

Effect of IBA, Kinetin, and Benzil Amino Purine on the Germination, Shoot Development, and Root Formation in Avocado Embryos Cultivated *in vitro*.

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Summary

Mature embryos of avocado seed (*Persea americana* Mill. var. *americana* R. Mexicana) were inoculated on Murashige and Skoog medium supplemented with two cytokinins: Kinetin (K) and benzil amino purine (BA) alone or in combination with an auxin, indolbutyric acid (IBA). The explants were incubated at a temperature of $25\text{ }^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 21 days in darkness and transferred to conditions of continuous light for seven days. The percentage of germination, and the number of shoots and roots increased when the explants were placed in a basic medium with 0.3 mg L^{-1} of IBA and 1.0 mg L^{-1} of K added; bud growth was better in the treatment with 1.0 mg L^{-1} of K and 1.0 mg L^{-1} of IBA. The length of the root was stimulated with the combinations of 0.01 and 0.3 mg L^{-1} of K with 0.3 and 1.0 mg L^{-1} of IBA. Negative effects of shoot growth were observed when benzil amino purine was added.

Introduction

The widespread acceptance of the avocado in international markets has resulted in a considerable increase in the amount of surface area that is being dedicated to this fruit tree in those areas of the world where the avocado can be established. However, due to the quality of the production areas or the management given to the orchards, problems frequently arise due to the excess of salts (principally Na^+ and Cl^-) in the water or soil, a shortage of water during the critical stages of development, or the lack of adequate drainage of the soils, with subsequent devastating attacks by *Phytophthora cinnamomi*. These problems may be the result of the preexisting conditions of the land under cultivation or they may be the result of improper management. The presence of any one of these conditions reduces the productivity and the longevity of the avocado orchards.

Genetic improvement programs for finding genotypes that are resistant to, or tolerant of, the above-mentioned problems usually take several years to complete; and on occasion, the expected results are not obtained.

The cultivation *in vitro* of embryos is an alternative that allows us to select genotypes

with outstanding characteristics in a shorter period of time. Tukey (1933) carried out the first such experiment on fruit trees by cultivating embryos of the sweet cherry (*Prunus avium*). In avocado, information concerning this technique is rare, aside from the studies of Pliego-Alfaro and Murashige (1987) who used the cultivation of embryos to evaluate the rooting capacity of cuttings from mature material, and those of Skene and Barlass (1983) who rescued hybrid embryos, cultivating them *in vitro*. In the selection of genotypes, it is necessary to study those aspects related to the behavior of the embryos in a chemically-defined medium, for their subsequent manipulation.

Therefore, the purpose of this project was to establish the hormonal requirements which permit the rapid obtainment of plantlets from mature embryos of criollo avocado by cultivating them *in vitro* in a chemically-defined medium.

Materials and Methods

Mature embryos from avocado seeds of Mexican race were cultured *in vitro* on Murashige and Skoog (1962) medium complemented with 2.0 mg L⁻¹ glycine, 100 mg L⁻¹ myo-inositol, 5 mg L⁻¹ pyridoxine-HCl, 1.0 mg L⁻¹ thiamine-HCl, 5.0 mg L⁻¹ nicotinic acid, 30.0 g L⁻¹ sucrose, and 7.0 g L⁻¹ bacto agar. The pH of MS medium was adjusted to 5.7 with 0.1 N of NaOH or HCl. Ten ml were poured into a culture tube (25 x 150 mm) and capped with aluminum foil; sterilized in autoclave at temperature of 120°C, 1.5 kg/cm² for 15 minutes.

The seeds were submerged in ethanol at 70% (v/v) for 1 minute, the embryo surface was sterilized during 15 minutes with 2% of sodium hypochloride, and rinsed in sterile, double distilled water. The embryos were excised with a small piece of cotyledon and inoculated with five levels of IBA alone or in combination with five levels of kinetin (K) or benzyl amino purine (BA). The levels used were: 0.01, 0.1, 0.3, 1.0, and 3.0 mg L⁻¹.

The explants were cultured at 25 °C ± 2°C in darkness for 21 days after incubation, and were subsequently exposed to continuous light (3000 lux Cool White fluorescent irradiance).

The evaluated variables were:

- a. Percentage of germination. Embryos were considered germinated when the plumule and the root had a minimum length of 0.5 cm, assuming that embryos not germinating within 35 days would no longer do so.
- b. Shoot number. When multiple shoots were formed in some treatments, they were considered separately in estimating the average per treatment.
- c. Root number. When adventitious roots appeared, they were used in estimating and establishing the average per treatment.
- d. Shoot and root length. The length of each embryo shoot and root was measured after the 35-day germination period.

The Tukey test with a significance level of 5 % was used for statistical comparison.

Results and Discussion

Percentage of germination, shoot, and root numbers.

The number of shoots and roots obtained in the treatments was closely related to the percentage of germination. It was observed that the individual addition of K or IBA to the medium did not significantly affect the results obtained in any of the three variables. In varying the concentration of the regulators, it was discovered that the level of IBA has a greater effect than that of K in terms of germination and shoot number; the greater the quantity of auxin (up to 0.3 mg L^{-1}), the higher the average per level, reaching an optimum germination of 78% with 1.24 shoots; greater concentrations reduced the percentage of germination. The values were statistically superior to those obtained with the IBA levels of 0.01 and 3.0 mg L^{-1} in both concentrations, and in addition, to the 1.0 mg L^{-1} shoot number.

It is possible that the germination obtained through the use of K and IBA is due to an increase in the quantity of endogenous hormones (Bewley and Black, 1978), or to a synthesis of an enzyme and/or an increase in enzymes as have been described by Salisbury and Ross (1985). It is also possible that the kinetin is acting during the period of darkness while the embryos are incubating. This view is supported by the results obtained by Miller (cited by Nickell, 1982).

The development of adventitious shoots in the embryos was stimulated when combinations of 3.0 mg L^{-1} of K + 0.3 mg L^{-1} of IBA and 1.0 mg L^{-1} of K + 0.3 mg L^{-1} of IBA were added to the medium. This relationship between the combinations of regulators (the higher concentration of K) concurs with the generally-accepted theory established by the research of Skoog and Miller (1962) (cited by Horgan, 1985) which determined that a higher level of cytokinin, as opposed to auxin, was more conducive to the development of stem shoots in tobacco cultivated *in vitro*.

Gonzalez-Rosas and Salazar-Garcia (1984) used a greater concentration of K (3.0 mg L^{-1}) to stimulate the development of axial bud in avocado. The concentrations used by those authors were different from those in the present study, possibly because of the type of explant and the concentrations of endogens which may vary from one to the other.

As far as the roots are concerned, none of the auxin or cytokinin levels significantly modified the number of root organs developed by the embryos.

Shoot length.

It is observed that the individual addition of K or IBA did not significantly affect the growth of shoots. However, when combined, the concentration of K had a greater effect than that of IBA. In the treatments with 1.0 mg L^{-1} kinetin, a greater average length was achieved than with other concentrations of cytokinin. At this same level and combined with 1.0 mg L^{-1} of IBA, the greatest average shoot length (12.5 cm) of all the treatments was achieved.

These results do not coincide with those mentioned by Schroeder (1967) who, in investigating the explants of the pericarp of avocado cultivated *in vitro*, discovered that a

higher level of auxin, in relation to cytokinin, is more conducive to tissue growth. Nevertheless, this situation is possible because the requirements of a substance vary considerably in different plant tissues. This may possibly be due to differences in the endogen levels.

Cytokinins are compounds generally related to cytokinesis, while auxins are associated with cellular lengthening (Bhojwani and Radzan, 1983). Therefore, the differential results may be due to the effect of these regulators on the processes of cell division and lengthening of the cells.

Root length.

In this variable, as in the previous ones, the addition of only one regulator to the medium did not significantly affect the length of the roots; however, when combined, both the K and IBA levels significantly affected the growth of these organs. Root length increased, with a level of 0.1 mg L⁻¹ or more of K showing that at this level and at the 1.0 mg L⁻¹ level, the averages were significantly higher than those recorded for 0.01 mg L⁻¹. Among the different levels tested for IBA it was discovered that, on the one hand, a 0.3 mg L⁻¹ level resulted in an average growth increase of 1.75 cm, above the growth rate recorded for the treatments in which this auxin was not used. On the other hand, with a higher concentration (3.0 mg L⁻¹), root length growth was 1.74 cm less than the best treatment given by 0.3 mg L⁻¹ (4.07 cm).

It was observed that the interactions between K and IBA which increased root length in comparison with the control (without auxin and without cytokinin), were 0.3 and 1.0 mg L⁻¹ of IBA combined with 0.1 and 3.0 mg L⁻¹ of K. Treatments with lower levels did not register any increases, possibly because the concentrations, even when taking the endogene hormones into consideration, were not sufficient to produce a positive effect. With the treatments at higher levels, lengthening begins to diminish, perhaps because of the biosynthesis of ethylene induced by the higher than the optimum auxin concentrations found in the tissues. This increase in the production of ethylene at high auxin levels was noted by Salisbury and Ross (1985).

The same relationship between the concentrations of K and IBA which stimulated shoot growth (higher auxin than cytokinin levels) also stimulated root growth. This suggests that the activating mechanism of the regulators is similar in both organs and that the K promotes cytokinesis whereas the auxin mainly stimulates lengthening.

Literature Cited

- Bewley, J. D., and M. Black. 1978. Physiology and biochemistry of seeds in relation to germination. Springer-Verlag, New York, pp. 88-105.
- Bhojwani, S. S., and M. K. Radzan. 1983. Zygotic embryo culture. In: Plant Tissue Culture: Theory and Practice. Ed. S. S. Bhojwani y M. K. Radzan. Elsevier. New York. pp. 199-236.
- Gonzalez-Rosas, H., and S. Salazar-Garcia. 1984. Root induction and vegetative development from avocado plantules (*Persea americana* Mill.). Calif. Avocado Soc. Ybk. 68: 167-171.

- Gonzalez-Rosas, H., S. Salazar-Garcia, and V. Vazquez Valdivia. 1985. Propagation *in vitro* of chinini (*Persea schiedeana* Ness). Calif. Avocado Soc. Ybk. 69: 125-131.
- Horgan, R. 1985. Cytokinins. In: Advanced Plant Physiology. Ed. M. Wilkins Pitman Publishing Limited. London, pp. 53-70.
- Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-497.
- Nickell, L. G. 1982. Plant Growth Regulators. Springer-Verlag. New York. pp. 6-7.
- Pliego-Alfaro, F., and T. Murashige. 1987. Possible rejuvenation of adult avocado by grafting onto juvenile rootstocks *in vitro*. *Horts.* 22: 1321-1324.
- Salisbury, F. G., and C. W. Ross. 1985. Plant Physiology. Wadsworth Publishing Co., Belmont, California, pp. 309-348.
- Schroeder, C. A. 1967. Fruit tissue culture with special reference to plant growth regulator. *Proc. Int. Symp. on Subtropical and Tropical Horticulture*, pp. 191-200.
- Skene, H. G. M., and M. Barlass. 1983. *In vitro* culture of abscised immature avocado embryos. *Ann. Bot.* 52: 667-672.
- Tukey, H. B. 1933. Artificial culture of sweet cherry embryos. *J. Hered.* 24: 7-12.