

## The effects of calcium and magnesium on carrot (*Dacus carota* cv. Nants) petiole somatic embryogenesis

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### Abstract

The present study was conducted in two different experiments based on complete randomized design to evaluate the effects of calcium (0, 1, 2, 4 mM) and magnesium (0, 1.02, 2.04, 4.08 mM) on carrot petiole somatic embryogenesis in B5 medium. A difference on somatic embryogenesis was observed among different levels of calcium and magnesium. The number of globular embryos in medium with low magnesium (0, 1.02 mM) was significantly low. The number of cotyledonary or plantlet embryos in medium containing 4.08 mM magnesium was significantly higher than those of other levels of magnesium. The result indicated that magnesium and calcium plays important roles in carrot embryogenesis. Generally it can be concluded that the presence of both calcium and magnesium in both induction and realization stages of carrot petiole embryogenesis is necessary.

**Keywords:** carrot; calcium; magnesium; somatic embryogenesis

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### Introduction

In Embryogenesis, somatic cells convert to vegetative embryo and finally a developed plant in different steps (Halprin, 1995). Carrot is extensively using as a model to study the biochemical and molecular changes during embryogenesis (Pierik, 1997). Among minerals, calcium plays as important regulator. In plants calcium acts as a secondary messenger to transfer environmental signals (Marshner, 1995; Harpner, 2004; Suparasasna et al., 2004; Mashyeki, 2007). Based on these findings it seems that the increase of calcium regulates calcium signaling and act as a good stimulator for somatic embryogenesis (Weisenseel et al., 1975; Montoro et al.; 1995; Vanderluit et al., 1999). Magnesium is vital in chlorophyll and with magnesium deficit the chlorophyll and carotenoid concentration reduce (Marchner, 1995). It has important role in carbohydrate synthesis which has important role in embryogenesis. Magnesium stimulates ribosome accumulation during protein synthesis and ATP production at phosphorylation process which are important for embryogenesis (Marchner, 1995). In a study on magnolia tree it has been showed that under high

concentration of magnesium embryogenesis and organogenesis were strongly increased (Valova et al., 1996).

### Materials and Methods

The disinfected seeds (ethanol 70% for 30 seconds and sodium hypochlorite 3% for 20 for 20 min) of carrot were cultured in hormonless solid B5 medium and petioles were collected as explant four weeks later. Five segments of petiole (1-2 cm) were kept in induction medium included B5 medium stock salts, 0.45 Mm 2, 4-D, 20g sucrose, and different levels of calcium(0, 1, 2, 3, 4mM) and magnesium (0, 1.02, 2.04, 4.08 Mm) under 26 °C and permanent light (2000 lux) conditions. 30 days later the samples were transferred to realization medium (hormonless) containing the same levels of calcium and magnesium. Next four weeks the number of pyramidal, hearty, torpedo, cotyledonary embryos, and the morphological changes like rooted callus and probable adventitious shoots were counted using stereoscope equipped with computer and monitoring apparatus model Sony. Experiments were carried out as a complete randomized design with four replications. The data normal distribution was done using root square conversion method and then was analyzed using SAS software and Duncan test in 5% probability.

### Results and Discussion

Calcium has significant effect on embryogenesis which is in agreement with Arruda et al. (2000). As calcium concentration increased (especially from 0 to 2 Mm), the total number of embryos was increased (the highest was for globular embryos). In the cotyledonary or plantlet embryos the highest embryos was observed with 2mM calcium (Table 1). These results show that calcium has a direct effect on somatic embryo. Montoro et al. (1995) showed that high concentration of calcium produces hard and breakable callus and for embryo formation the callus must transfer to a medium with lower concentration of calcium. They concluded that interaction between calcium and hormones is the main reason of their observation. Additionally Harpner et al. (2004) showed that hyper increasing of calcium limits calcium entrance into cell and the calcium accumulate on cell wall and vacuole membrane.

Table 1. Effect of calcium on somatic embryogenesis of carrot petiole in B5 medium.

Calcium (mM)	Globular embryos	Heart embryos	Torpedo embryos	Cotyledonary embryos	Total no. of embryos	Neumorphe
control	8.44 <sup>c</sup>	0.91 <sup>a</sup>	14.78 <sup>b</sup>	5.26 <sup>b</sup>	35.02 <sup>c</sup>	3.18 <sup>b</sup>
1	13.63 <sup>bc</sup>	4.83 <sup>a</sup>	18.1 <sup>b</sup>	51.4 <sup>ab</sup>	94.17 <sup>bc</sup>	3.95 <sup>b</sup>
2	41.1 <sup>ab</sup>	0.6 <sup>a</sup>	88.8 <sup>a</sup>	113.13 <sup>b</sup>	247.2 <sup>a</sup>	4.92 <sup>b</sup>
4	53.66 <sup>a</sup>	5.95 <sup>a</sup>	4.72 <sup>b</sup>	21.12 <sup>ab</sup>	125.38 <sup>ab</sup>	20.11 <sup>a</sup>

As is revealed in table 1, the response of embryo to calcium depends on embryo development, the more developed embryo the more reaction to calcium and vice versa. It seems that the presence of calcium at the end of induction phase or beginning of realization phase increase the number of embryo. Takada et al. (1998) showed that when explant

directly transferred to medium containing calcium the number of embryos was higher than that of explants which were transferred 7 days later. They concluded that their observation could be due to interaction of calcium and auxin at initiation phase. Calcium transduction into cell and high concentration of this element in cytosol collects in one part of cell and produces electrical potential. The produced electrical potential increases the polarity of cell and encourages cell differentiation (Mashayekhi, 2007). Takeda et al. (2003) in a study concluded that calcium increases embryogenesis at a certain level. They were believed that probably calcium can not control the embryogenesis at presence of high auxin concentrations. Different reasons like high concentration of ABA are involved in neumorphe (Figure 1. A) formation (John et al., 1995). Our results show that when the calcium increased to 4mM the number of neumorphe was increased (Table 1). Tagawa and Bonner (1957) indicated that additional calcium concentration reduces stem growth and cotyledons elongation. In compare with control increasing in the amount of magnesium increases the number of globular embryos. But no significant difference was observed among different shapes of embryos (Table 2). Our results indicate that magnesium has significant effect on embryogenesis is in agreement with Minyaka and et al. (2008).

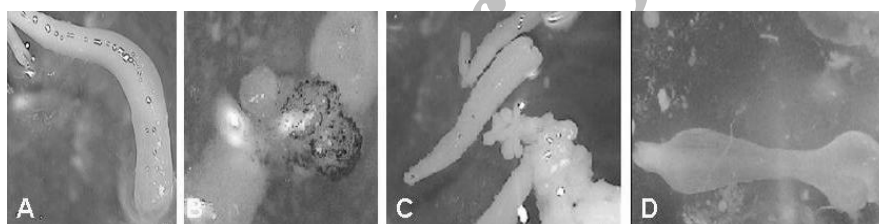


Figure 1. Neumorphs in B5 realization medium containing 4 mM calcium (A), Anthocyanin accumulation in B5 with 4.08 mM magnesium (B), whit embryos in B5 without magnesium (C), Undeveloped embryo in B5 with magnesium deficit (D).

Table2. Effect of magnesium on carrot embryogenesis.

Magnesium (mM)	Globular embryos	Heart embryos	Torpedo embryos	Cotyledonary embryos	Total no. of embryos	Neumorphe
control	11.26 <sup>b</sup>	0.48 <sup>a</sup>	8.08 <sup>b</sup>	0 <sup>b</sup>	20.20 <sup>c</sup>	6.26 <sup>a</sup>
1.02	20.57 <sup>ab</sup>	0.48 <sup>a</sup>	4.2 <sup>b</sup>	9.35 <sup>b</sup>	38.6 <sup>c</sup>	12.03 <sup>a</sup>
2.04	45.19 <sup>a</sup>	1.11 <sup>a</sup>	35.14 <sup>a</sup>	20.37 <sup>b</sup>	103.1 <sup>b</sup>	14.94 <sup>a</sup>
4.08	33.14 <sup>a</sup>	2.35 <sup>a</sup>	48.5 <sup>a</sup>	130.6 <sup>a</sup>	212.6 <sup>a</sup>	11.54 <sup>a</sup>

The most effect of magnesium was observed at cotyledonary stage (table 2). When the magnesium concentration was increased from 2.04 to 4.08 the number of cotyledonary embryo was increased from 20.4 to 130.6. It can be concluded that the magnesium has most effect on last stages of embryogenesis. Control samples did not produce any cotyledonary embryo and this observation is in accordance with the findings of Valova et al. (1996) who showed that in Magnolia high concentrations of magnesium are related to embryogenesis and organogenesis and low concentrations of this element only induce callus formation. Anthocyanin accumulation on the surface of samples was shown in Figure1. B. It could be suggested that anthocyanin accumulation might refer to tissue response to stress. Anthocyanin increasing under high concentration of magnesium causes a complex of

magnesium and antocyanin to avoid its hydrolysis (John et al., 1995; Liat et al., 2002). When the magnesium was removed from medium the colour of callus and embryos was turn to white and no plantlet was formed (Figure 1. C). Beside colorless embryos some undeveloped green embryos also was observed in our experiment (Figure 1. D). This observation indicates that magnesium has direct effect on organogenesis of embryos and their development. Norway spruce Ingestad (1979) showed that magnesium deficit reduces the vegetative growth rate of root. It has been showed that carbohydrate translocation in phloem is directly related to magnesium (Cakmak et al., 2008). Our results showed that deficit of both elements reduce the embryogenesis or limit embryo development. The interaction of two elements did not investigate in this study and elements were used in both induction and realization stages. It is not clear that in which stages elements are more necessary. Our additional unpublished data showed that if both elements supplied only in induction stage, the number of cotyledonary embryos and total embryos are significantly lower than that of the present results. Generally it can be concluded that presence of calcium and magnesium in both induction and realization stages of carrot petiole embryogenesis is necessary.

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