Industrial Crops and Products 54 (2014) 1–5

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Genome size variation in guayule and mariola: Fundamental descriptors for polyploid plant taxa

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ARTICLE INFO

Article history:
Received 23 October 2013
Received in revised form
29 December 2013
Accepted 30 December 2013
Available online 25 January 2014

Keywords:
Guayule
Mariola
Ploidy level
Nuclear genome size
Flow cytometry

ABSTRACT

Guayule (*Parthenium argentatum* A. Gray) has tremendous potential as a domestic source of natural rubber production in the southwestern United States. However, genetic improvement of guayule has been slowed by its complex mode of reproduction, natural ploidy series, and lack of genetic and genomic resources. The interspecific hybridization of guayule with its closest sister taxon mariola (*P. incanum* Kunth) offers an opportunity to access novel genetic variation for guayule breeding programs, but mariola accessions available from the U.S. National Plant Germplasm System (NPGS) have never been evaluated for natural variation in ploidy level. In addition, the nuclear genome sizes for guayule and mariola at any ploidy level are unknown. To that end, we examined the ploidy of 10 mariola accessions, which revealed a natural polyploid series ranging from triploid ($2n = 3x = 54$) to pentaploid ($2n = 5x = 90$). In contrast, a ploidy analysis of five guayule accessions uncovered a natural polyploid series that ranged from diploid ($2n = 2x = 36$) to hexaploid ($2n = 6x = 108$). More than one ploidy level among individual plants (mixed ploidy) and instances of aneuploid plants were observed for accessions of both guayule and mariola. The nuclear genome sizes of guayule and mariola were similar at identical ploidy levels, and the genome size of diploid guayule (1624 Mb) was almost twofold smaller than the genomes of sunflower (*H. annuus* L. $2n = 2x = 34$) and lettuce (*L. sativa* L.; $2n = 2x = 18$), two other Compositae (Asteraceae) species that are being genome-sequenced. The results from this study will serve as a foundation for interspecific breeding and genome sequencing of guayule and mariola.

1. Introduction

Guayule (*Parthenium argentatum* A. Gray) is a woody perennial shrub indigenous to the deserts of the southwestern United States and Northern Mexico. This member of the Compositae (Asteraceae) family has been commercialized as a renewable source of natural rubber and hypoallergenic latex (see review in Ray et al., 2005). Even though the rubber tree [*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.] is the predominant commercial source of natural rubber in the world, interest in guayule as a sustainable domestic source of natural rubber has intensified partly due to the projected global shortages of rubber by the next decade and the vulnerability of rubber tree plantations to South American leaf blight caused by the fungus *Microcyclus ulei* (Mann, 2009). Not only is guayule more amenable to traditional agronomic practices than the rubber tree, but it also produces a resin that resists termites when impregnated into wood (Holt et al., 2012). Guayule bagasse (plant residue after rubber and latex extraction) has the added potential of serving as a source of biofuel in the southwestern United States (Boateng et al., 2009).

Tremendous ploidy level variation exists within and among wild populations, cultivars, and germplasm lines of guayule (Bergner, 1946; Kuruvadi et al., 1997; Gore et al., 2011). This natural polyploid series typical ranges from diploid ($2n = 2x = 36$) to pentaploid ($2n = 5x = 90$), but even higher ploidy levels such as octoploid are possible (Powers, 1945; Thompson and Ray, 1988). However, tetraploid plants predominate in wild stands (Kuruvadi et al., 1997) and the U.S. germplasm collection (Gore et al., 2011). In addition, there is a low incidence of aneuploids within natural populations (Powers, 1945; Bergner, 1946; Kuruvadi et al., 1997).

Abbreviations: Mb, megabase; NPGS, National Plant Germplasm System.

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http://dx.doi.org/10.1016/j.indcrop.2013.12.052

Guayule diploid plants principally have a sexual mode of reproduction and are obligate outcrossers by virtue of a sporophytic self-incompatibility system (see review in Thompson and Ray, 1988; Ray et al., 2005). In contrast, the facultative expression of apomixis in polyploid plants results in the production of both apomictic and sexual offspring. This complex mode of reproduction and a highly variable number of chromosomes have unquestionably slowed the genetic progress for a crop that is still in the process of domestication.

Genetic improvement for rubber yield has primarily resulted from single-plant selections in genetically narrow populations of apomictic polyploid plants, thus new avenues for exploitation of genetic diversity should be considered (Ray, 1993). The extent to which interspecific hybridization has been used in guayule breeding programs is limited, but the 13 other species of the genus *Parthenium* (www.theplantlist.org) with only trace amounts of rubber are potential sources of genetic variation for increased vigor, resin content, biomass, disease resistance, and cold tolerance (Ray et al., 2010). Of these 13 species, the facultative apomictic species *P. incanum* Kunth (mariola) with a natural ploidy series is postulated to be the closest sister taxon of guayule (Powers and Rollins, 1945). Indeed, interspecific hybrids between guayule and mariola are found at low frequency where both species naturally coexist in the wild (Rollins, 1944) and have been produced by controlled crosses for broadening the genetic base of guayule (Rollins, 1945).

The genetic diversity of guayule and mariola can be more effectively exploited in an interspecific breeding program when the ploidy level and chromosome number variation of the germplasm are known. We have previously collected ploidy level data from guayule germplasm available in the U.S. National Plant Germplasm System (NPGS) (Gore et al., 2011), but identical information for mariola germplasm is still needed. In addition, having nuclear genome size estimates of guayule and mariola is critical knowledge for helping to understand genome evolution and develop genome sequencing projects. We conducted the present study to (i) examine ploidy level variation within and among mariola germplasm available from NPGS, and (ii) measure nuclear genome size of guayule and mariola accessions at several ploidy levels.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of six guayule and 10 mariola accessions, as well as an interspecific hybrid of these two species, were obtained from the National Arid Land Plant Genetics Resources Unit (NALPGRU) at Parlier, CA (Table 1). Of the six guayule accessions, CFS-21 and CFS-24 are wild material collected from Texas, while the other four accessions are improved lines developed by guayule breeding programs in California (W6 429, 11591, N566) and Arizona (AZ-5). The pedigrees of the four improved cultivars and breeding lines are available from Thompson and Ray (1988), Ray et al. (1999), and Gore et al. (2011). All of the mariola accessions are wild material collected from native stands in Texas. The natural interspecific hybrid was collected from the wild in the Mexican state of Coahuila. Seeds of pea (*Pisum sativum* L.; Ctirad) were obtained from J. Doležel at the Institute of Experimental Botany in the Czech Republic. Sunflower (*Helianthus annuus* L.; PI 642777; HA 412 HO) and lettuce (*Lactuca sativa* L.; PI 536851; Salinas) seeds were obtained from the North Central Plant Introduction Station in Ames, IA, and R. Michelmore at University of California, Davis, respectively.

Seeds were sown on moist vermiculite inside a growth chamber (14 h light, 250 μmol m^{-2} s^{-1} at 28 °C and 10 h dark at 21 °C). Seven-day-old seedlings were planted individually into 10 cm diameter pots containing moistened Sunshine Mix #1 (Sun Gro Horticulture Inc., Bellevue, WA) and perlite (4:1 ratio). Plants were fertilized every 2 weeks with 20–20–20 (50 ppm N) Peters Professional plant nutrient solution (The Scotts Company, Marysville, OH, USA). Two months later, seedlings were transplanted separately into 1 gal pots with Sunshine Mix #1. Plants were grown under natural light in a greenhouse with daytime and nighttime mean temperatures at 28 and 21 °C, respectively. Plants were fertilized every two-weeks with 20–20–20 (200 ppm N) Peters Professional plant nutrient solution.

2.2. Sample collection and preparation for ploidy analysis

Leaf tissue samples were collected and prepared for ploidy analysis as previously described (Gore et al., 2011). Briefly, young, fully expanded leaves were collected from immature flower stalks of individual plants with unknown ploidy and a *P. argentatum* diploid plant (PI 478663; W6 429) that was repeatedly used as an internal diploid standard and confirmed to be diploid ($2n = 2x = 36$) via mitotic chromosome counts in root tip cells in this (data not shown) and our previous study (Gore et al., 2011). Leaf tissue samples were collected the morning of the experiment and maintained at 4 °C until preparation later in the same day. To prepare each sample for ploidy analysis, we added 1 mL of woody plant buffer (Loureiro et al., 2007) to a Petri dish that contained equivalent amounts of leaf tissue from an individual plant with unknown ploidy and the *P. argentatum* diploid plant, followed by coarse chopping of the combined leaf tissue with a razor blade for 30 s. The resultant homogenate was filtered through a Partec 30 μm CellTrics (Partec GmbH, Münster, Germany) disposable nylon filter. Nuclei were stained with 4 μg mL^{-1} of 4′,6-diamidino-2-phenylindole (DAPI; Sigma–Aldrich, St. Louis, MO, USA) and incubated on wet ice for 10 min before immediate analysis by the flow cytometer.

2.3. Analysis of ploidy level with flow cytometry

A Partec Ploidy Analyser flow cytometer (Partec GmbH) was used to analyze the fluorescence of nuclei stained with DAPI as previously described (Gore et al., 2011). Briefly, the mean position of the G1 peak for the *P. argentatum* diploid standard was set at channel 100. The order in which samples were run on the instrument was randomized. In the first experiment, we evaluated 15 plants from each of 16 accessions ('unknowns') listed in Table 1. One plant from each accession was randomly assigned to one of 15 blocks or runs on the flow cytometer. The entire experiment was replicated twice, for a total of two measurements per plant. To assess the quality of the data, the Partec CA3 analysis software was used to calculate the coefficient of variation for each measurement.

The peak ratio for each plant of unknown ploidy was calculated per Doležel et al. (2007) as the mean position of the G1 peak for the unknown ploidy plant ('sample') divided by the mean position of the G1 peak for the *P. argentatum* diploid standard plant ('reference'). For each experiment, we screened for statistical outliers in SAS version 9.2 (SAS Institute, 2011) by examining the Studentized deleted residuals obtained from a mixed linear model fitted with peak ratio as the dependent variable, plant as a fixed effect, and replicate nested within plant as a random effect. Degrees of freedom were calculated via the Satterthwaite approximation. With outliers removed, the same mixed linear models were used to obtain least square means for peak ratios with the LSMEANS statement in PROC MIXED. For each accession, the overall average and standard deviation of least square means for peak ratios of plants within a ploidy level were calculated. The ploidy level within an accession was estimated by multiplying the average peak ratio by two (the ploidy of the *P. argentatum* diploid plant, $2x$).