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Cell division interference in newly fertilized ovules induces stenospermocarpy in cross-pollinated citrus fruit

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ABSTRACT

Seedlessness is a highly desirable characteristic in fresh fruits. However, post-fertilization seed abortion of cross-pollinated citrus fruit is uncommon. The factors regulating stenospermocarpy in citrus are unknown. In this research, we induced stenospermocarpy interfering in newly fertilized ovule cell division. The research also elucidates the most sensitive stage for ovule/seed abortion in citrus. Experiments were conducted with 'Afourer' mandarin that cross-pollinates with several cultivars and species. Cross-pollinated fruitlets were treated with maleic hydrazide (MH), a systemic growth regulator that specifically interferes in cell division. MH reduced ovule growth rate, the number of cell layers in nucella and inhibited embryo sac expansion; moreover, the treatment increased callose accumulation in nucella and surrounding the embryo sac. Fruits developed an early-aborted seed type with an immature, soft and edible seed coat. Seed number (−80%) and seed weight (−46%) were reduced in mature fruits. MH also hampered cell division in ovary walls, mesocarp and endocarp, thus reducing daily fruitlet growth and increasing fruit abscission. Stenospermocarpy could only be induced for a short period of time in the progamic phase of fertilization, specifically, when ovules are ready to be fertilized (7 days after anthesis) to early stages of embryo sac development (14 days after anthesis).

1. Introduction

Post-fertilization ovule abortion (stenospermocarpy) is due to abnormal meiosis in triploid plants or to defects in endosperm development [1]. In stenospermocarpic fruits, i.e. watermelon or grapes, pollination and fertilization occur but both the seed coat and endosperm cease their normal development at early stages, exhibiting quantitative and qualitative variation in the degree of seed development [2,3]. Although very uncommon, stenospermocarpy has also been observed in seedy fruit such as mango [4], pistachio [5] or avocado [6]. In citrus, stenospermocarpy is also uncommon and it has only been occasionally observed in 'Valencia' sweet orange (*Citrus sinensis*) [7] and recently described in 'Mukaku Kishu' cherry orange fruit (*Citrus kinokuni* hort. ex Tanaka) [8,9], a marginal species.

The citrus fresh fruit market is based on producing seedless cultivars. Most are homogenetic self-incompatible mandarins that produce parthenocarpic fruits. However, as a result of cross-pollination with compatible pollen from other cultivars or species grown nearby, fruits frequently contain seeds. Commercial quality of seedy fruit is much lower than seedless ones producing major economic losses. Therefore, several strategies have been explored to avoid seed formation in citrus under cross-pollination conditions, ranging from the regulation of pollen flow [10] to interfering in the fertilization process of the flower, either controlling pollen grain germination, pollen tube growth, and/or ovule development [11,12]. Moreover, a number of breeding strategies namely ploidy manipulation [13,14] or induced mutation [15] have been explored to produce new sterile varieties. However, given the permissive cross-compatibility between citrus species and cultivars, and the long flowering period that enable cross-pollination a definitive answer is still elusive.

In stenospermocarpic fruits, aborted seeds are very soft and present little inconvenience for consumers. To date, very few alternatives have been explored to interfere in early citrus seed development and/or to reduce seed size. Lewin and Monselise [16] sprayed trees with naphthalene acetic acid similar to that done to abort pome fruits [17–19], but treatments failed to produce seedless or stenospermocarpic fruit. More recently, Koltunow et al. [20] and García-Lor et al. [21] isolated

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specific genes of the embryo sac and endothelium to use their promoters in transgenic constructions designed to reduce seed size.

During stage I of citrus fruit development, the increase in seed size is low and mainly due to nucella expansion at the chalazal end of the seed. It is much later, during stage II, when the developmental stages of zygotic embryo, globular, heart and torpedo take place and seed increases in size [7,21]. Stenospermocarpic expression in 'Mukaku Kishu' cherry orange fruit may be caused by one factor of early arrested seed that inhibits zygote or embryo development at early stages [8]. However, it is unclear whether the interference in embryo development is caused by genetic, physiological or environmental factors. Thus, the factors regulating stenospermocarpy in citrus are still unknown.

Seed development and size involve complex interactions between the endosperm, the zygotic embryo and the seed coat derived from the integuments [22]. Final seed size has been directly linked to cell division. Seed size in monocots is often attributable to the extent of endosperm growth [23] while in eudicots it is attributed to cotyledon cell number [24]. However, the embryo is not the only factor which determines seed size and, recently, higher seed size has been directly linked to extra cell division in the integuments leading to the formation of enlarged seed coats [22].

In summary, there is a need to better our understanding of the processes controlling seed abortion in citrus. In this study, we aimed to impede cell division in newly fertilized ovule and during early seed development in order to induce stenospermocarpy in self-incompatible species grown under cross-pollination conditions. Maleic hydrazide (MH, 6-hydroxy-3(2*H*)-pyridazinone), a systemic synthetic plant growth regulator that specifically interferes in the S phase of the cell cycle [25], was used for the experiments. The research also elucidates the most sensitive stage for ovule/seed abortion in citrus.

2. Materials and methods

2.1. Plant material

Experiments were conducted over 2 consecutive years in commercial orchards of 'Afourer' tangor [*Citrus reticulata* × *C. sinensis* (L.) Osb.] located in Valencia and Huelva (Spain). Fifteen years old trees were used in the experiment. Trees grown in Valencia were grafted onto Carrizo citrange rootstock [*C. sinensis* (L.) Osb. *Poncirus trifoliata* (L.) Raf.], and trees grown in Huelva were grafted onto Citrumelo Swingle rootstock [*Citrus paradisi* Macf. × *P. trifoliata* (L.) Raf.]. The trees were planted 6 × 4 m apart in a loamy clay soil (Valencia) and sandy–loamy soil (Huelva) with drip irrigation. Fertilization, pruning, and pest management were in accordance with optimum commercial practice.

'Afourer' is a high quality parthenocarpic mandarin cultivated worldwide that originated from a seed of 'Murcott' fruit (a tangor of unknown parentage created by W.T. Swingle in Florida, USA) in Afourer, Morocco. Whether it is a natural hybrid, a nucellar selection or a bud mutation of 'Murcott' is not known [26]. 'Afourer' is also referred to as 'Nadorcott' and 'Delite'. Flowers are self-incompatible but cross-pollinate efficiently with the most important species, i.e. Clementine mandarin (*Citrus clementina* Hort ex. Tan.), lemon (*Citrus limon*), 'Valencia' sweet orange (*C. sinensis* L. Osb.), grapefruit (*C. paradisi*) and some hybrids like-mandarins [27]. Under cross-pollination conditions, the fruit may contain between 8 and 21 seeds depending on the cross [27], so it was previously used as a plant model to study the problem of cross-pollination in citrus [10,11].

2.2. Experimental design

2.2.1. Induction of stenospermocarpy: Treatment date and MH concentration

MH [6-hydroxy-3(2*H*)-pyridazinone, 60% potassium salt, Pamena S.L., Madrid, Spain], at a concentration of 1000 mg l^{-1}, was

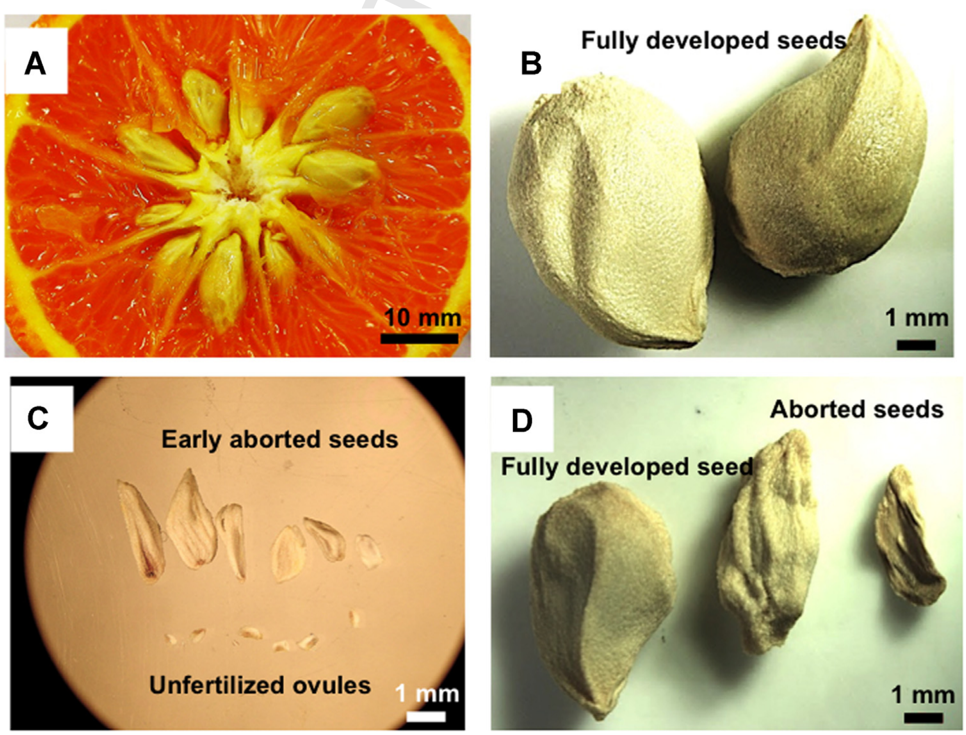

Fig. 1. Stenospermocarpy induced by maleic hydrazide (1000 mg l^{-1}) on 'Afourer' fruits under natural cross-pollination conditions. Treatment was applied to the entire Tree 14 days after anthesis. (A) Control cross-pollinated mature fruit; (B) seeds from control fruit; (C) unfertilized ovules from control and treated fruits and early aborted seeds from treated fruits; (D) seeds and aborted seeds from treated fruits.

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