Scientia Horticulturae 190 (2015) 36–42

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Development and utilization of an efficient cytoplasmic male sterile system for Cai-xin (*Brassica rapa* L.)

Ying Peng[a,1], Dianyi Shi[b,1], Tian Zhang[a], Xuehong Li[a], Tingdong Fu[c], Yuejin Xu[a,*], Zhengjie Wan[a,*]

[a] College of Horticulture and Forestry, Huazhong Agricultural University, Key Laboratory of Horticultural Plant Biology, Ministry of Education, Wuhan 430070, China

[b] National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

[c] College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

ARTICLE INFO

Article history:
Received 23 January 2015
Received in revised form 31 March 2015
Accepted 1 April 2015
Available online 29 April 2015

Keywords:
Cai-xin (*Brassica rapa* L. ssp. *chinensis* var. *utilis* Tsen et Lee)
Cytoplasmic male sterility
Anther development
Heterosis

ABSTRACT

Six cytoplasmic male sterile (CMS) lines of *Brassica* vegetable Cai-xin (*Brassica rapa* L. ssp. *chinensis* var. *utilis* Tsen et Lee) were newly developed by backcrossing of six local inbred lines with a new *eru* CMS line of the vegetable purple cai-tai (*B. rapa* L. var. *purpurea* Bailey) from South China. The sterility of these Cai-xin *eru* CMS lines was stable and complete. The line 4A represented typical sterility and produced six off-white and shriveled stamens without pollen grains in the anthers. Paraffin sectioning comparison of the anthers between the CMS line 4A and its maintainer line 4B showed that their developmental differentiations started at the early to middle mononucleate microspore stage. The tapetum in the CMS line 4A was vacuolized highly and expanded radically, which extruded the developing microspores and caused their adhesion with each other and failure to develope mature pollen. With six CMS lines as female parents and 6 fertile lines as pollen parents, 36 hybrids were produced and their potentials of heterosis were analyzed. In general, mid-parent heterosis was detected for most of the 14 agronomic, yield and quality traits, such as petiole length (19.01%), weight of main flower stalk (35.96%), and soluble protein content (1.20%). These results indicated that the six newly derived *eru* CMS lines of Cai-xin provided a novel CMS system for its breeding and heterosis utilization.

1. Introduction

The *Brassica* vegetable Cai-xin (*Brassica rapa* L. ssp. *chinensis* var. *utilis* Tsen et Lee) originated from China and is a variant of Chinese cabbage, which is still cultivated widely in south China. Its growth period is short and the multiple cropping index is high, with only about 40–56 days from germination to flowering and 80–90 days to seed maturation (Li et al., 2011). The stalk is tender and easy to cook and tastes well. Currently, the main cultivars of Cai-xin in China are conventional varieties (Li et al., 2011).

Brassicaceae crops display strong hybrid vigor, and have long been subject to F_1 hybrid breeding. Two pollination control mechanisms, self-incompatibility (SI) and male sterility are widely used for production of F_1 hybrid seeds in *Brassicaceae* crops. So far, majority of cruciferous hybrid cultivars have been developed by using SI system (Watanabe and Hinata, 1999). However, SI system has several disadvantages (Jirik, 1985; Kucera, 1990; Sharma et al., 2004). For example, the hybridity level reduced in F_1 hybrids of the SI system. The artificial pollination and high cost limited the utilization of SI parents in production (Wan et al., 2013). The most reliable system of F_1 seed production is based on cytoplasmic male sterility (CMS), and various types of CMS have been developed and adopted in practice to breed *Brassicaceae* oil seed and vegetable crops (Yamagishi and Bhat, 2014). CMS is common in angiosperms and is maternally inherited, resulting in the failure to produce functional pollen (Kaul, 1988). CMS is often associated with novel mitochondrial open reading frames, which interferes with the proper functioning of mitochondria and pollen development (Hanson, 1991; Hanson and Folkerts, 1992; Bonen and Brown, 1993). Several CMS systems have been reported in *Brassica* crops, including *Raphanus*/*ogu* (Ogura, 1968), *tour* (Mathias, 1985), *polima or pol* (Fu, 1981), *Moricandia-arvensis* (Bhat et al., 2006, 2008), *hau*

* Corresponding authors. Tel.: +86 27 8728056; fax: +86 27 87280566.
E-mail addresses: xyjho@mail.hzau.edu.cn (Y. Xu), wanzj@mail.hzau.edu.cn (Z. Wan).

[1] The authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scienta.2015.04.002
0304-4238/

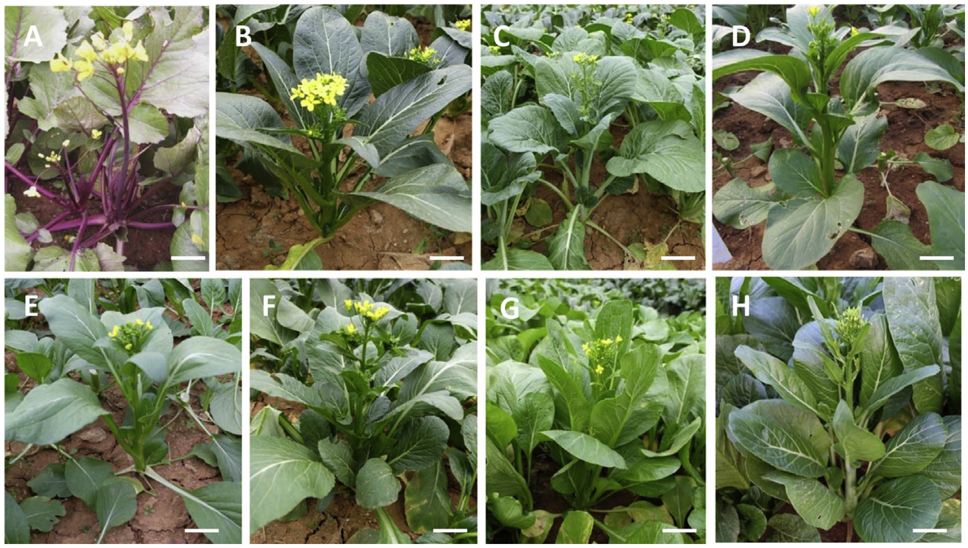

Fig. 1. *Phenotype of donor parent and six eru CMS lines.* (A) Purple cai-tai *eru* CMS line. (B–G) Six Cai-xin CMS lines 1A–6A. (H) F_1 hybrid with 35.96% MPH for weight of main flower stalk.

(Wan et al., 2008), *Shan* 2*A* (Li, 1980), Nsa (Hu et al., 2004), 681A, 126-1 (Sodhi et al., 2006) and *nap* CMS (Shiga and Baba, 1971, 1973; Thompson, 1972). The *pol* CMS is sensitive to environment in certain nuclear backgrounds, leading to breakdown of sterility and the reduction of hybridity levels in F_1 hybrids.

However, there are also some disadvantages for the utilization of CMS. When it was transferred to other species or variants with different nuclear backgrounds, some undesirable phenotypes would finally appear in the F_1 hybrids, such as malformed and degenerated anthers, poorly developed nectars, poorly exserted stigmas, dead buds and lack of petals (Wan et al., 2008). In addition, seedlings of these hybrids were sensitive to temperature sometimes. For instance, when radish *Ogura* CMS was introduced into *B. napus*, the resultant alloplasmic lines of *B. napus* showed male sterility, but all of them had chlorotic leaves, yellowing at low temperatures below 15 °C (Pelletier et al., 1983). Chlorophyll deficiency in these lines was suggested to result from functional incompatibility between the *B. napus* nucleus and *R. sativus* chloroplasts, which was undesirable for breeders. Therefore, the existing CMS lines should be constantly improved during breeding processes, and more CMS resources should be developed to meet the changing requirements (Yamagishi and Bhat, 2014; Wan et al., 2013).

At present, CMS resource for the vegetable Cai-xin is unavailable. During the past two decades, breeders had transferred some dominant or recessive genic male sterility (GMS) and CMS from *B. napus* L. to this variant by backcrossing. However, most GMS was featured by some degree of sensitivity to environments. While recessive GMS produced up to 50% fertile individuals which should be removed during hybrid production. Hence, it costs extra laboring and reduces breeding efficiency. In contrast, the parent line with CMS without need of artificial emasculation is easy to be propagated, which saves labors and improves breeding efficiency. More importantly, it assures high purity of F_1 hybrids. On the other hand, the breeding approach is also helpful for protecting germplasm resources and intellectual property. The edible parts of *Brassica* vegetable plants are vegetative organs, thus it does not demand well developed seeds, and restorer lines.

Recently, we developed a stable *eru* CMS line for vegetable purple cai-tai (*B. rapa* L. var. *purpurea* Bailey) by backcrossing with a *B. napus eru* CMS line, which showed 100% sterility degree and other superior traits (Wan et al., 2013). However, the absence of a restorer means that the *eru* CMS originally from *B. napus* is currently not feasible for rapeseed breeding or production. But for vegetables, the harvestable parts are usually vegetative organs, such as leaves, stalks or tubers, without requirement of seeds. Thus the *eru* CMS is suitable for vegetable breeding, and the associated gene of the CMS is under study (unpublished). In this study, six newly developed Cai-xin *eru* CMS lines were characterized for morphology, cytology and their utilization, which would broaden the CMS germplasm resources for this vegetable.

2. Materials and methods

2.1. Development of CMS lines and hybrids

The *eru* CMS line of purple cai-tai (*B. rapa* L. var. *purpurea* Bailey) was used as the donor parent (Fig. 1A), and six inbred lines (B1–B6) of Cai-xin (*B. rapa* L. ssp. *chinensis* var. *utilis* Tsen et Lee) were used as the recurrent parents. Six Cai-xin CMS lines were developed after five generations of backcrossing from 2010 to 2012 (Figs. 1B–G and 2).

The six CMS lines (female lines) and six representative cultivars (testers) of Cai-xin were used to produce 36 hybrids in

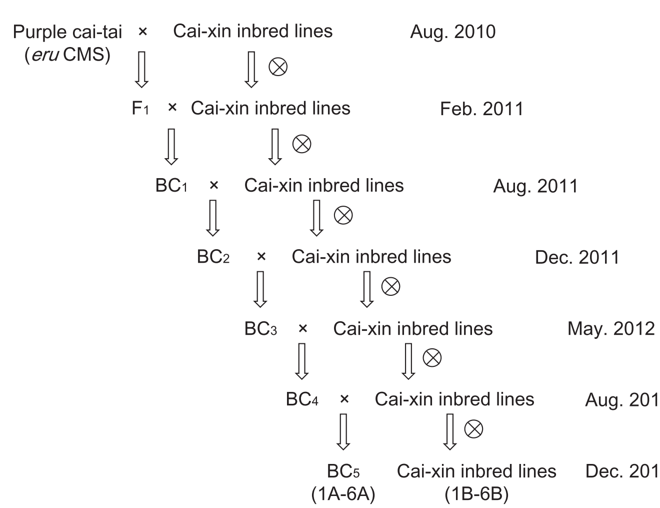

Fig. 2. *Breeding process of eru CMS lines.*