In vitro culture of mature avocado embryos

BE Llano-Agudelo,
H Gonzalez-Rosas
Centro de Fruticultura
Colegio de Postgraduados
Chapingo, Edo de Mex
56230, Mexico

S Salazar-Garcia
University of California
Botany and Plant Sciences
Riverside, CA 92521
USA

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ABSTRACT
Mature embryos excised from avocado seeds (*Persea americana* Mill, cv Americana, Mexican race) were cultured on Murashige and Skoog medium supplemented with kinetin (K), and/or indolebutyric acid (IBA) at 25 ± 2°C for 21 d in darkness followed by 14 d of continuous light. Embryo germination, production of a shoot and root of 0.5 cm in length minimum, was 100% for embryos cultured with 0.3 mg/l IBA and 0.1 to 0.3 mg/l K. Concentrations greater than 0.3 mg/l IBA reduced germination. The greatest nmber of adventitious shoots was obtained with 3.0 mg/l IBA and 0.1 mg/l K. Maximum mean shoot length (12.5 cm) was achieved with the combination of 1.0 mg/l IBA and 1.0 mg/l K. Maximum root length (4.9 to 6.6 cm) was obtained with IBA of between 0.3 and 1.0 mg/l combined with 0.1 to 1.0 mg/l K.

KEYWORDS
Persea americana, plant embryos, *in vitro* culture, culture media, germination.

Culture *in vitro* d'embryons matures d'avocats.

RÉSUMÉ
Des embryons matures extraits de noyaux d'avocats (*Persea americana* Mill, cv Americana, race mexicaine) ont été cultivés sur un milieu de Murashige et Skoog enrichi en kinétine (K) et/ou en acide indole-butyrique (IBA), à 25 ± 2°C, pendant 21 j à l'obscurité suivis de 14 j en lumière continue. La germination des embryons, mesurée par le taux d'embryons ayant produit une tige et une racine de 0,5 cm de longueur minimale, a été de 100% pour les embryons cultivés avec 0,3 mg/l d'IBA et 0,1 à 0,3 mg/l de K. Des concentrations supérieures à 0,3 mg/l d'IBA ont diminué ce taux de germination. Les milieux contenant 3,0 mg/l de K ont donné le plus grand nombre de pousses adventives. La plus longue tige (12,5 cm en moyenne) a été observée sur des milieux contenant 1,0 mg/l d'IBA et 1,0 mg de K. Les racines les plus longues (4,9 à 6,6 cm) ont été obtenues sur des milieux contenant de 0,3 à 1,0 mg/l d'IBA combinée avec 0,1 à 1,0 mg/l de K.

MOTS CLÉS
Persea americana, embryon végétal, culture *in vitro*, milieu de culture, germination.

Cultivo *in vitro* de embriones maduros de semillones de aguacate.

RESUMEN
Se sacaron embriones maduros de semillones de aguacate (*Persea americana* Mill., cv Americana, raza mejicana) y se cultivaron en un medio ambiente Murashige y Skoog (MS) al que se le añadió kinetina (K) y/o ácido indolbutírico (AIB) a 25 ± 2°C durante 21 d y en la obscuridad, seguidos de 14 d de luz continua. Se obtuvo la germinación de los embriones, la producción de retoños y raíces de 0,5 cm de largo como mínimo en el 100% de los embriones cultivados con 0,3 mg/l de AIB y 0,1 a 0,3 mg/l de K. Aquellas concentraciones de AIB superiores a 0,3 mg/l redujeron la germinación. Se obtuvo el mayor número de retoños con 3 mg/l de AIB y 0,1 mg/l de K. Aquellas concentraciones de 1 mg/l de AIB y 1 mg/l de K provocaron la largura máxima de los retoños (12,5 cm). La combinación de 0,3 a 1 mg/l de AIB y de 0,1 a 1 mg/l de K hizo posible obtener una longitud máxima de las raíces (4,9 a 6,6 cm).

PALABRAS CLAVES
Persea americana, embriones vegetales, cultivo *in vitro*, medio de cultivo, germinación.

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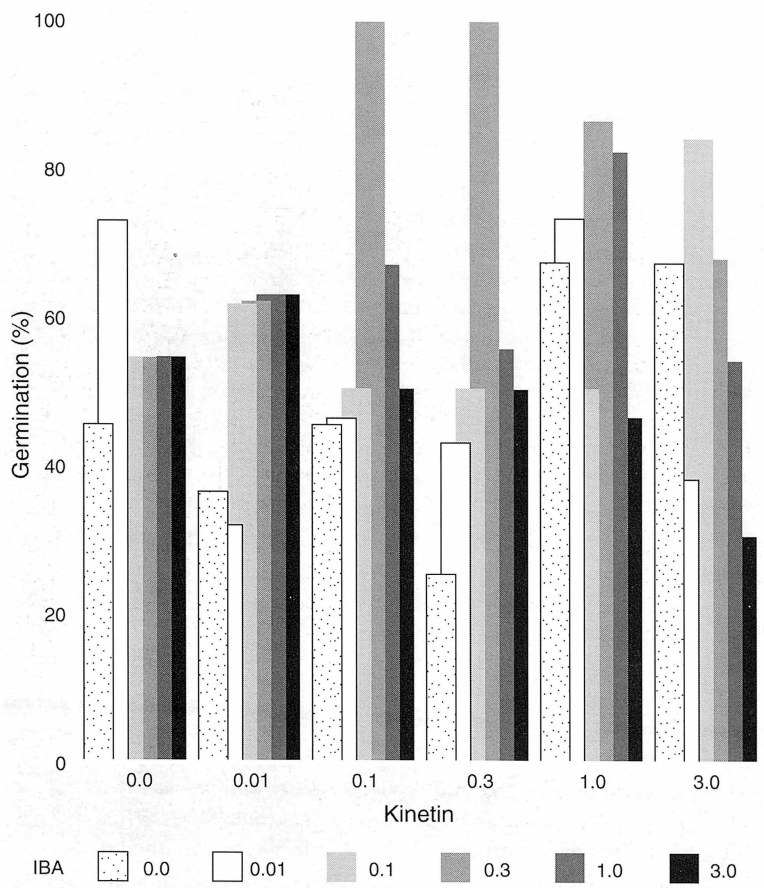
introduction

Widespread acceptance of avocados on international markets has resulted in a considerable worldwide increase in the amount of land dedicated to avocado production in areas where avocados can be grown. However, the presence of adverse production conditions at many new planting sites and/or inappropriate orchard management is resulting in low yields. Typical problems include those caused by excess salts (principally Na^+ and Cl^-) in the water or soil, a shortage of water during the critical stages of fruit development, or the lack of adequate soil drainage, leading to subsequent devastating attacks by *Phythophthora cinnamomi* Rands. The presence of any of these conditions reduces the productivity and longevity of an avocado orchard.

Finding genotypes with resistance or tolerance to the above-mentioned factors through classical genetic improvement programs usually takes several years, and sometimes the expected results are not obtained. *In vitro* embryo culture may provide a means to select genotypes with desired characteristics in a shorter period of time. For a fruit tree crop, the first successful *in vitro* embryo culture was the work of TUKEY (1933) with sweet cherry (*Prunus avium*). In avocado, there is very little information concerning this technique. PLIEGO-ALFARO and MURASHIGE (1987) used embryo cultivation to evaluate the rooting capacity of cuttings from mature material; SKENE and BARLASS (1983) rescued hybrid embryos by cultivating them *in vitro*. The objective of this study was to test the efficacy of adding IBA and/or K to basic MURASHIGE and SKOOG (1962) culture medium to increase germination and root and shoot growth for rapid production of *in vitro* plantlets from mature embryos of creole avocado.

Figure 1
Effect of kinetin and IBA (mg/l) on germination of mature avocado embryos.



materials and methods

Avocado seeds of the Mexican race were submerged in 70% ethanol (v/v) for 1 min. The seed was split and the embryo surface was sterilized for 15 min with 2% sodium hypochloride (v/v) and rinsed in sterile double-distilled water. The embryos were excised with a small piece of cotyledon (photo 1) and aseptically transferred to sterile culture tubes (25 x 150 mm) containing 10 ml of MURASHIGE and SKOOG (1962) (MS) supplemented with 2.0 mg/l glycine, 100 mg/l myoinositol, 5 mg/l pyridoxine-HCl, 1.0 mg/l thiamine-HCl, 5.0 mg/l nicotinic acid, 30.0 g/l sucrose and 7.0 g/l bacto agar. The MS medium was adjusted to pH 5.7 with 0.1 N NaOH or HCl. Culture tubes were capped with aluminum foil and sterilized by autoclaving at 120°C and 1.5 kg/cm^2 pressure for 15 min. The MS medium was supplemented with IBA and K alone or in combination, at concentrations from 0.0, 0.01, 0.1, 0.3, 1.0 and 3.0 mg/l.

The explants were incubated at 25 ± 2°C in darkness for 21 d, followed by continuous light (3 000 lux cool white fluorescent irradiance) for 14 d. At the end of 35 d of culture, the following parameters were evaluated: 1) germination, as

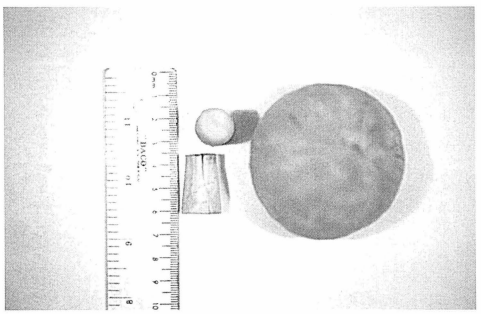


Photo 1
Seeds from the Mexican race of avocado. Whole seed (left) and seeds showing the cotyledons trimmed (right) to be inoculated in vitro.

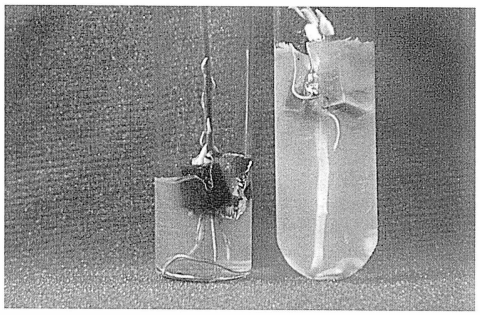


Photo 2
Seedlings obtained from mature avocado embryos cultured on MS medium supplemented with 0.3 mg/l IBA and 0.1 mg/l K.

the number of embryos in which both the plumule and root were a minimum of 0.5 cm in length expressed as a percent of the total number of embryos cultured; 2) shoot number and length. When multiple shoots were formed in some treatments, they were considered separately in estimating the average per treatment; 3) root number and length.

The results were analyzed as a randomized design, with a single embryo per treatment and 30 replications. The Tukey test with a significance level of 5% was used for statistical comparisons.

results and discussion

germination

The number of shoots and roots obtained in the treatments was closely related to the percentage of germination. It was found that separate addition of K or IBA to the medium did not significantly affect the results for any of these three variables. Germination of embryos cultured with 0.3 mg/l IBA mixed with 0.1 or 0.3 mg/l K was 100% and the resulting seedlings showed excellent development (photo 2). Greater concentrations of IBA (up to 3.0 mg/l) reduced embryo germination. Germination of embryos cultured with 0.3 mg IBA plus 1.0 mg/l K or 0.1 mg IBA plus 3.0 mg/l K was 85.7% and 83.3%, respectively (fig 1).

shoot number and length

The highest number of adventitious shoots (1.83) was obtained when the avocado embryos

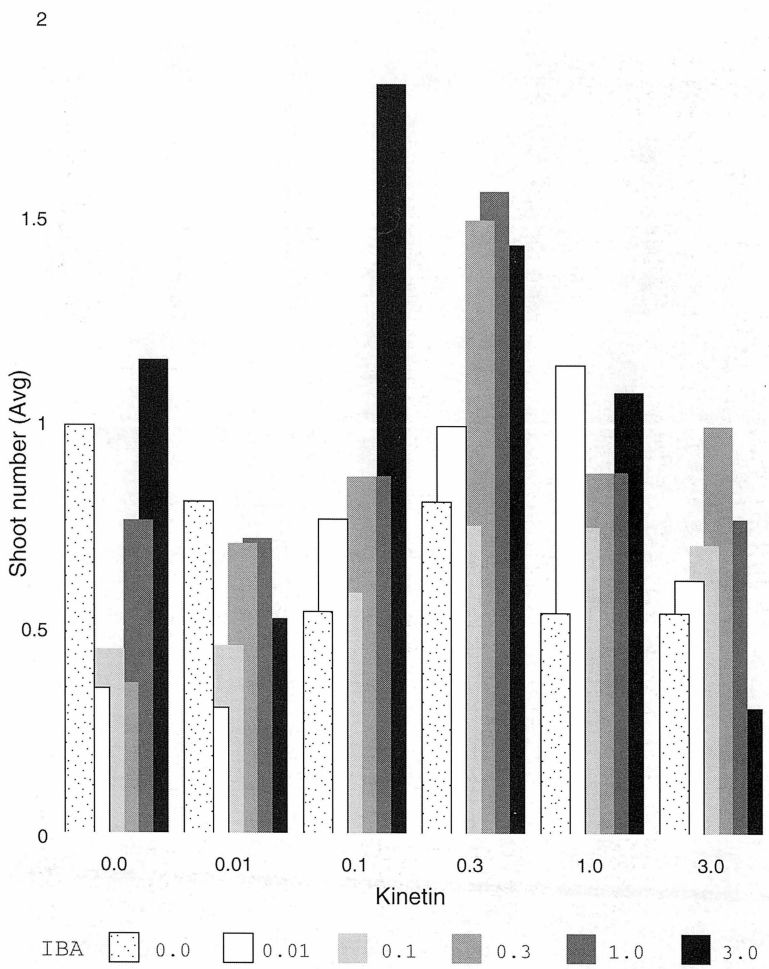


Figure 2
Effect of kinetin and IBA (mg/l) on shoot production.

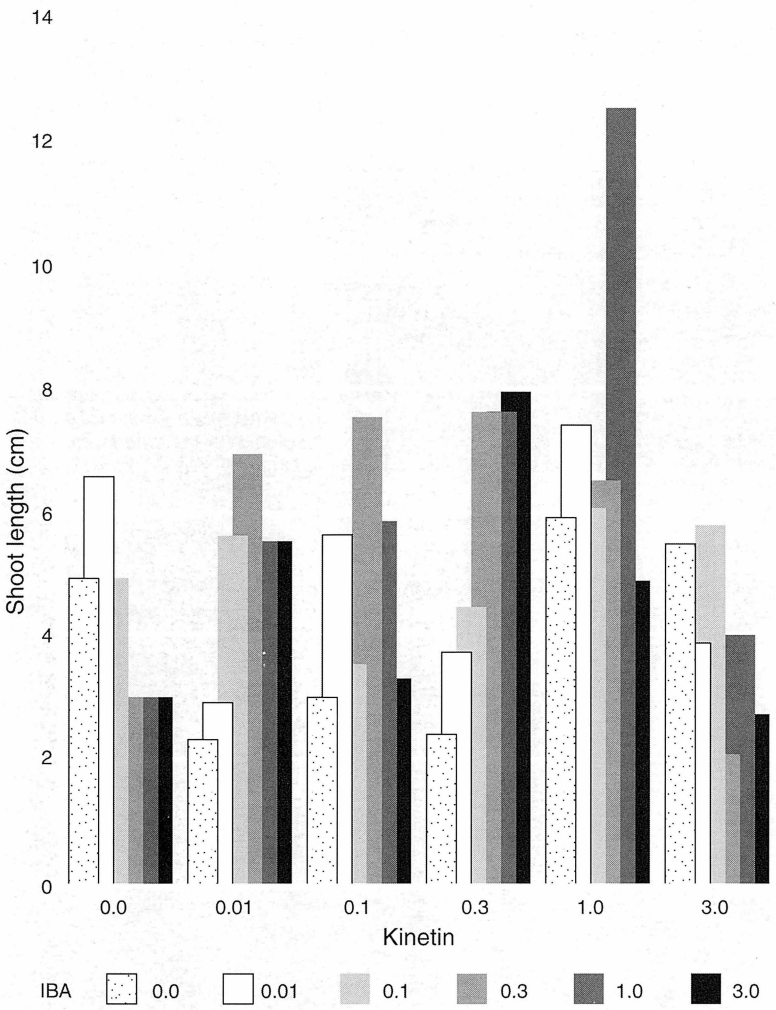


Figure 3
Effect of kinetin and IBA (mg/l) on shoot length.

were cultured in MS medium supplemented with 3.0 mg/l IBA and 0.1 mg/l K (fig 2). The addition of 0.3 mg/l K also provided good results when this concentration was combined with 0.3 (1.5 shoots), 1.0 (1.57 shoots) and 3.0 (1.44 shoots) mg/l IBA. However, an average of 2.6 shoots was observed in one of each of the four embryos. Even though under natural conditions multiple shoots occur in avocado embryos, this phenomenon has been observed in 2-10% of the Mexican race of avocado (SALAZAR-GARCIA and BORYS, 1983). This frequency contrasts with that obtained in the present experiment (25%). GONZALEZ-ROSAS and SALAZAR-GARCIA (1984) and GONZALEZ-ROSAS *et al* (1985) used a greater concentration of K (3.0/l mg) to stimulate the development of axillary buds in stem segments of avocado. The concentrations used and the results obtained by these authors differed from those in the present study, possibly because of the type of explant and the difference in its endogenous hormonal concentration. Conversely, it can be considered that a minimum portion of cotyledon tissue left with the embryos initially stimulates germination and later promotes both shoot development and the formation of axillary buds. The results of this study complement those of SKENE and BARLASS (1983) which established a possible role of cotyledons on shoot development.

Shoot length was not significantly affected by the separate addition of K or IBA. However, when combined, the concentration of K had a greater effect than that of IBA. The maximum mean shoot length (12.5 cm) was achieved with a combination of 1.0 mg/l IBA and 1.0 mg/l K (fig 3). This does not coincide with the results of SCHROEDER (1967), who in an experiment with explants of avocado pericarp cultivated *in vitro*, discovered that a higher level of auxin, in relation to cytokinin, was more conducive to tissue growth. Nevertheless, this is possible because the requirements of a substance vary considerably in different plant tissues. Cytokinins are compounds that are generally related to cytokinesis, while auxins are associated with cellular lengthening (BHOJWANI and RADZAN, 1983). Their effect on shoot length may thus be due to the influence of these growth regulators on the processes of cell division and lengthening.

root number and length

The number of roots produced by the embryos was not significantly modified by auxin or cytokinin levels, although greater root formation was obtained when embryos were cultured in MS containing either 0.3 mg/IBA plus 0.1 or 0.3 mg/l K, or 1.0 mg/l IBA with 1.0 mg/l K (data not shown). Our results on mature avocado embryos contrasted with those of SKENE and BARLASS (1983), who mentioned that only a few immature embryos produced roots.

As for the previous variables, the addition of only 1 growth regulator to the MS medium did not significantly affect root length. However, the combination of IBA and K levels affected

this characteristic. A significant increase in root length was obtained with kinetin levels of 0.1 mg/l and higher (table I; fig 4). Concerning the different levels tested for IBA, 0.3 mg/l IBA concentrations produced the highest mean root length (4.4 cm). The lowest values were recorded for the IBA levels of 0.0 and 3.0 mg/l (table I).

The combinations of IBA and K which produced the highest root length increases were 0.3 and 1.0 mg/l IBA combined with 0.1 and 0.3 mg/l K (fig 4). Treatments with lower levels of kinetin did not result in significant root length increases. When the levels of IBA and kinetin were increased beyond 1.0 and 0.3 mg/l, respectively, root length decreased (fig 4).

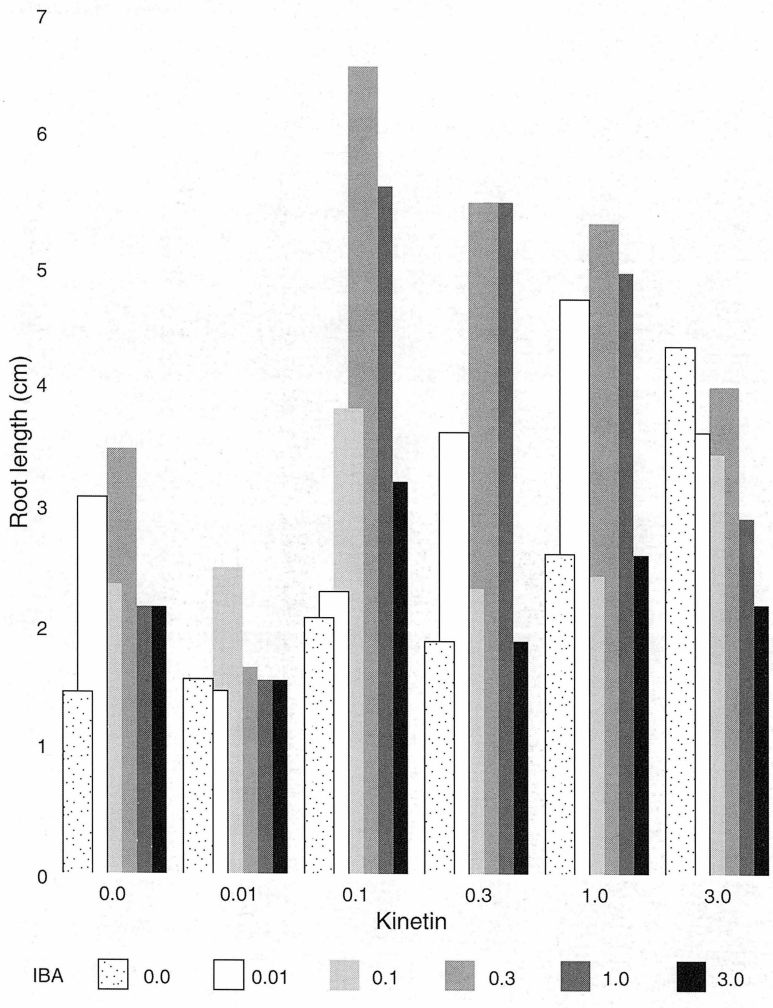


Figure 4
Effect of kinetin and IBA (mg/l) on root length.

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Table I
Root length (cm): effect of different levels (mg/l) of IBA and kinetin.

	Kinetin						*Root lenght*
IBA	*0.0*	*0.01*	*0.1*	*0.3*	*1.0*	*3.0*	*average*
0.0	1.5 [a] A	1.6 [a] A	2.1 [c] A	1.9 [b] A	2.6 [a] A	4.3 [a] A	2.3 [bc]
0.01	3.1 [a] AB	1.5 [a] B	2.3 [c] AB	3.6 [ab] AB	4.7 [a] A	3.6 [a] AB	3.1 [abc]
0.1	2.4 [a] A	2.5 [a] A	3.8 [abc] A	2.3 [b] A	2.4 [a] A	3.5 [a] A	2.8 [abc]
0.3	3.5 [a] BC	1.7 [a] C	6.6 [a] A	5.5 [a] AB	5.3 [a] AB	4.0 [a] ABC	4.4 [a]
1.0	1.5 [a] B	1.3 [a] B	5.6 [a] A	5.5 [a] A	4.9 [a] A	2.9 [a] AB	3.6 [ab]
3.0	2.2 [a] A	1.6 [a] A	3.2 [bc] A	1.9 [b] A	2.6 [a] A	2.2 [a] A	2.3 [bc]
Average	2.4 B	1.7 B	3.9 A	3.4 A	3.7 A	3.4 A	

The same lower case or capital letters in the same column or line are not significantly different (Tukey, 5%).