■■ 2016. Horticultural Plant Journal, ■■ (■■): ■■–■■.

Horticultural Plant Journal

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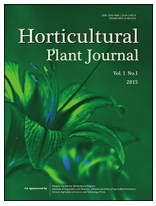

Karyotype Analysis of *Gazania rigens* Varieties

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Received 8 March 2016; Received in revised form 23 May 2016; Accepted 2 July 2016

Available online

Abstract

For studying species origin, systematic evolution and phylogenetic relationship of *Gazania rigens*, four different *G. rigens* varieties, with different flower colors, were subjected to chromosome karyotype analysis. The somatic chromosome number in three varieties 'Hongwen', 'Xingbai' and 'Richu' was 2n = 10, while in 'Zhongguo Xunzhangju' it was 2n = 20. We speculate that the cardinal number of chromosomes in *G. rigens* plants is x = 5, in which case 'Zhongguo Xunzhangju' is a tetraploid. The karyotype formulae of 'Hongwen', 'Xingbai' and 'Richu' were 2n = 8m + 2sm, 2n = 8m + 2sm and 2n = 10m respectively. The karyotype formula of 'Zhongguo Xunzhangju' was 2n = 18m + 2sm. The asymmetrical karyotype coefficients of the four *G. rigens* varieties ranged from 53.80% to 58.84%. Only 'Richu' had a '1A' karyotype, while the others were relatively symmetric '2A'. Karyotype analysis indicates that the three introduced varieties have a close genetic relationship.

Keywords: *Gazania rigens*; chromosome; karyotype analysis

1. Introduction

Gazania rigens (L.) Gaertn. is a member of the Asteraceae. It is a herbaceous perennial named for a flower shape resembling a medal. *G. rigens* is native to South Africa and grows best in warm, sunny locations. Two types, tufted and decumbent, were introduced into China as useful groundcovers (Xie et al., 2013). They are evergreen with colorful flowers with a long flower life and flowering season, and have a strong resistance to drought, heat, poor soils and moderately cold temperatures. *G. rigens* is strongly agamogenic, making it easy to propagate by cuttings, plant division, and tissue culture, and it rapidly colonizes roadbed landscapes. Li (2011) and Wang (2012) discussed potential landscape applications. Wang (2013) and Zhou (2014) noted plant, leaf, flower shape, and flower color variability of *G. rigens*.

The cytology of *G. rigens* is poorly known. Chen et al. (2003) reported that *Gazania* was diploid (2n = 20), and its chromosome cardinal number was x = 10, but Barkley (2006) suggested that the chromosome cardinal number was x = 9 (2n = 18). In the present research, we analyzed chromosome karyotypes of four *Gazania* varieties and found that the chromosome number in three introduced varieties was 2n = 10, and for the variety 'Zhongguo Xunzhangju,' the number was 2n = 20. We propose that the chromosome cardinal number in *Gazania* is x = 5, and that 'Zhongguo Xunzhangju' is a tetraploid.

2. Materials and methods

This research was conducted from June 2013 to September 2014. 'Zhongguo Xunzhangju' was cultivated by the Plant Cultivation and Physiology Laboratory, Soochow University, China. 'Xingbai', 'Hongwen' and 'Richu' varieties were introduced from Japan and were selected for karyotype analysis.

We used the methods of Li et al. (2008) and Zhu et al. (2011). Root samples were taken when the new root length of cuttings from the *Gazania* varieties reached 1–3 cm. Roots were pretreated for 20–24 h in ice water, then transferred into Carnoy's fluid (ethyl alcohol:glacial acetic acid = 3:1), immobilized for 20–24 h at 4 °C, washed three times with 90% alcohol, then transferred into 70% alcohol, at 4 °C. Root tips were sliced and subjected to acidolysis by immersing in 1 mol·L^{-1} hydrochloric

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Peer review under responsibility of Chinese Society for Horticultural Science (CSHS) and Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS)

The Chinese version of this paper is published in Acta Horticulturae Sinica. doi:10.16420/j.issn.0513-353x.2015-0301

http://dx.doi.org/10.1016/j.hpj.2016.07.004

Table 1 Chromosome parameters of 4 *G. rigens* varieties

Chromosome No.	Hongwen			Xingbai			Richu			Zhongguo Xunzhangju		
	Relative length/%	Arm ratio	Type	Relative length/%	Arm ratio	Type	Relative length/%	Arm ratio	Type	Relative length/%	Arm ratio	Type
1	12.24	1.34	m	11.51	1.98	sm	11.40	1.33	m	5.86	1.03	m
2	11.74	1.07	m	11.06	2.14	sm	11.29	1.05	m	5.79	1.06	m
3	10.74	1.19	m	11.48	1.28	m	11.00	1.21	m	5.69	1.09	m
4	10.55	1.42	m	11.41	1.18	m	10.50	1.11	m	5.61	1.27	m
5	10.54	1.27	m	10.30	1.36	m	10.04	1.15	m	5.61	1.25	m
6	9.67	1.39	m	10.01	1.37	m	9.90	1.27	m	5.46	1.07	m
7	9.55	2.05	sm	9.77	1.34	m	9.53	1.11	m	5.43	1.05	m
8	9.51	2.68	sm	9.06	1.17	m	9.17	1.18	m	5.38	1.08	m
9	8.27	1.53	m	7.91	1.17	m	9.01	1.21	m	5.33	1.62	m
10	7.19	1.15	m	7.47	1.57	m	8.15	1.04	m	5.23	1.65	m
11										5.10	1.44	m
12										5.01	1.16	m
13										5.02	2.40	sm
14										4.21	2.62	sm
15										4.74	1.02	m
16										4.62	1.19	m
17										4.50	1.15	m
18										4.16	1.32	m
19										3.77	1.07	m
20										3.49	1.13	m

acid for 8–10 min using a Thermostatic Water Bath at 60 °C, removed and washed three times with pure water (with each washing lasting 1–2 min). Roots were then dyed with aceto-carmine for 5–10 min, and 1–2 mm slices of root tip were placed on microscope slides and covered with coverslips. The bubble inside was extruded by tapping slightly. It was then dried with an alcohol lamp 1–2 times, and then observed and photographed using a Leica DM300 microscope.

About 40–50 clearly viewable cells of each variety were observed at the metaphase. We selected cells whose number of consistent chromosomes exceeded 85%. We photographed root tip cells, at mitosis metaphase, that had well-separated chromosomes and a clear centromere. Chromosomes were measured and matched using Photoshop CS 5.0 software and their relative length, arm ratio, chromosome length ratio were calculated (Zhou et al., 2009; Lu et al., 2013). The Li and Chen (1985) method was used for karyotype analysis and the Stebbins (1971) method was used for karyotype classification. The karyotype asymmetry coefficient (As. K, %) was defined as (length of long arm/length of the entire chromosome) × 100 (Arano, 1963).

3. Results

Metaphase karyotype analysis of root tip cells showed that centromeres were mainly located at the middle (m) or near the middle (sm) of chromosomes. There was little variation of chromosome length. The number of chromosomes with length ratios of 1.40–1.70, and arm ratios >2:1, accounted for 0–20.00% of the total and their asymmetrical karyotype coefficients were 53.80%–58.84%. In the karyotypes of the four *G. rigens* varieties, the chromosomes are arranged in descending order (Fig. 1, Table 1 and Table 2).

The chromosome number of ‘Hongwen’ was 2n = 10. The karyotype formula was 2n = 8m + 2sm, of which the fourth pair of chromosomes was the sm type (mean arm ratio = 2.34) (Fig. 1, Table 1). The ratio of the longest and shortest chromosome was 1.70, frequency of chromosomes with arm ratio >2 was 20.00%, and the karyotype was ‘2A’ (Table 2).

The chromosome number of ‘Xingbai’ was 2n = 10. The karyotype formula was 2n = 8m + 2sm. The first pairs of chromosomes were type sm (mean arm ratio = 2.06) (Fig. 1, Table 1). The asymmetrical karyotype coefficient was the maximum (58.84%) and the karyotype was ‘2A’ (Table 2).

The chromosome number of ‘Richu’ was 2n = 10. All chromosomes were type m, and the karyotype formula was 2n = 10m (Fig. 1, Table 1). The ratio of the longest and shortest chromosome, asymmetrical karyotype coefficient, and percentage of chromosomes exceeding 2:1 of the arm ratio were respectively 1.40%, 53.80% and 0. The karyotype was ‘1A’ (Table 2).

Table 2 Karyotype characteristics of 4 *G. rigens* varieties

Variety	Chromosome number	Centromere location		Chromosome length ratio (longest/shortest)	Asymmetrical karyotype coefficient/%	Percent with arm ratio >2	Arm index	Karyotype classification
		m	sm					
Hongwen	10	8	2	1.70	58.80	20.00	20	2A
Xingbai	10	8	2	1.54	58.84	10.00	20	2A
Richu	10	10		1.40	53.80	0	20	1A
Zhongguo Xunzhangju	20	18	2	1.68	55.86	10.00	40	2A

Please cite this article in press as: ZENG Jiashi, et al., Karyotype Analysis of *Gazania rigens* Varieties, Horticultural Plant Journal (2016), doi: 10.1016/j.hpj.2016.07.004