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INFLUENCE OF ACTIVATED CHARCOAL ON *IN VITRO* EMBRYO GERMINATION AND GROWTH OF PLANTLETS OF PISTACHIO (*Pistacia vera* L.)

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ABSTRACT

In the present study, the effect of activated charcoal on *in vitro* embryos germination and growth of *Pistacia vera* seedlings was investigated. Different concentrations (0, 0.5, 1, 2, 5 and 10 $g.l^{-1}$) were incorporated in Hormone-free MS basal medium. The results obtained after 30 days of culture showed that adding activated charcoal to the culture medium had no effect on *in vitro* germination of embryonic axes of *Pistacia vera* L. Indeed, the germination rate was 100% for different experimental treatment. However, activated charcoal in the medium culture improved seedlings growth. The best results were obtained with 2 g l-1 active charcoal. The improvement percentage of growth was 44.4% for aerial parts and 67.4% for roots.

KEYWORDS: *Pistacia vera* L, activated charcoal, embryonic axes, *in vitro* germination, seedling, growth.

INTRODUCTION

Under the *in vivo* conditions, the passage of fire in forest has a beneficial effect on natural regeneration and germination process of many plants. Its positive effect on seeds germination was often related to the presence of an adsorbent: charcoal produced by forest fires (Hille and Den Ouden, 2005). In *in vitro* conditions, the activated charcoal is commonly used in tissue culture media. Its addition to culture medium many promote or inhibit growth *in vitro*, depending on different factors. The positive or negative effects of activated charcoal depend especially on its concentration in the culture medium, species cultivated *in vitro* and their phases of multiplication (Fridborg and Eriksson, 1975; Ahuja, 1985; Pan and Staden, 1998). Use of activated charcoal *in vitro* culture many affect growth principally shoot elongation but also rooting (Fridborg and Eriksson, 1975; Damiano, 1978; Dumas and Monteuuis, 1995; Bousselmame *et al.*, 2001). To our knowledge, no study has been reported on the influence of activated charcoal on *in vitro* zygotic embryo germination of *Pistacia* species. This study focused on assessing the effects of activated charcoal on *in vitro* germination of embryonic axes and the plantlet growth of *Pistacia vera* L.

MATERIAL AND METHODS

Pistacia vera seeds were harvested at maturity (late September) in the El Fehoul orchard (Tlemcen, Algeria), released from their epicarp and dried naturally. Then, they were stored in plastic bags, in ambient and dark conditions. Seeds were disinfected by soaking in 70% ethanol (v/v H_2O) for 1 min, then for 10 min in a solution of sodium hypochlorite (NaClO 2.6% available chlorine) containing a few drops of a wetting agent. The seeds were then rinsed three times in sterile distilled water and left in the last rinse water overnight to increase cotyledon imbibition and facilitate embryonic axis removal (Benmahioul *et al.*, 2009). The basal medium contained macro- and micro-elements of Murashige and Skoog (1962) supplemented with B_5 vitamins (Gamborg *et al.*, 1968), myoinositol (100 $mg.l^{-1}$), casein hydrolysate (500 $mg.l^{-1}$), 3% (w/v) sucrose was used throughout this study. These growth regulator-free nutrient solutions solidified with 0.4% (w/v) Difco Bacto-Agar (Benmahioul *et al.*, 2009) was also supplemented with different activated charcoal concentrations (0, 0.5, 1, 2, 5 and 10 $g.l^{-1}$). Media were adjusted to pH 5.7 with KOH 0.1N prior to autoclaving at 113°C for 20 min. The embryos were cultured in glass tubes (22 mm x 150 mm) containing 15 ml nutrient medium and placed in a growth culture room under a photoperiod of 16 h light/8 h darkness, at temperature 22 ± 1°C, under 40µmol m^{-2} S^{-1} light intensity (Benmahioul, 2009) The *in vitro* response of the isolated embryos to different concentration of activated charcoal was evaluated according to the following parameters: percentage germination, length of aerial parts and roots, the biomass of the fresh matter of aerial parts and roots and mean number of leaves and internodes per seedling. The improvement growth percentage is calculated according to the equation: 100 x ((treatment – control)/treatment). The results obtained were analysed using Statgraphics Plus software. An analysis of variance

(ANOVA) was performed and for each significant difference, means were differentiated using Duncan's multiple range test. The significance level was set at ($P < 0.05$).

RESULTS

The results obtained showed that adding active charcoal to the culture medium had no effect on *in vitro* germination of embryonic axes of *Pistacia vera* L. The germination rate was 100% for different experimental treatment. However, the joint application of active charcoal had a positive effect on growth of plantlets. After 30 days of culture, significant difference was observed for a different parameter tested: length of the aerial and root parts, foliar organogenesis of isolated embryos, number of internodes per seedling and fresh matter weight of aerial parts and root system (Figure 1). The best results were obtained with 2 g l-1 active charcoal. The improvement percentage of growth was 44.4% for aerial parts and 67.4% for roots (Table 1).

TABLE 1. Improvement percentage of the growth of aerial parts and roots of seedlings after 1 month of culture on hormone-free MS containing a various concentrations of activated charcoal (AC).

[AC] g l^{-1}	Improvement of aerial parts (%)	Improvement of roots (%)
0,5	29,2	52,9
1	33,3	27,9
2	56,4	72,5
5	50,7	74,9
10	43,3	76,2
Mean	44,4	67,4

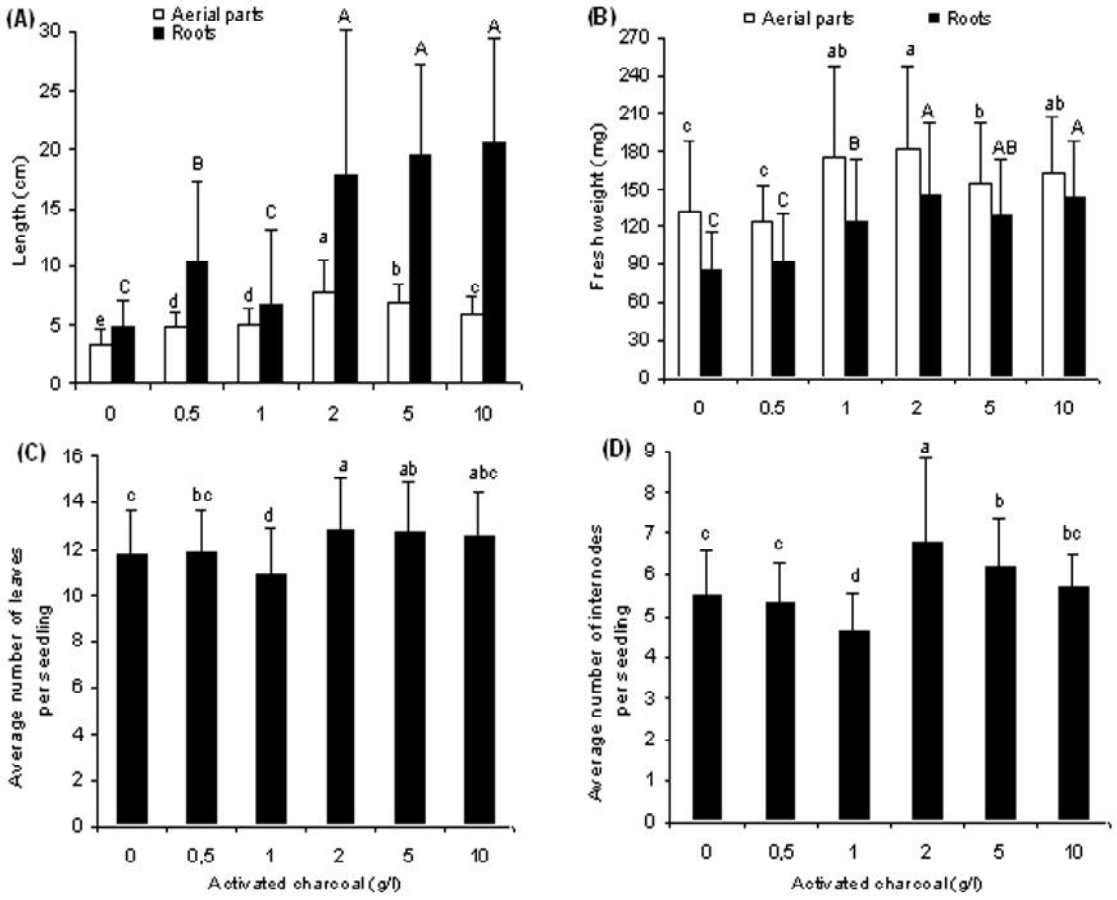

FIGURE 1. Effect of activated charcoal on the growth of *Pistacia vera* seedling after 30 days of culture on the basic MS medium (hormone-free). Length (**A),** Fresh biomass of the aerial part and of the root **(B)**, Average number of leaves (**C**) and internodes (**D**) per seedling. Values represent the mean of 40 measures per treatment. Bars with the same letters are not significantly different at $P < 0.05$.

DISCUSSION AND CONCLUSION

The addition of active charcoal to culture medium has a beneficial effect on seedling growth of *Pistacia vera* L. This improvement is more significant for the root system (Figure 2).

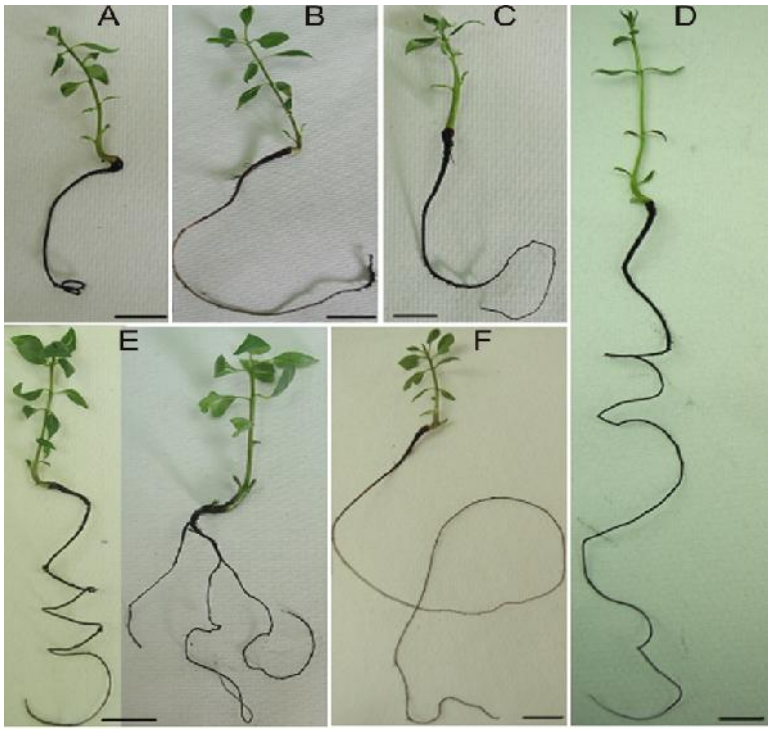

FIGURE 2. Morphology of *Pistacia vera* seedlings obtained after 1 month of culture on hormone-free MS containing various concentrations of activated charcoal (g l^{-1}): **A-** Control (0) **B-** (0.5) **C-** (1) **D-** (2) **E-** (5) and **F-** (10) (Bar = 1 cm)

Many research workers have show that the addition of activated charcoal often has a promotive effect on the growth and organogenesis of different plants (Mensuali-Sodi *et al*., 1993**)**. Addition of active charcoal in culture medium may affect especially rooting. Dumas and Monteuuis (1995) observed that activated charcoal in the medium culture improves not only rooting rates, but also roots growth of *Pinus pinaster*. Similar results were reported by others authors (Misson *et al.*, 1983; Margara, 1989; Side and Staden, 1998; Bousselmame *et al.*, 2001). Charcoal prevented discoloration by adsorbing phenolics and rendered polyphenol oxidase and peroxidase inactive (Maene and Debergh, 1985; Pan and Staden, 1998). However, charcoal in culture medium had a marked negative effect on cultured explants. Webb et al. (1988) reported that active charcoal inhibited shoot proliferation and rooting of *Pinus strobus* L. The negative effect of active charcoal has also been reported. Van Waes (1987) noted that germination rates and shoot development of European orchids were decreased by activated charcoal. In our experiment, activated charcoal used in medium for *Pistacia vera* L. had a marked positive effect on growth *in vitro*. Seedling growth especially roots elongation was promoted by charcoal. Charcoal provides a degree of darkness during *in vitro* culture. The activated charcoal can affect the activity and stability of micrplant growth regulators by reducing or excluding light.

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