Journal of Medicinal Plants Research Vol. 5(15), pp. 3488-3499, 4 August, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Development of a stable male-sterile line and its utilization in high yield hybrid of *Platycodon Grandiflorum*

Feng-Hua Shi[1,2#], Chun Sui[1#], Cheng-Min Yang[1], Jian-He Wei[1]*, Yue Jin[1], Hong-Jiang Chen[1], Lu-Qi Huang[3], Yu-Kai Zhao[1], Xiang-Sheng Zhao[1], Xu-Dong Xu[1], Qing-Long Chu[1] and Jing Yu[1]

[1]Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, China.
[2]Guangxi Branch, Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College, Jinghong, Guangxi 666100, China.
[3]Institute of Chinese Material Medical, China Academy of Chinese Medical Sciences, Beijing 100700, China.

Accepted 19 May, 2011

Bell flower (*Platycodon grandiflorum* (Jacq.) A. DC.) has been widely cultivated for medicinal, edible and ornamental purposes. In this study, a stable male-sterile line, GP1BC1-12, was developed based on the discovery of original male-sterile plants of bell flower from cultivated populations in 2004. And three hybrid cultivars, "Zhonggeng No. 1", "Zhonggeng No. 2" and "Zhonggeng No. 3", were further bred using GP1BC1-12 as female parent in producing hybrids seeds. Deduced from the assay of crosses between GP1BC1-12 and 18 self-cross lines, GP1BC1-12 belonged to the nucleo-cytoplasmic interaction type of male sterility and its maintainers exited extensively. Over-standard heterosis of the hybrids was common and evident, and average over-standard heterosis in the weight of root, the weight of main root per plant and the rate of taproot was 47.50, 44.33 and 25.45%, respectively. The strain comparison and regional yield trials of three hybrid cultivars were conducted. The results showed that the hybrids had higher yields than the mainly cultivated populations in China. This is the first report about the development of a stable male-sterile line and the breeding of new hybrid cultivars for bell flower. It can be predicted that the extension and utilization of the new hybrid cultivars with high yield and high disease resistance will benefit the growers of bell flower. Moreover, the consistent genetic background of hybrid cultivars will contribute to the quality control of medicinal materials.

Key words: *Platycodon grandiflorum* (Jacq.) A. DC., male-sterile line, cytoplasmic male sterility (CMS), heterosis, hybrid cultivar.

INTRODUCTION

Platycodon grandiflorum A. DC., known as bell flower or balloon flower, is the only species in the genus *Platycodon* L. of the family Campanulaceae. It is herbaceous perennial plant and native to the Northeast of Asia (Hong et al., 1983). China is the main production area of bell flower and its output is not only to satisfy domestic demand, but also to supply for the markets of Korea, Japan and other countries. Bell flower is a highly valued plant in medicine, food and ornamental. Its roots are the traditional Chinese medicine for the treatment of cough with much phlegm, sore throat, hoarseness and pulmonary abscesses with pus running abscess difficult to burst after suppuration (Chinese Pharmacopoeia Commission, 2005). Modern pharmacological studies showed that the saponins from bell flower are the main active components with antioxidant and anticancer activities (Lee et al., 2004; Kim et al., 2005; Fu et al., 2009; Hu et al., 2010; Jeong et al., 2010). The root extracts of bell flower have effect on many disorders such as hyperlipidemia, hypercholesterolemia, diabetes, hepatic steatosis and amnesia (Kim et al., 2000; Lee et al., 2007; Moon et al., 2010; Noh et al., 2010). As food,

*Corresponding author. E-mail: wjianh@263.net.

#Both authors contributed equally to this work.

bell flower is very popular and has been paid much attention as functional food (Choi et al., 2010; Kang et al., 2008). In Europe and the USA, many cultivars of bell flower, with purple, white or light-pink color flowers, are often grown as garden and bedding plants. There are also some cultivars used for pot and cut flowers (Park et al., 1998; Song et al., 1993; Halevy et al., 2002).

Except for ornamental cultivars aforementioned, only two cultivars of bell flower, which were bred for its medicinal and dietary usage, were reported so far. One was a high-yield and white color cultivar, "Jangbaek", developed by pureline selection from some native strains in Korea (Kim et al., 2004). The other was a Chinese cultivar purified from a native cultivated population by artificial weeding and rouging (Zhu et al., 2009). Besides, some reports could be found on the cultivation techniques of bell flower (Hosoda et al., 1992; Liu et al., 2006). Usually, the cultivated populations of bell flower in China were spontaneously domesticated from wild types by local farmers from 1970's. The long time use of native populations has resulted in a remarkable frailty with population deterioration and severe diseases shown in most bell flower growing districts of China. It is obvious that breeding and utilization of new cultivars will be a choice to settle these realistic problems.

Heterosis is a synonym for hybrid vigor: The increased size, performance, resistance, and strength of hybrids (Lippman and Zamir, 2007; Springer and Stupar, 2007). Recently, the underlying mechanism of heterosis has been studied with the aid of QTL mapping, transcriptome profiling and other molecular biological techniques (Hochholdinger and Hoecker, 2007; Meyer et al., 2010; Song et al., 2010). Heterosis, as an effective approach, has been widely used to produce cultivars for crops, vegetables and grasses (Pradhan et al., 1993; Vogel and Mitchell, 2008; Patel et al., 2009). Whereas, the breeding practice of medicinal plants lagged far behind that of other crops. It is estimated that only 5% of 300 traditional medicinal plant species cultivated in China have bred cultivars, among which only 20% were derived from cross breeding. This does not match with the demand of consistency and stableness for medicinal materials, especially for those used as decoction pieces. Indeed, to assure the identical genetic background of cultivated populations is the underlying basis of modernization and standardization of traditional Chinese medicine. Breeding hybrid cultivar is an effective pathway to receive identical cultivated populations. The presence of substantial heterosis and economic hybrid seed production are the two most desirable components for success of any commercial hybrid breeding programme (Shukla and Pandey, 2008). There are several ways available for hybrid seed production, such as cytoplasmic male sterility (CMS), genetic male sterility (GMS), self-incompatibility (SI) and chemical hybridizing agents (CHA) (Shen et al., 2005). Male sterility is an ideal method to produce hybrid seed (Havey, 2004; Sodhi et al., 2006; Zhao and Gai, 2006). Although, there was a report on the discovery of bell flower male sterility in China (Wu et al., 2008), no further development of male-sterile line and its utilization were found until now.

In this study, a stable male-sterile line was developed using the original male-sterile plants which we discovered from bulk wild and cultivated germplasms in 2004. It was determined to belong to the type of CMS. Using the male-sterile line, 18 cross combinations were prepared and the agronomic traits of their progenies were assayed. From those, three crosses were selected to produce excellent hybrids cultivars. The successful development of hybrid cultivars will promote the planting and management of bell flower. Also the breeding practice will be expected to provide valuable information for the breeding of most other medicinal plants, especially for those of rare and staple medicinal materials, such as *Bupleurum chinense* DC., *Scutellaria baicalensis* Georgi and *Salvia miltiorrhiza* Bunge which have strong demand for quality stability.

MATERIALS AND METHODS

Male-sterile plants and their potential maintainers' identification

To identify potential male-sterile resources, about 100 bell flower germplasms containing cultivated populations, wild accessions and self-cross lines from different ecological regions of China (including Shandong, Anhui, Hebei, Gansu, Inner Mongolia and Beijing) were screened during 2004 to 2005. A total of 15 individual male-sterile plants were selected. The characters of male-sterile plants' flowers were observed. The pistil and stamen were observed using Olympus-SZX9 stereomicroscope. TTC method was used to assay the pollen vigor. Pollens placed on a microscope slide were dipped in a drop of 0.5% TTC, bathed in 35°C for 15 min and then observed using Olympus-BX51 microscope. The anther and pollen from male-sterile and normal plants were observed after staining with hematoxylin using paraffin section method. To investigate the female fertility of those 15 original male-sterile plants and the male fertility of their progenies, 8 fertile plants, JA88, JA143, JA71, JA64, JC52, P, W1 and W2, were used as male parents for test crosses. The number of pollinated flowers (NPF), the number of fruits setting (NFS), the rate of fruits setting (RFS), the number of seed per fruit (NS), and the sterile plant rate of F1 (RMS) were recorded. To explore the potential uses of the male-sterile plants in future breeding practice, the main agronomical traits of the male-sterile plants were compared with those of their source populations and the traits of the progenies were compared with those of the male test parents' self-cross progenies. Simultaneously, the fertile plants with good performance in agricultural traits were self-crossed to develop into self-cross lines.

Male-sterile line GP1BC1-12 development

Further test or sister crosses were performed to evaluate the seed set for the male-sterile progenies of the 6 original male-sterile plants' test crosses. According to the second generation progenies' performance on fertility, growth vigor and roots characters in the test or sister crosses, the original male-sterile plant, JA162, and the test parents, JA71 and JA88, were promising materials for further development of steady male-sterile line and its corresponding maintainers. Therefore, three additional generations test crosses were conducted between male-sterile progenies of JA162 and the self-cross lines of JA88 and JA71 successively in Hainan (a tropical province in the south of China, where is preferred as a plant adding-generation place in winter for Chinese crop breeders) and Beijing. During the course of test crosses, the fertility and agronomy performance of progenies were investigated and eventually, a male-sterile line GP1BC1-12 with 100% sterility was developed.

Hybrid combinations performance trial between male-sterile line GP1BC1-12 and 18 self-cross lines

To evaluate the performance of the male-sterile line GP1BC1-12 in producing high yield hybrids, a total of 18 self-cross lines were selected as test parents for the combinations in Beijing in July 2007. The cultivated population from Chifeng, a main bell flower production region of Inner Mongolia in China, was used as the control. The comparing experiment was conducted in a completely randomized design with four replicates in Beijing in April 2008. Each replicate was planted in a plot of 1 m^2 with row distance 25 cm and plant distance 5 cm. During the full-bloom stage, the pollen fertility of F1 hybrids was investigated. Five flowers from each of 100 randomly selected plants were observed for each replicates. The fertility for each plant was classified into three grades: Complete sterile (S: Flowers examined are all sterile), partial fertile (PF: at lest one of flowers examined is sterile) and complete fertile (F: Flowers examined are all fertile).

At medicinal roots harvest time, main agronomical and economic traits of F1 hybrids were investigated. A total of 20 plants were randomly sampled for each replicates. The root weight (RW), the main root weight (MRW), the rate of lateral root weight (RLRW), the lateral root number (LRN), the root up diameter (RUD, on the root of 1 cm down away from the interface of root and stem), the root middle diameter (RMD), the rate of taproot per plot (RT) and the drying rate of root (DRR) were recorded. Heterosis performance of each hybrid derived from the male-sterile line GP1BC1-12 was evaluated by examining the heterosis of each trait in comparison to that of the control. The data were evaluated by analysis of variance, and the means were compared using Dunnett's test to determine significance.

Three high yield and disease resistant hybrid cultivars breeding

Three cross combinations which showed excellent performance among the 18 combinations were selected for further investigation. Two Chinese main cultivated populations were used as control in strain comparison trial which was conducted with 30 m^2 plot area in Beijing. Since bell flower was often planted as biennial in China, the character assay was separately conducted in the first and second years for the strain comparison trial. The sowing date was May 17, 2009 and the planting density was ca. 1,500,000 plants/ha for the first growing year. The first harvest time was October 15, 2009. On the early second growing year, the density was regulated to 900,000 plants/ha and the second harvest time was October 22, 2010.

After harvest, the main agronomic traits such as root weight, stem length, stem diameter, root length, root up diameter, root middle diameter were measured for 40 randomly collected individual plants and the average values were calculated. Total roots from 50 plants were used to assay the quality. The contents of total saponin and Platycodin D were determined using weight method and high-performance liquid chromatography-UV (HPLC-UV) method, respectively. The content of the polysaccharide was determined using phenol-H_2SO_4 colorimetry method. The content of the crude fiber was determined using acid hydrolysis combined with ashing method according to the national standard GB/T5009.10-2003 ("Determination of crude fiber in vegetable foods"). All data were statistically analyzed by ANOVA method. The significant difference was assayed by LSD method. The different letters listed after the data showed the significant difference at $P \leq 0.05$. The fresh and dry root yields were calculated from the plot total output. In 2010, two hybrids were planted in two Chinese main producing areas of bell flower, Shandong and Anhui provinces to evaluate the yield trait and adaptability.

RESULTS

Male-sterile plants discovery in wild and cultivated populations of bell flower

The male-sterile resources play an important role in production of hybrid seed. We discovered the male-sterile plants of bell flower for the first time in 2004. Compared with that of the normal plants, the anther of the male-sterile plants was wilting and yellowing with little or without pollens (Figure 1). The stamen was only half the length of its stigma. But the stigma was morphologically normal. The vigor of those few pollens from the male-sterile plants was assayed using TTC method and the result showed the pollens had no vigor indeed. Observed under microscope, abnormal microspores and tetrads instead of normal pollen grains filled in the anther sacs in one kind of the male-sterile plants and some analogous leftover filled in that of the other kind of the male-sterile plants (Figure 2).

The comparison on the main characters of some male-sterile individual plants with their source populations showed that no significant difference existed between them and no disadvantage existed in the growth and development of the male-sterile plants (data not shown). The male sterility of the progenies and the pistil development of the male-sterile plants were investigated in the test crosses. The result showed the pistils of most male-sterile plants could accept pollens and complete fertilization and seed setting (Table 1). The highest rate of fruits setting was produced in the combinations, JA109×JA88 (4/4, 100%) and JC259×JC52 (12/14, 85.7%) and the lowest rate was in the combinations, JA221×JA137 (1/29, 3.4%) and JC194×W2 (1/26, 3.8%). The most number of seed per fruit was in the combinations JA110×JA137 (137) and JA110×W2 (115), JA115×JA71 (115) and the fewest number was in the combination of JC194×JA143 (4), JC68×P (5). Considering the fruit and seed setting together, 9 of the 15 male-sterile individual plants (JA37, JA73, JA75, JA109, JA110, JA115, JA162, JA204 and JS11) were suitable for further investigation. The sterility percents for the first generation progenies of 12 natural-crossed original male-sterile individual plants were from 53.3 to 87.9%. For the test-crossed progenies, the percents were from 33.3 to 95.2%. Over 90% male-sterile rates were obtained in the four combinations, JA162×JA71 (35: 2, 94.6%), JA162×JA88 (40: 2, 95.2%), JA115×JA88 (12: 1, 92.3%, JA75×JA143 (13: 1, 92.9%) (Table 2). The test progenies of the male-sterile plants had different performance in characters assayed. Generally, no

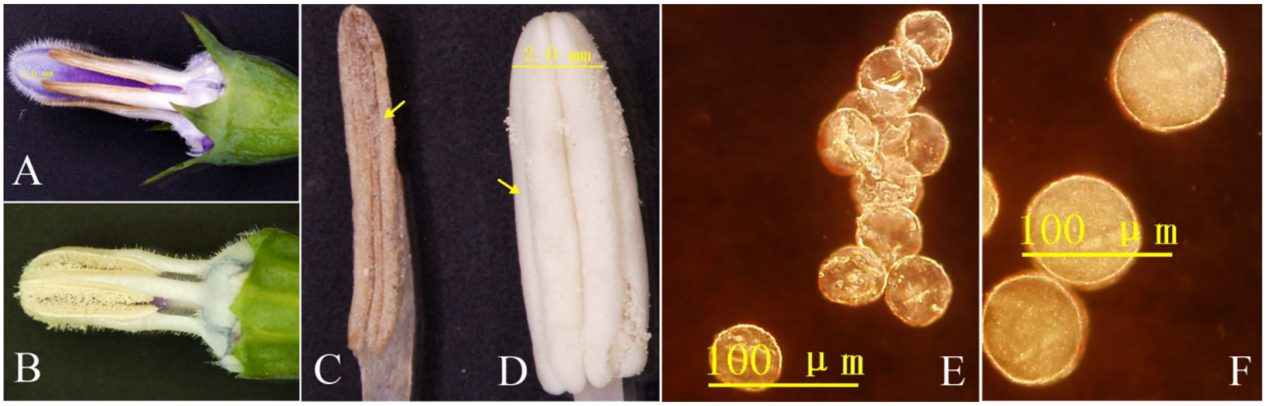

Figure 1. Comparison of anther, stigma and pollen from normal and male-sterile plants. *A, C* and *E* shows flower after removing petals, a wilting and yellowing anther and abnormal pollens from a male-sterile plant, respectively. *B, D* and *F* show a flower after removing petals, an anther and pollens from a normal plant, respectively.

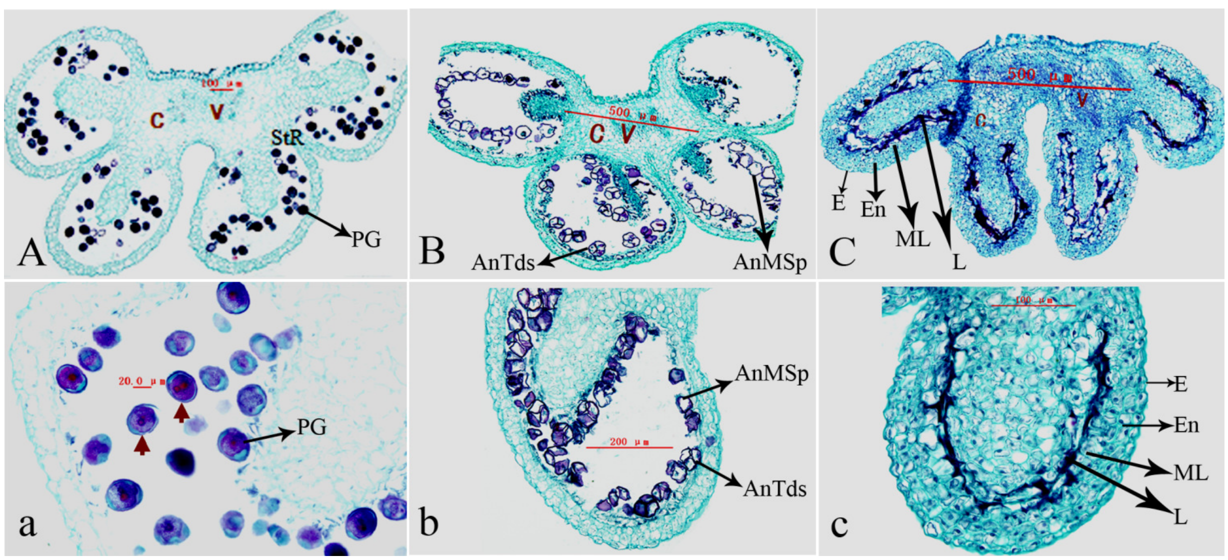

Figure 2. Comparison of the anther and pollens from the normal and male-sterile plants. *A and a* shows anther and pollens from a normal plant. *B and b* shows and abnormal microspores from a male-sterile plant JA37. *C and c* shows anther from a male-sterile plant JA162 showing the abnormal existing of the leftover. AnMSp, abnormal microspores; AnTds, tetrads; C, connective; E, epidermis; En, endothecium; L, leftover; ML, middle layer; PG, pollen grains; StR, stomium region; V, vascular region.

apparent inferiorities were observed. For the medicinal and dietary uses of bell flower, the roots yield was an important trait. In exception of the crosses, JA162×JA71, JC194×JA71 and JC194 × JA88, all test crosses' progenies had much higher roots weights (Table 3). According to these primary results, it is practicable to develop stable male-sterile lines and use them in producing high yield hybrids for bell flower.

Development of the male-sterile line GP1BC1-12

According to the primary results of the test crosses, the male-sterile progenies of JA162 were continuously test crossed with the self-cross progenies of JA71 and JA88. Since vernalization is not required for the germination of *Platycodon*, generation adding could be used to accelerate the development of male-sterile line. The crosses were successively performed in February in Hainan and in July in Beijing from 2005 to 2007. The fourth cross seeds of the male-sterile line, designated GP1BC1-12, were obtained in April, 2007. The male-sterile rates were increased with the increasing of crossing generations and the 100% sterility of the progenies was finally achieved. JA88 was finally selected to use as maintainer.

Table 1. Seed setting performance of crosses derived from 15 male-sterile plants by artificial pollination.

No.	MS[a]	TMP[b]	NPF[c]	NFS[d]	RFS[e] (%)	NS[f]
01	JC13	JA71	3	2	66.7	21
		JA88	2	0	0.0	0
		JA143	4	0	0.0	0
		JC52	13	2	15.4	32
		P	14	3	21.4	20
		W1	3	1	33.3	25
		W2	13	1	7.7	7
02	JC68	JC52	5	0	0.0	0
		W1	2	0	0.0	0
		W2	11	0	0.0	0
		P	16	2	12.5	5
03	JC194	JA71	11	6	54.5	88
		JA88	11	7	63.6	44
		JA143	2	1	50.0	4
		JC52	27	7	25.9	44
		W1	5	0	0.0	0
		W2	26	1	3.8	15
		P	25	3	12.0	11
04	JC259	JC52	14	12	85.7	47
		W1	8	3	37.5	60
		W2	22	5	22.7	9
		P	23	9	39.1	29
05	JA37	JA71	31	20	64.5	80
		JA88	39	24	61.5	77
		JA143	24	3	12.5	38
		JS64	17	1	5.9	33
		JA137	21	16	76.2	66
		W2	37	2	5.4	24
		P	29	3	10.3	31
06	JA73	JA137	20	7	35.0	78
		W2	32	7	21.9	68
		P	39	9	23.1	18
07	JA75	JA71	16	6	37.5	59
		JA88	20	11	55.0	72
		JA143	8	5	62.5	52
		JS64	10	1	10.0	11
		JA137	21	10	47.6	81
		W2	24	2	8.3	38
		P	23	4	17.4	26
08	JA78	JA71	6	3	50.0	88
		JA88	7	3	42.9	79
		JA143	6	0	0.0	0
		JS64	7	0	0.0	0
09	JA109	JA71	3	2	66.7	35
		JA88	4	4	100.0	73
		JS64	4	0	0.0	0
10	JA110	JA137	30	21	70.0	63
		W2	32	10	31.3	40
		P	21	12	57.1	21
		JA137	23	18	78.3	137
11	JA115	W2	23	8	34.8	115
		P	20	11	55.0	22
		JA71	14	6	42.9	115
12	JA162	JA88	15	4	26.7	94
		JA143	11	1	9.1	31
		JS64	5	2	40.0	67
		JA71	33	18	54.5	77
		JA88	24	12	50.0	95
		JA143	19	7	36.8	94
		JS64	14	1	7.1	28
		JA137	23	7	30.4	85
13	JA204	W2	23	4	17.4	69
		P	21	2	9.5	22
		JA71	23	12	52.2	61
14	JA221	JA88	13	9	69.2	57
		JA143	8	2	25.0	46
		JS64	10	0	0.0	0
		JA71	12	0	0.0	0
		JA88	11	2	18.2	20
		JA143	6	0	0.0	0
		JS64	9	0	0.0	0
15	JS11	JA137	29	1	3.4	18
		W2	27	0	0.0	0
		P	28	3	10.7	12
		JA71	3	2	66.7	39
		JA88	4	2	50.0	68
		JS47	23	17	73.9	61
		W2	20	0	0.0	0
		P	22	4	18.2	11

[a] MS: Male-sterile individual plant. [b] TMP: Test male parent. [c] NPF: Number of pollinated flowers. [d] NFS: Number of fruits setting. [e] RFS: Rate of fruits setting. [f] NS: Number of seed per fruit.

Table 2. Fertility segregation of natural-crossed and test-crossed progenies of male-sterile plants.

No.	MS[a]	Fertility segregation of natural-crossed progenies			TMP[e]	Fertility segregation of test-crossed progenies		
		NMF[b]	NMS[c]	RMS[d] (%)		NMF	NMS	RMS (%)
01	JC13	10	21	67.7	JA71	1	0	-[f]
02	JC68	7	27	79.4				
03	JC194				JA71	13	16	55.2
					JA88	4	10	71.4
					JA143	0	3	-[f]
05	JA37	12	14	53.8	JA71	21	27	56.3
					JA88	16	33	67.3
					JA143	0	1	-[f]
					JS64	1	1	-[f]
07	JA75	4	29	87.9	JS64	0	2	-[f]
					JA71	10	8	44.4
					JA88	5	14	73.7
					JA143	1	13	92.9
08	JA78	7	20	74.1	JA71	14	7	33.3
					JA88	0	1	-[f]
09	JA109	10	16	61.5	JA88	5	8	61.5
					JA71	1	2	-[f]
11	JA115	6	24	80.0	JA71	2	1	-[f]
					JS64	2	4	-[f]
					JA88	1	12	92.3
12	JA162	6	31	83.8	JA71	2	35	94.6
					JA88	2	40	95.2
					JS64	0	4	-[f]
					JA143	9	21	70.0
13	JA204	12	22	64.7	JA143	1	1	-[f]
					JA71	6	15	71.4
					JA88	4	9	69.2
14	JA221	14	16	53.3				
15	JS11	11	20	64.5				

[a] MS: Male-sterile individual plant. [b] NMF: Number of male-fertile progenies. [c] NMS: Number of male-sterile progenies. [d] RMS: Rate of male-sterile progenies. [e] TMP: Test male parent. [f]: too few progenies to calculate the male-sterile rate.

The stability of the male-sterile line, GP1BC1-12, was confirmed in Hainan and Beijing from 2008 to 2009.

Hybrids performance and the male-sterile type determination of GP1BC1-12

To detect the heterotic performance of the hybrids derived from the male-sterile line GP1BC1-12, the mean square values and over-standard heterosis indices for main agricultural traits were calculated on the basis of the difference between 18 hybrids and the control. The mean squares for the seven traits are shown in Table 4. Variability among crosses was highly significant ($P \leq 0.01$) for root weight, main root weight, rate of lateral root weight, lateral root number, roots up diameter and rate of taproot. No significant difference was observed for root middle diameter and drying rate of root (Table 4).

Table 3. Comparison of characters between the one-year old progenies of test crosses and the self-cross progenies of the test male parents.

Combination/line	Upground weight (g)	Fresh root weight (g)	Dry root up diameter (cm)	Root length (cm)	Lateral root no.	Fruit no.
JA37×JA71	16.4	19.6	1.5	16.8	6.7	44.6
JA75×JA71	19.4	14.9	1.3	16.7	6.5	37.8
JA115×JA71	27.8	19.7	1.3	18.3	7.0	66.8
JA162×JA71	22.1	13.6	1.4	14.0	8.5	64.4
JC194×JA71	15.4	12.3	1.3	13.7	5.8	35.8
GS116[a] (JA71[b])	13.5	14.8	1.3	18.2	6.8	30.8
JA37×JA88	25.3	24.6	1.5	18.2	9.7	65.2
JA75×JA88	31.1	21.9	1.4	16.9	6.8	187.0
JA115×JA88	41.2	25.8	1.5	14.3	10.8	71.0
JA162×JA88	29.8	22.0	1.5	14.2	10.2	81.8
JC194×JA88	8.1	12.4	1.2	14.1	4.8	23.0
GS125[a] (JA88[b])	14.3	13.6	1.4	17.9	6.2	14.6
JA162×JA143	24.3	18.9	1.3	14.2	7.6	57.4
GS139[a] (JA143[b])	11.4	10.7	1.1	17.7	4.4	27.2

[a] Self-cross progenies lines of the test male parents. [b] Test male parents.

Table 4. Average heterosis and main agronomic and economic traits variance analyses of progenies of GP1BC1-12 and 18 self-cross lines.

No.	Male parent	RW[a] (%)	MRW[b] (%)	RLRW[c] (%)	LRN[d] (%)	RUD[e] (%)	RMD[f] (%)	RT[g] (%)	DRR[h] (%)
1	GS262	45.79 **	43.60 **	40.18	63.68	10.13	24.85 *	23.77	9.10 **
2	GSPW-W3	55.28 **	54.59 **	14.23	56.02	15.93 *	30.63 **	9.53	7.18
3	GSNH2-W3	103.30 **	91.40 **	97.21	235.70	23.97 **	278.55	-32.71	-5.87 *
4	GSNH3-M1	82.24 **	71.79 **	83.20	175.75	22.81 *	32.92	-13.41	4.75
5	GS171-W1	77.68 *	71.02 **	77.45	71.15	26.11 **	28.60	-12.31	9.47 **
6	GS217-3-3	48.52 **	43.19 *	80.14 *	158.67 *	19.81 **	4.99	-77.44 **	5.52
7	GS111-1-4	24.05	25.48	-23.95	-3.97	9.32	12.22	46.98	2.49
8	GS171-9-1	63.35 **	56.26 **	78.82	44.94	21.15 **	20.97 **	50.69	6.56
9	GS204-3-5	115.10 **	103.92 **	97.99 *	217.84 *	19.30	25.78	-15.56	10.37 *
10	GS259-5-3	-6.79	-4.99	-38.35	-76.23 **	-7.77	-6.91	67.20 *	1.61
11	GS150-1	14.00	13.62	24.39	3.25	0.08	19.97 *	60.30 *	1.15
12	GS228-1	53.84 *	48.40 *	62.88	77.16	14.24 *	18.38 *	34.36	9.31
13	GS218-1	4.79	5.09	-8.25	-25.54	0.86	12.10	51.67	-3.83
14	GS107-1	-1.78	-0.02	-36.13 *	-59.04 *	-9.45	-1.41	78.67 **	2.85
15	GS206-1	40.73 *	38.69 *	21.21	16.58	28.43	15.86	91.36 **	8.50
16	GS109-1	36.11 *	37.11 **	-15.57	17.01	14.31 *	11.40	38.80 *	4.57
17	GS266	33.21 *	34.33 *	-9.12	25.56	12.25 *	6.34	36.21	29.45
18	GS205-1	65.56 **	64.51 **	15.45	50.01	23.42 **	21.56 **	20.02	15.19 *
	Average	47.50	44.33	31.21	58.25	13.60	30.93	25.45	6.58
					Means square values				
S.O.V.	**df**	**RW**	**MRW**	**RLRW**	**LRN**	**RUD**	**RMD**	**RT**	**DRR**
Crosses	17	14.854**	10.51 **	20.81 **	0.81 **	0.06 **	0.13	782.68 **	8.14
Error	54	2.612	1.69	9.00	0.26	0.02	0.11	112.18	9.09

[a]RW: Root weight. [b] MRW: Main root weight. [c] RLRW: Rate of lateral root weight. [d] LRN: Lateral root number. [e] RUD: Root up diameter. [f] RMD: Root middle diameter. [g] RT: Rate of taproot per plot. [h]DRR: Drying rate of root.

Analyses on the degrees of dominance for eight traits of the 18 hybrids showed that all traits in the hybrids had positive over-standard heterosis, and the trait of root weight had the highest heterosis (Table 4). It was suggested that heterosis in agricultural traits was common among hybrids of bell flower.

Table 5. Rate of fertility segregation of hybrids derived from GP1BC1-12 and 18 self-crossed lines.

No.	Male parent	NPI [a]	S [b] (%)	PS[c] (%)	F[d] (%)
1	GS262	92	83.7	7.6	8.7
2	GSPW-W3	59	100	0	0
3	GSNH2-W3	77	96.1	3.9	0
4	GSNH3-M1	83	94.0	6.0	0
5	GS171-W1	67	91.1	5.9	3
6	GS217-3-3	87	94.3	3.4	2.3
7	GS111-1-4	83	81.8	10.8	8.4
8	GS171-9-1	93	91.4	2.1	6.5
9	GS204-3-5	50	100	0	0
10	GS259-5-3	100	75	12	13
11	GS150-1	85	89.5	4.7	5.8
12	GS228-1	85	84.8	7.0	8.2
13	GS218-1	95	88.5	4.2	7.3
14	GS107-1	110	91.0	7.2	1.8
15	GS206-1	86	69.8	15.1	15.1
16	GS109-1	90	58.9	23.3	17.8
17	GS266	91	97.9	2.1	0
18	GS205-1	67	95.6	4.4	0

[a] NPI: Number of plants investigated. [b] S: Complete sterile. [c] PS: Partial sterile. [d] F: Complete fertile

Sixteen hybrids out of 18 crosses displayed positive heterosis in both the root weight and the main root weight, among which 12 hybrids displayed significant positive heterosis. The range of heterosis was between -6.79 and 115.10% in the root weight and between -4.99 and 103.92% in the main root weight, respectively. Higher heterosis was noted in cross 9 followed by cross 3. No significant negative heterosis was observed in all 18 crosses. Six crosses exhibited negative heterosis in the rate of lateral root weight over the control ranging from -38.15 to -8.25%. Cross 14 gave significant and minimum heterosis (-36.13%). Only four crosses displayed negative heterosis in the number of lateral root per plant, and among which two crosses were significant. Sixteen of 18 crosses showed positive heterosis in root up diameter and root middle diameter ranging from -9.45 to 28.43% and -6.91 to 278.35%, respectively. And 10 crosses in the root up diameter and six in the root middle diameter had significant positive heterosis over the control. For the rate of taproot per plot, five crosses exhibited significant positive heterosis, while only one cross indicated significant negative heterosis. Sixteen crosses showed positive heterosis in drying rate of root ranging from -5.87 to 29.45%, and four crosses was significant. Above all, heterosis was embodied in most agricultural traits relating to the yield and morphology of F1 crosses root, and the male-sterile line is feasible to improve the yield and shape of root by F1 in bellflower.

It was shown that the rates of male-sterile progenies from two crosses reached 100%, those from 15 crosses more than 80%, and that from one cross less than 60% (Table 5). Based on the rate of fertility segregation of the 18 crosses' progenies, it was predicted that the male-sterile line, GP1BC1-12, belonged to the nucleo-cytoplasmic interaction type (CMS). It also can be deduced that maintainers of GP1BC1-12 existed extensively and the male parents of Cross 2 and 9 could thoroughly keep its sterility.

Characters of three high yield hybrid cultivars

Three of the aforementioned 18 cross combinations were screened for further hybrids assessment. The seeds of the male-sterile line GP1BC1-12 and its maintainer JA88 were propagated and more hybrid seeds of the three crosses were produced for strain comparison and regional yield trials. Now the three hybrids, which were designated as "Zhonggeng No. 1", "Zhonggeng No. 2" and "Zhonggeng No. 3", had been officially identified as new cultivars of bell flower by Beijing Municipal Seed Management Station. The strain comparison trail was conducted in Beijing with two cultivated populations from Chinese main producing areas as controls in 2009. The results of the investigation on the agronomic characteristics of one-year and two-year harvested plants are shown in Tables 6 and 8. The average single root weight of the three hybrids was 8.0, 7.5 and 9.8 g for one-year harvested plants and 27.8, 25.3 and 26.3 g for two-year harvested plants, respectively. For one-year harvested roots, the fresh and dry root yields of the three hybrids were all much higher than those of the two controls. For two-year harvested roots, the fresh root yield of "Zhonggeng No. 1" was significantly higher than

Table 6. Agronomic characteristics of one-year and two-year harvested three hybrid cultivars.

Cultivar	Root weight (g)	Stem length (cm)	Stem diameter (mm)	Rate of lateral root weight (%)	Lateral root No.	Root length (cm)	Root up diameter (mm)	Root middle diameter (mm)
One-year planting density: ~ 1,500,000 plants/ha								
Zhonggeng No. 1	8.0 c	38.4 b	2.7 ab	6.3 a	0.6 ab	26.6 b	14.1 b	3.5 a
Zhonggeng No. 2	7.5 bc	45.6 c	2.9 bc	4.0 a	0.6 ab	25.3 ab	12.4 ab	3.2 a
Zhonggeng No. 3	9.8 d	45.6 c	3.0 c	4.5 a	1.4 bc	25.0 ab	14.1 b	3.5 a
CK1	5.4 a	34.4 a	2.4 a	4.8 a	0.5 a	24.2 ab	10.9 a	3.1 a
CK2	5.9 ab	35.3 ab	2.4 a	4.7 a	0.5 a	24.1 a	11.2 a	3.0 a
Two-year planting density: ~ 900,000 plants/ha								
Zhonggeng No. 1	27.8 c	111.9 cd	6.5 b	11.6 b	3.3 b	26.3 a	20.6 b	11.1 b
Zhonggeng No. 2	25.3 bc	107.8 c	5.9 b	17.5 ab	3.8 b	25.4 a	21.4 b	9.8 b
Zhonggeng No. 3	26.3 c	115.6 d	6.0 b	5.8 c	3.0 b	28.6 b	21.9 b	10.2 b
CK1	21.1 b	87.7 a	5.0 a	16.1 b	3.1 b	26.6 a	17.2 a	8.5 a
CK2	16.2 a	95.5 b	4.9 a	14.4 b	1.8 a	26.1 a	15.9 a	7.4 a

significant difference existed among the fresh root yields of "Zhonggeng No. 2", "Zhonggeng No. 3" and the two controls. The dry root yields of the three hybrids were all higher than those of the two controls. For the annual plants of three hybrid cultivars, "Zhonggeng No. 1" showed significantly higher single root weight and root up diameter than controls. "Zhonggeng No. 2" showed significantly higher stem length and stem diameter than controls. "Zhonggeng No. 3" showed significantly higher single root weight, root up diameter, lateral root number, stem length and stem diameter than controls. For the biennial plants of three hybrid cultivars, "Zhonggeng No. 1" showed significantly higher single root weight, stem length, stem diameter, root up diameter and root middle diameter than controls. Zhonggeng No. 2" showed significantly higher stem length, stem diameter, root up diameter and root middle diameter than the two controls. "Zhonggeng No. 3" showed significantly higher values in all tested characters except lateral root number than the two controls. Considering two years performance, CK2 had relative fewer lateral root numbers, while "Zhonggeng No. 3" had fewer lateral root weight than other cultivars. The crude saponin, platycodin D, polysaccharide and crude fiber of one-year and two-year harvested roots of the three hybrid cultivars were assayed and listed in Table 7. The contents of crude saponin and platycodin D were lower for the two-year harvested roots than the one-year harvested roots, while the contents of crude fiber were vice versa except that of "Zhonggeng No. 3" which had no apparent different. The content of polysaccharide in one-year harvested roots of "Zhonggeng No. 3" was significant higher than those of other two cultivars and two controls. To be noticed, significant difference existed in the platycodin D content of the biennial roots of all tested cultivars and controls.

The plants in the 2009 comparison trial were regulated to density of ca. 900,000 plants/ha and were harvested in October, 2010. The one-year and two-year root yields are listed in Table 8. The root yields of three hybrid cultivars were all higher than those of the two controls. Although "Zhonggeng No. 1" had a relative lower root yield than other two hybrid cultivars for the first year, its yield for the second year was the highest among all the tested populations. "Zhonggeng No. 2" and "Zhonggeng No. 3" had the highest yields for the first year, but for the second year their fresh root yields had no significant difference with those of the two controls; their dry root yields significantly higher than those of the two controls and lower than that of "Zhonggeng No. 1". According to the results of strain comparison trail, "Zhonggeng No. 2" and "Zhonggeng No. 3" can be sown on spring and harvest on autumn In the same year, while "Zhonggeng No. 1" is more suitable for two-year planting. This phenomenon will be further confirmed in subsequent comparison trails conducted in different ecological environment. The hybrid cultivars of "Zhonggeng No. 2" and "Zhonggeng No. 3" were planted in Shandong and

Table 7. Saponin, polysaccharide and crude fiber contents in dried roots of the three hybrid cultivars.

Cultivar	One year root				Two years root			
	Crude saponin (%)	Crude saponin (%)	Polysaccharide (%)	Crude fiber (%)	Crude saponin (%)	Platycodin D (%)	Polysaccharide (%)	Crude fiber (%)
Zhonggeng No. 1	4.65 [a]	0.635 [a]	10.9 [d]	5.0 [a]	2.45 [a]	0.09 [d]	16.5 [ab]	6.5 [b]
Zhonggeng No. 2	4.81 [a]	0.655 [a]	17.3 [b]	5.5 [a]	2.89 [b]	0.10 [d]	19.5 [c]	6.7 [b]
Zhonggeng No. 3	4.01 [c]	0.524 [b]	19.4 [a]	5.9 [a]	2.59 [ab]	0.08 [c]	17.1 [b]	5.8 [a]
CK1	4.62 [ab]	0.661 [a]	14.8 [c]	5.4 [a]	2.47 [a]	0.05 [b]	15.0 [a]	6.3 [b]
CK2	4.18 [bc]	0.557 [b]	6.6 [e]	5.1 [a]	2.91 [b]	0.04 [a]	18.4 [c]	8.0 [c]

Table 8. Root yield of three hybrid cultivars in strain comparison and regional yield trials.

Cultivar	Strain comparison trial in Beijing				Regional yield trial in Shandong province	Regional yield trial in Anhui province
	One year roots (Density: ~ 1,500,000 plants/ha)		Two years roots (Density: ~ 900,000 plants/ha)		One year roots (Density: ~ 1,000,000 plants/ha)	One year roots (Density: ~ 1,000,000 plants/ha)
	Fresh (kg/ha)	Dry (kg/ha)	Fresh (kg/ha)	Dry (kg/ha)	Fresh (kg/ha)/single root weight (g)	Fresh (kg/ha)/ single root weight (g)
Zhonggeng No. 1	12,300 [b]	2,512 [b]	28,170 [b]	7,043 [c]	-	-
Zhonggeng No. 2	15,030 [c]	3,506 [c]	21,405 [a]	5,565 [b]	8,235/9.5	16,983/16.6
Zhonggeng No. 3	17,865 [c]	3,926 [c]	20,100 [a]	5,226 [b]	9,180/11.9	12,987/12.4
CK1	8,085 [a]	1,395 [a]	20,625 [a]	4,538 [a]	-	5,994/6.0 (native)
CK2	8,100 [a]	1,612 [a]	19,005 [a]	4,371 [a]	4,635/6.0 (native)	-

Anhui provinces of China in 2010 with the native cultivated population as control. those of the two controls and the other two hybrids. No The two hybrid cultivars showed high disease resistant and had nearly two times of root yield than control in Shandong province and more than two ("Zhonggeng No. 3") or three ("Zhonggeng No. 2") times in Anhui province. The average single root weight of the two hybrid cultivars ("Zhonggeng No. 2" 9.5 g in Shandong, 16.6 g in Anhui; "Zhonggeng No. 3" 11.9 g in Shandong, 12.4 g in Anhui) were also much higher than that of their native control (both were 6.0 g in two provinces) (Table 8 and Figures 3B, b, C and c).

DISCUSSION

Utilization heterosis is an effective method to improve yield or quality of crops and vegetables. However, to the best of our knowledge, no other practice on hybridization was conducted for bell flower, a plant species with multiple functions in edible, medicinal and ornamental fields. Root is the main economic part of bell flower for edible and medicinal uses. Long and thick main root with less lateral root is desired in the market. In our present study, the root traits of hybrids derived from male-sterile line GP1BC1-12 were improved not only in the weight but also in the shape. There were 88% (16/18) hybrids in our investigated cross combinations displaying positive heterosis in root weight (RW), 100% (18/18) hybrids displaying positive heterosis in root up diameter (RUD) and root middle diameter (RMD) over the control. Though an increase in the number of lateral root (LRN) was comparatively common (14/18; 83.33%) in the hybrids, 33.33% (6/18) hybrids displayed finer lateral roots. Moreove

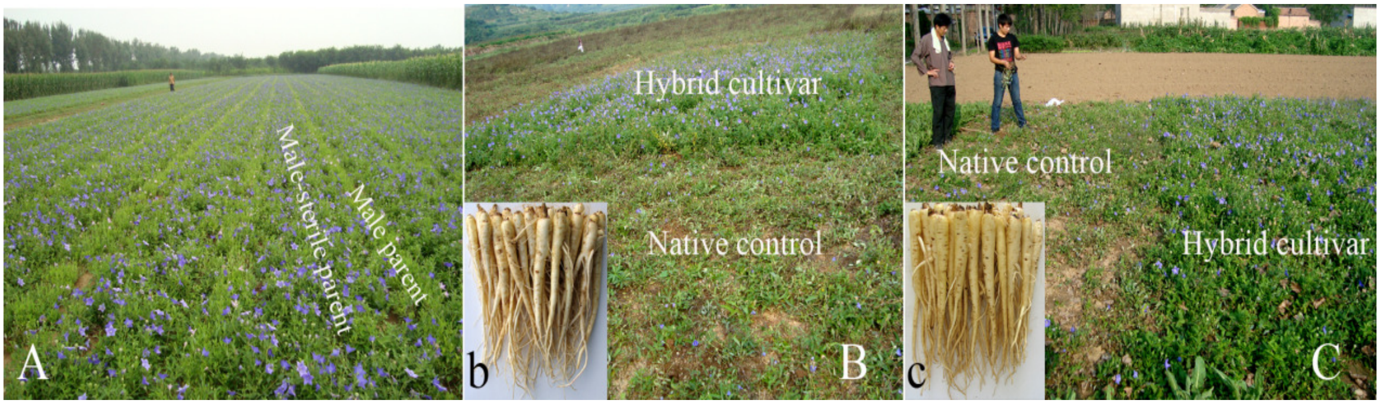

Figure 3. Hybrid seed production and hybrid cultivar regional trials. *A*, field producing hybrid seeds planted with male-sterile parent showing purple flowers and male parent showing pink flowers; *B* and *b*, regional trial field and the produced roots in Shandong province; *C* and *c*, regional trial field and the produced roots in Shandong province.

the rate of taproot per plot (RT) had a positive heterosis in 72.22% (13/18) hybrids. Our results indicated that it is promising to exploit hybrid vigor for the improvement of economic organ of bell flower. For our firstly officially identified three hybrid cultivars, the high root yield and high disease resistance were their predominant virtues. Adding the hybrids were prepared with the male-sterile line, the production of hybrid seeds was relatively easier. At present, we are energetically releasing and promoting the new cultivars. Compared with the reports on the pure line selected cultivar "Jangbaek" from Korea (Kim et al., 2004), the yields of our three hybrid cultivars (28,170, 21,405 and 20,100 kg/ha) were almost one time higher than those of "Jangbaek" and its control (11,600 and 9,990 kg/ha) for two years planting. The contents of Platycodin D in the two-year planted roots of our hybrid cultivars were near those in the four-year planted roots of "Jangbaek" and its control (0.072 and 0.086%), and were much lower than those in the one-year planted roots of our hybrid cultivars. While the contents of crude saponin in the four-year planted roots of "Jangbaek" and its control (3.69 and 3.85%) were between those in the one-year and two-year planted roots of our hybrid cultivars (ca. 4 to 5% and 2 to 3%, respectively). Therefore, we initiated researches on hybrid cultivar breeding for bell flower, although more works were needed to be done, such as evaluating the combining ability and heterosis over main traits of yield, pharmacological components contents and disease resistance, introducing the male sterility from the male-sterile plants to the elite cultivars or cultivated types.

In the regional yield trials of the three hybrid cultivars derived from three of 18 crosses in Shandong and Anhui in 2010, the yields of hybrid cultivars were much more than those of their individual native controls (more than two times). In a certain extent, one of the reasons was that the hybrid cultivars showed stronger disease resistance. The disease happened in mid-July and earlier August when raining was much. The stems of diseased plants were firstly rotting and withering and then the whole aerial parts of plants wilted, but new shoots of most diseased plants sprouted in late August. This led to smaller roots of diseased native control plants although the roots numbers per unit area did not reduced. In harvest season, some rotted roots were found in both hybrid cultivars and native controls. Therefore, more pathogens researches and disease resistant cultivars breeding are in need.

In China, most of medicinal materials are derived from wild resources or cultivated types that farmers spontaneously tended and domesticated in recent years. The diverse and heterologous planting populations, adding the regulation-lacking management during cultivation and harvesting, have been considered as the fundamental origin of quality unstable medicinal materials. Our successful works are an example in attempting to improve the stability, consistency and controllability of Chinese medicinal materials by breeding and utilization of hybrid cultivar with the same genetic background. For the edible and decoction slices usage of bell flower, large root with fewer lateral branches for easier preprocessing, high polysaccharide and low crude fiber may be the desired traits. For the medicinal extraction usage of bell flower, high root yield and high content of pharmacological components, such as saponins, are desired. Indeed, the output of total roots, the bulk of single root, and the amount of lateral root branches are three associated traits. Usually, in higher planting density, the yield of harvested total roots is higher and the bulk of single root and the amount of lateral root branches are relatively lower. Therefore, it will be our resent research goal to breed specialized cultivars for edible, decoction slices or medicinal chemical extraction purpose and to determine the necessary cultivation techniques for best displaying the heterosis of new hybrid cultivars (Xu et al., 2004).

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 30772728), the Important National Science and Technology Specific Projects (2009ZX09308-002) and the National Department Public Benefit Research Foundation (201107011; 201107009-05).

REFERENCES

Chinese Pharmacopoeia Commission (2005). Pharmacopoeia of the People's Republic of China. People's Medical Publishing House, I: 227.

Choi YH, Yoo DS, Cha MR, Choi CW, Kim YS, Choi SU, Lee KR, Ryu SY (2010). Antiproliferative effects of saponins from the roots of *Platycodon grandiflorum* on cultured human tumor cells. J. Nat. Prod., DOI: 10.1021/np100496p

Fu XJ, Liu HB, Wang P, Guan HS (2009). A study on the antioxidant activity and tissues selective inhibition of lipid peroxidation by saponins from the roots of *Platycodon grandiflorum*. Am. J. Chin. Med., 37(5): 967-975.

Halevy AH, Shlomo E, Ziv O (2002). Improving cut flower production of balloon flower. Hort Sci., 37(5): 759-761.

Havey MJ (2004). The use of cytoplasmic male sterility for hybrid seed production. In: Daniell H, Chase C (eds) Molecular biology and biotechnology of plant organelles, Springer, Dordrecht, The Netherlands, pp. 617-628.

Hochholdinger F, Hoecker N (2007). Towards the molecular basis of heterosis. Trends Plant Sci., 12(9): 427-432.

Hong DY, Lian YS, Shen LD (1983). Flora Reipublicae Popularis Sinicae. Sci. Press, 73(2): 76-77.

Hosoda K, Noguchi M, Kanaya T, Nishijima A (1992). Studies on the cultivation and preparation of *Platycodon* root. III: Effect of picking flower and fruit on the quality of skin peeled root. Chem. Pharm. Bull., 40(7): 1946-1947.

Hu Q, Pan RL, Wang LW, Peng B, Tang JT, Liu XM (2010). *Platycodon grandiflorum* induces apoptosis in SKOV3 human ovarian cancer cells through mitochondrial-dependent pathway. Am. J. Chin. Med., 38(2): 373-386.

Jeong CH, Choi GN, Kim JH, Kwak JH, Kim DO, Kim YJ, Heo HJ (2010). Antioxidant activities from the aerial parts of *Platycodon grandiflorum.* Food Chem., 118(2): 278-282.

Kang YS, Hong KP, Jung DC, Hong S, Lee JH, Nah SY, Lim Y, Bae DH (2008). Calcium channel-blocking activity of Chinese balloon flower (*Platycodon grandiflorum*) for producing blood pressure-lowering functional foods. Food Sci. Biotechnol., 17(1): 156-160.

Kim HT, Kim GS, Lim GJ, Seong JD, Han SI, Kwack YH, Suh DY, Kim SS (2004). A new high-yielding and white color balloon flower cultivar, "Jangbaek". Korean J. Breed, 36(1): 69-70.

Kim KS, Seo EK, Lee YC, Lee TY, Cho YW, Ezaki O, Kim CH (2000). Effect of dietary *Platycodon grandiflorum* on the improvement of insulin resistance in obese Zucker rats. J. Nutr. Biochem., 11: 420-424.

Kim YS, Kim JS, Choi SU, Kim JS, Lee HS, Roh SH, Jeong YC, Kim YK, Ryu SY (2005). Isolation of a new saponin and cytotoxic effect of saponins from the root of *Platycodon grandiflorum* on human tumor cell lines. Planta Med., 71(6): 566-568.

Lee JY, Hwang WI, Lim ST (2004). Antioxidant and anticancer activities of organic extracts from *Platycodon grandiflorum* A. De Candolle roots. J. Ethnopharmacol., 93(2-3): 409-415.

Lee YH, Kim YJ, Kim HI, Cho HY, Yoon GH (2007). Potent HAT inhibitory effect of aqueous extract from bellflower (*Platycodon grandiflorum*) roots on androgen receptor-mediated transcriptional regulation. Food Sci. Biotechnol., 16: 457-462.

Lippman ZB, Zamir D (2007). Heterosis: revisiting the magic. Trends Genet., 23(2): 60-66.

Liu ZG, Zhang Y, Wang XY, Yu HS, Wu Z (2006). The research progress of breeding in *Platycodon grandiflorum*. Chin. Trad. Herbal Drugs, 37(6): Appendix 6-8.

Meyer RC, Kusterer B, Lisec J, Steinfath M, Becher M, Scharr H, Melchinger AE, Selbig J, Schurr U, Willmitzer L, Altmann T (2010). QTL analysis of early stage heterosis for biomass in *Arabidopsis*. Theor. Appl. Genet., 120(2): 227-237.

Moon MK, Ahn JY, Kim S, Ryu SY, Kim YS, Ha TY (2010). Ethanol extract and saponin of *Platycodon grandiflorum* ameliorate scopolamine-induced amnesia in mice. J. Med. Food, 13(3): 584-588.

Noh JR, Kim YH, Gang GT, Yang KJ, Kim SK, Ryu SY, Kim YS, Lee CH, Lee HS (2010). Preventative effects of *Platycodon grandiflorum* treatment on hepatic steatosis in high fat diet-fed C57BL/6 mice. Biol. Pharm. Bull., 33(3): 450-454.

Park BH, Oliveira N, Pearson S (1998). Temperature affects growth and flowering of the balloon flower [*Platycodon grandiflorum* (Jacq.) A. DC. cv. Astra Blue]. Hort. Sci., 33 (2): 233-236.

Patel SJ, Desai RT, Bhakta RS, Patel DU, Kodappully VC, Mali SC (2009). Heterosis studies in cowpea (*Vigna unguiculata* (L.) Walp) Legume Res., 32(3): 199-202.

Pradhan AK, Sodhi YS, Mukhopadhyay A, Pental D (1993). Heterosis breeding in India mustard (*Brassica juncea* L. Czern and Coss): Analysis of component characters contributing to heterosis for yield. Euphytica, 69(3): 219-229.

Shen JX, Fu TD, Yang GS, Ma CZ, Tu JX (2005). Genetic analysis of rapeseed self-incompatibility lines reveals significant heterosis of different patterns for yield and oil content traits. Plant Breeding, 124: 111-116.

Shukla SK, Pandey MP (2008). Combining ability and heterosis over environments for yield and yield components in two-line hybrids involving thermosensitive genic male sterile lines in rice (*Oryza sativa* L.). Plant Breeding, 127: 28-32.

Sodhi YS, Chandra A, Verma JK, Arumugam N, Mukhopadhyay A, Gupta V, Pental D, Pradhan AK (2006). A new cytoplasmic male sterility system for hybrid seed production in Indian oilseed mustard *Brassica juncea* Theor. Appl. Genet., 114(1): 93-99.

Song CY, Roh MS, Chung SK, Lawson RH (1993). Effect of temperature and light on growth and flowering of potted plant production of *Platycodon*. J. Korea Soc. Hort. Sci., 34(6): 446-453.

Song GS, Zhai HL, Peng YG, Zhang L, Wei G, Chen XY, Xiao YG, Wang L, Chen YJ, Wu B, Chen B, Zhang Y, Chen H, Feng XJ, Gong WK, Liu Y, Yin ZJ, Wang F, Liu GZ, Xu HL, Wei XL, Zhao XL, Ouwerkerk PBF, Hankemeier T, Reijmers T, Vander Heijden R, Lu CM, Wang M, Vander Greef J, Zhu Z (2010). Comparative transcriptional profiling and preliminary study on heterosis mechanism of super-hybrid rice. Mol. Plant Doi:10.1093/mp/ssq046

Springer NM, Stupar RM (2007). Allelic variation and heterosis in maize: how do two halves make more than a whole? Genome Res., 17: 264-275.

Vogel KP, Mitchell RB (2008). Heterosis in switchgrass: biomass yield in swards. Crop Sci., 48(6): 2159-2164.

Wu JR, Yan YZ, Quan XL (2008). Discovery and identification of male sterility *Platycodon grandiflorum* germplasm JXB-1. Bull. Bot. Res., 28: 716-719.

Xu BJ, Zheng YN, Sung CK (2004). Quality control and evaluation of *Platycodon grandiflorum* implications for future management. Nat. Prod. Sci., 10: 141-151.

Zhao TJ, Gai JY (2006). Discovery of new male-sterile cytoplasm sources and development of a new cytoplasmic nuclear male-sterile line NJCMS 3A in soybean. Euphytica, 152: 387-396.

Zhu YW, Shan CG, Ni DP, Zhang JH, Su XH, Yan SL, Wang ZF (2009). Breeding and cultivation techniques of new balloonflower variety Lugeng 1. Shandong Agric. Sci., 1: 115-116.