Nuclear DNA Content, Selected Morphological and Anatomical Traits of Narcissus Cultivars and Breeding Clones

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Abstract: The genus Narcissus belongs to the family Amaryllidaceae. This genus has been the subject of numerous cytological and cytometric studies and have shown enormous variation in terms of genome size, ploidy level, and even the basic chromosome number. The basic chromosome numbers are 5 or 7, but 10, 11, and 12 have been recorded as well. Most narcissus cultivars are euploid tetraploids. There are also numerous triploids. Some cultivars are aneuploid such as tetraploids or triploids, with missing chromosomes or possessing additional chromosomes. Due to their very complex parentage, cultivars have various numbers of chromosomes not found in the species. In this publication, we present a study on the genome size and assessment of the likely ploidy level of 38 cultivars and breeding clones of Narcissus in relation to their selected morphological traits and information on their parental forms. For the first time, 12 Polish cultivars and breeding clones of narcissus were the subject of such an evaluation. Perianth diameter, leaf length, and width were evaluated and rated with notes according to the descriptor of the International Union for the Protection of New Varieties of Plants. Stomatal density and stomata length were measured using light microscopy. Analysis of genome size was carried out using flow cytometry. For three selected genotypes, the chromosome number was counted. Our results lead to the general conclusion that the morphological traits studied and nuclear DNA content can be useful for determining the possible ploidy level of narcissi. The information on the origin and parental forms of narcissi can be helpful in determining the ploidy level of narcissi. However, clear confirmation of ploidy level requires verification of chromosome number and preferably karyotyping. The results obtained are a prelude to further studies.

Keywords: chromosome; daffodils; flow cytometry; genome size; light microscopy; phenotype; stomata

1. Introduction

Narcissus L. (narcissus, daffodil) is one of the most important spring-flowering bulbous crops cultivated worldwide. Uses of Narcissus includes bulb production, the cultivation of cut flowers from the ground, cut and potted flowers from greenhouses, and the use of bulbs in gardens and urban green areas. The largest producer of Narcissus bulbs is the United Kingdom with an area of over 4000 ha [1]; second are the Netherlands with an area of 1280 ha [2]. The narcissus industry in United Kingdom is estimated to have an annual output value of around 45 million pounds [1]. Bulbs of this crop on the area larger than 50 ha are also produced in Australia, Canada, Israel, USA, and Poland [3]. A huge number of cultivars of the genus Narcissus are grown worldwide. The last International Daffodil Register and Classified List of Royal Horticultural Society, London, UK, contains 26 thousand names of genotypes ordered in 13 groups (divisions) of horticultural classification [4]. Commercial cut-flower production is dominated by cultivars from the trumpet (i.e., ‘Dutch
Master’, ‘Golden Harvest’) and large-cupped (i.e., ‘Carlton’) daffodil divisions, and the most important cultivar in pot production is ‘Tête-à-Tête’ from division 12 (other daffodil cultivars; former division 6—cyclamineus daffodil cultivars). More than 50% of newly bred and registered cultivars in recent years also belong to the trumpet and large-cupped daffodil divisions [5].

The genus Narcissus belongs to the family Amaryllidaceae and has a mainly Mediterranean distribution. The species of Narcissus originate primarily from the Iberian Peninsula, they also occur in south-western France, northern Africa and eastwards to Greece, in Asia Minor and Syria [3,6]. N. tazetta L. originated from the Mediterranean region, is doubtless subspontaneous in Persia and Cashmir [7] and was also introduced to China and Japan in ancient times and known now as N. tazetta var. chinensis (M. Roem.) Masam. & Yanagih [6].

In horticultural production, narcissi are propagated vegetatively by means of daughter bulbs (offsets), but also by bulb segmentation (chipping), twin-scaling, and micropropagation in vitro [3,6]. Production from seeds is, of course, the method used by breeders and is also important for species that form few offsets [3]. In nature, species reproduce in both ways (i.e., by producing progeny bulbs and by seeds). There are great taxonomic difficulties with the genus Narcissus since many species have been cultivated for a long time, there has been extensive selection of cultivars and ecotypes, and there has been extensive natural hybridization in their original environment, with intentional breeding by humans for several hundred years [8]. This situation has promoted intensive genetic changes, including those related to chromosome number, DNA content, and plant ploidy levels. Marques et al. even suggest that natural hybrid populations are composed of a mixture of markedly different hybrid genotypes produced either by structural chromosome changes, consistent with classic cytogenetic studies in Narcissus, or by transposon-mediated events [9].

The total number of Narcissus species is estimated from 27 according to Flora Europaea [10], 36 [11] to 41 [12]. Chromosome number in the Narcissus species and cultivars was first assessed almost 100 years ago [13], but much of the pioneering cytogenetic research was carried out by Fernandes starting in 1934 [7,14]. Then, in the 1980s and 1990s, the chromosome number was known in more than 1000 genotypes, species, and cultivars of Narcissus [15,16]. This genus has been the subject of numerous cytological and cytometric studies which have shown enormous variation in terms of genome size, ploidy level, and even their basic chromosome number [9,11,16–19]. In the majority of species, the basic chromosome number is 7 (x = 7) including, e.g., N. poeticus L. and N. hispanicus Gouan, but 10, 11 and 12 have been also recorded in other species [9,16,19]. The second basic chromosome number is 5 (x = 5) for subgenus Hermione [20]. According to the Kew Database [12], in diploid species, the most common chromosome number is 14 (N. pseudonarcissus L. [17], N. poeticus [21], N. hispanicus, N. cyclamineus DC. [11]), but there are also 20 in (N. tazetta), 22 (N. papyraceus Ker Gawl.) [17] and even 19 (N. × alentejanus Fernandes Cacas) or 29 (N. × perezlarae Font Quer) [22]. Some species such as N. poeticus and N. hispanicus occur in the form of diploids and triploids, and N. pseudonarcissus as diploid and hexaploid as N. pseudonarcissus subsp. bicolor (L.) Willk. & Lange. Recently, more polyploids such as tetraploid N. papyraceus Ker Gawl. (2n = 4x = 22) and hexaploid N. dubius Gouan (2n = 6x = 50) have been reported [17]. Within the Narcissus species, 2C value for the same chromosome number (2n = 2x = 14) is also very diverse from 13.00 pg in N. hedraeanthus (Webb & Held.) Colmeiro [23] to 38.20 pg in N. nevadensis Pugsley [17].

Most narcissus cultivars are euploid tetraploids. However, there are also numerous triploids [15,16]. Some cultivars are either aneuploid tetraploids or triploids with missing chromosomes or possessing additional chromosomes. According Ramanna et al. [24], among ornamental geophytes there is a tendency to replace diploids with polyploids cultivars, which is especially visible in Narcissus and Lilium. Due to the very complex parentage, cultivars have various numbers of chromosomes not found in the species, e.g., some diploid cultivars have been reported to possess 24 chromosomes (‘Dutch Master’) and 28 or 29 chromosomes (‘Beersheba’) [19,24]. In addition, a higher number of chromosomes (28) was
reported by other researchers [25,26]. On the other hand, the number of 24 chromosomes minus or plus 1-2 chromosomes have been recorded for triploid cultivars, e.g., ‘Tête-à-Tête’ and ‘Cheerfulness’ based on karyotype analysis [18]. Thus, knowing the chromosome number and/or genome size it difficult to state clearly the ploidy level without knowledge of cultivar parentage and appearance.

It is common knowledge that, in many cases, higher ploidy level of ornamental plants manifests itself in bigger flowers, bigger leaves, and better plant vigour in relation to their diploid counterparts (which was confirmed for *Hemerocallis* [27,28] and *Tulipa* [24,29]). Many studies also confirm the relationship between the size of stomata and stomata density with the level of ploidy of ornamental plants, including geophytes. There are reports that as ploidy levels increase, the stomatal density decreases [30] but stomata size increases [27,28,30,31]. It can also be assumed that for the genus *Narcissus*, such relationships exist and morphological traits can be found that can become markers of ploidy level.

In this publication, we present a study on the genome size and assessment of the likely ploidy level of 38 cultivars and breeding clones of narcissus (*Narcissus* L.) in relation to their selected morphological and anatomical traits, and information on their parental forms. For the first time, 12 Polish cultivars and breeding clones of narcissus were the subject of such studies.

Studying the genome size and assessing the ploidy level of *Narcissus* cultivars collected in the genebank, representing most groups of the horticultural classification of the genus *Narcissus*, has a scientific but also a practical aspect for use in breeding aimed at obtaining plants with more robust and showy flowers. Finding morphological markers related to the ploidy level of plants may be helpful in developing a rapid identification method useful for cultivar recognition and/or breeding as a preliminary test for ploidy assessment.

2. Material and methods

2.1. Plant Material

Thirty-eight genotypes (cultivars and breeding clones) of narcissus (*Narcissus* L.), including twelve Polish ones, derived from the genebank located at the National Institute of Horticultural Research, Skierniewice, Poland, were used for the study. All genotypes were grown in the field and included in the genebank at least five years before the study. Evaluated narcissus accessions belonged to different horticultural classification’s divisions according to The International Daffodil Register & Classified List of the Royal Horticultural Society (RHS), London, UK [4] (Table 1). All cultivars and clones included in the study are listed alphabetically in Table 1 together with the names of the horticultural classification division, the flower color code, and information on their parental forms and year of registration or breeder’s creation. The used international flower color code according to RHS [4] provides the information about the basic colors of the flower perianth segments and the corona: W, white or whitish; G, green; Y, yellow; P, pink; O, orange; R, red. The letter(s) before the hyphen describe the perianth segments, and after the hyphen they describe the corona. The genotypes tested represented 6 out of 13 divisions of the RHS horticultural classification, and most belonged to the divisions of large-cupped daffodil cultivars and trumpet daffodil cultivars, which are still the most abundantly represented in the horticultural market and still bred for the most part [5].

2.2. Phenotype Evaluation of Plants

The phenotype evaluation of 38 genotypes of narcissus based on three selected characteristics of the International Union for the Protection of New Varieties of Plants (UPOV) descriptor for the genus *Narcissus* was made [32]. The perianth diameter, leaf length, and width of fifteen plants of each genotype were evaluated and rated with notes according to the UPOV descriptor [32]. Perianth diameter and notes were determined according to the following rules: <6 cm (small, note 3); 6–9 cm (medium, 5); >9 cm (large, 7), leaf length: <20 cm (short, note 3); 20–30 cm (medium, 5); >30 cm (long, 7) and leaf width:
<1 cm (narrow, note 3); 1–2 cm (medium, 5); >2 cm (broad, 7). The three morphological traits above were chosen due to their frequent correlation with the ploidy level of many ornamental plant species.

Table 1. Phenotype evaluation of 38 genotypes of *Narcissus* according three characters of UPOV descriptor [25] (UPOV 1983) and their origin (if known).

| Cultivar/Breeding Clone | Year of Registration or (Year of Crossing/First Flowering) | Division of Horticultural Classification and Color Code ¹ (Based on [4] and on Our Own Observations in Relation to Polish Cultivars and Clones) | Origin/Parents (Seed Parent at the First Place) (Based on [4, 25] and on Breeder’s Book in Relation to Polish Cultivars and Clones) | Perianth Diameter ² | Leaf Length ³ | Leaf Width ⁴ |
|-------------------------|----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|-------------------|---------------|---------------|
| 'Alayne' (pre-1947)     | 2 W-Y ¹ (long-cupped)                                     | unknown                                                                                                         | 'White Knight' (1 W-W) × seedling                                                                                                     | 5 (medium)       | 7 (long)     | 5 (medium)   |
| 'Beersheba' (pre-1923)  | 1 W-W (trumpet)                                           | 'White Knight' (1 W-W)                                                                                           | 7 (large)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Bridal Crown' (pre-1949) | 4 W-Y (double)                                           | Sport of 'L'Innocence' (8 W-Y; Poetaz = N. poeticus × N. tazetta)                                               | 3 (small)                                                                                                                           | 5 (medium)       | 5 (medium)   |
| 'Broadway Star' 1975    | 11b W-WOO (split-corona)                                   | unknown                                                                                                         | 'William de Silente' × open pollination                                                                                              | 5 (medium)       | 7 (long)     | 5 (medium)   |
| 'Bryza' (1970)          | 1 Y-Y (trumpet)                                           | 'Majorda' × open pollination                                                                                       | 7 (large)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Bursztyniec' 1996      | 2 Y-O (long-cupped)                                       | 'Majorda' × open pollination                                                                                       | 7 (large)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Carlton' (pre-1927)    | 2 Y-Y (long-cupped)                                       | unknown                                                                                                         | 'King Alfred' (1 Y-Y)                                                                                                               | 7 (large)        | 7 (long)     | 5 (medium)   |
| 'Caruso' (= 'Richard Tauber') (pre-1930) | 8 W-GOO or W-Y (tazetta)                               | unknown                                                                                                         | 5 (medium)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Chanterelle' 1962      | 11a Y-Y (split-corona)                                    | unknown                                                                                                         | 'Majorda' × open pollination                                                                                                        | 5 (medium)       | 7 (long)     | 5 (medium)   |
| 'Dick Wilden' 1962      | 4 Y-Y (double)                                            | Sport of 'Carlton' (2 Y-Y)                                                                                         | 7 (large)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Dutch Master' (pre-1938)| 1 Y-Y (trumpet)                                           | unknown                                                                                                         | 'King Alfred' (1 Y-Y)                                                                                                               | 7 (large)        | 7 (long)     | 5 (medium)   |
| 'Fortissimo' 1964       | 2 Y-O (long-cupped)                                       | unknown                                                                                                         | 7 (large)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Golden Ducat' (pre-1947)| 4 Y-Y (double)                                            | Sport of 'King Alfred' (1 Y-Y)                                                                                   | 5 (medium)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Heweliusz' 1997        | 2 Y-O (long-cupped)                                       | 'Aranjuez' (2 Y-YYO) × open pollination                                                                       | 7 (large)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Ice Follies' (pre-1953) | 2 W-W, opens W-Y (long-cupped)                          | 'John Evelyn' (2 W-Y) × unknown                                                                                  | 5 (medium)                                                                                                                           | 5 (medium)       | 5 (medium)   |
| 'John Evelyn' (pre-1920) | 2 W-Y (long-cupped)                                       | 'Tunis × 'Therapia'                                                                                               | 5 (medium)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Joseph MacLeod' (pre-1946) | 1 Y-Y (trumpet)                                         | unknown                                                                                                         | 7 (large)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'King Alfred' (pre-1899) | 1 Y-Y (trumpet)                                           | 'Maximus' (1 Y-Y) × auto-tetraploid or 'Emperor' (1 Y-Y) × 'Maximus'                                            | 5 (medium)                                                                                                                           | 5 (medium)       | 5 (medium)   |
| 'Lajkonik' (1977)       | 1 Y-Y (trumpet)                                           | 'Emperor' (1 Y-Y) × 'Maximus'                                                                                    | 5 (medium)                                                                                                                           | 5 (medium)       | 5 (medium)   |
| 'Lemon Beauty' 1962     | 11b W-WYW or W-Y (split-corona)                           | unknown                                                                                                         | 'Split' × 'Callarosa' (or California Rose? 4 W-P)                                                                                     | 5 (medium)       | 7 (long)     | 5 (medium)   |
| 'Marie-jose' 1974       | 11b W-WYW (split-corona)                                  | 'Papillon Blanc' (11b W-W) × 'Eddy Canzony' (2 W-YYO)                                                           | 5 (medium)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Moneymaker' 1980       | 2 Y-Y (trumpet)                                           | unknown                                                                                                         | 7 (large)                                                                                                                           | 7 (long)         | 5 (medium)   |
| 'Orange Prince' 1933    | 8 Y-O (tazetta)                                           | Poetaz (N. poeticus × N. tazetta)                                                                                | 5 (medium)                                                                                                                           | 7 (long)         | 3 (narrow)   |
| 'Palmares' 1973         | 11a W-P (split-corona)                                    | 'Split' × 'Callarosa' (or California Rose? 4 W-P)                                                               | 5 (medium)                                                                                                                           | 5 (medium)       | 5 (medium)   |
| 'Papillon Blanc' 1960   | 11b W-W (split-corona)                                    | 'Redmarley' (2 Y-O) × open pollination                                                                          | 5 (medium)                                                                                                                           | 7 (long)         | 5 (medium)   |
Table 1. Cont.

| Cultivar/Breeding Clone | Year of Registration or (Year of Crossing/First Flowering) (Based on [4] and on Breeder’s Book in Relation to Polish Genotypes) | Division of Horticultural Classification and Color Code 1 (Based on [4] and on Our Own Observations in Relation to Polish Cultivars and Clones) | Origin/Parents (Seed Parent at the First Place) (Based on [4,25] and on Breeder’s Book in Relation to Polish Cultivars and Clones) | Perianth Diameter 2 | Leaf Length 3 | Leaf Width 4 |
|-------------------------|-----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|----------------|----------------|
| ‘Passat’ 1998           | 2 W-Y (long-cupped)                                                                                           | ‘Redmarley’ (2 Y-O) × open pollination                                                                                                         | 5 (medium)                                                                                                                  | 7 (long)                                                                 | 5 (medium)    |
| ‘Poseidon’ 1992         | 2 Y-Y (long-cupped)                                                                                           | From ‘Majarda’                                                                                                                                | 5 (medium)                                                                                                                  | 7 (long)                                                                 | 5 (medium)    |
| ‘Roseate Hues’ (pre-1944)| 2 W-YP (long-cupped)                                                                                        | unknown                                                                                                                                       | 5 (medium)                                                                                                                  | 7 (long)                                                                 | 5 (medium)    |
| ‘Salome’ 1958           | 2 W-PPY (long-cupped)                                                                                         | ‘Salmon Trout’ (2 W-P) × ‘Rose Caprice’                                                                                                       | 5 (medium)                                                                                                                  | 5 (medium)                                                                 | 5 (medium)    |
| ‘Tête-à-Tête’ (pre-1949) | 12 Y-Y (miscellaneous)                                                                                       | ‘Cyclataz’ (N. tazetta × N. cyclamineus) (8 Y-O) × open pollination                                                                          | 3 (small)                                                                                                                   | 3 (short)                                                               | 5 (medium)    |
| ‘Unsurpassable’ (pre-1923)| 1 Y-Y (trumpet)                                                                                              | unknown                                                                                                                                       | 5 (medium)                                                                                                                  | 7 (long)                                                                 | 5 (medium)    |
| ‘Yellow Cheerfulness’ (1937)| 4 Y-Y (double)                                                                                               | ‘Sport of ‘Cheerfulness’                                                                                                                      | 3 (small)                                                                                                                   | 7 (long)                                                                 | 5 (medium)    |
| 0.1138-a (1976)         | 1 Y-Y (trumpet)                                                                                               | Seedling 639 × ‘Tintoretto’                                                                                                                   | 5 (medium)                                                                                                                  | 7 (long)                                                                 | 5 (medium)    |
| 0.919-a (1971)          | 1 Y-Y (trumpet)                                                                                               | Seedling 639 × ‘Tintoretto’                                                                                                                   | 7 (large)                                                                                                                   | 7 (long)                                                                 | 7 (broad)     |
| 0.985G (1972)           | 2 Y-O (long-corona)                                                                                           | Seedling 606 × ‘Burgem. Gouverner’                                                                                                            | 7 (large)                                                                                                                   | 7 (long)                                                                 | 5 (medium)    |
| 0.985T (1972)           | 1 Y-O (trumpet)                                                                                               | Seedling 606 × ‘Burgem. Gouverner’                                                                                                            | 7 (large)                                                                                                                   | 7 (long)                                                                 | 5 (medium)    |
| 7/97 (1997)             | 1 W-Y (trumpet)                                                                                               | unknown                                                                                                                                       | 7 (large)                                                                                                                   | 7 (long)                                                                 | 7 (broad)     |
| 8/97 (1997)             | 1 Y-Y (trumpet)                                                                                               | unknown                                                                                                                                       | 5 (medium)                                                                                                                  | 7 (long)                                                                 | 5 (medium)    |

1 Color code: W, white or whitish; G, green; Y, yellow; P, pink; O, orange; R, red. The letter(s) before the hyphen describe the perianth segments, after the hyphen—describe the corona; 2 ≤6 cm (small, note 3); 6–9 cm (medium, 5); >9 cm (large, 7); 3 ≤20 cm (short, 3); 20–30 cm (medium, 5); >30 cm (long, 7); 4 ≤1 cm (narrow, note 3); 1–2 cm (medium, 5); >2 cm (broad, 7).

2.3. Stomatal Density and Stomata Length

Stomata were measured using light microscopy. The leaf samples (2 cm in length) were collected from the part distant 10 cm from the leaf tip, in mid-May, after the flowering period. The abaxial epidermis was isolated with a transparent adhesive tape and stained with toluidine blue and next mounted on slides for microscopic observations according to the procedure of Dyki and Habdas (1996) [33]. The stomata lengths were determined for three leaves (×10 stomata) of each genotype counted and measured using a Nikon Eclipse 80i microscope with the program NIS-Elements BR 2.30 at 200 times magnification. For each sample, the stomatal density (i.e., number of stomata per field of view) was counted on the basis of the 15 fields of view.

2.4. Nuclear DNA Content

Analysis of genome size was carried out using flow cytometry (FCM/PI) (CyFlow PA, Partec, Münster, Germany). Samples were taken in mid-May from six leaves collected from six plant of each analyzed cultivar. Leaf tissue (0.5–1 cm²) was chopped together with a piece (1 cm³) of plant internal standard in a Petri dish in 0.5 mL nuclei isolation Galbraith’s buffer [34] to which propidium iodide (50 µg/mL) and RNasa (50 µg/mL) were added [35]. As an internal standard, the young leaves of Vicia faba ‘Inovec’ (2C = 26.9 pg DNA) were used. The exception was the cultivar with the smallest genome, ‘Bridal Crown’ (27.69 pg), which was analyzed with the standard of Agave americana (15.9 pg) since the peaks of V. faba ‘Inovec’ overlapped with those of the narcissus genotype tested. After adding 1.5 mL of the isolation buffer, the samples were filtered through a 30 µm filter and incubated for...
30 min in room temperature. The fluorescence of the nuclei was measured using CyFlow Ploidy Analyser with CyView software (CyFlow PA, Partec, Münster, Germany) with an Nd-YAG green laser at 532 nm. Data were analysed by means of CyView software. The 2C DNA content of a sample was calculated as the sample peak mean divided by the standard plant peak and multiplied by the amount of DNA of the standard plant. Samples with at least 5000 nuclei were measured for six leaves (one leaf of each plant) with two runs from each nuclei isolation extract.

2.5. Chromosome Number

For three selected genotype the chromosome number were counted. Our selection included three cultivars representing different ploidy levels (‘Dutch Master’ as a diploid, ‘Yellow Cheerfulness’ as a triploid and ‘Ice Follies’ as a tetraploid). Bulb roots were dipped in a 0.25% aqueous solution of colchicine for 20–24 h. The root tips were macerated with 0.1 M HCl for 30 min at 60 °C, followed by isolation and fixation of them for 24 h in a mixture of acetic acid and ethanol (1:3). Crushed, microscopic slides of the roots with dry ice were stained with 2% aceto-orcein for 24 h. The chromosome counts were made using a Nikon Eclipse 80i microscope. The evaluation of metaphase chromosomes in the cells of root meristems was carried out. At least three metaphases were observed and compared in one root.

2.6. Statistical Analyses

To compare the degree of variation in nuclear DNA content, the standard deviation (SD) was determined. The results of number and length of stomata were analyzed by the analysis of variance (ANOVA) and post hoc Duncan’s Multiple Range test at the 5% significance level, using SPSS, the version PS IMAGO 4.0 (IBM Statistics 24).

3. Results and Discussion

3.1. Phenotype Evaluation of Plants

Among all of the assessed genotypes of narcissus, only three cultivars were characterized by a small diameter of the perianth (<6 cm): double (division 4) ‘Bridal Crown’ and ‘Yellow Cheerfulness’, and dwarf ‘Tête-à-Tête’ from division 12 (other daffodil cultivars), large (6–9 cm) by 13 genotypes, and medium (>9 cm) by the remaining 22 genotypes (Table 1). Only one genotype, the dwarf cultivar ‘Tête-à-Tête’ had short leaves (<20 cm in length). Five genotypes had medium length leaves (20–30 cm), and thirty-two genotypes had long leaves. Regarding leaf width, the dwarf ‘Orange Prince’ from division 8 (tazetta daffodil cultivars) had narrow leaves (<1 cm), 2 breeding clones (0.919-a and 7/97) had wide leaves (>2 cm), all other genotypes had medium leaf width (1–2 cm). Some of the traits described appear to be related to the level of ploidy of the genotype (Tables 1 and 2). Among others, the diploid cultivar ‘Bridal Crown’ and triploid cultivars ‘Yellow Cheerfulness’ and ‘Tête-à-Tête’ were characterized by small perianth diameter. Additionally, the cultivar ‘Tête-à-Tête’ had short leaves and the triploid ‘Orange Prince’ was characterized by narrow leaves. Tetraploids had medium to high values of the morphological traits studied. This correlation was not confirmed only for the triploid cultivar ‘Caruso’, which had medium perianth diameter and long and medium leaf length and width, respectively. Obtained results confirmed other reports concerning phenotype evaluation of higher ploidy level of ornamental geophytes in relation to their diploid counterparts. Leaves and flowers of obtained tetraploid plants of daylilies (Hemerocallis) were significantly larger in comparison to their diploid parent cultivars [27,28]. Also, polyploid tulips, especially triploid cultivars, proved to be superior to their diploid parents, usually having larger flower size, sturdier stems, broader and thicker leaves, or more compact plants [24,29]. Among ornamental geophytes, there is a tendency to replace diploids with polyploid cultivars, a trend which is especially visible in Narcissus, Lilium, and Hemerocallis [24,36,37]. At present, nearly 75% of Narcissus cultivars are tetraploids while the diploids and triploids amount to only about 12% each [38]. Polyploids, including tetraploids of many ornamental crops, have taken a
leading position among the cultivars due to desirable traits such as vigorous growth and larger flowers, sometimes with more intense color or other interesting characteristics. In the case of polyploidization of diploid cultivar of *Tulipa* [31] smaller flowers and fragile stems were obtained in tetraploids. Despite these disadvantages, the obtained tetraploids are characterized by a compact plant habit and more fringed tepals, which can be considered advantages. Our results do not always strongly indicate a relationship between the morphological traits studied and the level of ploidy, but they do indicate such a tendency.

3.2. Stomatal Density and Stomata Length

The stomatal density ranged from 5.80 (for tetraploid ‘Moneymaker’) to 18.40 (for diploid ‘Bridal Crown’) (Table 2). This confirms other reports that as ploidy levels increase, the stomatal density decreases. The stomatal density was reduced in tetraploid plants of *Lilium regale* compared to diploid [30] and in polyploids of *Tagetes erecta* [39]. A relatively high number of stomata (12.47) was also recorded for the cultivar ‘Lajkonik’, which could be considered a tetraploid based on the amount of cDNA. This could indicate either a different level of ploidy in this cultivar or a lack of correlation between a higher level of ploidy and a lower number of stomata. The predominant majority of the values obtained are between 6.73 and 8.73 pcs.

The size of stomata (the average length of 30 measurements) ranged from 46.1 for diploid cultivar ‘Bridal Crown’ to 60.9 and concern tetraploids (Figure 1A–C; Table 2). The difference between the shortest stomata and the longest was nearly 34%. The smallest stomata was less than in 34 of the 38 genotypes tested. Our results confirm other reports. Stomata of tetraploid plants of daylilies (*Hemerocallis*) were longer by 30–35% than those of diploid parent cultivars [27,28]. In the case of tulips (*Tulipa*) stomata were longer by 30% in tetraploids as compared to diploids [31]. An increase in the length of stomata was found also in polyploid plantlets of lilies (*L. regale*) [30] and African marigold (*T. erecta*) [39]. The study of McGoey et al. [40] in the case of *Crataegus* shows that stomata size may be useful in differentiating between tetraploid *C. douglasii* and diploid and triploid *C. suksdorfii*. They found that stomata differed between the two species, with *C. douglasii* having larger average stomata sizes than *C. suksdorfii*.

![Microscopic images of the stomata seen on the abaxial side of narcissus leaves, along with their marked length: (A) breeding clone 0.1138-a; (B) cultivar ‘Beersheba’; and (C) cultivar ‘Roseate Hues’.](image)

**Figure 1.** Microscopic images of the stomata seen on the abaxial side of narcissus leaves, along with their marked length: (A) breeding clone 0.1138-a; (B) cultivar ‘Beersheba’; and (C) cultivar ‘Roseate Hues’.

3.3. Nuclear DNA Content

For 33 tested genotypes (12 Polish cultivars and breeding clones and 21 foreign cultivars), the DNA content ranged from 46.89 to 52.2 pg (Table 2), which indicates that they can be preliminary considered tetraploids based on the results for the reference cultivar ‘Ice Follies’ with a known from the literature number of chromosomes 2n = 4x = 28 [16,17,26] and 48.80 pg of nuclear DNA [17]. For four cultivars, the nuclear DNA content ranged from 39.21 pg to 41.42 pg and these cultivars can be considered triploids. In reference triploid cultivars ‘Tête-à-Tête’ and ‘Yellow Cheerfulness’, having 2n = 3x = 24 chromosomes, 2C value was 39.21 and 40.80 pg, respectively, which were very close to the reported by Zonneveld [11]. In addition, more recent studies confirmed that cultivar ‘Tête-à-Tête’ (originated...
from crossing diploid hybrid Cyclataz $2n = 2x = 17$, what means crossing of *Narcissus cyclamineus* ‘Soleil d’Or’ and *N. tazetta*, with unknown diploid parent with $2n = 14$), has indeed two genomes of *N. cyclamineus* and one genome of *N. tazetta* together with a B chromosome ($2n = 3x = 24 + 1B$) [14,34]. Based on the 2C value, two other cultivars ‘Caruso’, ‘Orange Prince’ could be also triploids. On the other hand, in the cultivar ‘Orange Prince’ with a DNA content of 41.42 pg, having in its pedigree *N. poeticus* and *N. tazetta*, the number of chromosomes may be $2n = 26$.

The lowest content of nuclear DNA (27.69 pg) was recorded for the cultivar ‘Bridal Crown’ from the group of double narcissi, which may confirm the literature data on the 2C DNA value of 28.2 pg and the number of chromosomes $2x = 17$ [11]. This cultivar may be considered a diploid. Most of the diploid cultivars possess 14 chromosomes. However, in some diploid cultivars, the number of chromosomes ranges from 10 to 28. This is due to the fact that the origin of the majority of cultivars is very complex. The cultivar ‘Bridal Crown’ is a sport of the cultivar ‘L’Innocence’ with $2n = 17$, which originated from a cross between *N. poeticus* ($2n = 14$) and *N. tazetta* ($2n = 4x = 20$) or cultivars derived from these species. None of the Polish cultivars or breeding lines turned out to be diploid.

Surprisingly, two of the cultivars—‘Dutch Master’ and ‘Fortissimo’—showing 2C values of approximately 48 pg having 24 [19] or 28 [25,26] chromosomes (‘Dutch Master’) and 28 [19] chromosomes (‘Fortissimo’) have been considered diploids based on karyotype analysis [19]. The origin of both cultivars is unfortunately unknown. The explanation why the plants with a high 2C value and a large number of chromosomes indicating a tetraploid level appears to be diploid can be found in the phenomenon of diploidization. This phenomenon involves many changes in genome organization that ultimately restore bivalent chromosome pairing and disomic inheritance [41].

Examples of the flow cytometry histograms for selected *Narcissus* genotypes with different nuclear DNA contents and thus with probably different ploidy levels are shown in Figure 2.

![Flow Cytometry Histograms](image-url)

**Figure 2.** Histograms of flow cytometry of selected *Narcissus* genotypes: (A) cultivar ‘Bridal Crown’, assumed to be diploid; (B) ‘Caruso’, assumed to be triploid; and (C) ‘King Alfred’ and (D) ‘Pappillon Blanc’, assumed to be tetraploids.
Table 2. Nuclear DNA content, chromosome number, and stomata number and length of 38 cultivars and breeding clones of narcissus.

| Cultivar/Breeding Clone | Nuclear DNA Content [pg] | Chromosome Number | Ploidy Level | Stomatal Density [pcs.] | Lenght of Stomata [µm] |
|-------------------------|--------------------------|-------------------|--------------|--------------------------|-------------------------|
|                         | Authors’                  | References        | Acc. References |                         |                         |
| 'Bridal Crown'          | 27.69 ± 0.14              | 28.2 [15]         | 17 [15,24,42]  | 2n = 2x [17,26]          | Diploid 18.40 ± s       |
| 'Tè-te-à-Tè'           | 39.21 ± 0.29              | 39.7 [9]          | 24 [15,16] 24 + 1B [12,24,43] | 2n = 3x [15] | Triploid 8.33 b–j 53.8 d–j |
| 'Caruso'                | 40.62 ± 0.16              | -                 | -            | -                        | -                       |
| 'Yellow Cheerfulness'   | 40.80 ± 0.17              | 40.9 [11]         | 24 [15,17,25] | 2n = 3x [11]            | -                       |
| 'Orange Prince'         | 41.42 ± 1.11              | -                 | -            | -                        | -                       |
| 'Moneymaker'            | 46.89 ± 0.53              | -                 | -            | -                        | -                       |
| 'Unsuspassable'         | 46.94 ± 0.19              | -                 | -            | -                        | -                       |
| 'Joseph MacLeod'        | 47.07 ± 0.79              | -                 | -            | -                        | -                       |
| 'King Alfred'           | 47.26 ± 0.54              | 48.9 [15,19]      | 28 [15,25]   | -                        | 8.53 i–k 54.8 e–k       |
| 'Dutch Master'          | 47.18 ± 0.37              | 46.9 [15]         | 24 [19], 28 [25,26] | 2n = 2x 4x = 28 [26] | Diploid/Tetraploid 7.20 c–f 52.6 c–i |
| '7/97'                  | 47.96 ± 0.64              | -                 | -            | -                        | -                       |
| 'Palmas'                | 48.10 ± 0.52              | -                 | -            | -                        | -                       |
| 'Golden Ducat'          | 48.12 ± 0.19              | 47.7 [15]         | 28 [15,17,25] | -                        | -                       |
| 'Ice Follies'           | 48.23 ± 0.73              | 48.80 [15]        | 28 [16,17,26], 14 or 28 [25] | 2n = 4x [17,26] | Tetraploid 9.27 k–l 55.1 e–k |
| 'Dick Wilden'           | 48.33 ± 0.69              | -                 | -            | -                        | -                       |
| 'Chantarelle'           | 48.37 ± 0.36              | -                 | -            | -                        | -                       |
| 'Beersheba'             | 48.40 ± 0.31              | -                 | -            | -                        | -                       |
| 'Carlton'               | 48.41 ± 0.31              | 47.6 [15]         | 28 [16,17,25] | -                        | -                       |
| 'Fortissimo'            | 48.44 ± 0.32              | -                 | 28 [19]      | 2n = 2x [19]            | Diploid 8.27 g–j 56.8 h–l |
| 'Roseate Hues'          | 48.48 ± 0.28              | -                 | -            | -                        | -                       |
| 0.1138-a                | 48.48 ± 0.18              | -                 | -            | -                        | -                       |
| 'Heveliuss'             | 48.49 ± 0.43              | -                 | -            | -                        | -                       |
| 0.985G                  | 48.65 ± 0.19              | -                 | -            | -                        | -                       |
| 'Salome'                | 48.68 ± 0.23              | -                 | 28 [15,25]   | -                        | -                       |
| 'Lajkonsk'              | 48.69 ± 0.50              | -                 | -            | -                        | -                       |
| 0.98ST                  | 48.75 ± 0.36              | -                 | -            | -                        | -                       |
| 0.919-a                 | 48.82 ± 0.39              | -                 | -            | -                        | -                       |
| 'Posejdon'              | 48.90 ± 0.15              | -                 | -            | -                        | -                       |
| 'Bryza'                 | 48.92 ± 0.65              | -                 | -            | -                        | -                       |
| 'Marie-joie'            | 49.05 ± 0.58              | -                 | -            | -                        | -                       |
| 'Alayne'                | 49.09 ± 0.18              | -                 | -            | -                        | -                       |
| 'Passat'                | 49.13 ± 0.60              | -                 | -            | -                        | -                       |
| 'John Evelyn'           | 49.31 ± 0.18              | -                 | 28 [16,25]   | -                        | -                       |
| 'Bursztyniek'           | 49.60 ± 0.22              | -                 | -            | -                        | -                       |
| 'Lemon Beauty'          | 50.02 ± 1.41              | -                 | -            | -                        | -                       |
| 'Broadway Star'         | 50.17 ± 0.42              | -                 | -            | -                        | -                       |
| 'Papillon Blanc'        | 51.61 ± 0.32              | -                 | -            | -                        | -                       |
| 8/97                    | 52.20 ± 0.24              | -                 | -            | -                        | -                       |

1 ± means standard deviation; 2 Means in a column followed by the same letter do not differ significantly at α = 0.05 according Duncan’s test.

3.4. Chromosome Number

The microscopic images make it possible to estimate the number of chromosomes in diploid cultivar ‘Dutch Master’ at 28 (Figure 3A), which confirms the literature reports on chromosomes number [25,26]. However, this is also different from that (5) as reported by Sun et al. [19]. For the triploid cultivar ‘Yellow Cheerfulness’, the number of chromosomes could be estimated at 24 (Figure 3B), which confirms the literature reports [15,17,25] and
our own research by flow cytometry analysis carried out for this cultivar. For the reference
tetraploid cultivar ‘Ice Follies’, the number of chromosomes could be estimated at 28
(Figure 3C), which confirms the literature reports on chromosomes 2n = 4x = 28 [16,17,26]
and studies conducted independently by flow cytometry. Our studies on chromosome
number should be regarded as preliminary only. Further studies will be carried out on all
cultivars and breeding clones evaluated. In addition, as seen in the example of the cultivar
‘Dutch Master’ previously considered a tetraploid, it would be very important to carry out
karyotype analyses of all cultivars.

![Microscopic view of chromosomes during mitotic divisions](image)

Figure 3. Microscopic view of chromosomes during mitotic divisions: (A) diploid cultivar ‘Dutch
Master’; (B) triploid cultivar ‘Yellow Cheerfulness’; and (C) tetraploid cultivar ‘Ice Follies’.

4. Conclusions

Our results lead to the general conclusion that the morphological traits studied and
nuclear DNA content can be helpful for determining the possible ploidy level of narcissus,
as well the information on the origin and parental forms. The results obtained are a prelude
to further studies, especially in the assessment of chromosome number and karyotype,
in order to ascertain in cases of doubt the true level of ploidy. In detail, we propose the
following conclusions:

1. Flower diameter, leaf length and width, and density and size of stomata are in many
cases correlated or indicated a tendency towards the ploidy level of *Narcissus* geno-
types evaluated and could be used as a part of the system of morphological markers
(but absolutely after additional research).

2. Our results of nuclear DNA content confirm the literature reports, which are known for
7 of the 38 genotypes studied. The remaining genotypes without literature references,
where relatively high values of 2C DNA were obtained that may tentatively suggest
tetraploids, should be verified by chromosomes counting.

3. Clear confirmation of ploidy level requires verification of chromosome number and
preferably karyotyping.

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References

1. Warwick Crop Centre. Narcissus (Daffodil). 2017. Available online: http://www2.warwick.ac.uk/fac/sci/lifesci/wcc/research/narcissus/ (accessed on 22 February 2022).

2. BKD. Bloembollenkultuurdienst. Voorlopige Statistiek Voorjaarsbloeiers. 2022. Available online: https://www.bkd.eu/wp-content/uploads/2022/02/voorlopige-statistiek-voorjaarsbloeiers-2021-2022-versie-2-17-02-2022-website.pdf (accessed on 22 February 2022).

3. Hanks, G.R. Narcissus and daffodil—The genus Narcissus. In Medicinal and Aromatic Plants—Industrial Profiles 21, 1st ed.; Hanks, G.R., Ed.; CRC Press: Boca Raton, FL, USA; Taylor & Francis: Boca Raton, FL, USA, 2002; p. 428.

4. Kington, S. The International Daffodil Register & Classified List; Royal Horticultural Society: London, UK, 2008; p. 1414.

5. Marasek-Ciolakowska, A.; Sochacki, D.; Marciniak, P. Breeding aspects of selected ornamental bulbous crops. Agronomy 2021, 11, 1709. [CrossRef]

6. Okubo, H.; Sochacki, D. Botanical and horticultural aspects of major ornamental geophytes. In Ornamental Geophytes: From Basic Science to Sustainable Production, 1st ed.; Kamenetsky, R., Okubo, H., Eds.; CRS Press: Boca Raton, FL, USA; Taylor & Francis Group: Boca Raton, FL, USA, 2012; pp. 77–121.

7. Fernandes, A. Sur la phylogene des especes du genre Narcissus L. Bol. Soc. Broteriana 1951, 25, 113–192.

8. Rees, A.R. Pests, diseases and disorders. In Ornamental Bulbs, Corms and Tubers; CAB International: Wallingford, UK, 1992; p. 220.

9. Marques, I.; Nieto Feliner, G.; Martins-Louçã, M.A.; Fuertes Aguilar, J. Genome size and base composition variation in natural and experimental Narcissus (Amaryllidaceae) hybrids. Ann. Bot. 2012, 109, 257–264. [CrossRef] [PubMed]

10. Webb, D.A. Narcissus. In Flora Europea; Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A., Chater, A.O., Richardson, I.B.K., Eds.; Cambridge University Press: Cambridge, UK, 1980; Volume 5, pp. 78–84.

11. Zonneveld, B.J.M. The involvement of Narcissus hispanicus Gouan in the origin of Narcissus bujei and of cultivated trumpet daffodils (Amaryllidaceae). Real Jardín Botánico An. Del Jardín Botánico Madr. 2010, 67, 29–39. [CrossRef]

12. Leitch, I.J.; Johnston, E.; Pellicer, J.; Hidalgo, O.; Bennett, M.D. Angiosperm DNA C-Values Database (Release 9.0, Apr 2019). 2019. Available online: https://cvalues.science.kew.org/ (accessed on 22 September 2021).

13. De Mol, W.E. The disappearance of the diploid and triploid Magnicoronatae Narcissi from the larger cultures and the appearance in their place of tetraploid forms. Proc. Sect. Sci. K. Ned. Akad. Van Wet. 1922, 25, 216–220.

14. Fernandes, A. Nouvelles etudes caryologiques sur le genre Narcissus L. Bol. Soc. Broteriana 1934, 9, 1–198.

15. Brandham, P.E.; Kirton, P.R. The Chromosomes of species, hybrids and cultivars of Narcissus L. (Amaryllidaceae). Kew Bull. 1987, 42, 65–102. [CrossRef]

16. Brandham, P.E. Chromosome numbers in narcissus cultivars and their significance to the plant breeder. Planta 1992, 14, 133–168.

17. Zonneveld, B.J.M. The systematic value of nuclear DNA content for all species of Narcissus L. (Amaryllidaceae). Plant Syst. Evol. 2008, 275, 109–132. [CrossRef]

18. Wang, C.; Luo, F.; Li, Y.; Liu, W. Karyotype analysis of four exotic daffodil cultivars. Acta Botanica Boreali-Occidentalia Sinica 2011, 31, 1577–1581.

19. Sun, X.M.; Sun, Q.; Yang, H.G.; Zhang, L.J.; Wang, Y.B. Karyotype analysis in seven cultivars of Narcissus spp. Caryologia 2015, 68, 63–68. [CrossRef]

20. Marques, I.; Aguilar, J.F.; Martins-Louçã, M.A.; Moharrek, F.; Feliner, G.N. A three-genome five-gene comprehensive phylogeny of the bulbous genus Narcissus (Amaryllidaceae) challenges current classifications and reveals multiple hybridization events. Taxon 2017, 66, 832–854. [CrossRef]

21. Siljak-Yakovlev, S.; Pustahija, F.; Šolic, E.M.; Bogunic, F.; Muratovic, E.; Bašić, N.; Catrice, O.; Brown, S.C. Towards a genome size and chromosome number database of Balcan flora: C-values for 242. Adv. Sci. Lett. 2010, 3, 190–213. [CrossRef]

22. Marquez, I.; Feliner, G.N.; Munt, D.D.; Martins-Louçã, M.A.; Aguilar, J.F. Unraveling cryptic reticulate relationships and the origin of orphan hybrid disjunct populations in Narcissus. Evolution 2010, 64, 2353–2368.

23. Gonzalez-Aguilera, J.J.; Ludena Reyes, P.; Fernandez-Peralta, A.M. Intra- and interspecific variants in nuclear parameters of two closely related species of Narcissus L. Genetica 1990, 82, 25–31. [CrossRef]

24. Rode, S.; Kirton, P.R. The Chromosomes of species, hybrids and cultivars of Narcissus L. (Amaryllidaceae). Caryologia 1995, 48, 257–264. [CrossRef] [PubMed]

25. Van Tuyl, J.M.; Arens, P.; Marasek-Ciolakowska, A. Breeding and Genetics of Ornamental Geophytes. In Ornamental Geophytes: From Basic Science to Sustainable Horticultural Production, 1st ed.; Kamenetsky, R., Okubo, H., Eds.; CRC Press: Boca Raton, FL, USA, 2012; pp. 131–158.
30. Jeloudar, N.I.; Chamani, E.; Shokouhian, A.-A.; Zakaria, R.A. Induction and identification of polyploidy by colchicine treatment in *Lilium regale*. *Cytologia* **2019**, *84*, 271–276. [CrossRef]

31. Podwyszyńska, M.; Trzewik, A.; Marasek-Ciolakowska, A. In vitro polyploidisation of tulips (*Tulipa gesneriana* L.)—Phenotype assessment of tetraploids. *Sci. Hortic.* **2018**, *242*, 155–163. [CrossRef]

32. UPOV. *Guidelines for the Conduct of Tests for Distinctness, Homogeneity and Stability; Narcissus* L. TG/87/2; International Union for the Protection of New Varieties of Plants: Geneva, Switzerland, 1983.

33. Dyki, B.; Habdas, H. Metoda izolowania epidermy liści pomidora i ogórka dla mikroskopowej oceny rozwoju grzybów patogenniczych [The method of isolation of epidermis of tomato and cucumber leaves for microscopic investigation of pathogenic fungus development]. *Acta Agrobot.* **1996**, *49*, 123–129. (In Polish) [CrossRef]

34. Galbraith, D.W.; Harkins, K.R.; Maddox, J.M.; Ayres, N.M.; Sharma, D.P.; Firoozabady, E. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science* **1983**, *220*, 1049–1051. [CrossRef] [PubMed]

35. Śliwińska, E. Zastosowanie cytometrii przepływowej do oznaczania zawartości DNA u roślin [Estimation of DNA content in plants using flow cytometry]. *Postępy Biol. Komórki* **2008**, *35* (Suppl. S24), 165–176. (In Polish)

36. Gatlin, F.L.; Brennan, J.R. Tetraploid daylilies. In *The New Daylily Handbook*; American Hemerocallis Society Inc.: Jackson, TN, USA, 2002; pp. 251–264.

37. Gulia, S.K.; Singh, B.P.; Carter, J.; Griesbach, R.J. *Daylily: Botany, Propagation, Breeding*; Janick, J., Ed.; Horticultural Reviews; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2009; Volume 35, pp. 193–220.

38. Brandham, P.E. Evolution of polyploidy in cultivated *Narcissus* subgenus *Narcissus*. *Genetica* **1986**, *68*, 161–167. [CrossRef]

39. Sajjad, Y.; Jaskani, M.J.; Mehmood, A.; Ahmad, I.; Abbas, H. Effect of colchicine on in vitro polyploidy induction in African marigold (*Tagetes erecta*). *Pak. J. Bot.* **2013**, *45*, 1255–1258.

40. McGoey, B.V.; Chau, K.; Dickinson, T.A. Stomata size in relation to ploidy level in North American hawthorns (*Crataegus*, Rosaceae). *Madroño* **2014**, *61*, 177–193. [CrossRef]

41. Li, Z.; McKibben, M.T.W.; Finch, G.F.; Blischak, P.D.; Sutherland, B.L.; Barker, M.S. Patterns and processes of diploidization in land plants. *Annu. Rev. Plant Biol.* **2021**, *72*, 877–410. [CrossRef]

42. Karihaloo, J.L.; Koul, A.K. Cytogenetic studies in the genus *Narcissus*. L. VII. Karyotype and nucleolar condition in some *N. pseudonarcissus* L. cultivars. *Cytologia* **1989**, *54*, 589–595. [CrossRef]

43. Wu, H.; Ramanna, M.S.; Arens, P.; van Tuyl, J.M. Genome constitution of *Narcissus* variety ‘Tête-à-Tête’ analysed through GISH and NBS profiling. *Euphytica* **2011**, *181*, 285–292. [CrossRef]