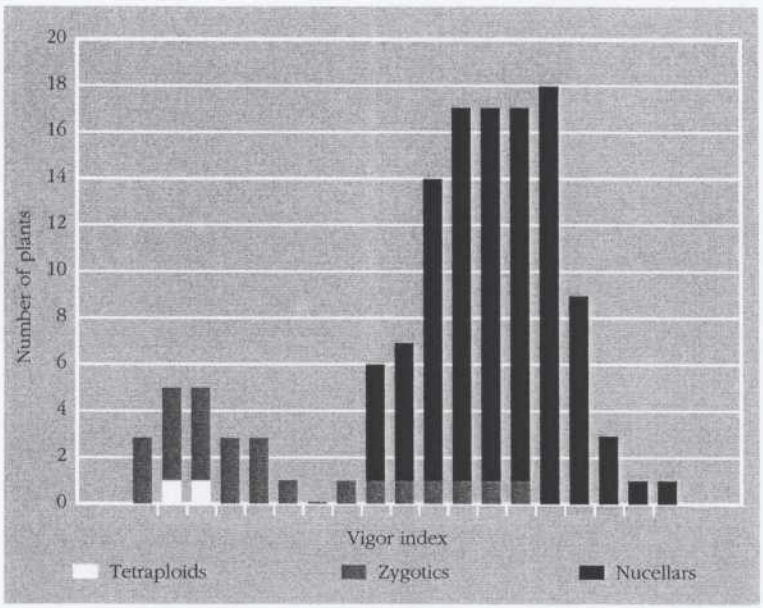

Figure 2
Vigor indexes for C. volkameriana *seedlings of three genetic origins.*

presence of several unlinked mutations of this kind. However, the morphology of some zygotic seedlings was very similar to the nucellar morphology. For agronomic or varietal tests, it is thus necessary to certify the nucellar origin of seedlings by molecular analysis (OLLITRAULT *et al.*, 1992).

The low proportion and vigor of spontaneous *C. volkameriana* tetraploids was in agreement with results obtained in other *Citrus* species (IWAMASA and NITO, 1989). Despite inbreeding, the proportion of zygotic seedlings was relatively high (>20%). This could indicate that the rate of spontaneous tetraploidization of nucellar tissues was higher than that evaluated for tetraploid plantlets. Indeed, the emergence of zygotic seedlings must be favoured by the very low vigor of tetraploid embryos. ●

references

ESEN A., SOOST R.K., 1977.
Relation of unexpected polyploids to diploid megagametophytes and embryo: endosperm ploidy ratios in *Citrus*. *In*: I. Congreso mundial de Citricultura, Murcia, Valencia, 29 April-10 May 1973. Vol. II, O. Carpena ed., International Society of Citriculture, p. 53-63.

IWAMASA M., NITO N., LING J.T., 1989.
Intra- and intergeneric hybridization in the orange subfamily, *Aurantioideae*. *In*: Proc. 6th Int. Citr. Cong. Middle-East, Tel Aviv, Israel, 6-11 March 1988. R. Goren and K. Mendel eds., Margraf Scientific Publishers, Germany, p. 123-130.

KHAN I.A., ROOSE M.L., 1988.
Frequency and characteristics of nucellar and zygotic seedlings in three cultivars of trifoliate orange. J. Amer. Soc. Hort. Sci. 113 (1): 105-110.

LURO F., LORIEUX M., LAIGRET F., BOVE J.M., OLLITRAULT P., 1995.
Genetic Mapping of an Intergeneric *Citrus* Hybrid using Molecular Markers. Fruits, vol. 49 (5-6): 402-406.

OLLITRAULT P., FAURE X., NORMAND F., 1992.
Citrus rootstock characterization with bark and leaf isozymes; application for distinguishing nucellar from zygotic trees. In: Proc. of VII Int. Citrus Cong., Acireale, Italy, 3-8 March 1992. Int. Soc. of Citriculture, p. 338-341.

OLLITRAULT P., MICHAUX-FERRIERE N., 1992.
Application of flow cytometry for *Citrus* genetics and breeding. In: Proc. VII Int. Citrus Congr., Acireale, Italy, 3-8 March 1992. Int. Soc. of Citriculture, p. 193-198.

SOOST R.K., CAMERON J.W., 1975.
Citrus. In: Advances in Fruit Breeding, Purdue University Press, Indiana, p. 507-540.