

A Novel Double-flowered Cultivar of *Gypsophila paniculata* Mutagenized by ^{60}Co γ -Ray

Fan Li, Xijun Mo, Lifang Wu, and Chunmei Yang

National Engineering Research Center for Ornamental Horticulture, Key Laboratory for Flower Breeding of Yunnan Province, Floriculture Research Institute, Yunnan Academy of Agricultural Sciences, Kunming 650200, China

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Gypsophila paniculata, widely known as baby's breath, is a flowering plant of the Caryophyllaceae family (Li et al., 2019). Because of its ornamental value as one of the most important cut flowers in global commercial floriculture, *G. paniculata* ranks as one of the top-ten best-selling cut flower species globally (Zvi et al., 2008). However, classical hybridization is seriously hampered by the sterility of *G. paniculata* (Wang et al., 2013). As a consequence, very few variations exist among commercial cultivars used as fresh and dried cut flowers for fillers in flower arrangements (Zvi et al., 2008). Therefore, there is massive demand in the floriculture market for new varieties with novel characteristics.

Considering the challenges associated with genetic engineering of specific ornamental traits, the proven technique of radiation mutagenesis is an ideal alternative. Effectively, it induces a variety of mutagenesis responsible for alteration traits. Indeed, numerous traits have been generated through the use of radiation mutagenesis techniques (e.g., X-rays and γ -rays), including flower type and color, all of which determine the quality and popularity of cut flowers (Lin et al., 2019; Singh, 2014; Singh and Bala, 2015; Vasko and Kyrychenko, 2016). Flower type is the key ornamental trait and the top interest in *G. paniculata* breeding. Several commercial varieties of *G. paniculata* have been released by breeders, such as 'Million Stars', 'Bristol Fairy', 'Snowball', and 'Cloudstar 5' (Li et al., 2019; Shibuya et al., 2017; Zvi et al., 2008). To develop new cultivars with a novel double-flower type, we conducted gamma radiation muta-

genesis using a variety of 'Cloudstar 4', which has a semidouble flower. In 2018, a new *G. paniculata* cultivar was selected from the mutagenized population and was named 'Huixing 1'. Compared with its progenitor ('Cloudstar 4'), 'Huixing 1' has bigger flowers and more flower petals.

Origin

'Huixing 1' originated from a γ -ray mutants screen using the seedlings of 'Cloudstar 4', which has semidouble flowers. The plant seedlings were obtained from Yuxi Yunxing Biological Technology Co., Ltd. (Yuxi, China) and were planted separately

in 128-well plant trays (well diameter, 3.0 cm; well height, 4.7 cm) filled with commercial potting medium (a peat and perlite mixture). In May 2017, the seedlings were mutagenized using gamma radiation (3 kR, 4 kR, and 5 kR). Thereafter all the mutagenized plants were grown in a solar greenhouse (without heating) using a natural photoperiod at the experimental farm of Yunnan Academy of Agricultural Sciences in Yuxi, China. In Jan. 2018, a unique mutant showing bigger and double flowers was observed and isolated from the 4-kR gamma radiation mutagenized population, which was distinct from 'Cloudstar 4'. This mutant plant was then separated into several individuals by cutting propagation and the clones were grown at the same experimental farm. During the flowering period in Dec. 2018, the field performance of this mutant population displayed stable and consistent morphological traits as the mother mutant, demonstrating the flower trait of this mutant is fixed and can be inherited stably. To speed up the propagation of this mutant, the stout plants were selected as explants for tissue culture to generate a larger number of clone plants. In July 2019, 9000 individual seedlings were divided into three repetitions and grew at the production base of Lumeiyi Village, Kunming, for further field performance testing. In Apr. 2020, the new cultivar was officially authorized to be released as 'Huixing 1' by the Yunnan Forestry and Grassland Bureau with accession no. 20200013.



Fig. 1. The flower phenotype of (A) 'Huixing 1' and (B) 'Cloudstar 4'. The flower of 'Huixing 1' has a bigger flower size than 'Cloudstar 4'. The flower type of 'Huixing 1' is a double flower, whereas 'Cloudstar 4' is a semidouble flower. 'Huixing 1' has more flower petals than 'Cloudstar 4', which is consistent with the flower type. Bar = 1 cm.

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L.W. and C.Y. supervised this work jointly.

C.Y. is the corresponding author. E-mail: yangcmfri@foxmail.com.

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Table 1. Morphological trait differentiation of the new cultivar Huixing 1 and cultivar Cloudstar 4.²

Cultivar	Petal no.			Flower diam (cm)			Leaf length (cm)		
	Mean	SD	Sig.	Mean	SD	Sig.	Mean	SD	Sig.
Huixing 1	132.73	8.20	a	1.30	0.05	a	8.70	0.50	a
Cloudstar 4	55.77	9.17	b	1.11	0.06	b	10.22	0.50	b

²The data were collected from 30 plants (10 plants of each replication) planted in Lumeiyi Village, Kunming, China. One-way analysis of variance was used to assess statistical significance, and *P* values were calculated with Tukey's honestly significant difference test ($\alpha = 0.05$). Sig. = significance.

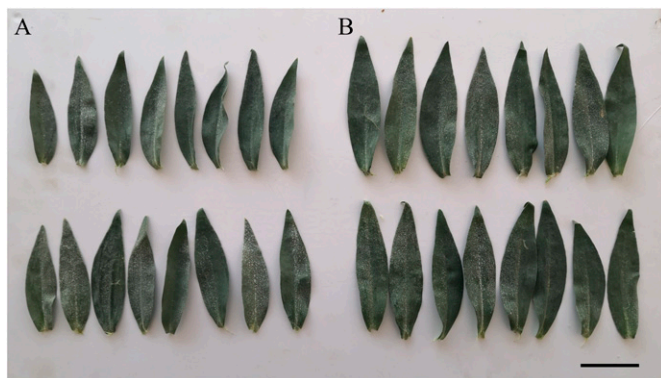


Fig. 2. The leaf phenotype of (A) 'Huixing 1' and (B) 'Cloudstar 4'. The leaves of 'Huixing 1' are smaller than those of 'Cloudstar 4'. Bar = 5 cm.

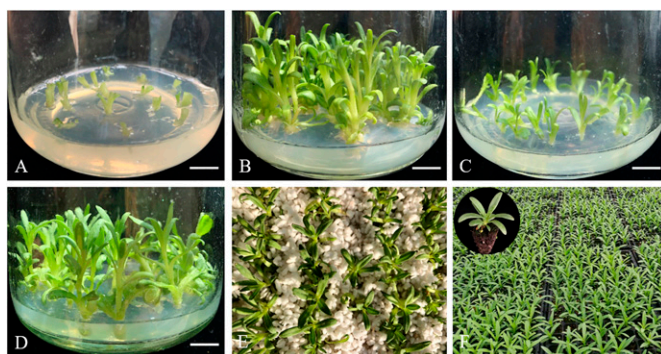


Fig. 3. The progress of commercial propagation of 'Huixing 1' by tissue culture to produce numerous standard seedlings. (A) Short explant shoots were inoculated in induction MS medium. (B) Clustered shoots formed after 30 d. (C) The clustered shoots were cut into individual shoots with stem tips, and were cultured in root primordium induction MS medium. (D) The seedlings were grown in a 10-h photoperiod (light intensity, 5000 Lx) at 22 °C. (E) The seedlings were transplanted into perlite for root formation culture. (F) The commercial standard seedlings were planted in 128-well plant tray. Bar = 1 cm.

Description

The flower of 'Huixing 1' has white double petals that are distinguishable from those of 'Cloudstar 4' (Fig. 1). The petal number of 'Huixing 1' was around 133 ($n = 30$), which was more than 2-fold compared with 'Cloudstar 4' (55.77 ± 9.17 , $n = 30$) (Table 1). Moreover, the flower size of 'Huixing 1' was about 1.30 cm, which was a little bit larger than that of 'Cloudstar 4' (1.11 cm, $n = 30$). In addition to these two distinctly different ornamental traits, the leaf length of 'Huixing 1' was shorter than that of 'Cloudstar 4' (Fig. 2). Therefore, the phenotype of 'Huixing 1' is very

different from its progenitor ('Cloudstar 4'), especially with regard to flower type.

Commercial propagation of 'Huixing 1' can use tissue culture to produce numerous standard seedlings (Fig. 3). The detailed protocol is as follows. The stout vegetative shoots were selected as explants and sterilized in 0.14% mercury-containing solution for 15 min in the erlenmeyer flask (Guangzhou Jet Bio-Filtration Co., Ltd., TAB101250). The sterilized shoots were then cut 1.0 cm long and inoculated in the induction MS medium (1.5 mg/L BA, 0.1 mg/L NAA). After 30 d, clustered shoots were cut into individual shoots along with the stem tip (1.2 cm). The

shoots were transferred into root primordium induction MS medium (0.1 mg/L IAA, 0.2 mg/L NAA) for 10 d and grown in a 10-h photoperiod (light intensity, 5000 Lx) at 22 °C. Last, the seedlings were first transplanted into perlite for root formation culture and then were transplanted into the 128-well plant tray filled with mixed substrate (perlite:peat = 1:1) after 30 d. The commercial standard seedlings were produced after 20 to 25 d, which were ready to sell.

Availability

An application for Plant Variety Protection has been filed for 'Huixing 1' by the Floriculture Research Institute of Yunnan Academy of Agricultural Sciences and Yuxi Yunxing Biological Technology Co., Ltd. Commercial production of this cultivar is required to have licensing agreements with the agency. The seedlings can be obtained from the Yuxi Yunxing Biological Technology Co., Ltd.

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