Scientia Horticulturae 209 (2016) 132–138

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Nuclear DNA content variation within the genus *Daucus* (Apiaceae) determined by flow cytometry

Anna Nowicka[a], Elwira Sliwinska[b], Dariusz Grzebelus[c], Rafal Baranski[c], Philipp W. Simon[d], Thomas Nothnagel[e], Ewa Grzebelus[c,*]

[a] Department of Cell Biology, The Franciszek Gorski Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, PL 30-239 Krakow, Poland
[b] Department of Genetics, Physiology and Biotechnology, UTP University of Science and Technology, Prof. S. Kaliskiego Ave. 7, PL 85-789, Bydgoszcz, Poland
[c] Institute of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Al. 29 Listopada 54, PL 31-425 Krakow, Poland
[d] United States Department of Agriculture – Agricultural Research Service, Vegetable Crops Research Unit, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706-1590, USA
[e] Institute for Breeding Research on Horticultural Crops, Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Erwin-Baur-Str. 27, DE 06-484 Quedlinburg, Germany

ARTICLE INFO

Article history:
Received 13 April 2016
Received in revised form 16 June 2016
Accepted 17 June 2016
Available online 29 June 2016

Keywords:
DNA C-value
Genome size
Carrot
Interspecific variation
Intraspecific variation

ABSTRACT

The genus *Daucus* (Apiaceae) comprises species from around the world, covering a wide climatic range, and showing great morphological plasticity. Both cultivated and wild forms are described within the genus. The aim of the present study was to estimate the genome size variability in the collection of diploid *Daucus* species differing in chromosome number (2n = 18, 20 or 22) and originating from various regions of the world. In total, the 2C DNA content in 19 accessions of nine wild *Daucus* species, as well as in 22 wild and 26 cultivated accessions of *Daucus carota* L. was measured by flow cytometry. The 2C DNA content varied over 3-fold, from 0.920 pg in *D. carota* subsp. *maximus* (2n = 18, Spain) to 3.019 pg in *D. littoralis* (2n = 20, Israel). The interspecific genome size differences within wild *Daucus* species were pronounced, e.g. *D. carota* (2n = 18, Greece) possessed 0.940 pg/2C DNA, *D. broteri* (2n = 20, Cyprus) 2.218 pg/2C and *D. montevidensis* (2n = 22, South America) 1.295 pg/2C. In the group of 22-chromosome *Daucus* species, high similarity in nuclear DNA content among different accessions of one species was observed, in contrast to the 20-chromosome *Daucus* species, suggesting possible taxonomical misclassifications of accessions within the latter group. The nuclear DNA content of non-cultivated forms of *D. carota* varied from 0.920 pg/2C in subspecies *maximus* (Spain) to 1.154 pg/2C in subspecies *halophilus* (Portugal), which reflects 20% difference. In turn, in the group of cultivated carrots we observed a high homogeneity among the studied accessions, the 2C DNA content ranged between 0.950 pg ('Kuroda type II', China; 'Imperial Long Scarlet', Japan; 'Koral', Poland) and 0.977 pg ('Cape Market', South Africa). The mean 2C value calculated for both wild and cultivated forms of *D. carota* amounted 0.973 pg.

1. Introduction

The genus *Daucus* is a member of the Apiaceae family (previously Umbelliferae) and includes about 20 recognized species (Spooner et al., 2014) that are widespread in the Northern Hemisphere, with few species in South America and Australia (Grzebelus et al., 2011). They are usually herbaceous biennials, rarely annuals, growing from thin to very stout taproots (Rubatzky et al., 1999). The ancestral area of the genus *Daucus* encompasses the Mediterranean region, particularly North Africa, where extensive speciation took place (Camadro et al., 2007). The most recognizable species belonging to this genus is *Daucus carota* L., which exists in both cultivated and wild forms. Because of a high level of morphological variation, several subspecies of *D. carota* were identified. However, the proposed classifications differed in the number of recognized subspecies from four (Sáenz Laín, 1981), to ten (Pujadas Salvà, 2003), to eleven (Heywood, 1986). The nominative subspecies *D. carota* L. subsp. *carota* is the most widespread species of the genus *Daucus*, presently appearing in temperate climatic zone (Grzebelus et al., 2011). Most of *Daucus* species found so far were diploids, with the chromosome number 2n = 2x = 18, 20, or 22 for wild species and

* Corresponding author.
E-mail address: e.grzebelus@ogr.ur.krakow.pl (E. Grzebelus).

http://dx.doi.org/10.1016/j.scienta.2016.06.023
0304-4238/

2n = 2x = 18 for wild and cultivated *D. carota* (Rubatzky et al., 1999; Iovene et al., 2008; Grzebelus et al., 2011; Arbizu et al., 2014a).

Daucus carota L. subsp. *sativus* Hoffm. is the only cultivated form of the genus *Daucus* and simultaneously the most notable umbellifer cultivated worldwide (Rong et al., 2010; Arbizu et al., 2014a). With a current annual world production reaching above 37 million tons harvested in 2013 (FAOSTAT, 2015), it ranks among the top ten vegetable crops. Recently, application of advanced methods of molecular biology and bioinformatic tools pointed that Central Asia was the primary center of carrot domestication (Iorizzo et al., 2013), which is in accordance with records in historical documents reporting that Central Asia and Asia Minor were the first regions of carrot cultivation. Subsequently, its cultivation spread into Western Europe and other regions of the world (Stolarczyk and Janick, 2011). Edible carrot is generally divided into either eastern or western type. Eastern carrots generally grown in Asia, have root color other than orange due to the presence of anthocyanin (purple), lutein (yellow), or lycopene (red), and lack or show only low content of beta-carotene. They are poor in pro-vitamin A carotenoids and may be rich in phenolic compounds that is particularly well exhibited in purple roots containing anthocyanins (Leja et al., 2013). Western carrots, likely selected from yellow cultivated carrots (Iorizzo et al., 2013), are characterized by high content of pro-vitamin A carotenoids, mostly β-carotene responsible for the orange root color (Simon, 2000). Additionally, they are sweeter, having on average 18% higher sugar content than eastern carrots (Baranski et al., 2012a). Also, western cultivars are better adapted for modern commercial production and processing.

In order to improve existing cultivars of carrot or to develop novel ones with better taste, increased level of phytonutrients or tolerant against biotic/abiotic stress factors, plant breeders often need to access a wider range of diversity. Such diversity can be found in seed collections of plant genetic resources preserved in gene banks (Baranski et al., 2012a). Availability of materials from gene banks has enabled extensive evaluation of both cultivated carrots and wild members of *Daucus* considering many aspects (Baranski et al., 2012a,b; Arbizu et al., 2014a,b; Grzebelus et al., 2014; Spooner et al., 2014). Although, numerous studies have been carried out within the genus *Daucus,* the nuclear DNA content, which is a sort of basic, genetic information characterizing species/accession, has been reported only for a few *D. carota* subspecies (Bennett and Smith, 1976; Arumuganathan and Earle, 1991; Bennett and Leitch, 1995; Bai et al., 2012; Pustahija et al., 2013; Tavares et al., 2014) and for several wild *Daucus* species (Bennett and Smith, 1976).

Genome size is a distinguishable character of living organisms, of high importance in biological and biodiversity research (Bennett et al., 2000; Zavesky et al., 2005). Numerous works showed that estimates of genome size were vital for systematic and evolutionary considerations (Knight et al., 2005; Bancheva and Greilhuber, 2006; Naganowska et al., 2006; Bainard et al., 2013). The nuclear DNA content is probably a feature under strict genotypic control within defined limits (Bennett et al., 2000). There is an ongoing discussion on factors determining and controlling plant genome size, and especially on mechanisms responsible for the DNA gain or loss during evolution, domestication and breeding (Bennett et al., 2000; Knight et al., 2005).

Among the diverse methods used for estimation of plant nuclear DNA content, flow cytometry (FCM) is a fast and accurate method, and nowadays it is frequently applied due to its reliability, ease and relatively low cost (Doležel et al., 2007). Despite many published contributions to this field, the proportion of angiosperms with known genome size is still low, below 2% [ca. 6300 species out of 360,000 species belonging to this group] (Valles et al., 2014).

In the present paper, FCM was used to estimate the 2C DNA content in a broad collection of wild *Daucus* species as well as in wild and cultivated accessions of *D. carota.* To our knowledge, this is the first study on the DNA content in the genus *Daucus* based on data collected from such a large number of accessions originating from various regions of the world. Also, this is the first estimation of genome size for five wild species and eight *D. carota* subspecies.

2. Materials and methods

2.1. Plant material

The 2C DNA content was estimated for 67 accessions: nine wild *Daucus* species (19 accessions), 14 wild *D. carota* subspecies (22 accessions, hereinafter referred to as 'wild *D. carota*'), and 26 cultivated, edible carrots (*Daucus carota* L. subsp. *sativus* Hoffm.). Seeds were obtained from gene bank collections, research institutes or commercial sources (Table 1). According to seed providers' information, the accessions originated mostly from Europe (8 wild *Daucus* and 15 wild *D. carota* accessions, 8 cultivated carrots) and Asia (6 wild *Daucus* and 2 wild *D. carota* accessions, 13 cultivated carrots). Accessions were also collected in Africa (3 cultivars), South America (4 wild *Daucus* and 2 wild *D. carota*), North America (1 wild and 1 cultivated carrots), New Zealand (1 cultivar), and the Mediterranean (1 wild *Daucus* accession). For two accessions of wild *D. carota* there was no information available regarding their origin. In the case of edible carrots, most of the accessions used in the present research were described as advanced cultivars (16) with orange (15) or purple (1) roots. A less numerous group was represented by landraces (9) producing orange (5), yellow (3), or purple (1) roots. One accession was described as a breeding line with orange root color. A more detailed description of that material can be found in Baranski et al. (2012a).

Plants were grown in 3:1 sand and commercial humus mixture in 10 or 19 cm pots. Glasshouse conditions were optimized for carrot growth, i.e. 20–25/10–15 °C (day/night), and ca. 60% relative humidity.

2.2. Flow cytometry

For nuclear DNA content estimation *Brassica napus* L. cv. 'Bor' (2C = 2.180 pg; estimated using male human leukocytes with 2C = 7 pg) was used as an internal standard for most of the accessions, and for some species *D. carota* subsp. *sativus* cv. 'Dolanka' (2C = 0.955 pg; estimated in the present research) served as an intermediate standard (Table 1). Nuclei were isolated from young leaves by chopping in 1 ml of Galbraith's buffer (Galbraith et al., 1983), supplemented with 1% (w/v) polyvinylpyrrolidone (PVP-10), propidium iodide (PI; 50 μg ml^{-1}), and RNase A (50 μg ml^{-1}). Then, the suspension was passed through a 50-μm mesh nylon filter and analyzed using a CyFlow SL Green (Partec GmbH, Münster, Germany) flow cytometer, equipped with a high-grade solid-state laser with green light emission at 532 nm, long-pass filter RG 590 E, DM 560 A, as well as with side (SSC) and forward (FSC) scatters. For each sample, the DNA content of 2000–5000 nuclei was measured. Analyses were performed on 6–26 individuals per accession (depending on plants availability; Table 1), using a linear amplification. Histograms (CV = 3.31-6.44%) were evaluated using the FloMax program (Partec GmbH, Münster, Germany). Nuclear DNA content was calculated using the linear relationship between the ratio of the 2C peak positions of the target species/internal standard on the histogram of fluorescence intensities.

2.3. Statistical analysis

Mean values, standard deviation (SD), coefficient of variation (CV) for the 2C DNA content, as well as 1C-value (Mbp) for each accession, were calculated using Excel 7.0. A one-way analysis