COMUNICAÇÃO

'PONKAN' MANDARIN (*Citrus reticulata* Blanco) IMMATURE FRUITS STORAGE

Armazenamento de frutos imaturos de tangerineira 'Ponkan' (*Citrus reticulata* Blanco)

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ABSTRACT

The aim of this work was to evaluate the effect of 'Ponkan' mandarin (*C. reticulata*) x 'Pêra' sweet orange (*C. sinensis*) immature fruits storage and sucrose concentrations on embryos *in vitro* culture. Fruits with 3 to 4 cm in diameter were harvested and placed inside black polyethylene bags with lateral openings and stored at 5±1ºC during 135 days. Every 15 days a sample was removed, its embryos were excised and individually inoculated in test tubes containing 15 mL of MS medium (MURASHIGE & SKOOG, 1962) with sucrose (0, 1.5, 3, 6, 12, 18 and 24 g L^{-1}) and 0.3 mg L^{-1} GA_3 and 1 g L^{-1} activated charcoal. Those treatments rested 48 hours in the dark and later in a growth room at 27 ± 1ºC with a 16-h photoperiod and 32 µmol m^{-2} s^{-1} light intensity. Immature fruits can be stored for posterior excision and embryos culture. Fruits with 120 days after the pollination can be stored for at most 135 days without damaging the embryos viability. It was observed a better development of the aerial part and root system of plantlets from 'Ponkan' mandarin x 'Pêra' sweet orange embryos in MS medium with 12-18 g L^{-1} sucrose.

Index terms: Embryo culture, nutrient medium, breeding.

RESUMO

Objetivou-se com este trabalho avaliar o efeito do armazenamento de frutos e concentrações de sacarose no cultivo *in vitro* de embriões imaturos de tangerineira 'Ponkan' (*Citrus reticulata* Blanco) x laranjeira 'Pêra' [*Citrus sinensis* (L.) Osb.]. Frutos com 3 a 4 cm de diâmetro foram coletados e colocados em sacos pretos de polietileno perfurados e armazenados a 5±1ºC por um período de 135 dias. A cada 15 dias, uma amostra foi retirada, seus embriões foram excisados e inoculados individualmente em tubos de ensaio contendo 15 mL de meio MS acrescido de sacarose (0; 1,5; 3; 6; 12; 18 e 24 g L^{-1}), 0,3 mg L^{-1} GA_3 e 1 g L^{-1} de carvão ativado. Os tratamentos permaneceram 48 horas no escuro e em seguida foram transferidos para sala de crescimento a 27±1ºC com fotoperíodo de 16 horas e 32 µmol m^{-2} s^{-1} de intensidade luminosa. Frutos imaturos podem ser armazenados e posteriormente utilizados na excisão e cultura de embriões. Frutos com 120 dias após a polinização podem ser armazenados por mais de 135 dias sem afetar a viabilidade dos embriões. Observou-se melhor desenvolvimento da parte aérea e sistema radicular de plântulas oriundas de embriões de tangerineira 'Ponkan' x laranjeira 'Pêra' em meio MS com 12-18 g L^{-1} de sacarose.

Termos para indexação: Cultura de embriões, meio nutritivo, melhoramento.

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In any breeding program, successive hybridizations are carried out in order to achieve the desired objective. In citrus breeding, immature embryos culture provides conditions for both zygotic and nucelar embryos development besides allowing an early identification of plantlets produced by zygotic embryos. The storage of fruits allow the embryos to be excised and cultivated for a long period. The low temperature during the storage delays the maturation process and the early fruit senescence. When storing seeds of *Citrus paradisi* Macf. in different conditions (CHACKO & SINGH, 1969), noticed that the viability was kept for more than 13 months when the seeds were stored with high humidity (above 80%), under 5-8°C. The longevity of *C. karna* Raf., *C. jambhiri* Lush., *C. limonia* Osb., and 'Rusk' citrange [(*C. sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.] seeds after storage was studied under environmental temperatures and 8°C, in hermetic containers, in plastic or paper bags, with or without dissecting during five months (KRISHNA & SHANKER, 1977). *C. karna* and 'Rusk' citrange presented 100% seed viability, while *C. jambhiri* and *C. limonia* showed 84% and 80% viability, respectively, when stored in plastic bags containing $CaCl_2$, under low temperatures. Generally the excised embryos from temperate region plants require lower temperatures than those ones from

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plants of tropical or sub-tropical regions (NARAYANASWAMI & NORSTOG, 1964). Light must be avoided during the first days of culture, once it leads to early embryos germination. The growth of most cultures is supported by the source of carbohydrate added to the medium, which supplies metabolic energy and carbonic skeletons for the amino acids biosynthesis and production of structural proteins, and polysaccharides as the cellulose (CALDAS et al., 1998). Sucrose is the best carbon source to be used, besides offering an optimum growth, it is also a low cost source (MELLO, 1998), and for many species it is applied in the culture medium at 2-4% concentration. Below this concentration, generalized chlorosis may occur in the plantlets, and above that problems related to excessive osmotic potential of the medium may result in culture decay (GRATTAPAGLIA & MACHADO, 1990). Multiplication and growth of *in vitro* plants also depend on the explant and the sucrose concentration restraining the nitrate reductase activity, which is responsible for the cell nitrate utilization (CALDAS et al., 1998). Various concentrations are used with *Citrus* species for several purposes (BELOUALY, 1991; GAVISH et al., 1991). Concentrations higher than 5% have presented a good result for *Citrus* micrografting (NAVARRO et al., 1975). The 3% concentration presented satisfactory development to the axillary buds (PASQUAL & ANDO, 1989 a,b). The present study aimed to evaluate the effect of 'Ponkan' mandarin (*C. reticulata*) x 'Pêra' sweet orange (*C. sinensis*) immature fruits storage and sucrose concentrations on embryos *in vitro* culture.

'Ponkan' mandarin immature fruits obtained from crosses with 'Pêra' sweet orange, with 3 to 4 cm diameter were used. Fruits were harvested and placed into black polyethylene bags with lateral openings and then stored at 5±1°C for a maximum of 135 days. Every 15 days a sample was removed, its embryos were excised and cultured *in vitro*. The embryos, independently of their development stage, were excised and inoculated in test tubes containing 15 mL of the MS medium (MURASHIGE & SKOOG, 1962) with increasing concentrations of sucrose (0, 1.5, 3, 6, 12, 18 and 24 g L^{-1}), 0.3 mg L^{-1} GA_3 (giberelic acid) and 1 g L^{-1} activated charcoal. These treatments rested 48 hours in the dark and later in a growth room at 27±1°C, with a 16-h photoperiod and 32 µmol m^{-2} s^{-1} light intensity. The storage periods were 0, 15, 30, 45, 60, 75, 90, 105, 120 and 135 days. The plantlets variables observed were: aerial part height, number of leaves, aerial part dry matter weight and length of the roots.

The storage of fruits increased the aerial part height for all sucrose concentrations (Figure 1A). Greater number of leaves was obtained with 12 g L^{-1} sucrose at 90 days storage (Figure 1B). Aerial part dry matter weight was identical for 12 and 18 g L^{-1} sucrose at 135 days storage (Figure 1C). The dry matter weight with 18 g L^{-1} sucrose without storing the fruits, was significantly higher than 12 g L^{-1} sucrose for the same storage period. Better results for roots length were observed at 12 as well as 18 g L^{-1} sucrose at 135 days storage (Figure 1D).

Temperature and humidity affect the seeds longevity, either under natural environment or controlled conditions (BARTON, 1961; ROBERTS, 1972). In the fruit storing with viable embryos by more than 4 months (CHACKO & SINGH, 1969) the best conditions for seed storage of *C. paradisi* were high humidity and temperatures between 5 and 8°C. According to our results, this high embryo viability value after 135 days of storage can also be attributed to the absence of light, once the fruits were being stored. Under these conditions the early embryo germination was avoided which turn possible them to complete their development. The temperature utilized for the fruits storage was also an important factor in preserving the embryo characteristics. The fact that in a same fruit one can find embryos at different stages of development, and that they consequently have different requirements concerning the osmotic potential (SHARMA et al., 1996), a greater sucrose demand would be needed in order to avoid the embryos early germination. In this paper intermediate sucrose concentrations (12 to 18 g L^{-1}) showed the best results.

Fruits with 120 days after the pollination can be stored for at most 135 days without damaging the embryos viability.

Better development of the aerial part and root system of plantlets from 'Ponkan' mandarin x 'Pêra' sweet orange embryos can be obtained in MS medium with 12-18 g L^{-1} sucrose.

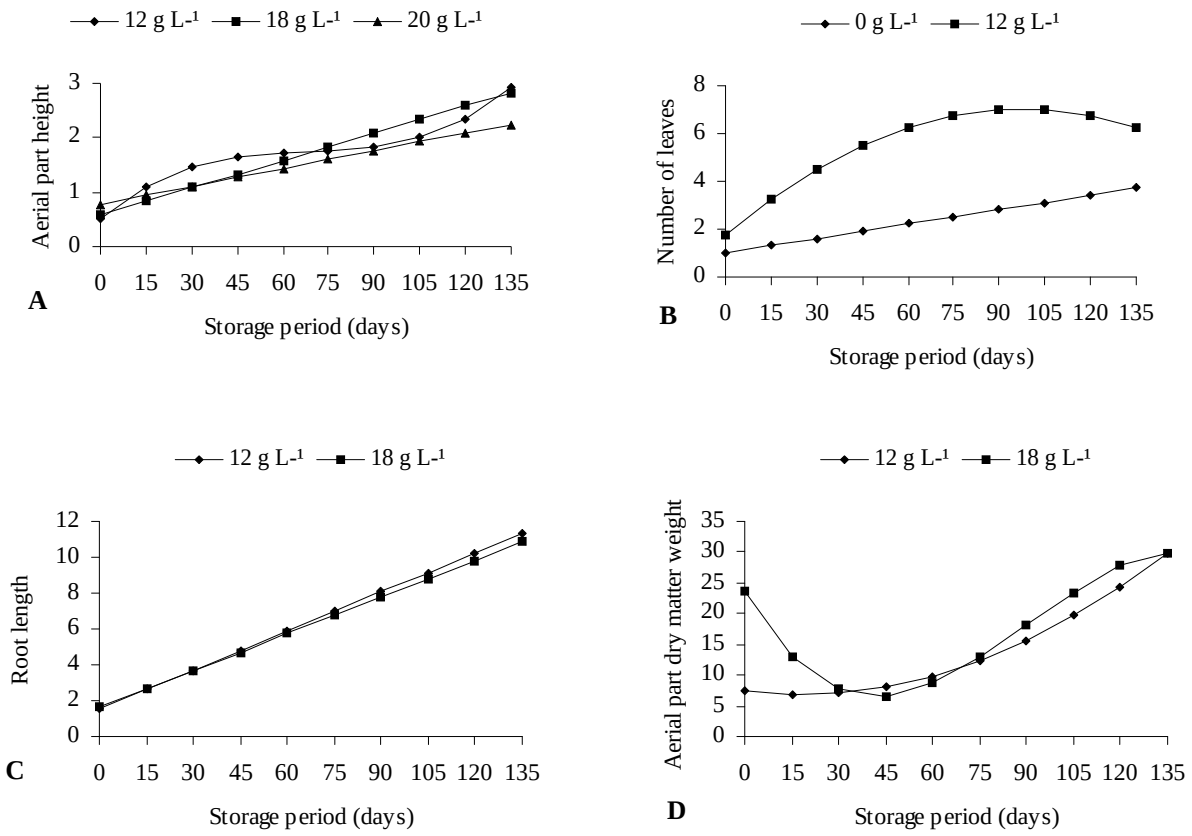

FIGURE 1 – Aerial part height (cm) - A, Number of leaves - B, Aerial part dry matter weight (mg) -C, Root length (cm) - D, of plantlets from 'Ponkan' mandarin [(*Citrus reticulata* Blanco) x 'Pêra' sweet orange (*C. sinensis* (L.) Osb.] *in vitro* culture immature embryos, with different sucrose concentrations from stored fruits.

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