Scientia Horticulturae 217 (2017) 73–83

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Abnormal megagametogenesis results in seedlessness of a polyembryonic 'Meiguicheng' orange (*Citrus sinensis*) mutant created with gamma-rays

Jing-Hao Huang[a,b], Shou-Xing Wen[a], Yan-Fang Zhang[a], Qiu-Zhen Zhong[a], Ling Yang[a], Li-Song Chen[b,c,*]

[a] *Pomological Institute, Fujian Academy of Agricultural Sciences, Fuzhou 350013, China*
[b] *Institute of Plant Nutritional Physiology and Molecular Biology, College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou 350002, China*
[c] *Fujian Provincial Key Laboratory of Soil Environmental Health and Regulation, College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou 350002, China*

A R T I C L E I N F O

Article history:
Received 13 September 2016
Received in revised form 17 January 2017
Accepted 19 January 2017
Available online 24 January 2017

Keywords:
Megasporocyte
Meiosis
Microsporocyte
Sterility
Seediness

A B S T R A C T

Gamma radiation has been widely applied in citrus breeding to create seedless cultivar. However, little is yet known regarding the effect of γ-radiation mutagenesis on seed development in genus *Citrus*. In this report, a seedless mutant created from polyembryonic 'Meiguicheng' orange (*Citrus sinensis*) with γ-rays and its wild type were used to investigate the relationship between γ-radiation and the mechanism of seedlessness. Results indicated that both the seedless mutant and its wild type were diploid, producing highly similar flowers. Radiation-induced chromosomal aberrations significantly reduced pollen quantity and viability in the seedless mutant. However, cytological observation on pollen mother cells showed that there was no significant difference in irregular chromosomal behaviors at each given meiotic stage between the mutant and its wild type, indicating that the reduction of pollen viability in the seedless mutant might not be caused by meiotic disturbances. Also, γ-radiation-induced chromosomal aberrations caused a significantly higher abortion rate (86.21%) of megagametophyte during megasporogenesis and early megagametogenesis, resulting in female sterility in the seedless mutant. Intercrossing between the seedless mutant and its wild type revealed that pollen grains from the mutant efficiently fertilised the wild type, suggesting that the mutant is male fertile thereby could be used as male parent in further hybridization breeding programs. However, fruits developed from the flowers of cross-pollinated with wild-type pollen still produced very few seeds, most of which were much smaller in size and contained only nucellar embryos inside. Thus, the seedlessness of the mutant was caused by strong female sterility. Nucellar embryos could develop independently to produce seeds in this cultivar, though with low incidence.

1. Introduction

'Meiguicheng' orange (*Citrus sinensis*) was imported from Taiwan in 2000 by Wen et al. (2011). Previous studies have shown that under the long term evaluation, it displays a stable high-yield, and a wide adaptability to diverse agro-climatic conditions, and is tolerant to citrus bacterial canker disease when cultivated in Fujian, China. 'Meiguicheng' orange fruits are well accepted by consumers due to the excellent fruit shape, rosy inner pericarp layer, tender fresh texture with few slags, low acidity, as well as the rose-flavor (Wen et al., 2011). However, extending the cultivation of this cultivar is still limited due to its seediness. Each fruit contains as many as 20 polyembryonic seeds, in which many embryos develop directly from nucellar cells (Bruck and Walker, 1985).

Nucellar embryony is one of pathways for apomictic reproduction, which eliminates the need for events that were considered essential for the formation of a seed (Koltunow 1993). In polyembryonic *Citrus*, nucellar embryos are initiated autonomously and develop with or without pollination in both unfertilized ovules and developing seeds (Wakana and Uemoto, 1987, 1988). Therefore, the

* Corresponding author at: Institute of Plant Nutritional Physiology and Molecular Biology, College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou 350002, China.
E-mail address: lisongchen2002@hotmail.com (L.-S. Chen).

http://dx.doi.org/10.1016/j.scienta.2017.01.034
0304-4238/

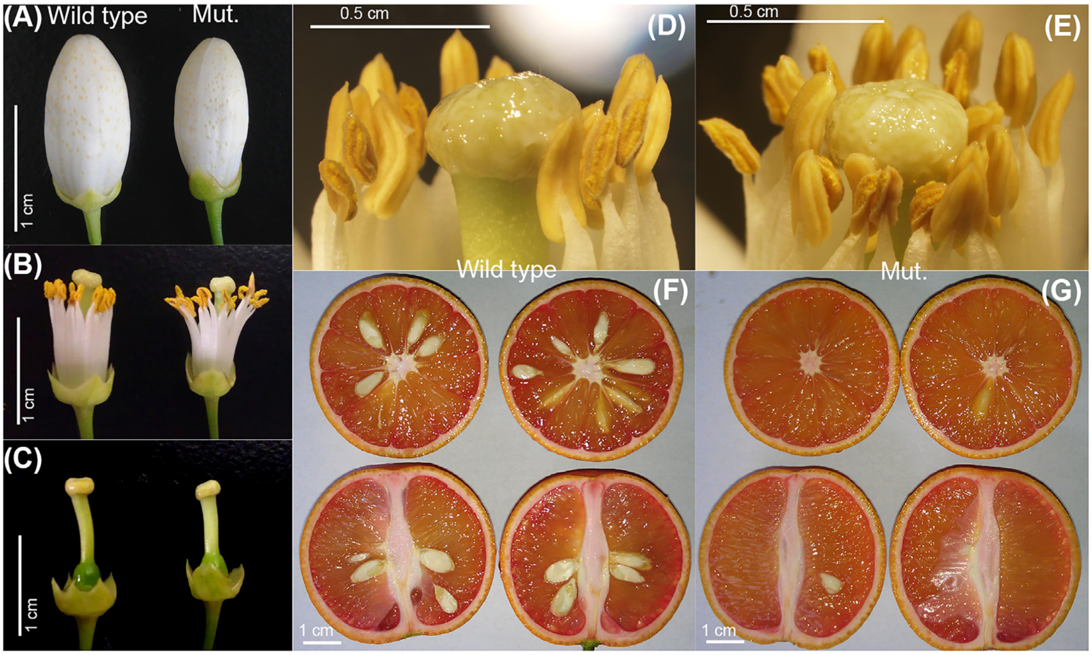

Fig. 1. Flower morphology (A–E) and seeds in fruits (F–G) in the seedless mutant (Mut.) and the wild type. Flower bud size was reduced **t**ransversely in the seedless mutant (A), while male (B) and female (C-E) reproductive organs remained highly similar between the seedless mutant and its wild type. Seed numbers per fruit in the seedless mutant were significantly reduced (F and G).

existence of nucellar embryony raises questions about how seeds develop in polyembryonic *Citrus* plants and how we could control seed formation in *Citrus*.

Seedlessness is one of desirable economic traits for both the fresh and processed citrus markets, since seedless fruits make consumption and processing more convenient and easier when compared to seedy ones (Qin et al., 2015). Currently, the most commercial citrus fruits such as oranges, mandarins and lemons (Vardi et al., 2008), and citrus hybrid (Zhang and Deng, 2006; Yamasaki et al., 2007; Qin et al., 2015) are seedless. And breeding seedless cultivars has become a major objective for citrus breeders.

In plants, one practical method for obtaining new cultivars is artificial mutation breeding (Broertjes et al., 1976; Polat et al., 2015). For the past few decades, mutation induction has been a routine tool to generate genetic variation in crop germplasm and has been overwhelmingly used in crop improvement (Oswaldo, 2007). This technique is particularly efficient for creating novel cultivars in plants with long juvenile periods (Predieri, 2001) and has been widely used in vegetatively propagated woody plants (Polat et al., 2015). In genus *Citrus*, radiation has been proven efficient for cultivar improvement by Haskin and Moore as early as 1935 (Cameron and Frost, 1968). Since then, γ- radiation has been applied in citrus breeding programs worldwide, because it avoids insurmountable difficulties, such as apomixis and polyembryony, in conventional sexual hybridization. At present, there are several examples of seedless cultivars induced by radiation in *Citrus* (Gulsen et al., 2007; Bermejo et al., 2011; Goldenberg et al., 2014).

Seedlessness in citrus can be caused by many factors, such as male- and/or female-sterility, defective ovules and embryo-sac abortion, pollen self-incompatibility, polyploidy, abnormal climate, as well as application of plant-growth regulators (Bermejo et al., 2011). In polyembryonic *C. sinensis*, a viable seed is not produced because the nucellar embryos were arrested after the globular stage in unfertilized ovules, even though the seed coat differentiates (Koltunow et al., 1995). Nevertheless, little is yet known regarding the effect of γ-radiation mutagenesis on seeds development in genus *Citrus*, despite the efforts invested in pollen germination (Bermejo et al., 2011) and fruit quality (Bermejo et al., 2011; Goldenberg et al., 2014) of mutated seedless varieties.

Table 1
Flower morphology of the seedless mutant and its wild type.

	Wild type	Seedless mutant
Transverse flower bud diameter/mm	7.70 ± 0.45	7.21 ± 0.54 **
Longitudinal flower bud diameter/mm	14.59 ± 1.27	13.97 ± 1.50
Sepal/petal numbers per flower	5.10 ± 0.45	4.65 ± 0.49 **
Anther numbers per flower	21.50 ± 1.91	21.45 ± 1.85
Filament length/mm	10.40 ± 1.00	10.16 ± 1.15
Style length/mm	8.97 ± 1.04	8.62 ± 1.21
Ovary diameter/mm	2.90 ± 0.23	2.84 ± 0.24

"**" indicated significant difference at $P < 0.01$ level (independent sample *t*-test).

To improve fruit properties of the 'Meiguicheng' orange, artificial mutation was carried out on budwood using ^{60}Co γ-rays radiation (Huang et al., 2010a, 2010b) and finally a seedless mutant was obtained. Research into this material and the wild type is essential for understanding the relationship between γ-radiation and the mechanism of seedlessness, and the relationship between nucellar embryogenesis and seed development. In this report, chromosomal ploidies as well as floral organ formation of both the seedless mutant and its wild type were examined. The processes of sporogenesis and gametogenesis were described histologically in details. Contributions of male and female fertility to seedlessness in the seedless mutant were also examined using intercrossing experiment.

2. Materials and methods

2.1. Plant materials and growth conditions

Four-year old 'Meiguicheng' orange (*Citrus sinensis*) and a seedless mutant produced from a seedy normal 'Meiguicheng' orange bud radiated by ^{60}Co γ-rays (Huang et al., 2010a,b) were used as materials. All plants were grafted on *Poncirus trifoliata* and cultivated in a greenhouse under natural photoperiod in cultivar