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## Introduction

Knowledge of the nuclear DNA content and genome size estimates are fundamental parameters in many genetic and molecular studies. This information is particularly useful in evaluating somatic and reproductive compatibility for breeding programs that are based on interspecific crosses. Differences in ploidy level lead to genetic barriers to obtain interspecific hybrids.

The joint *Prunus* rootstock breeding program between EEAD-CSIC and CEAF includes interspecific hybrids between different species belonging to different *Prunus* subgenera (*Euamygdalus* and *Prunophora*). The determination of the ploidy level in parental lines and elite candidates under selection is a priority for our breeding programs. Therefore, this study aimed to determine the ploidy level of different accessions of *Prunus* rootstocks included in the germplasm bank and in the breeding programs of EEAD-CSIC and CEAF.

## Materials and Methods

We determined ploidy level of 7 rootstocks from Myrobalan plums (*Prunus cerasifera*), 21 from common plums (*Prunus domestica*, including subsp. *insititia*), 2 from sour cherry (*Prunus cerasus*) and 58 from interspecific hybrids of the genus *Prunus*. Commercial cultivars were used as reference standards of well-known ploidy level.

Leaf samples were collected early in the summer from mature orchard trees. Ploidy levels were determined by flow cytometry (PAS, PARTEC). For isolation of nuclei, leaf tissue (approx. 0.5 cm<sup>2</sup>) was chopped with a scalpel using the UV CyStain ploidy (DAPI) solution. Data were displayed as histograms of the number of nuclei vs. the relative fluorescent intensity using a logarithmic scale along the x-axis.

## Results

We obtained different ploidy levels among the rootstocks of genus *Prunus* (Figure 1). One hundred percent of the Myrobalan plums were diploids (Figure 1A) and 100 % of common plums were hexaploids (Figure 1D). The interspecific hybrids showed different ploidy levels according to those observed in their parental genotypes. 88 % of the interspecific hybrids were diploids (most of them coming from almond x peach hybrids). 7 % were tetraploid, including White Blackthorn (*P. spinosa*) (Figure 1B), Jaspi (*P. salicina* x *P. Cerasifera*) x *P. spinosa*) and Cab6P (*P. cerasus*). Pentaploid rootstocks were also identified (2 %), including Damas GF 1869 (*P. domestica* x *P. spinosa*) (Figure 1C).

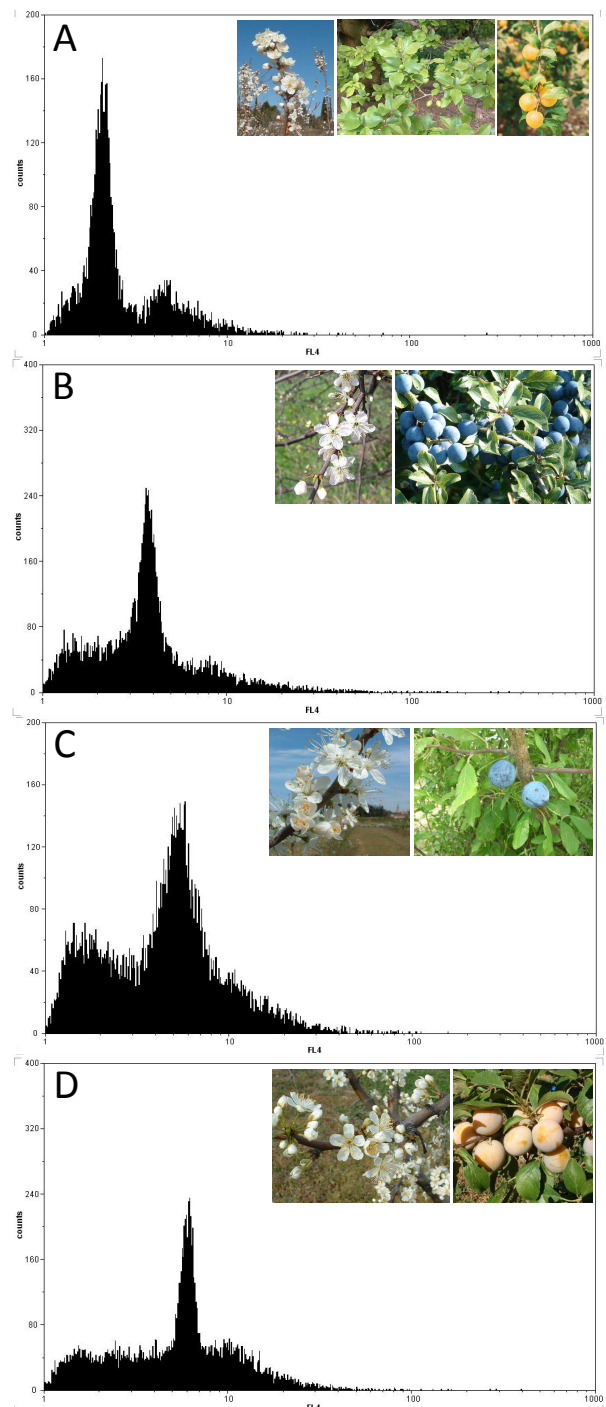
## Conclusion

For most varieties used as a reference, ploidy level obtained coincide with those published by other authors and/or with the estimate obtained from the parallel characterization performed with microsatellites. However, in some cases differences were observed with ploidy levels inferred from microsatellite profiles. These results will be corroborated by counting chromosomes (karyotype).

### References:

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**Figure 1. Ploidy analysis of *Prunus* rootstocks by flow cytometry.** Histograms show the number of nuclei ('counts') versus the amount of fluorescence emitted by the fluorochrome DAPI (FL4). **Fig. 1A:** Adara (*P. cerasifera*, diploid), **Fig. 1B:** Wild Blackthorn (*P. spinosa*, tetraploid), **Fig. 1C:** Damas GF 1869 (*P. domestica* x *P. spinosa*, pentaploid) and **Fig. 1D:** RC GF 1380 (*P. domestica*, hexaploid).