Research Article

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# Effect of $GA_3$ concentrations in basal medium on embryos germination of Cleopatra mandarin x carrizo citrange and Cleopatra mandarin x Flying Dragon

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**ABSTRACT**

The gametic sterility, the long duration of the young stage, species incompatibilities and especially the high rate of polyembryony are common difficulties in citrus breeding. Polyembryonic cultivars usually contain a zygotic embryo at the earlier stages of seeds. By embryo rescue techniques, the zygotic embryos further development can be secured. In this study, suitable $GA_3$ concentration (0; 1; 2 mg/L) and embryo development stages (days after pollination 105, 115, 125) for embryo germination at Cleopatra mandarin x Carrizo citrange and Cleopatra mandarin x 'Flying Dragon' trifoliate orange were examined. The results indicated that the highest germination rate was observed from 2 mg/L $GA_3$ containing M&T media which were harvested 115 days after pollination. The highest germination rates of embryos taken 115 days after pollination and germinated on 2 mg/L $GA_3$ containing media in Cleopatra mandarin x Carrizo citrange and Cleopatra mandarin x Flying Dragon were obtained with 65% and 30%, respectively.

***Keywords***: *citrus*, embryo rescue, $GA_3$, germination, hybridization, immature embryo

## Introduction

Citrus, having major importance in the world and Turkey, is propagated by vegetative and generative methods. However, rootstocks in citrus have to be used because of particular diseases and the some soil and climatic conditions.

One of the major problems in citrus breeding is competition between zygotic and nucellar embryos (Soost and Roose 1996). This challenge is eliminated by in vitro embryo rescue techniques of developing embryos. The success of embryo rescue depends on ingredient of medium and embryo developing stages (Jaskani et al. 2005). The germination capacity of citrus embryos can be affected by embryo's genetic structure and embryo developing stage (Viloria et al. 2005).

Various studies have reported that the addition of 0.01 mg/L $GA_3$ (Chagas et al. 2005), 1 mg/L $GA_3$ (Ollitrault et al. 2007), and 2 mg/L $GA_3$ (Gmitter et al. 1990) in growing media for embryos is important in developing citrus plants.

In this study, we aimed to obtain hybrid individuals from Cleopatra mandarin (*Citrus reshni* hort. ex Tanaka) x Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.], and Cleopatra mandarin (*Citrus reshni* hort. ex Tanaka) x 'Flying Dragon' trifoliate orange (*Poncirus trifoliata* (L.) Raf.).

## Material and methods

Cleopatra mandarin, 'Flying Dragon' and Carrizo citrange were used as materials. Carrizo citrange and 'Flying Dragon' trifoliate orange were used as father plant and Cleopatra mandarin used as mother plant in the crossing combinations.

Murashige and Tucker (1969) medium was used as basic culture medium and 50 g/L sucrose, 25 mg/L adenine sulfate, 500 mg/L malt extract were put in medium. Then, 0, 1 and 2 mg/L $GA_3$ were supplemented to the prepared medium and 8 g/L agar was added. The fruits were taken 105, 115 and 125 days after pollination (DAP), were

surface sterilized (Ollitrault et al. 2007). Then, the seeds were removed from the fruit by forceps and immature embryos were taken from the microphyl parts of seeds under binocular. Two embryos were placed into Petri dishes separately containing culture medium. And then, the Petri dishes were incubated in growth chamber. Germinated embryos were counted and rate of germination of embryos was determined. Later, embryos were sub-cultured in Murashige and Skoog (1962) medium containing 0.02 mg/L NAA and 20 g/L sucrose in culture tubes to provide seedling growing (Perez-Tornero and Porras 2008), and then they were incubated. The plantlets in sub-culture were transferred to pots. The experiment was conducted as randomized plot design with 10 replications and each replication have two embryos. Data were subjected to analysis of variance with mean separation by LSD's test.

## Results

Embryo development stages, $GA_3$ concentrations in the medium and their interactions were significant on germination rate of Cleopatra mandarin x Carrizo citrange (Table 1) and Cleopatra mandarin x 'Flying Dragon' trifoliate orange (Table 2) hybrid embryos ($p \leq 0.05$).

Table 1. Germination rate (%) of Cleopatra mandarin x Carrizo citrange

| Embryo development stage | $GA_3$ Concentration | | | |
|---|---|---|---|---|
| | Control 0 mg/L $GA_3$ | 1mg/L$GA_3$ | 2mg/L $GA_3$ | Means (DAP) |
| 105 DAP | 15.00 e* | 35.00 c | 40.00 bc | 45.00 |
| 115 DAP | 25.00 d | 45.00 b | 65.00 a | 30.00 |
| 125 DAP | 10.00 e | 45.00 b | 25.00 d | 26.67 |
| Means ($GA_3$) | 16.17 | 41.67 | 43.33 | |

* Different letters indicate significant differences (P<0.05) according to the LSD test
* (LSD: 5.1462)

In Table 1, the highest germination rate of Cleopatra mandarin x Carrizo citrange were obtained from embryos taken 115 days after pollination and germinated on 2 mg/L GA3 containing media with 65.00%. In Table 2, the highest germination rates of Cleopatra mandarin x Flying Dragon were obtained from embryos taken 115 days after pollination and germinated on 2 mg/L $GA_3$ containing media with 30.00%.

Table 2.Germination rate (%) of Cleopatra mandarin x Flying Dragon

| Embryo development stage | $GA_3$ Concentration | | | |
|---|---|---|---|---|
| | Control 0 mg/L $GA_3$ | 1mg/L$GA_3$ | 2mg/L $GA_3$ | Means (DAP) |
| 105 DAP | 5.00 d * | 10.00 cd | 20.00 b | 11.67 |
| 115 DAP | 5.00 d | 5.00 d | 30.00 a | 13.33 |
| 125 DAP | 5.00 d | 10.00 cd | 15.00 bc | 10.00 |
| Means (GA3) | 5.00 | 8.33 | 21.67 | |

* Different letters indicate significant differences (P<0.05) according to the LSD test
* (LSD: 5.1462)

## Discussion

According to our results, the highest germination rate in embryo development stages were obtained 115 days after pollination in two hybridization combinations. Similarly, 118 days after pollination for embryo germination has been reported by Chagas et al. (2005). On the other hand, there were reports indicating good embryo germinations after 50 days (Wang et al. 1999), 80 days (Tan et al. 2007), 100 days (Deng et al. 1996), 105 days (Scarano et al. 2003) and 120 days (Carimi et al. 1998; Das et al. 2000; Kurt and Ulger 2014).

2 mg/L $GA_3$ dose was appropriated for embryo germination. Gmitter et al. (1990) studied different citrus species and cultivars, and they indicated that adding 2 mg/L $GA_3$ to the medium gave positive effects in increasing citrus embryo germination. Some reports also showed the addition of 0.01 mg/L $GA_3$ (Ribeiro et al. 2000), 0.1 mg/L $GA_3$ (Pasqual et al. 1990), 1 mg/L $GA_3$ (Kurt and Ulger 2014) and to growing on the medium alone resulted in good embryo germination of citrus. These differences may be due to the growing location and cultivars used.

As a result, it was determined that the best embryo rescue stage was 115 days after pollination. In addition, 2 mg/L $GA_3$ was determined the appropriate dose in media for embryo rescue.

### References


Carimi F, Pasquale F and Puglia AM (1998). *In vitro* rescue of zygotic embryos of sour orange (*Citrus aurantium* L.) and their detection based on RFLP analysis. Plant Breeding 117:261–266.

Chagas EA, Pasqual M, Ramos JD, Pio LAS, Dutra LF and Cazetta JO (2005). Activated charcoal and gibberellic acid concentrations on immature embryos culture. Ciencia e Agrotecnologia 29(6):1125–1131.

Das A, Paul AK and Chaudhuris S (2000). Micropropagation of sweet orange (*Citrus sinensis* Osbeck) for the development of nucellar seedling. Indian J. Expt. Bot.38(3):269–272.

Deng XX, Yi HL, Li F and Guo WW (1996). Triploid plants regenerated from crossing diploid pummel and tangerine with allotetraploid somatic hybrid of citrus. Proc. Int. Soc. Citricult. 1:189–192.

Gmitter FG, Ling XB and Deng XX (1990). Induction of triploid Citrus plants from endosperm cali in vitro. Theor. Appl. Genet. 80:785–790.

Jaskani MJ, Khan IA and Khan MM (2005). Fruit set, seed development and embryo germination in interploid crosses of Citrus. Sci. Hort. 107:51–57.

Kurt S and Ulger S (2014). Production of Common Sour Orange × Carrizo Citrange Hybrids Using Embryo Rescue. *International Journal of Fruit Science*, 14:42–48.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Murashige T and Tucker PH (1969). Growth factor requirements of citrus tissue culture. Proc. 1st Intl. Citrus Symp. 3:1155–1161.

Ollitrault P, Guo W and Grosser JW (2007). Somatic hybridization, p. 235–260. In:I. Khan (ed.). Citrus genetics, breeding and biotechnology. CABI, New York.

Pasqual M, Riberio VG and Ramos JD (1990). Influencia do $GA_3$ e do carvao ativado sobre o enraizamento in vitro de embrioes de laranja 'Natal'. Pesquisa Agropeciaria Brasileira, Brasilia 25(10):1477–1482.

Perez-Tornero O and Porras I (2008). Assessment of polyembryony in lemon: rescue and in vitro culture of immature embryos. Plant Cell Tissue Organ Cult.93:173–180.

Ribeiro VG, Sanábio D, Souza CN de, Lopes PSN, Bocardo MR and Pasqual M (2000). Efeitos de ácido giberélico e carvão ativado no cultivo in vitro de *Citrus limonia* Osbeck x *Poncirus trifoliata* (L.) Raf. Pesquisa Agropecuária Brasileira, Brasília 35:27–30.

Scarano MT, Tusa N, Abbate L, Lucretti S, Nardi L and Ferrante S (2003). Flowcytometry, SSR and modified AFLP markers for the identification of zygotic plantlets in backcrosses between 'Femminello' lemon cybrids (2n and 4n) and a diploid clone of 'Femminello' lemon (*Citrus limon* L. Burm. F.) tolerant to mal secco disease. Plant Sci. 164(6):1009–1017.

Soost RK and Roose M (1996). Citrus, In: Fruit breeding: Tree and tropical fruits, Janick J and Moore JN (eds.), Wiley, New York, pp: 257–323.

Tan M, Song J and Deng X (2007). Production of two mandarin x trifoliate orange hybrid populations via embryo rescue with verification by SSR analysis. Euphytica 157:155–160.

Viloria Z, Grosser JW and Bracho B (2005). Immature embryo rescue, culture and seedling development of acid citrus fruit derived from interploid hybridization. Plant Cell, Tissue and Organ Cult. 82:159–167.

Wang JF, Chen ZG and Lin TX (1999). Observation on the embryonic development in citrus after cross pollination. Chin. Dev. Reprod. Biol. Soc. 8(2):57–63.