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# Acquirement of a new male sterile germplasm of Chinese jujube

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**Abstract** A male sterile germplasm of Chinese jujube (*Ziziphus jujuba* Mill.), named ‘male sterile No.1’ (JMS1), was firstly identified from a natural population through studies of pollen amount and vitality and its anatomy. Its microspores got massed and then disaggregated after the tetrad stage during pollen development. Then its anthers became empty, or only pollen vestiges remained in the yellow buds. The pollen became abortive after the tetrad stage. Hypertrophy and hyperplasia of tapetum at the tetrad stage were related to pollen abortion. In view of its moderate embryo fertility, big fruit-size and early maturing, ‘JMS1’ could be used as a worthwhile female parent in the cross breeding of Chinese jujube.

**Keywords** Chinese jujube, male sterility, new germplasm, male sterile No.1

## 1 Introduction

No crossbred cultivar in Chinese jujube has been released up to now because of the difficulties in stamen removal, which is the key obstacle in its cross breeding. Its male organs mature earlier than female ones during flower development, so manual emasculation must be completed before bud cracking. However, Chinese jujube buds are very small, only 3–5 mm

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in diameter. Flowers are usually damaged during emasculation manipulation. Pollination is also very difficult to successfully carry out. If male sterile germplasm is available and utilized, the manual emasculation will not be necessary and cross breeding will be greatly promoted. So far, there has been no special research on male sterility in references of Chinese jujube pollen and hybridization (Lu, 2003). In our studies from 2002 to 2005, a male sterile germplasm unable to produce pollen was firstly identified, and its cytological characteristics during pollen abortion were also observed.

## 2 Methods

Fifty-two cultivars planted in the germplasm garden of Chinese jujube located in Cangxian County, Hebei, China were sampled for pollen vitality test from June 3 to 7 in 2002. Flowers in the middle of bearing shoot were collected at sepal flat stage and pollen vitality was determined by I<sub>2</sub>-IK staining method (Zhang, 1990).

Buds from cultivar ‘*Hunanjidanzao*’ and ‘male sterile No.1 (JMS1)’ were collected and fixed in FAA liquid every 7 days from the end of May to the end of June in 2002, 2003 and 2004. Their changes of anatomical structure during pollen development were observed by the method of paraffin section and with light microscopes.

Pollen amount per anther in yellow buds of ‘JMS1’, ‘*Pingguozao*’, ‘*Lajiaozao*’, and ‘*Mingshandazao*’ from the counties of Cangxian and Mancheng in florescences in 2004 and 2005 was checked by counting chambers method (Zhang, 2001).

## 3 Results

### 3.1 Comparison of pollen viability among cultivars

Pollen viabilities were obviously different among the cultivars (14.1%–76.8%) (Table 1) with coefficient of variation of 40.20%. ‘*Wuhezao*’ showed the lowest pollen viability (14.1%). Pollen viabilities of ‘*Huluchanghong*’, ‘*Banzao*’

**Table 1** Pollen vitality of Chinese jujube cultivars

Cultivars	Vitality/%	Cultivars	Vitality/%	Cultivars	Vitality/%
Wuhezao	14.1	Yuanzao	30.2	Mayizao	42.8
Banzao	15.2	Huizao	32.2	Duanguochanghong	43.0
Huluchanghong	15.4	Dasuanzao	32.5	Chaoyangyuanzao	44.5
Dongzao	16.7	Huairoudacui	32.7	Dalingzao	45.3
Lingbaodazao	17.4	Chengtuoazao	32.9	Yuanlingzao	47.5
Zhuibuluo	17.5	Jianzao	33.0	Xuezao	49.0
Jiuyueqing	19.0	Ruanhezao	33.3	Chahuzao	50.1
Shangdonglizao	20.3	Mamazao	33.4	Mosaizao	50.3
Xiaozizao	23.1	Junzao	34.0	Suanmianzao	55.6
Linxexiaozao	23.2	Damuzao	34.1	Pingguozao	57.1
Dunhuangdazao	24.0	Gutouxiaozao	34.2	Changjixinzao	58.5
Jinsixiaozao	24.3	Langjiayuanzao	34.5	Wutouzao	58.5
Mianxiaozao	25.0	Popozao	36.1	Dalilongzao	62.6
Yiwuzao	25.2	Lizao	38.2	Mingshandazao	65.5
Jiebuqi	25.2	Henandamaya	41.1	Malianxiaozao	76.8
Niujiaozao	26.5	Hunanjidanzao	41.1	Material No.1	No pollen
Jinsixin-2	27.3	Xiangzao	41.1		
Manmanzao	27.5	Lianxianmuzao	42.3		

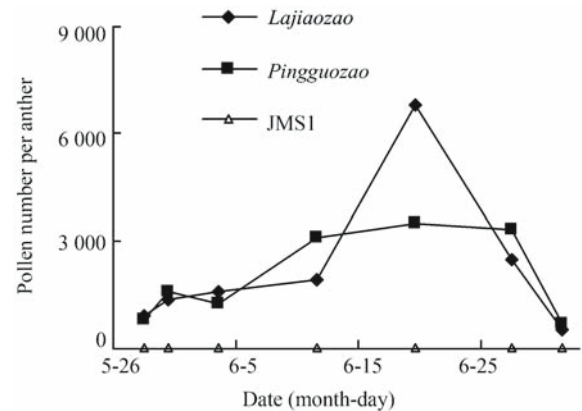
and ‘Dongzao’ were lower than 17.0%. In contrast, the highest of 76.8% was observed in ‘Malianxiaozao’ which was higher than 55.0% obtained in ‘Dalilongzao’, ‘Mingshandazao’, ‘Pingguozao’, ‘Wutouzao’, ‘Changjixinzao’ and ‘Suanmianzao’. Especially, a germplasm without pollen was found and named male sterile No.1 of Chinese jujube (JMS1). This germplasm from natural Chinese jujube population could be used as the key material in male sterility research.

### 3.2 Anatomical observation of anther of different cultivars

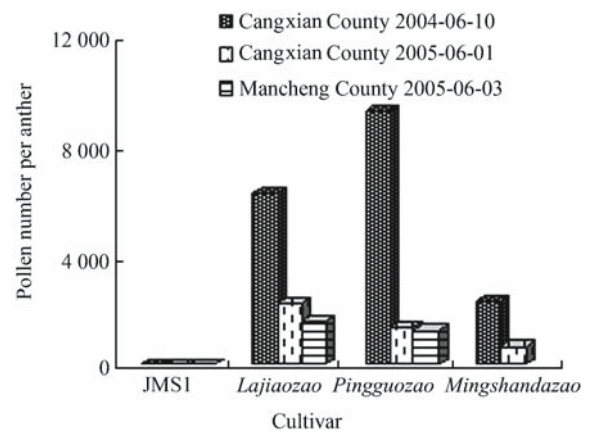
The anther anatomy structure of ‘JMS1’ was similar to ‘Hunanjidanzao’ at early stages and obvious differences appeared after the middle stage of pollen development. Anther tapetum of ‘JMS1’ showed abnormal hypertrophy and hyperplasia at tetrad stage (Plate I A–B), microspores got massed and then became disaggregated after tetrad (Plate I C–D) making anthers empty or being left only with pollen vestiges in the yellow buds (Plate I E–F). Therefore, compared with ‘Hunanjidanzao’, the pollen of ‘JMS1’ became abortive after tetrad and no normal pollen survived in the yellow buds (Plate I G–H).

### 3.3 Stability of male sterile trait in ‘JMS1’

While no pollen was observed in the yellow buds of ‘JMS1’ collected from Mancheng County in 2005, pollen amount per anther of ‘Lajiaozao’ and ‘Pingguozao’ was low at the end of May or early June, but reached the highest value in mid-June and then decreased (Fig. 1). Pollen amount per anther of ‘Lajiaozao’, ‘Pingguozao’, and ‘Mingshandazao’ in Cangxian County was higher in 2004 than in 2005. Pollen amount per anther of ‘Lajiaozao’, ‘Pingguozao’ in Cangxian County was higher than in Mancheng County in 2005 (Fig. 2). Thus, pollen amount was found related to sampling time and site. However, no pollen was observed in the yellow buds of

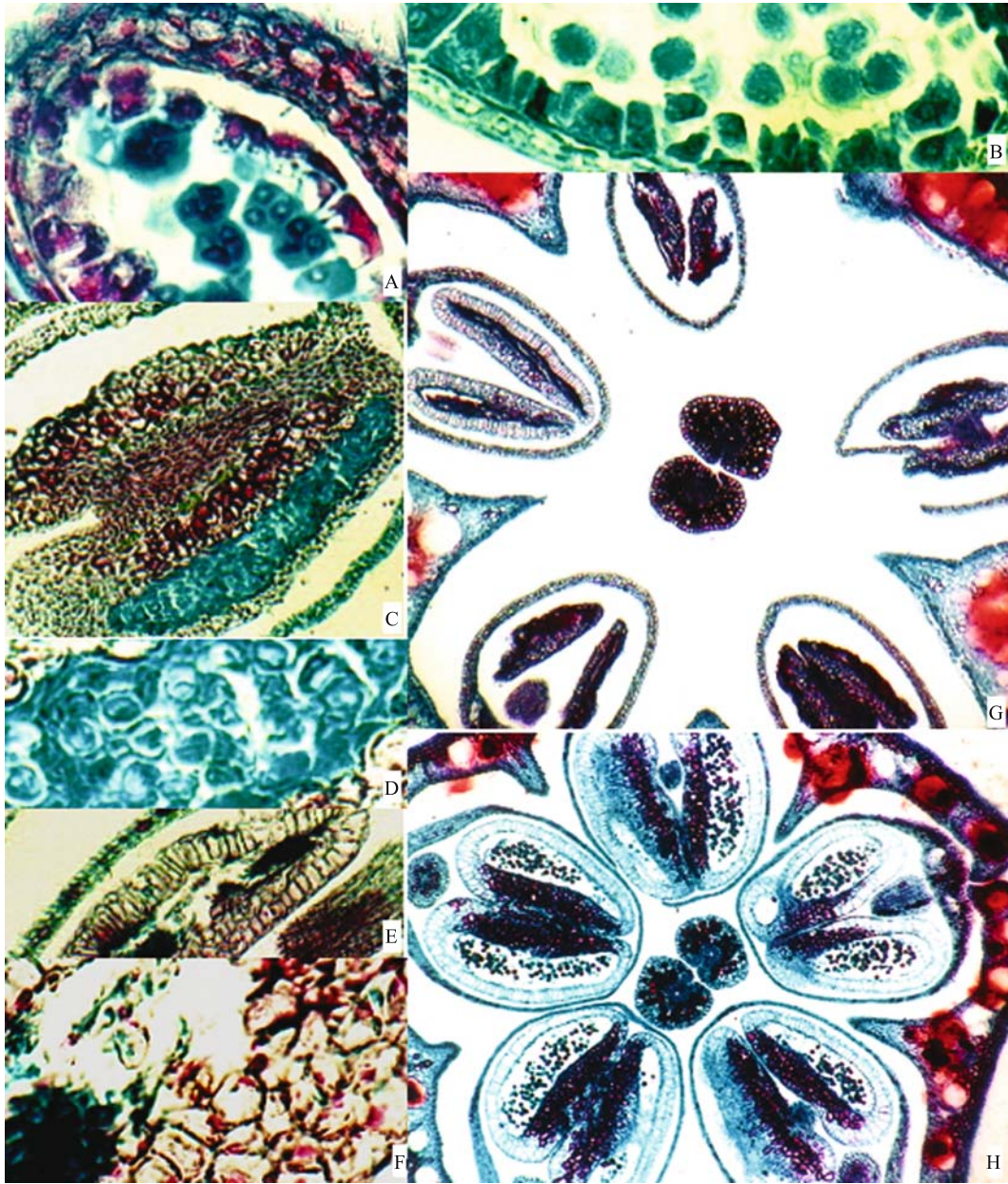


**Fig. 1** Changes of pollen number of Chinese jujube during flower season (Mancheng County, 2005)



**Fig. 2** Changes of pollen number of Chinese jujube in different years

‘JMS1’ collected from Cangxian County on May 29, June 1, June 10, and June 15 in 2004 and June 1, June 23, June 28, and August 29 in 2005. The results from Cangxian and



A: Tetrads of 'JMS1'  $\times 400$ ; B: Microspores of 'JMS1'  $\times 400$ ; C: 'JMS1' anthers with empty pollen sac  $\times 100$ ; D: Degenerated pollen sacs of 'JMS1'  $\times 400$ ; E: Cross section of 'JMS1' yellow buds  $\times 100$ ; F: Cross section of 'JMS1' yellow buds  $\times 400$ ; G: Cross section of 'JMS1' yellow buds  $\times 40$ ; H: Cross section of 'Hunanjidanzao' yellow buds  $\times 40$

**Plate I** Anatomical structure of pollens in 'JMS1'(A-G) and 'Hunanjidanzao'(H)

Mancheng Counties in 2004 and 2005 indicated that the trait of male sterility in 'JMS1' was stable.

#### 4 Discussion

'JMS1', a male sterile germplasm unable to produce pollen, was firstly identified from a natural population of Chinese jujube. In view of its moderate embryo fertility, big fruit-size, and early maturing, 'JMS1' could be used as a valuable female parent in cross breeding. Several abnormal changes of tapetum including hypertrophy, getting vacuolated, and early

or delayed disorganization are involved in plant male sterility (Singh and Kaul, 1991; Ilarslan et al., 1999). Hypertrophy and hyperplasia of tapetum were obvious at the tetrad stage of pollen development in 'JMS1'. After that, microspores got massed and then disaggregated. It is concluded, therefore, that hypertrophy and hyperplasia of tapetum at tetrad stage may be associated with pollen abortion in 'JMS1'.

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