

Heterosis and genetic analysis of branching in cut-flower chrysanthemums

Yunyang Yang · Chao Wen · Nan Ma ·
Liangjun Zhao

Received: 22 November 2014 / Accepted: 30 March 2015 / Published online: 9 April 2015
© Springer Science+Business Media Dordrecht 2015

Abstract The single-head cut-flower chrysanthemum is one of the most important and popular varieties of Chinese export cut flowers, but the less collateral varieties are very rare in the market. To produce new, high-quality varieties, we generated two hybrid combinations from the varieties Fukashi (SZ), Jinba (SM), and FengWofen (FW; “SM × SZ” and “SZ × FW”, respectively). A detailed analysis of morphological tests revealed the heredity and variation in the F₁ populations. Hybrid plant height showed significant negative heterosis. Flower diameter and the number of small flowers were both diminished, but the ornamental traits of the population overall showed wide variation. The results provided a basis of genetic differences for subsequent analysis. In both crosses, Fukashi played an important regulatory role in determining branching traits in the middle zone and basal zone of the progeny plants: it reduced hybrid branching traits in the middle zone to a certain extent, however, it increased those in the basal zone and there were some transgressive progeny. These results indicate that hybridization is an effective way to contribute to the phenotypic variation of cut-flower chrysanthemums. The inheritance of branching traits

identified in this study will be important in improving the plant architecture of chrysanthemum cultivars.

Keywords Branches · Cut-flower chrysanthemums · Genetic analysis · Heterosis

Introduction

Chrysanthemum (*Dendranthema grandiflorum*) is an important commercial cut flower, and the single-head cut chrysanthemum is the most popular of the four major cut flowers in China (Sun et al. 2010; Zhu et al. 2013). However, most cultivars of cut-flower chrysanthemums produce a number of branches, and these need to be removed to maintain plant architecture; this practice increases production costs (Liang et al. 2010). Hence, the promotion of varieties without axillary buds (non-germinated axillary buds) or with short axillary buds will reduce the cost, difficulty, and the labor intensity of production, and improve the commodity rate and competitive ability of cut chrysanthemums. Because less collateral varieties (i.e. cultivars with fewer branches) are very rare in the market, it would be of significant economic benefit to develop such cultivars through intervarietal hybridization.

Intervarietal hybridization, in which satisfactory branch characteristics or genes are transferred from one variety into another, is a promising strategy for improving plant architecture. For example, an axillary branched somaclonal mutant (*SbABM*) was crossed

Y. Yang · C. Wen · N. Ma · L. Zhao (✉)
Beijing Key Laboratory of Development and Quality
Control of Ornamental Crops, Department of Ornamental
Horticulture and Landscape Architecture, China
Agricultural University, No.2 Yuanmingyuan West Road,
Haidian District, Beijing 100193, China
e-mail: zhaolj5073@sina.com

with five normal sorghum cultivars in order to elucidate the inheritance of axillary branching, which helped in the genetic modification of plant architecture (Maralappanavar et al. 2000; Immadi et al. 2014). Similarly, low-tillering barley mutants (*als*) were crossed with six other tillering mutants (Dabbert et al. 2009), and reciprocal crosses were made with guar (Liu et al. 2006). However, no such detailed studies have been reported for hybridization in less collateral chrysanthemum varieties, with the exception of research concerning the outgrowth of axillary buds into branches, particularly under hormonal regulation (auxin, cytokinins, strigolactones, etc.) and the genes related to this process (Jiang et al. 2010; Liang et al. 2010).

Dendranthema grandiflorum cv. Fukashi (study code: SZ) is a typical less collateral cut chrysanthemum with non-germinating axillary buds, however, *Dendranthema grandiflorum* cv. Jinba (study code: SM) is the most popular single-head cut chrysanthemum with high axillary buds germination rate (Huh et al. 2011). *Dendranthema grandiflorum* cv. Fengwofen (study code: FW) is a ground-cover chrysanthemum cultivar with branch traits similar to SM. Consequently, the cost of manual removal is high; axillary bud germination also influences plant architecture and leads to the consumption of excessive soil nutrients. As a mean to enhance chrysanthemum cut-flower production significantly and to improve its market economics, the hybridization of these two high-quality cultivars is of particular interest because of the complementarity of desirable architectural features and ornamental value.

In this study, we performed two cross combinations, SM × SZ and SZ × FW, in order to examine the morphology of the hybrid progeny; ornamental and branch traits were then compared with those of their parents. The heredity analysis of ornamental and branch traits in F₁ populations provided a basis for parent selection and matching. Furthermore, it established a foundation for the development of cut chrysanthemum varieties with fewer (or no) branches.

Materials and methods

Plant materials and crossing experiments

SZ is a typical less collateral cut chrysanthemum with non-germinating axillary buds, its number of branches

is far below that of SM (Fig. 1b). However, its easy-falling tubiform petals lead to a low degree of market acceptance. As the most popular single-head cut chrysanthemum on the international (Asia) market, SM possesses large, pretty, and pure white flowers; in China, it is cultivated widely in Liaoning, Shandong, Jiangsu, Fujian, Guangdong, and Hainan. However, its axillary buds germination rate is high and thus it develops a large number of branches (Fig. 1a). In contrast, FW is a ground-cover chrysanthemum cultivar with a flowering time similar to SZ but with branch traits similar to SM (Fig. 1c). Plants of the three parents used in this study were maintained in the experimental field of the China Agricultural University (CAU), Beijing, China (Table 1). During the year 2012–2013, “SM,” “SZ,” and “FW” were used as parents to generate the two inter-varietal hybrids SM × SZ and SZ × FW. From September 2012, the female parents were pollinated using three replicates per inflorescence. About 60 days after pollination, the hybrid seeds were harvested. Seeds were sown in March 2013; on May 20th, the potted seedlings were transplanted into the field and water and fertilizer were applied fortnightly thereafter. From July 2013 onwards, the morphological characteristics of the parent and the hybrid plants were recorded.

Morphological tests for hybridity

Ornamental traits included: plant height, stem diameter, internode length, leaf shape (leaf length, width, thickness, leaf index), and flower shape (inflorescence diameter, the number of ligulate and tubular flowers, petal length and width, peduncle length and roughness). All traits were recorded according to the distinctness, uniformity and stability (DUS) test (Furones-Pérez and Fernández-López 2009; UPOV 1989), and flower color was recorded according to the standard RHS color chart (The Royal Horticulture Society, London).

For the branch traits of each plant, we counted “number of nodes,” “number of branches,” and “length of branches” at full flowering. From these observations, we derived a number of additional measures. First, we divided each plant into top, middle, and basal zones according to the number of nodes falling within the upper quarter, middle half and lower quarter, and counted the number of branches in each zone. If the number of branches was greater than

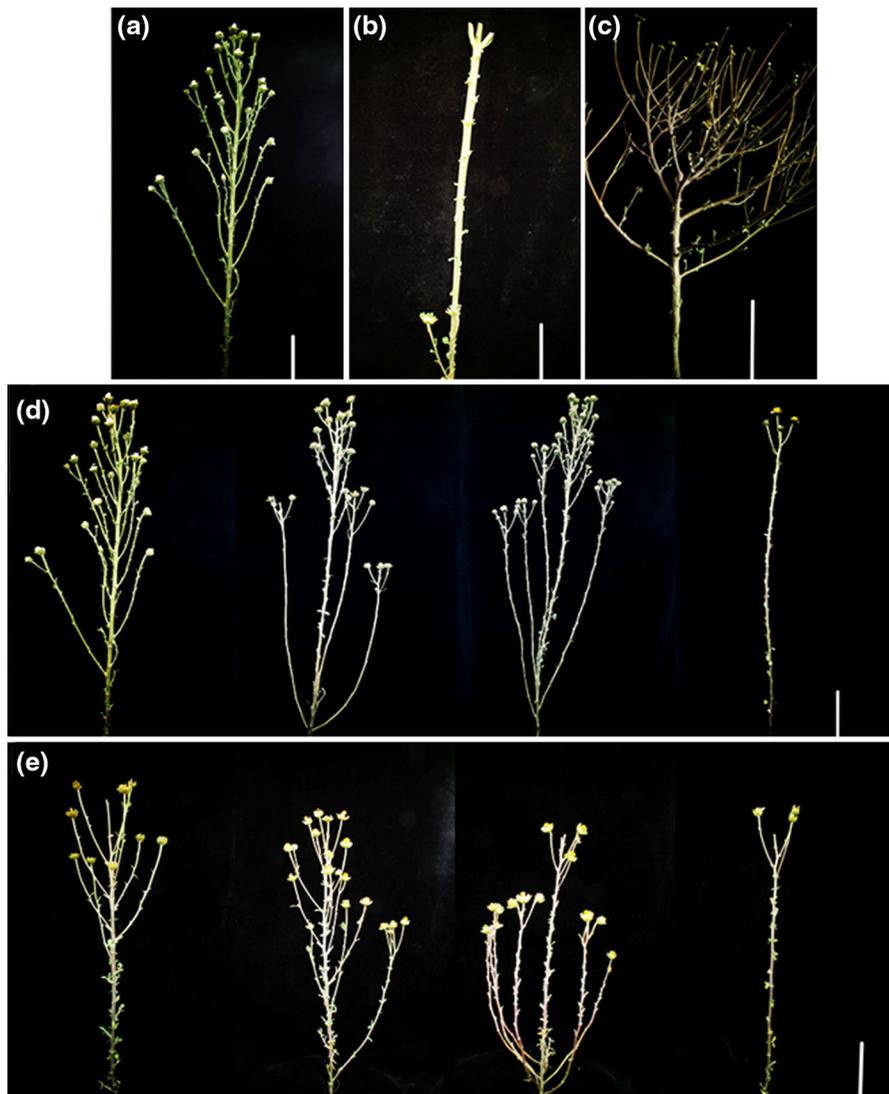


Fig. 1 Branching type in three parent chrysanthemums of *Dendranthema grandiflorum* cv. Jinba (SM), *Dendranthema grandiflorum* cv. Fukashi (SZ), *Dendranthema grandiflorum* cv. Fengwofen (FW) and 2 F_1 hybrid populations of *Dendranthema grandiflorum* cv. Jinba \times cv. Fukashi (SM \times SZ) and *Dendranthema grandiflorum* cv. Fukashi \times cv. Fengwofen (SZ \times FW). **a** SM (MT&NB type); **b** SZ (LT&LB type);

c FW (MT&NB type); **d** the 2 F_1 hybrid populations of SM \times SZ (MT&NB type, MT&LB type, MT&MB type, and LT&NB type; from left to right); **e** SZ \times FW (MT&NB type, MT&LB type, MT&MB type, and LT&NB type; from left to right). Leaves and petals were removed at full flowering; Scale bar = 10 cm

3, we regarded it as “much;” if the number was less than or equal to 3, we regarded it as “little;” and if the number was zero, we regarded it as “none.” According to this scale, the branch type of cut chrysanthemums can be divided into nine groups [“none” in the top and basal zones (NT&NB), “none” in the top zone and “much” in the basal zone (NT&MB), “none” in the top zone and “little” in the basal zone (NT&LB),

“little” in the top zone and “none” in the basal zone (LT&NB), “little” in the top and basal zones (LT&LB), “little” in the top zone and “much” in the basal zone (LT&MB), “much” in the top zone and “none” in the basal zone (MT&NB), “much” in the top and basal zones (MT&MB), and finally “none” in the top zone and “little” in the basal zone (NT&LB)]. Then we divided the number of branches by the

Table 1 Some identifying characteristics of the cut chrysanthemum varieties (*Dendranthema grandiflorum*) used in cross-breeding

Variety	Code	Natural flowering	Color	Petal shape	Axillary bud characteristics
Jinba	SM	Mid October	White	Flat	Axillary buds sprouting
Fukashi ^a	SZ	Late September	Yellow	Tubiform	Axillary buds not germinating
FengWofen ^b	FW	Early October	Pink	Flat	Axillary buds sprouting

^a Fukashi was obtained from the Liaoning Academy of Agricultural Sciences Institute of Flowers, Shenyang, China

^b Fengwofen was obtained from Professor Bo Hong (Department of Ornamental Horticulture and Landscape Architecture, CAU, Beijing, China)

number of nodes in the same zone, and expressed them as a ratio to denote the branch proportions; a similar measurement was described by Van Minnebruggen et al. (2013). Finally, by adding up the lengths of the branches for each plant, we obtained the total branch length for each plant and for each of the three zones.

Data analysis

Heterosis is expressed as mid-parent heterosis (Hm; Li and Wu 1997). Values of Hm for ornamental traits was defined as the difference between the mean value of that trait in F₁ populations (Fm) and the mid-parent value (MPV), that is, $Hm = Fm - MPV$; MPV is the mean value of that trait in the two parents. All data were analyzed by a one-way analysis of variance using the software package SPSS 18.0. A one-sample *t* test at the level of 0.05 and 0.01 was applied to discriminate the difference between Fm and MPV in ornamental traits.

Results

Statistical analysis of ornamental traits

The heredity for all ornamental traits was analyzed using heterosis, based on the comparison of the hybrids and their parents in the SM × SZ combination. There were distinguishable differences between the F₁ hybrids and parents. The heterosis values for the principle traits were all significant and negative (Table 2). For example, the values for plant height and inflorescence diameter were −10.5 and −15.1, respectively. However, the significantly positive heterosis of internode length is noteworthy; its proportion relative to the ultra-high parent was quite high (72.3 %). While the tubular flower number and peduncle length showed no significant difference in

heterosis, their individual coefficient of variation (CV) was relatively large (79.8 and 55.2 %, respectively). In overall terms, there was a large range of CV from 15.5 to 79.8 % in F₁ populations, which suggests a remarkable potential for the use of hybridization in the improvement of cut chrysanthemums.

For the SZ × FW combination, the genetic characteristics of all ornamental traits, comparing the hybrids and the parents, are presented in Table 3. The F₁ hybrids showed a similar trend in heterosis to that of the SM × SZ hybrid. Characteristics such as hybrid plant height, stem diameter, leaf index, inflorescence diameter, ligulate and tubular flower number, petal length, peduncle length, and peduncle roughness all showed significantly negative heterosis. On the other hand, leaf and petal width were each significantly positive in heterosis; in the F₁ hybrids, these characteristics were intermediate between those of their parents. Internode length, leaf length, and leaf thickness in the hybrids showed no significant difference in heterosis. Overall, the CV of all traits had a range from 9.2 to 62.6 %, which reflected a remarkable genetic variation.

Study of the inheritance of branching traits

The objective of this section was to determine the genetic branching mode of the less collateral variety, SZ. We analyzed the differences between hybrids and parents to ascertain the inheritance of branch traits using four aspects as follows: branch type, branch number (and node number on the whole plants), branch distribution (the number and the proportion of branches in three zones) and branch length (total length and zone length for each plant).

The statistical results for branching type in the two cross combinations are shown in Table 4 and Fig. 1. As parental plants, SM and FW were both MT&NB types, while SZ was an LT&LB type (Fig. 1a–c). In

Table 2 Genetic characteristics of ornamental traits in *Dendranthema grandiflorum* cv. Jinba (SM; ♀), *Dendranthema grandiflorum* cv. Fukashi (SZ; ♂) and their F₁ hybrid population

Ornamental traits	Parent plants		Hybrids F ₁				Comparison between hybrids and parents (%)				
	♀	♂	MPV	X ± δ	SD	CV (%)	Extreme value	Hm ^a	<Low parent value	Between parent value	>High parent value
Plant height (cm)	81.4	72.9	77.2	66.6 ± 1.6	11.3	16.9	42.3–90.3	-10.5**	72.3	19.2	8.5
Stem diameter (mm)	6.7	8.4	7.5	4.5 ± 0.1	0.8	18.2	2.9–6.2	-3.1**	100.0	0.0	0.0
Internode length (mm)	15.9	12.4	14.2	18.6 ± 0.8	5.2	27.8	2.4–29.5	4.4**	12.8	14.9	72.3
Leaf length (mm)	71.5	75.2	73.3	54.0 ± 1.5	10.3	19.1	27.2–85.6	-19.4**	95.7	2.1	2.1
Leaf width (mm)	44.1	51.1	47.6	44.3 ± 1.6	10.9	24.6	21.1–82.1	-3.3*	53.2	25.5	21.3
Leaf index	1.6	1.5	1.6	1.3 ± 0.0	0.2	15.3	0.9–1.9	-0.3**	91.5	4.3	4.3
Leaf thickness (mm)	0.5	0.7	0.6	0.5 ± 0.0	0.1	16.8	0.4–0.8	-0.1**	72.3	23.4	4.3
Inflorescence diameter (mm)	92.1	99.8	95.9	80.8 ± 1.9	12.7	15.7	51.9–113.8	-15.1**	83.0	10.6	6.4
Ligulate flower number	179.5	224.5	202.0	174.8 ± 11.9	81.4	46.6	43.0–411.0	-27.2*	66.0	4.3	29.8
Tubular flower number	76.5	92.0	84.3	92.6 ± 11.0	73.9	79.8	4.0–354.0	8.3	44.7	14.9	40.4
Petal length (mm)	62.6	50.3	56.4	43.4 ± 11.0	7.5	17.3	19.7–58.1	-13.1**	80.9	19.2	0.0
Petal width (mm)	12.7	11.1	11.9	9.9 ± 0.4	2.8	28.0	5.4–16.2	-2.0**	63.8	14.9	21.3
Peduncle length (mm)	35.6	26.6	31.1	30.7 ± 2.1	14.7	47.8	10.2–82.6	-0.4	44.7	25.5	29.8
Peduncle roughness (mm)	6.2	4.8	5.5	3.0 ± 0.1	0.7	21.8	1.5–4.9	-2.5**	100.0	0.0	0.0

* and ** indicate a significant difference at the 0.05 and 0.01 level, respectively

^a The difference between mean values in F₁ and mid-parents value (MPV) was analyzed by one-sample *t* test

Table 3 Genetic characteristics of ornamental traits in *Dendranthema grandiflorum* cv. Fukashi (SZ; ♀), *Dendranthema grandiflorum* cv. Fengwofen (FW; ♂) and their F₁ hybrid population

Ornamental traits	Parent plants		Hybrids F ₁		Comparison between hybrids and parents (%)						
	♀	♂	MPV	X ± δ	SD	CV (%)	Extreme value	Hm ^a	<Low parent value	Between parent value	>High parent value
Plant height (cm)	72.9	34.2	53.6	44.8 ± 1.3	9.8	21.8	26.3–69.9	-8.8**	17.5	82.5	0.0
Stem diameter (mm)	8.4	6.5	7.4	3.2 ± 0.1	0.7	18.0	2.6–5.2	-3.7**	100.0	0.0	0.0
Internode length (mm)	12.4	16.6	14.5	15.7 ± 0.7	5.0	32.2	2.2–26.1	1.2	24.6	28.1	47.4
Leaf length (mm)	75.2	42.1	58.7	57.6 ± 1.4	10.3	17.8	32.1–79.2	-1.1	5.3	87.7	7.0
Leaf width (mm)	51.1	35.2	43.2	49.1 ± 1.2	9.3	19.0	27.6–73.3	5.9**	8.8	49.1	42.1
Leaf index	1.5	1.2	1.3	1.2 ± 0.0	0.1	9.2	1.0–1.5	-0.2**	59.7	40.4	0.0
Leaf thickness (mm)	0.7	0.3	0.5	0.5 ± 0.0	0.1	17.3	0.3–0.8	0.0	0.0	94.7	5.3
Inflorescence diameter (mm)	99.8	43.2	71.5	61.3 ± 0.9	6.9	11.2	52.1–82.6	-10.2**	0.0	100.0	0.0
Ligulate flower number	224.5	202.0	213.3	178.5 ± 7.3	55.1	30.8	69.0–311.0	-34.7**	64.9	17.5	17.5
Tubular flower number	92.0	58.0	75.0	63.5 ± 5.23	39.8	62.6	1.0–159.0	-11.5*	52.6	26.3	21.1
Petal length(mm)	50.3	20.7	35.5	30.5 ± 0.8	5.7	18.6	22.4–53.2	-5.0**	0.0	98.3	1.8
Petal width (mm)	11.1	5.0	8.0	9.2 ± 0.3	2.1	23.0	4.8–13.6	1.2**	3.5	79.0	17.5
Peduncle length (mm)	26.6	82.1	54.3	32.0 ± 2.1	15.5	48.5	2.8–83.2	-22.3**	43.9	54.4	1.8
Peduncle roughness (mm)	4.8	2.7	3.8	2.1 ± 0.1	0.5	21.7	1.3–3.5	-1.7**	93.0	7.0	0.0

* and ** indicate significant difference at the 0.05 and 0.01 level, respectively

^a The difference between mean values in F₁ and mid-parents value (MPV) was analyzed by one-sample *t* test

Table 4 Heredity of branch type in *Dendranthema grandiflorum* cv. Jinba (SM), *Dendranthema grandiflorum* cv. Fukushima (SZ), *Dendranthema grandiflorum* cv. Fengwofen (FW) and their F₁ hybrid populations

Cross combination (♀ × ♂)	Matroclinal	Paternal	Hybrids F ₁			
			MT&NB	MT&LB	MT&MB	LT&NB
SM × SZ	MT&NB	LT&LB	25	10	17	2
Percentage (%)			46.3	18.5	31.5	3.7
SZ × FW	LT&LB	MT&NB	21	14	21	1
percentage (%)			42.0	28.0	42.0	2.0

Figures are mean (n = 54 plants for the SM × SZ hybrid and n = 57 plants for the SZ × FW hybrid)

Table 5 Heredity of the number of nodes and branches in *Dendranthema grandiflorum* cv. Jinba (SM), *Dendranthema grandiflorum* cv. Fukushima (SZ), *Dendranthema grandiflorum* cv. Fengwofen (FW) and their F₁ hybrid populations

Code	Number of nodes	Number of branches
SM	49.0 ± 2.1	21.6 ± 3.5
SZ	31.4 ± 2.3	3.2 ± 1.2
FW	17.0 ± 1.0	9.0 ± 0.6
F ₁ hybrid population of SM × SZ	38.0 ± 0.9	13.0 ± 0.8
F ₁ hybrid population of SZ × FW	31.3 ± 0.8	13.0 ± 0.8

Figures are mean ± SE (n = 15–18 for the plants of SM, SZ, and FW respectively; n = 54 plants for the SM × SZ hybrid and n = 57 plants for the SZ × FW hybrid)

contrast, the branching type of the two F₁ populations showed a similar trend (Fig. 1d, e): the number of MT&NB and MT&MB types was the greatest, followed by the MT&LB type, with the LT&NB type being the least frequent. However, the LT&NB type was the only type that was similar to SZ.

To analyze the heredity with respect to the numbers of nodes and branches, we focused on the comparison of the parents and their hybrids (Table 5). The number of nodes for the two F₁ populations was on average 38.0 and 31.3, respectively, with the greatest distribution being in the range of 30–40 and 20–30 (Fig. 2a, b), this figure was closer to SZ (31.4) and thus was greater than FW (17.0). On the other hand, the number for the two F₁ populations averaged 13.0, and the largest number of branches in the SM × SZ hybrid was in the range of 10–15, and that in the SZ × FW hybrid was in the range of 5–10. As compared to SZ, the number of branches for the F₁ hybrids increased significantly, especially in the SZ × FW combination.

The branch distribution for the two cross combinations was further analyzed for differences with respect to the numbers and the proportions of branches within the three zones (Table 6). In the SM × SZ combination, the number of branches in the top zone for the F₁ populations was on average 7.8, and the proportion was 0.8, mostly located in the range 0.8–1.0 (Fig. 3c), which was greater than in SZ and thus more similar to SM. In the middle zone, the average number of branches in F₁ populations was 2.7, and it was mainly in the range of 0–3. It was noticeable that for the hybrids, the number of branches decreased significantly, in contrast to SM. In the basal zone, there were some transgressive progenies with respect to the number of branches, based on comparison of the hybrids (2.5) and parent (0.0 and 1.2, respectively) plants; in the hybrid plants, the proportion of branches was similar to that in SZ.

Similar results for branch distribution were obtained in the combination SZ × FW, where in the top zone the number of branches in the hybrids (6.3) was greater than in the parents (2 and 4.7, respectively). Furthermore, the proportion in the top zone was 0.8, mostly located in the range 0.8–1.0 (Table 6; Fig. 3d), which was significantly greater than that for the other zones; this suggests that increasing the branch number of hybrids impacted mainly the top zone, associated with an increase in plant height and node number. In the middle zone, the average number of branches for the hybrids lay between that for the parents. However, in the hybrids, the proportion of branches in the middle zone was mostly in the range of 0.0–0.2, which was strikingly decreased in comparison to the parents. In the basal zone, the average number of branches in the hybrids increased significantly in comparison to the parents, and the proportion in the basal zone was

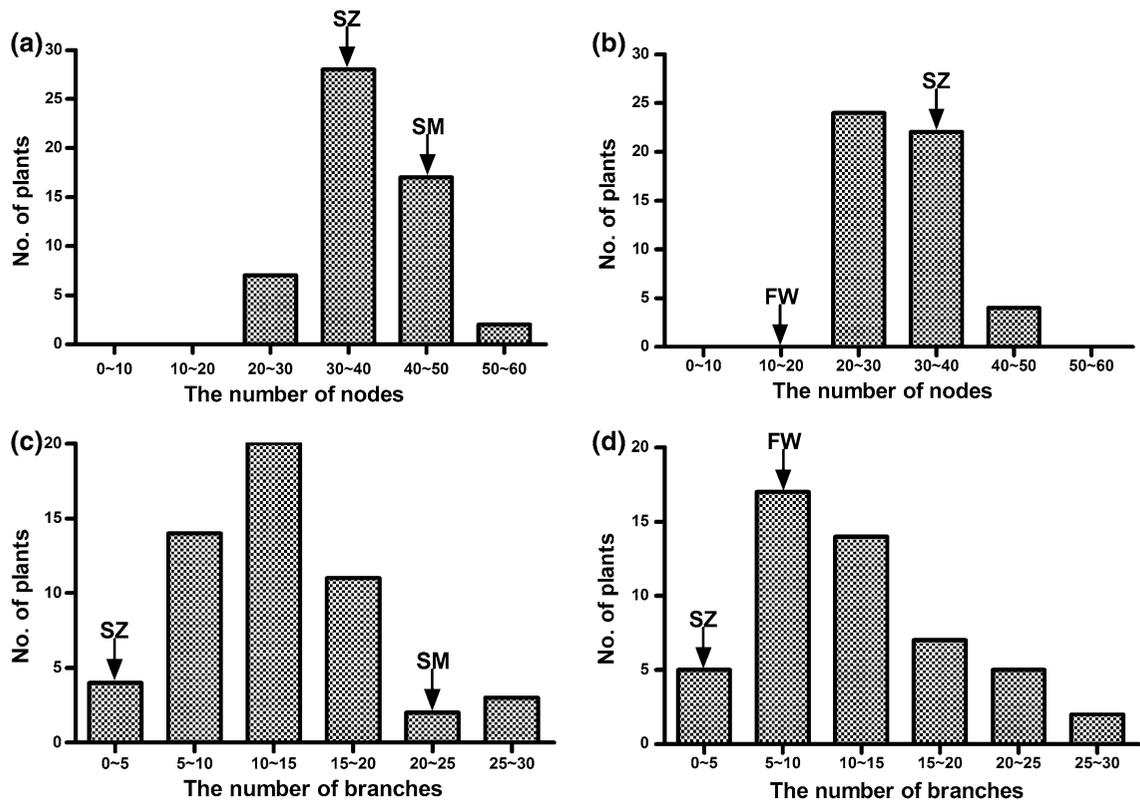


Fig. 2 The number of nodes and branches in the 2 F_1 hybrid populations of *Dendranthema grandiflorum* cv. Jinba × cv. Fukashi (SM × SZ) and *Dendranthema grandiflorum* cv.

Fukashi × cv. Fengwofen (SZ × FW). The number of nodes and branches are given for whole F_1 plants and for the crosses SM × SZ (a, c) and SZ × FW (b, d)

Table 6 Heredity of branch distribution in *Dendranthema grandiflorum* cv. Jinba (SM), *Dendranthema grandiflorum* cv. Fukashi (SZ), *Dendranthema grandiflorum* cv. Fengwofen (FW) and their F_1 hybrid populations

Code	Top zone		Middle zone		Basal zone	
	Number	Proportion	Number	Proportion	Number	Proportion
SM	9.9 ± 1.5	0.8 ± 0.1	11.7 ± 2.0	0.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
SZ	2.0 ± 0.7	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.6	0.2 ± 0.1
FW	4.7 ± 0.3	1.0 ± 0.0	5.0 ± 1.0	0.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
F_1 hybrid population of SM × SZ	7.8 ± 0.3	0.8 ± 0.0	2.7 ± 0.4	0.1 ± 0.0	2.5 ± 0.4	0.2 ± 0.0
F_1 hybrid population of SZ × FW	6.3 ± 0.2	0.8 ± 0.0	3.4 ± 0.5	0.2 ± 0.0	2.6 ± 0.5	0.4 ± 0.1

Figures are mean ± SE ($n = 15$ – 18 for the plants of SM, SZ, and FW respectively; $n = 54$ plants for the SM × SZ hybrid and $n = 57$ plants for the SZ × FW hybrid)

mostly located in the range 0.0–0.2, followed by the range 0.8–1.0; it was clear that there were also some transgressive progenies in the basal zone.

The data on the total length of branches is presented in Table 7. The total branch length for the two F_1 hybrids was 98.2 and 86.8 cm, respectively; thus it was greater than that of SZ (14.4 cm). In addition, the

total branch length for the hybrids lay largely in the range 0–50 (Fig. 4a, b), which was a significant decrease compared to the taller of the two parents. Overall, for the three zones, the length of the branches in the hybrids lay mainly in the range 0–35 cm and, in the top zone, 35–70 cm. In contrast, there was a rather sharp reduction in the middle zone, as the total branch

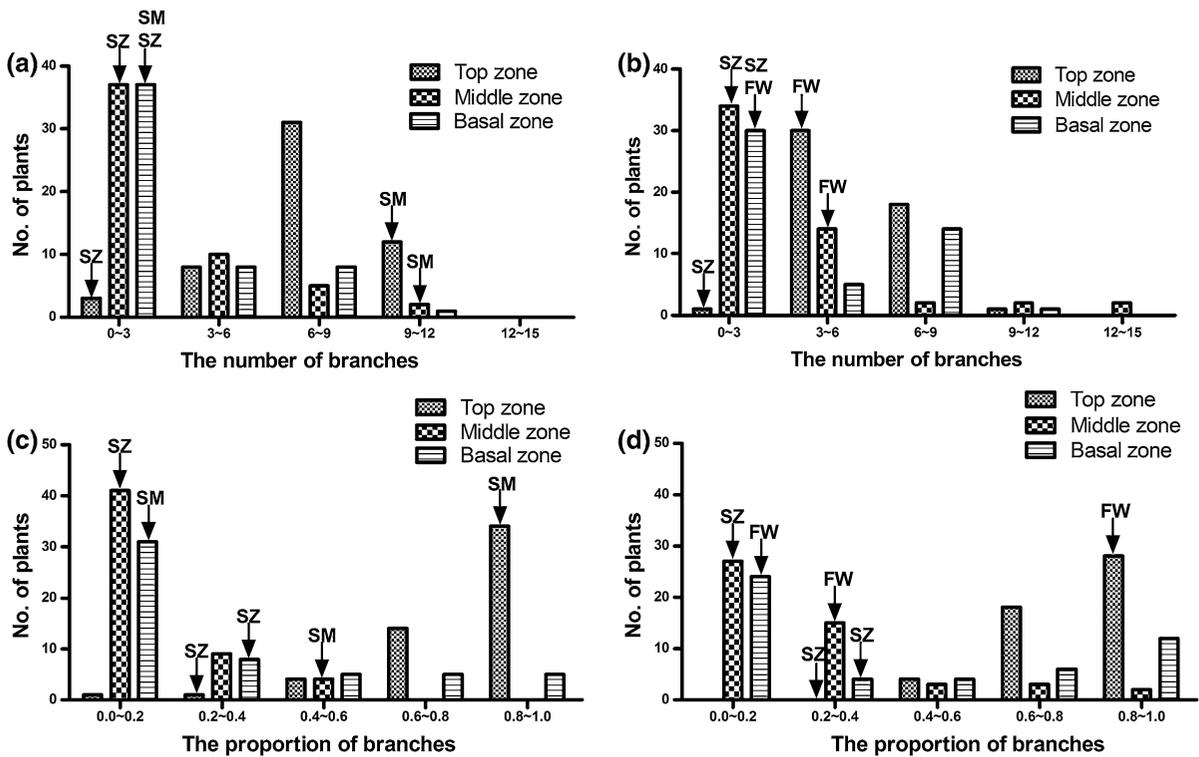


Fig. 3 The branch distribution (the number of branches and the proportion of branches) in 2 F₁ hybrid populations of *Dendranthema grandiflorum* cv. Jinba × cv. Fukashi (SM × SZ) and *Dendranthema grandiflorum* cv. Fukashi × cv.

Fengwofen (SZ × FW). The number and proportion of branches are given for whole F₁ plants in three zones (*top*, *middle* and *basal* zones), and for the crosses SM × SZ (a, c) and SZ × FW (b, d)

Table 7 Heredity of branch length in *Dendranthema grandiflorum* cv. Jinba (SM), *Dendranthema grandiflorum* cv. Fukashi (SZ), *Dendranthema grandiflorum* cv. Fengwofen (FW) and their F₁ hybrid populations

Code	Total length (cm)	Zone length (cm)		
		Top zone	Middle zone	Basal zone
SM	184.5 ± 34.6	83.0 ± 17.0	101.6 ± 21.9	0.0 ± 0.0
SZ	14.4 ± 6.0	2.8 ± 1.2	0.0 ± 0.0	11.6 ± 5.0
FW	213.6 ± 14.2	95.4 ± 8.0	118.3 ± 20.7	0.0 ± 0.0
F ₁ hybrid population of SM × SZ	98.2 ± 12.3	44.8 ± 3.1	8.5 ± 2.5	45.0 ± 9.7
F ₁ hybrid population of SZ × FW	86.8 ± 9.8	32.6 ± 2.1	13.0 ± 3.7	41.2 ± 8.3

Figures are mean ± SE (n = 15–18 for the plants of SM, SZ, and FW respectively; n = 54 plants for the SM × SZ hybrid and n = 57 plants for the SZ × FW hybrid)

length was only 8.5 and 13.0 cm respectively. In the basal zone, the total branch length for the two F₁ populations was about four times greater than that for SZ. As with the number of branches discussed above, it was clear that there were some transgressive progenies in branch length which exhibited heterosis in the basal zone.

Discussion

Heterosis of ornamental traits

Chrysanthemums possess important and popular ornamental traits. In our study, there was a similar trend in the heterosis of ornamental traits in two F₁

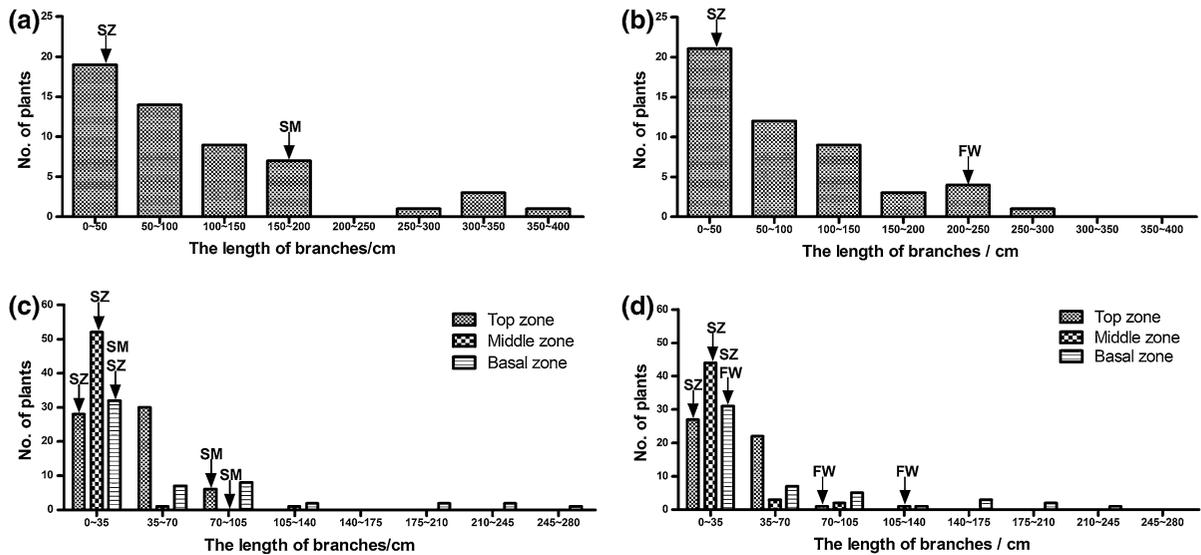


Fig. 4 The branch length in 2 F_1 hybrid populations of *Dendranthema grandiflorum* cv. Jinba \times cv. Fukashi (SM \times SZ) and cv. Fukashi \times cv. Fengwofen (SZ \times FW).

populations. These traits differed remarkably from those of the parents, and most exhibited significant heterosis (Cheng et al. 2010). With respect to hybrid plant height, there was significantly negative heterosis; however, in the SZ \times FW combination, compared to FW, there was a pronounced increase. The results confirmed that the character of SZ plant height was highly heritable (Zhu et al. 2013). While flower diameter and the number of ligulate and tubular flowers of hybrids showed some decline, there was a large range of CV in the F_1 populations, indicating that the phenotype values varied greatly between individuals. Furthermore, there was a range of variation in ornamental characters throughout the whole group, which will be helpful for further elucidating mechanisms of heterosis. The variability of morphology demonstrated that extensive hybridization is an effective breeding method in the genetic improvement of cut chrysanthemums (Tang et al. 2009).

Genetic analysis of branch traits

Most of the F_1 hybrids displayed a branch type similar to their parents (SM and FW; i.e. an MT&NB type), and only a few hybrids were of the LT&NB type, which was similar to SZ. It was evident that the less collateral phenotype could be heritable, though weakly so. The details of the expression of the phenotype

Branch lengths are given for whole F_1 plants and for their three zones (*top*, *middle*, and *basal* zones) and for the crosses SM \times SZ (a, c) and SZ \times FW (b, d)

remain unknown. Similarly, Van Minnebruggen et al. (2013) proved that branching patterns in red clover were likely to be determined by bud formation and bud outgrowth; this was of great reference value in our research. Regarding the inheritance of node number, two F_1 hybrids were similar to SZ, which implied that SZ had a higher level of heritability, whether as the male parent or as the female (Immadi et al. 2014). With regard to the genetic analysis of number of branches, the number of branches was significantly increased in the SZ \times FW hybrid; this may be because SZ improved strikingly both plant height and node number in the hybrids. A similar pattern was obtained in red clover, proving that a highly branched phenotype might have a connection with the formation of more buds (Van Minnebruggen et al. 2013). Other evidence showed that auxin locally regulated branch outgrowth at nodes (Chen et al. 2013). Therefore, we concluded that the presence of more branches resulted from the formation of more nodes.

The analyses of branch distribution and branch length were based on the comparison of two cross combinations. Whether SZ was the male or the female parent, it had a similar genetic effect in F_1 populations: In the top zone, the number, the proportion, and the length of branches for the F_1 hybrids were much higher than for SZ; the influence of SZ was weaker than that of SM or FW. One

explanation may lie in the fact that SM and FW were highly branched phenotypes and thus produced more branches to suppress the less collateral effects of SZ (Van Minnebruggen et al. 2013). In the middle zone, the number, the proportion and the length of branches in the F₁ hybrids were reduced to a certain extent; this suggests that the less collateral traits of SZ were well inherited by inter-varietal hybridization. In the basal zones, there were some transgressive progenies that expressed heterosis of branching traits. This phenotype was caused by SZ, as only SZ had a number of branches in the basal zone; *SbABM* (an axillary branched somaclonal mutant), also had the ability to produce hybrid plants with different numbers of branches (Immadi et al. 2014).

In this study, two cross combinations both had SZ as a parent and the branching traits of SZ were highly heritable. Whether SZ was the male or female parent, it played an important regulatory role in the middle and basal zones of the plants. This work provides the foundation for the further analysis of specific genetic mechanisms in SZ that are related to its collateral behavior. It is important to note that the inheritance of branching characteristics was affected by a variety of internal factors (hormonal, genetic, etc.) and external factors (light, temperature, humidity, etc.; Beveridge 2006; Leduc et al. 2014). The manner in which these factors affect the ultimate phenotypic value of the number of branches will be addressed in further studies of the genetic mechanisms involved.

Acknowledgments This work was supported by the 863 project of the Ministry of Science and Technology of the People's Republic of China (2011AA100208), and the National 948 Key Project of Ministry of Agriculture (Grant No. 2011-G17).

Conflict of interest The authors declare that they have no conflict of interest.

References

- Beveridge CA (2006) Axillary bud outgrowth: sending a message. *Curr Opin Plant Biol* 9:35–40
- Chen XL, Zhou XY, Xi L, Li JX, Zhao RY, Ma N, Zhao LJ (2013) Roles of *DgBRC1* in regulation of lateral branching in chrysanthemum (*Dendranthema × grandiflora* cv. Jinba). *PLoS ONE* 8(4):e61717
- Cheng X, Chen SM, Chen FD, Fang WM, Deng YM, She LF (2010) Interspecific hybrids between *Dendranthema morifolium* (Ramat.) Kitamura and *D. nankingense* (Nakai) Tzvel. achieved using ovary rescue and their cold tolerance characteristics. *Euphytica* 172:101–108
- Dabbert T, Okagaki RJ, Cho S, Boddu J, Muehlbauer GJ (2009) The genetics of barley low-tillering mutants: *absent lower laterals* (*als*). *Theor Appl Genet* 118:1351–1360
- Furones-Pérez P, Fernández-López J (2009) Usefulness of 13 morphological and phenological characteristics of sweet chestnut (*Castanea sativa* Mill.) for use in the DUS test. *Euphytica* 167:1–21
- Huh YJ, Lim JH, Park SK, Choi SR, Kim SH, Pak CH (2011) Endogenous indole-3-acetic acid and trans-zeatin ribosides in relation to axillary bud formation in standard chrysanthemum. *Hortic Environ Biotechnol* 52:128–132
- Immadi S, Patil S, Maralappanavar M, Sajjanar G (2014) Penetration, expressivity and inheritance of axillary branching in somaclonal mutant of sorghum (*Sorghum bicolor* L.). *Euphytica* 196:449–457
- Jiang BB, Miao HB, Chen SM, Zhang SM, Chen FD, Fang WM (2010) The *Lateral Suppressor-Like* gene, *DgLsL*, alternated the axillary branching in transgenic chrysanthemum (*Chrysanthemum × morifolium*) by modulating IAA and GA content. *Plant Mol Biol* 28:144–151
- Leduc N, Roman H, Barbier F, Péron T, Huché-Théliér L, Lothier J, Demotes-Mainard S, Sakr S (2014) Light signaling in bud outgrowth and branching in plants. *Plants* 3:223–250
- Li B, Wu R (1997) Heterosis and genotype × environment interactions of juvenile aspens in two contrasting sites. *Can J For Res* 27:1525–1537
- Liang JL, Zhao LJ, Challis R, Leyser O (2010) Strigolactone regulation of shoot branching in chrysanthemum (*Dendranthema grandiflorum*). *J Exp Bot* 61:3069–3078
- Liu WX, Hou AF, Peffley EB, Auld DL, Powell RJ (2006) The inheritance of a basal branching type in guar. *Euphytica* 151:303–309
- Maralappanavar SM, Kuruvinashetti S, Chandrashekar CH (2000) Regeneration, establishment and evaluation of somaclones in *Sorghum bicolor* (L.) Moench. *Euphytica* 115:173–180
- Sun CQ, Chen FD, Teng NJ, Liu ZL, Fang WM, Hou XL (2010) Interspecific hybrids between *Chrysanthemum grandiflorum* (Ramat.) Kitamura and *C. indicum* (L.) Des Moul. and their drought tolerance evaluation. *Euphytica* 174:51–60
- Tang FP, Chen FD, Chen SM, Teng NJ, Fang WM (2009) Intergeneric hybridization and relationship of genera within the tribe Anthemideae Cass. (I. *Dendranthema crassum* (kitam.) kitam. × *Crossostephium chinense* (L.) Makino). *Euphytica* 169:133–140
- UPOV (1989) Guidelines for the conduct of tests for distinctness, homogeneity and stability. *Chrysanthemumspec*. In: TG/26/4 (ed) International union for the protection of new varieties of plants
- Van Minnebruggen A, Cnops G, Saracutu O, Goormachtig S, Van Bockstaele E, Roldán-Ruiz I, Rohde A (2013) Processes underlying branching differences in fodder crops. *Euphytica* 195:301–313
- Zhu WY, Jiang JF, Chen SM, Wang L, Xu LL, Wang HB, Li PL, Guan ZY, Chen FD (2013) Intergeneric hybrid between *Chrysanthemum × morifolium* and *Artemisia japonica* achieved via embryo rescue shows salt tolerance. *Euphytica* 191:109–119