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In Vitro Germination of Avocado Pollen

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Abstract. **Avocado pollen was germinated *in vitro* without recourse to germination on the style. The technique employed involved inclusion of pollen grains in liquid medium of 15% sucrose and minerals and application of 1 or 2 drops on 1% agar plus 15% sucrose and minerals. No germination was obtained on agar plus sucrose without placement of pollen in liquid first. Of the cultivars tested, 'Ettinger' and 'Nabal' pollen germinated best, and 'Fuerte' was the poorest. The optimum temperature for germination was 25° to 27°C. Addition of Ca to the liquid proved beneficial.**

One of the difficulties in avocado breeding is that no germination medium has been available to assess pollen viability. Sedgley (14) states in 1981: no successful *in vitro* germination technique has been described for avocado. A similar statement is contained in Oppenheimer's comprehensive chapter on avocado (7). Attempts to germinate avocado pollen *in vitro* have been met with no success (3, 12), with the exception of unpublished and undefined results by Makino (1). Pollen germination has been achieved thus far only on stigma, but not on artificial media (8, 12, 14). Pollen storage of avocado has been reported (14, 16). Avocado races and cultivars often do not overlap in bloom (7). In Israel, flowering of cultivars of the Mexican race usually precede by weeks the flowering period of some Guatemalan cultivars. Assessment of pollen viability is important in ensuring success of controlled crosses between cultivars with a different flowering period, and in the use of pollen after storage.

Avocado flowers of the cultivars 'Topa Topa', 'Ettinger', 'Fuerte', 'Nabal', and 'West Indian' were cut and anthers immediately were placed in a liquid medium (pH = 7), containing 15% sucrose and (in mg/liter) $Ca(NO_3)_2$-H_20, 1000; $MgSO_4$, 300; KNO_3, 100; and H_3BO_3, 100. In each test tube (9 mm diameter) anthers from 15 flowers were placed in 6 ml liquid. After maceration, the anthers were submerged; pollen grains were recovered by Pasteur pipettes. The solution containing pollen grains was placed either on top of a cover slide in a Petri dish or 1-2 drops (30-60µl) containing the pollen were pipetted onto 1% solid agar (also containing 15% sucrose plus minerals). The dishes were sealed with Parafilm and placed in an incubator at 26°C. The percentage of pollen germinated was counted after 3 hr at 75.6 magnification.

In all experiments, no germination was obtained on solid medium alone (1% agar plus 15% sucrose and minerals), although such a medium proved highly satisfactory for a large number of other fruit tree species (4). Germination was achieved only when drops of solution containing 15% sucrose and the pollen were placed on agar, also containing 15% sucrose (Fig. 1). Germination was rather low when only sucrose was used. Increased germination percentages were obtained when minerals, especially B and K

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(in case of 'West Indian') were added to the solution (Table 1). The inclusion of Ca seemed to raise the percentage of germination of 'Topa Topa', whereas B and K were more important for 'West Indian'. The addition of growth substances and of coconut milk (CCM) also was investigated. CCM at 10% accelerated elongation of pollen tubes.

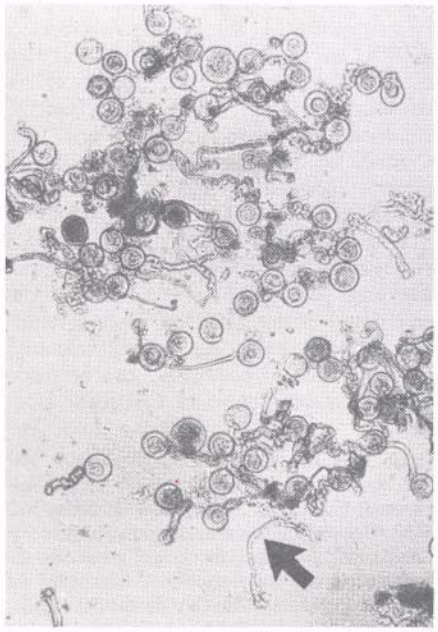

Fig. 1. Germination of avocado pollen in solution with 15% sucrose and minerals, placed on solid agar (1%). Some pollen grains get detached in the process (arrow). X235

Table 1. Percentage of germination of avocado pollen with the number of pollen grains counted in brackets.

Medium composition	Germination (%)	
	'West Indian'	'Topa Topa'
Sucrose only	8.0 ± 0.1[z] (567)	---
Sucrose + B	10.0 ± 0.2 (604)	---
Sucrose + B & K	29.0 ± 0.2 (738)	---
Sucrose + B, K, Ca	---	64.0 ± 0.8 (427)
Sucrose + B, K, Mg	21.0 ± 0.2 (653)	48.0 ± 0.7 (409)
Sucrose + B, K, Ca, Mg	30.0 ± 0.2 (420)	67.0 ± 0.7 (437)
Sucrose + B, Ca, Mg	---	65.0 ± 0.8 (462)
Sucrose + K, Ca, Mg	10.0 ± 0.2 (627)	---

[z] ± SE.

Important differences in germination percentage were noted among cultivars (Table 2). The liquid medium on top of agar was used, both containing 15% sucrose and minerals. 'Ettinger' pollen germination was highest, 'Topa Topa' and 'West Indian' were intermediate, and 'Fuerte' was the lowest.

The effect of temperature on germination percentage is summarized in Fig. 2. No germination was evident at 6°C. The optimum temperature was 25°, with 29° only slightly inferior. With 'Fuerte', no noticeable differences were found in the 19° to 29° range. Germination of 'Nabal' pollen at 19° and 37° was only slightly inferior to that at 27° or 30°.

The percentage of pollen grains stained by acetocarmine (2%) and by fluorescein diacetate (FDA) also was determined (5, 10). Twenty mg of FDA powder was dissolved in 3 ml acetone, and 20µl of this was added to a 50µl solution containing pollen grains. The percentage of pollen grains stained bright yellow was determined. Pollen grains stained by FDA are shown in Fig. 3. Fig. 4 shows a germinating pollen tube after staining by FDA. The data in Table 2 indicate that pollen germination on the artificial medium (drop of liquid on agar, both containing 15% sucrose + B, K, Ca, Mg salts) was consistently lower than the number of pollen grains effectively stained by either acetocarmine or FDA.

Table 2. Percentage of pollen grains (±SE) of avocado cultivars germinating on artificial medium, and percentage stained by acetocarmine or fluorescein diacetate (FDA).

Cultivar	Germination on artificial medium (%)	"Viable" pollen stained (%) by acetocarmine	"Viable" pollen stained by FDA (%)
Ettinger	64 ± 0.3 (879)	---	---
Fuerte	14 ± 1.5 (2017)[z]	62 ± 5 (922)	33 ± 4 (1525)
Topa-Topa	47 ± 6.2 (1053)	70 ± 8 (726)	83 ± 6 (1766)
West Indian	44 ± 7.7 (1666)	77 ± 9 (942)	78 ± 6 (1756)

[z]Number of pollen grains counted given in parentheses.

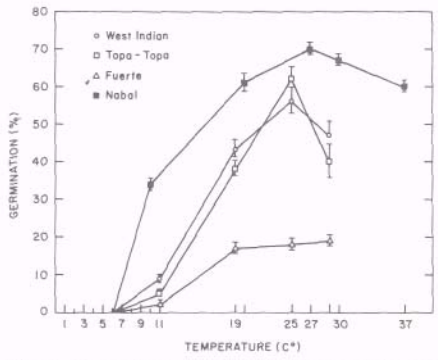

Fig. 2. Percentage of germination of avocado pollen, at different temperatures *in vitro*.

Satisfactory results were obtained for the first time germinating avocado pollen *in vitro.* Avocado pollen has a highly reduced exine (13), and the pollen grain has little protection from external conditions. Germination *in vitro* was obtained only when the delay between collection of fresh flowers and placing them in liquid with sucrose and minerals before germination did not exceed one hr. With a 4 hr lapse, no germination occurred. Previously, germination has been reported on the style only (8, 12, 14). Prolonged pollen storage has been reported recently (14). However, stored pollen has been germinated only on the style. No indication was given whether fertilization subsequently took place.

In our experiment, percentage of germination varied between 14% and 44%, according to cultivar. Increased rates of germination were achieved at temperatures up to 29°C, and in one case ('Nabal') up to 37°. Successful germination may have been due to certain procedures in the method employed, especially the placing of the avocado pollen grains, which are most sensitive to desiccation, in solution and applying a few drops of the solution containing the pollen on top of the agar. So far, correlation of the

percentage of pollen grains germinating *in vitro* and the percentage of pollen grains stained has not been satisfactory. Unsatisfactory correlation between the percentage of pollen grains stained and germination on an artificial medium has been reported with other species (6, 9, 11, 15). There was some indication in our work that the addition of calcium and, possibly, of boron to sucrose increases the number of germinated pollen tubes. Brewbaker and Kwack (2) reported on the beneficial effect of Ca on pollen germination.

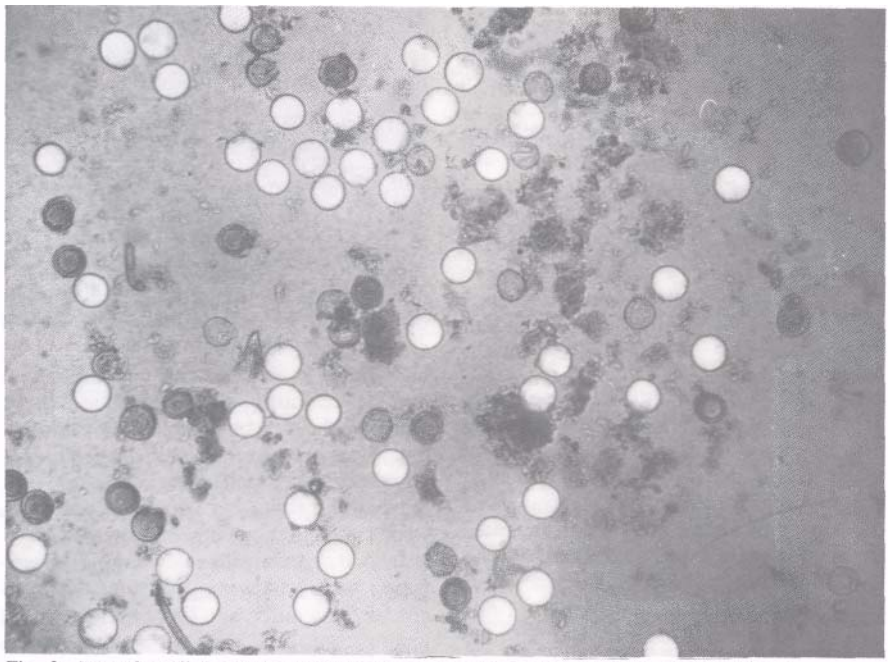

Fig. 3. Avocado pollen grains stained by fluorescein diacetate (light colored grains) X235.

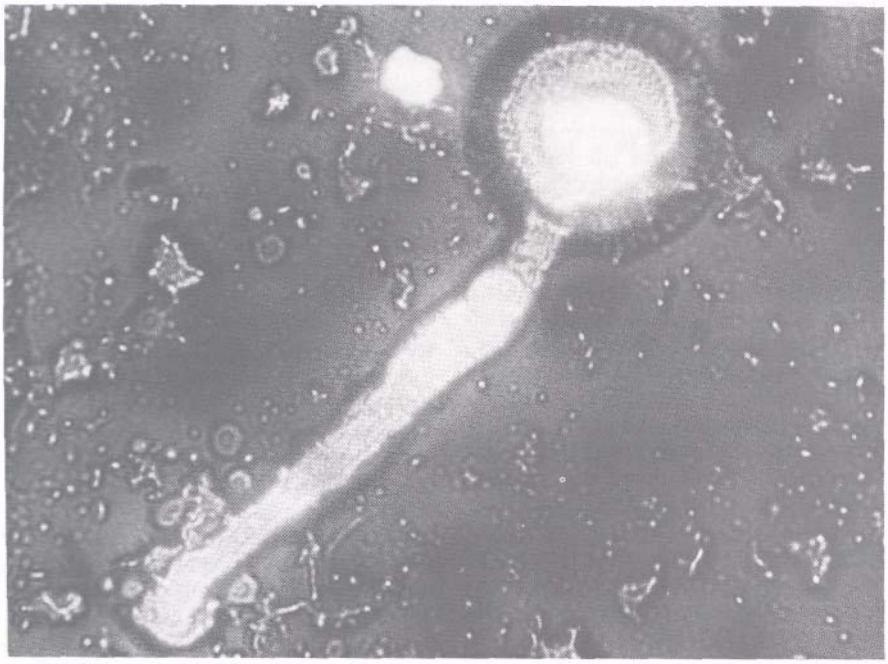

Fig. 4. Germinating pollen tube of avocado, after staining with fluorescein diacetate X750.

It is hoped that this method developed for germination of avocado *in vitro* will be

perfected further. In conjunction with the storage of pollen, this procedure will be helpful in improving breeding techniques for the avocado, by allowing controlled pollinations between seedlings or cultivars that do not flower at the same period. The method also may be of potential use in studies aimed at determining pollinizer requirements and efficacy.

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