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Short Communication

Influence of different cytokinins used in *in vitro* culture on the stoma morphology of pistachio (*Pistacia vera* L. cv. Siirt)

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Three different cytokinins (BA, kinetin and TDZ at 1 mg/l) were tested in order to study their effects on pistachio stoma morphology. Additional BA concentrations of 0.25, 0.625 and 2 mg/l were also tested. The stoma morphology of pistachio leaves *in vitro* was affected by cytokinins. Stomas obtained from 1 mg/l BA medium were similar to those developed *in vivo*. TDZ, kinetin and other BA concentrations resulted in abnormal stoma development.

Key words: *Pistacia vera*, cytokinins, stoma morphology.

INTRODUCTION

In vitro multiplication is the most common proliferation technique for many fruit species like pistachio (Onay, 2000a). Micropropagation is a plant tissue culture technique used for obtaining a large number of genetically identical plantlets. The special conditions during *in vitro* culture result in plantlets of abnormal growth and development. After *ex vitro* transfer, these plantlets might easily be impaired by sudden changes in environmental conditions and so they need a period of acclimatization to correct the abnormalities. This technique is mostly used for many garden, fields, decorative and woody plants (Salarova and Pospisilova, 1990a; Nguyen and kozai, 1998; Mansuroğlu and Gürel, 2001). However, the application of micropropagation on a large scale is restricted because of the plantlet damage after *in vivo* transplanttation and the large plantlet lost (Hayashi and Kozai, 1988; Kozai, 1991; Zobayed et al., 1999; Soon et al., 2000). The major reasons of this loss are the reduced leaf wax cover, thin cuticles formation, reduced formation of secondary roots and reduced stomata function, resulting in water loss and reduced photosynthesis capacity (Zobayed et al., 1999).

After the transfer from *in vitro* conditions to the garden or field conditions, changes are often noticed in the plantlets morphology and anatomy. The anatomical changes seen in *in vitro* cultivated plants mainly affect the CO_2 diffused in leaves and the net photosynthesis rate. Due to fact that the leaves of plantlets obtained by micropropagation are small and thin with undeveloped palisade cells, weak mesophylle layer and intercellular gab, the useability of CO_2 in mesophile and photosynthesis are affected.

Pistachio (*Pistacia vera* L.) is a dioeciously tree species cultivate widely in the Mediterranean regions of Europe and North Africa, the Middle East, China and California. Pistachio nut tree requires very special climatic conditions: cold winters and warm summers. Therefore the areas suitable for pistachio nuts production are limited in Turkey and elsewhere in the world (Ayfer, 1990). Due to its quite rich nutrient value as well as being an important export product, pistachio nut is a very important fruit for Turkey. Turkey is also an important center of genetical diversity for pistachio tree.

There are several reviews on the *in vitro* requirements for pistachio shoot tips and nodal culture (Hansman and Owens y de Novoa, 1986; Onay and Jeffree, 2000; Barghchi and Alderson, 1989). In these reviews the procedures for rejuvenating successfully mature pistachio shoots by micrografting cv Siirt shoot tips from *in vitro* seedlings are described. Moreover, the shoot tips of micrografts were proliferated and the plants recovered (Onay et al., 2004). There is a single report on axillary shoot initiation from explants of mature pistachio trees. Somatic embryogenesis of pistachio was first achieved using immature kernels as explants (Onay et al., 1995). Subsequently, somatic embryogenesis of several genotypes was reported using immature and mature zygotic em-

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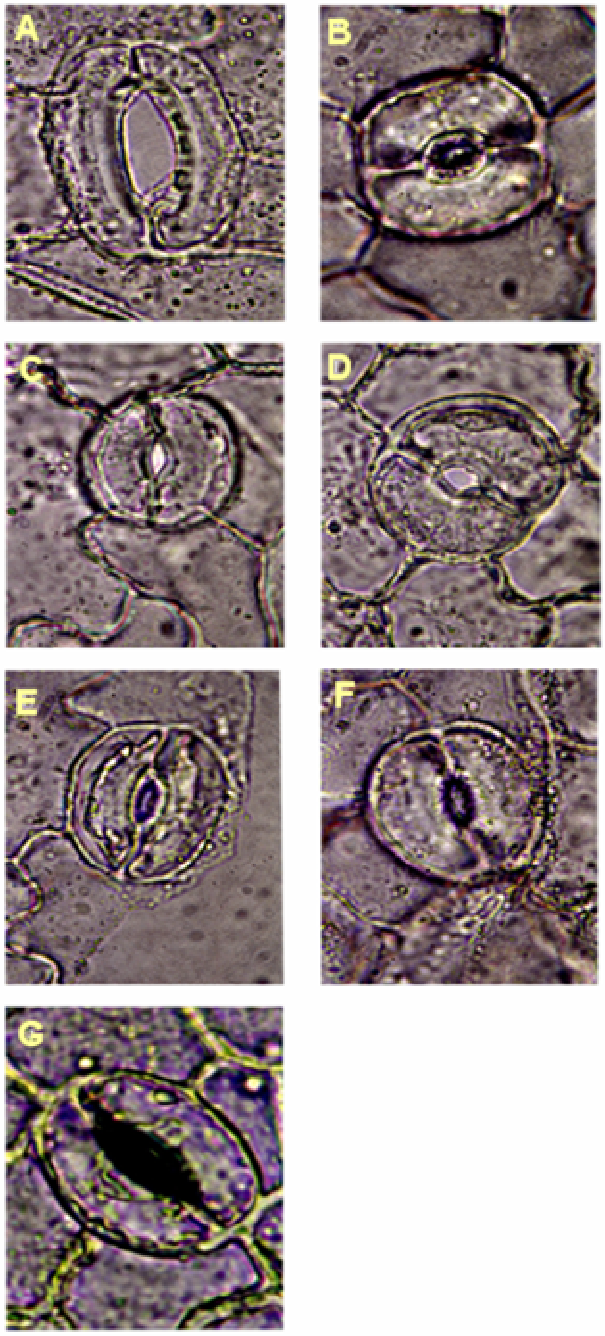

Figure 1. Influence of different cytokinins used in *in vitro* culture on the stoma morphology of pistachio (*Pistacia vera* L. cv. Siirt). (A) Stoma from 1 mg/l BA media. (B) Stoma from 1 mg/l kinetin media. (C) Stoma from 1 mg/l TDZ media. (D) Stoma from 0.25 mg/l BA media. (E) Stoma from 0.625 mg/l BA media. (F) Stoma from 2 mg/l BA medium. (G) *In vivo* developed stoma.

bryos, cotyledons and juvenile leaf tissues as explants (Onay and Jeffree, 2000; Taskin, 1995; Onay, 1996; Taskin et al., 1996; Onay, 2000b; Onay et al., 1997; Onay and Namli, 1998; Onay and Firat, 1999).

In this study, the influence of cytokinins added to the growth medium used in artificial conditions of tissue culture technique on the stoma morphology of pistachio was investigated.

MATERIALS AND METHODS

In this study, apical buds of pistachio cv. Siirt were used as source of plant material. The material used was obtained from G. Antep on South East of Turkey. The apical buds were first washed with tap water for 15 min, and depending upon their development they were kept in 3 or 5% sodium hypochlorite (NaOCl) for 30 min. Later, they were cleaned from (NaOCl) by rinsing them in sterilized water five times each time for 5 min. The buds isolated were transferred separately into MS media (Murashige and Skoog, 1962). The dedia were supplemented with different cytokinins (BA, K and TDZ at 1 mg/l) and later different concentrations of BA were tested. The medium pH was adjusted to 5.7 and was sterilized by autoclaving for 20 min. Explants were left to grow under 3000 lux at 16/8 photoperiod and at 25 + 2°C. Leaved explants were obtained from aseptically germinated seedlings after 4 weeks of culture in *in vitro* conditions from apical buds. Leaved explants were examined to establish the stoma morphology.

RESULTS AND DISCUSSION

The up and down-side epidermal sections from leaves obtained from apical sprouts at *in vitro* conditions were examined under the optical microscope. Three sections were prepared for each parameter. For each section 7 stomas were examined, meaning that in total 21 stomas were examined.

When the stomas of pistachio obtained in cytokine media (BA, kinetin, TDZ 1 mg/l) (Figures 1A, B and C) were compared to the stomas of pistachio leaves obtained *in vivo*, it could be noticed that the ones obtained with kinetin and TDZ (1 mg/l) media showed a continuously swelling of stoma cells and the pores of the stoma forms ring. Furthermore, intercellular gabs were reduced and distorted. The stomas of plantlets developed in BA (1 mg/l) had an elliptic form similar to those developed *in vivo*. This is in contrast to the stomas developed in TDZ and kinetin solutions, which showed deformation and reductions in intracellular spaces. The stoma cells developed in BA (1 mg/l) solutions are very comparable to the ones developed *in vivo*.

Different concentrations of BA (0.625, 0.25, 1, 2 mg/l) in the media were further attempted. It was observed that the stoma cells from 0.25 mg/l BA medium are edematous and the pores took the form of chain (Figure 1D). A reduction and a distortion of the extracellular spaces were also noticed. At a concentration of 0.625 mg/l BA, the results were the same with an extended increase of distortion and reduction of extracellular spaces (Figure 1E). At a concentration of 2 mg/l BA, obtained results were similar to those at of 0.625 and 0.25 mg/l BA solutions (Figure 1F).

Micropropagation is an intermediary stage for adaptation between *in vitro* and *in vivo* cultures. It is very impor-

tant for the capacity of plants to make use of the process of photosynthesis to create favorable phototrophic conditions (which can be achieved by addition of CO_2 to the culture medium, a sucrose feeding medium, using of aerate culture boxes and an extra light addition). Growth regulators like the cytokinins are also important additives to the growth media.

In conclusion, addition of cytokinins to media during *in vitro* propagation affects the development of *P. vera* stoma cells. Of all the cytokinins used, only 1 mg/l BA gives desirable result comparable to stomas developed *in vivo* (Figure 1G). Abnormal stoma development was observed in other BA concentrations as well as with TDZ and kinetin. Thus it can be concluded that the best cytokine for *P. vera* L. cv. Siirt stoma development is 1 mg/l BA.

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