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PLOIDIA LEVELS IN PYRENIC AND APYRENIC "PITANGUEIRA" ACCESSIONS

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ABSTRACT - Pitangueira (*E. uniflora*) is considered a diploid species with n = 11 and 2n = 22 chromosomes, in genotypes with the presence of seeds in the fruits. With the pitangueira production of apyrenic fruits existence, such behavior may be related to the ploidy level. The objective of this study was to determine the ploidy level of the pitangueira accessions producing fruit with and without seed, as well as to observe the meiotic behavior and possible chromosomal abnormalities. To check the ploidy level of the pitangueira, freshly expanded mature leaves were collected from the pyrenic pitangueira and from two pyrenic accessions, with suspensions of intact nuclei being prepared. Samples were analyzed in a flow cytometer equipped with multiple parameters data acquisition and UV laser. All analyses were performed using peak-height detection (>6000 fluorescent events, for example, nuclei, were analyzed per sample) and logarithmic amplification. The data were presented as histograms of the number of nuclei along the y-axis and the relative fluorescence intensity on the x-axis. The pyrenean pitangueira and other two pyrenic accessions were characterized as diploid.
Keywords: *Eugenia uniflora*, Myrtaceae, polyploidy, seedless fruit.

NÍVEIS DE PLOIDIA EM ACESSOS DE PITANGUEIRA PIRÊNICAS E APIRÊNICAS

RESUMO - A pitangueira (*E. uniflora*) é considerada espécie diplóide com n=11 e 2n=22 cromossomos, em genótipos com presença de sementes nos frutos. Com a existência de pitangueira produtora de frutos apirênicos, tal comportamento pode estar relacionado ao nível de ploidia. O objetivo deste estudo foi determinar o nível de ploidia dos acessos de pitangueira produtora de frutos sem e com semente, bem como, observar o comportamento meiótico e possíveis anormalidades cromossômicas. Para verificar o nível de ploidia da pitangueira, folhas maduras recém-expandidas foram coletadas da pitangueira apirênica e de dois acessos pirênicos, sendo preparados suspensões de núcleos intactos. As amostras foram analisadas em citômetro de fluxo equipado com aquisição de dados de múltiplos parâmetros e de laser UV. Todas as análises foram realizadas utilizando detecção de pico-altura (>6000 eventos fluorescentes, por exemplo, núcleos, foram analisados por amostra) e amplificação logarítmica. Os dados foram apresentados como histogramas do número de núcleos ao longo do eixo-y e a intensidade da fluorescência relativa no eixo-x. A pitangueira apirênica e outros dois acessos pirênicos, foram caracterizadas como diplóides.
Palavras-chave: *Eugenia uniflora*, Myrtaceae, poliploidia, fruto sem semente.

INTRODUCTION

The Brazilian flora is rich in fruitful species that have tasty edible fruits. In the south of Brazil, those of the Myrtaceae family stand out, with a broad potential for economic exploitation, which is still incipient, mainly due to the lack of knowledge about these species (SARMENTO et al., 2012).

Among the fruit species of Myrtaceae, with economic potential, those of the *Eugenia* genus stand out. Many species of this genus are appreciated for their fruits, as they are used as food (LAMARCA et al., 2013), like *E. uniflora*, popularly known as Surinan cherry, considered to be one of the best-known species of the genus (KOSERA NETO et al., 2018).

However, as a consequence of the anthropic action, many species genotypes with desirable characteristics have been lost, therefore, to reverse or minimize such damage it is essential to prospect for genotypes *in loco* in the occurrence areas, in order to analyze and characterize the properties of interest present in the fruits, for later selection and asexual propagation, in which they will be useful in commercial orchards or as genitors in breeding programs (DONAZZOLO et al., 2020).

In this latter case, it is also important to characterize the Surinan cherry ploidy levels, allowing to infer whether it is possible to obtain intraspecific hybrids. Polyploidy is a common phenomenon in plants, but occurs at low a frequency and it is considered a mechanism of speciation and adaptation (BROWNFIELD; KÖHLER,

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2011). In citrus, there are records of triploids, tetraploids, hexaploids and octaploids that occur spontaneously (USMAN et al., 2006).

In Myrtaceae, Silveira et al. (2006), performed chromosome counting in two species of jabuticaba tree, *Plinia trunciflora* and *P. cauliflora*, in which they presented two situations, one 2n = 22 and another 2n = 48 chromosomes. Therefore, differences were found in the number of chromosomes between material from the same Surinan cherry family.

In another Myrtaceae, but from the *Campomanesia* genus, the number of chromosomes was 2n = 22. In *Psidium* sp. there was variation between species, with *P. guajava* 2n = 22 and three others with 2n = 44 chromosomes, in addition to observing in *P. glomerata* (cabeludinha) 2n = 22 chromosomes (COSTA; FORNI-MARTINS, 2006).

Costa (2004) described the Surinan cherry (*E. uniflora*) as a diploid species with n = 11 and 2n = 22 chromosomes. In addition to this, this author verified that in a similar morphology kind with *E. uniflora*, the *E. pitanga*, as being a diploid but with individuals presenting n = 22 and 2 n = 44 chromosomes, having several cytotypes.

The morphological similarity makes it very difficult for the correct accession's identifications, when the number of chromosomes is obtained by cytogenetic techniques, the preponderant parameter for the species identification. The lack of studies in the cytogenetics of the species field leads to the belief that many samples are mistakenly identified.

The crossing seed production is possible, regardless of ploidy, being higher among diploids, followed by tetraploids and triploids (SILVA et al., 2013). As there are Surinan cherry which produce fruit with and without seeds, then it could be characterized in relation to polyploidy, especially the apyrenic, discarding the hypothesis that it was triploid, since some triploid species are characterized as plants that produce seedless fruits. The aim of this study was to determine the ploidy level of the accessions of Surinan cherry producing pyrene and apyrenic fruits.

MATERIAL AND METHODS

Forty newly expanded mature leaves of Surinan cherry tree producing apyrenic fruits and two accessions of pyrenic fruits were collected from UTFPR's collection of Native Fruit Trees at the Dois Vizinhos Campus. Afterwards, the leaves were placed on moist filter paper, kept in a polystyrene box at 4 °C. The studied plants were obtained by semi-mineral route, from a rural producer array in Honório Serpa – Paraná State, Brazil.

After collecting the leaves, suspensions of intact nuclei were prepared by cutting the material with a 0.5 cm^2 scalpel blade in a Petri® plate, containing 0.5 mL of CyStain UV ploidy, which contains 4', 6-diamidino-2-phenylindole (DAPI), followed by maceration. The suspensions were filtered through a 20 µm filter (to remove tissue residues and whole cells) and then kept on ice for at least 3 minutes. Finally, 1 mL of filtered Milli-Q water was added to the suspended nuclei, which were dyed with DAPI solution. The samples were analyzed in a flow cytometer (cytometer PAS, PARTEC) from Estación Experimental de Aula Dei (CSIC), in Zaragoza - Spain. The flow cytometer is equipped with multi-parameter data acquisition and UV laser, using peak-height detection (>6000 fluorescent events were analyzed per sample) and logarithmic amplification. The data were presented as histograms of the nuclei number (or frequency) along the y-axis and the relative fluorescence intensity on the x-axis.

RESULTS AND DISCUSSION

The results showed that both the apyrenic Surinan cherry and the two pyrenic accessions are diploids (Figure 1). Costa (2004), also described for *E. uniflora* as being a diploid species. It is believed that this result can be considered as a starting point for studies involving the genetic improvement of this apyrenic access, which can be used as a genitor in directed crossing, increasing the chance of obtaining hybrids with superior agronomic characteristics, associated to apyrenia.

In order to obtain desired agronomic characteristics, such as apyrenia, for example, within any breeding program, it is necessary to carry out hybridizations, involving superior genitor genotypes and with a wide genetic base, thus increasing the probability of obtaining hybrids with good performance for the characteristics desired by the breeder (WAGNER JÚNIOR et al., 2011). However, one must study the type of inheritance of this characteristic, so it will be possible to estimate the time and generations in order to achieve the genetic gains, as well as, the success in carrying out the selection in a specific directed crossing.

Thus, this apyrenic genotype could be incorporated into the Surinan cherry breeding programs, as a possible genitor, increasing the variability and the chances of obtaining hybrids with seedless fruits, a desirable characteristic for the consumer and industry.

In diploid species, the importance of pre-improvement lies in the fact that the vast majority of agronomic characteristics of interest, such as parthenocarpy, are dispersed in a large number of wild diploids, making hybridization and the development of improved diploids necessary. (SILVA et al., 2011).

Polyploidy reproduction has been successfully applied in many plants to obtain lines with new agronomic characteristics. This procedure provided seedless plants, with larger fruits and flowers - longer duration flowers - which overcome the hybridization barriers, show resistance to pests and tolerance to physical stress (PREDIERI, 2001). These effects can vary according to the species, degree of heterozygosity and ploidy level (ZHANG et al., 2008), requiring cytogenetic monitoring to verify its chromosomal stability over the generations.

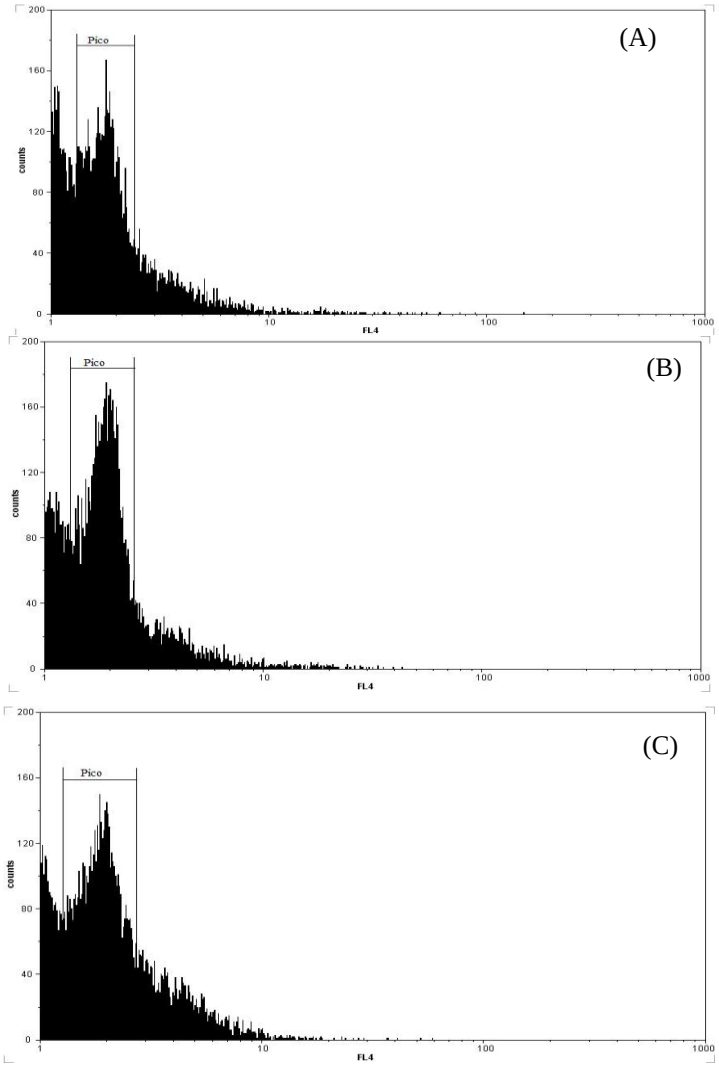

FIGURE 1 - Flow cytometry of the DNA content for Surinan cherry in pyrenic leaves (A), pyrenic, access 1 (B) and access 2 (C), on a logarithmic scale.

In general, it was observed in the present study that the apyrenia that occurred in Surinan cherry of a single genotype is not related to the number of chromosomes. New studies should be carried out seeking to elucidate the involved genes with the expression of such a characteristic in the Surinan cherry, since it becomes advantageous for commercial production, increasing the pulp yield and facilitating being handled by the industry.

CONCLUSIONS

The pyrenean Surinan cherry and two other pyrenic accessions were characterized as diploid.

REFERENCES

BROWNFIELD, L.; KÖHLER, C. Unreduced gamete formation in plants: mechanisms and prospects. **Journal of Experimental Botany**, v.62, n.5, p.1659-68, 2011.

COSTA, I.R. **Estudos cromossômicos em espécies de Myrtaceae Juss. no sudeste do Brasil.** 2004. 80p. Dissertação (Mestrado em Biologia Vegetal) - Universidade Estadual de Campinas, Campinas, 2004.

COSTA, I.R.; FORNI-MARTINS, E.R. Chromosome studies in species of *Eugenia*, *Myrciaria* and *Plinia* (Myrtaceae) from south-eastern Brazil. **Australian Journal of Botany**, v.54, n.4 p.409-415, 2006.

DONAZZOLO, J.; STEFENON, V.M.; GUERRA, M.P.; NODARI, R.O. On farm management of *Acca sellowiana* (Myrtaceae) as a strategy for conservation of species genetic diversity. **Scientia Horticulturae**, v.259, [s.n], p.1-6, 2020.

KOSERA NETO, C.; SILVA, M.; MOURA, G.C.; RADAELLI, J.C.; WAGNER JÚNIOR, A.; TARTAS, P.L. Armazenamento de sementes pré-germinadas de pitangueira. **Cadernos de Agroecologia**, v.13, n.1, p. 7-13, 2018.

LAMARCA, E.V.C.; BAPTISTA, W.; RODRIGUES, D.S.; OLIVEIRA JÚNIOR, C.J.F. Contribuições do conhecimento local sobre o uso de *Eugenia* spp. em sistemas de policultivos e agroflorestas. **Revista Brasileira de Agroecologia**, v.8, n.3, p.119-130, 2013.

PREDIERI, S. Mutation induction and tissue culture in improving fruits. **Plant Cell Tissue Organ Culture**, v.64, n.1-2, p.185-210, 2001.

SARMENTO, M.B.; SILVA, A.C.S.; SILVA, C.S. Recursos genéticos de frutas nativas da família Myrtaceae no sul do Brasil. **Magistra**, v.24, n.4, p.250-262, 2012.

SILVA, S.O.; AMORIM, E.P.; SANTOS-SEREJO, J.A.; FERREIRA, C.F.; RODRIGUEZ, M.A.D. Melhoramento genético da bananeira: estratégias e tecnologias disponíveis. **Revista Brasileira de Fruticultura**, v.35, n.3, p.919-931, 2013.

SILVA, S.O.; SANTOS-SEREJO, J.A.; AMORIM, E.P. Pré-melhoramento da banana. In: LOPES, M.A.; FÁVERO, A.P.; FERREIRA, M.A.J.F.; FALEIRO, F.G.; FOLLE, S.M.; GUIMARÃES, E.P. (Eds.). **Pré-melhoramento de plantas:** estado da arte e experiências de sucesso. Brasília: Embrapa Informação Tecnológica, 2011. p.317-350.

SILVEIRA, F.T.; ORTOLANI, F.A.; MATAQUEIRO, M.F.; MORO, J. R. Caracterização citogenética em duas espécies do gênero Myrciaria. **Revista de Biologia e Ciências da Terra**, v.6, n.2, p.327-333, 2006.

USMAN, M.; SAEED, T.; KHAN, M.M.; FATIMA, B. Occurrence of spontaneous polyploids in *Citrus*. **Horticultural Science**, v.33, n.3, p.124-129, 2006.

WAGNER JÚNIOR, A.; BRUCKNER, C.H.; CANTÍN, C.M.; SÁNCHEZ, M.A. M.; CRUZ, CD. Divergência genética entre progênies de pessegueiro em Zaragoza, Espanha. **Revista Brasileira de Fruticultura**, v.33, n.1, p.303-310, 2011.

ZHANG, Z.; DAI, H.; XIAO, M.; LIU, X. *In vitro* induction of tetraploids in *Phlox subulata* L. **Euphytica**, v.159, n.1-2, p.59-65, 2008.