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Notes on the Growing of Seedlings for Physiological Experimentation.

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For some years past we have carried on rather extensive experimental work[1] with seedlings of the canteloup *(Cucumis melo, var.)*. Much effort has been devoted to the working out of a physically accurate, and at the same time biologically optimal technique. Considerable progress has been made towards the achievement of these desiderata, and it is the purpose of the present paper to report upon the main features of the standard basic technique which we are now using in our seedling work.

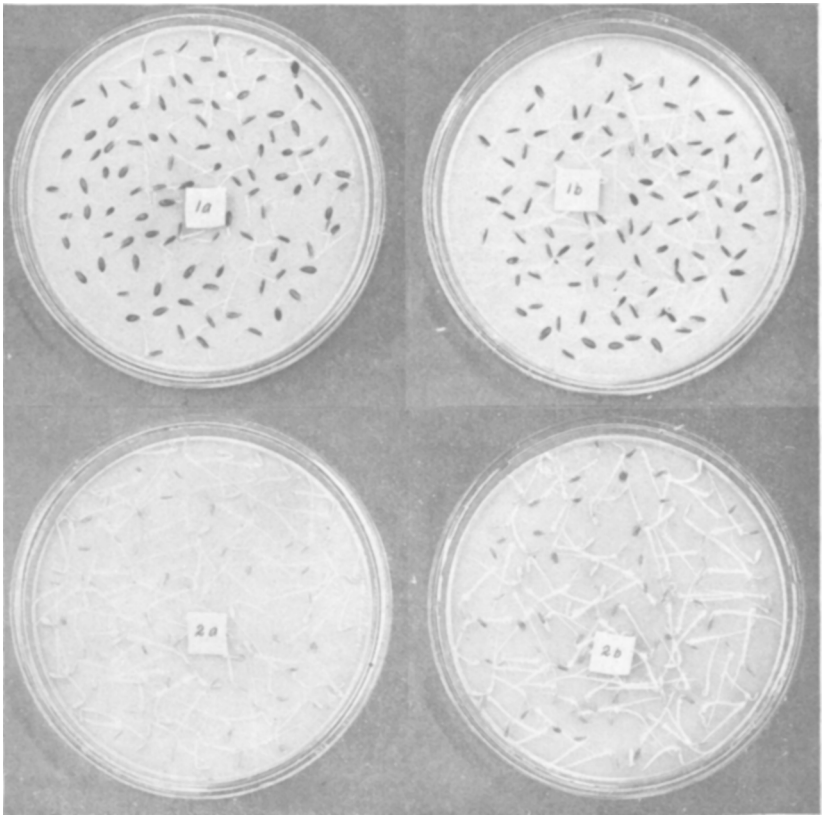
Most of our experiments are done under conditions of total darkness, with consequent complete etiolation of the seedlings. In the earlier stages of the work the seeds were germinated in the usual way between layers of moistened filter paper, cotton batting, or cloth, after preliminary soaking in water or some solution. They were then grown in water cultures in accordance with standard plant physiological technique.

All this procedure has now been abandoned in favor of a much better one. The seeds are germinated and grown upon a substratum of agar-agar. The best grade of bacteriological agar is bought at wholesale in bales of 100 or 200 lbs. in order to have a uniform supply over a long period of time. With the bale at present in use in this laboratory a concentration of 1.5 per cent gives the right consistency for the seedling work, making a firm, but not too firm jelly. For germination tests with this material, poured in Pyrex glass pie plates to a depth of about one inch, it is only necessary to strew the seeds to be tested on the surface of the agar jelly, and cover the dish with a glass plate to prevent evaporation. The 1.5 per cent agar is made with distilled water, and sterilized in the autoclave. With any given sample of commercial seeds we uniformly get a higher percentage of germination on these agar plates than with any standard germination technique, and we have tried them all. The use of agar in this way in seedling experimentation is not original with us. It has been employed by various persons, notably by Terroine,[2] who has used the method extensively in his studies on plant nutrition.

The most important advance which has been made in seedling

technique in this laboratory has to do with the effect of removing the outer coat or testa of the seed before germination. This can be done mechanically, splitting the testa along its edges by compression in the plane of the margins of the seed. If carefully done, the embryo can be removed uninjured. The tegmen is usually not removed. From this point on the procedure is exactly as though one were dealing with the intact, entire seed. The shelled seed is soaked in water, sown on the agar plate for germination and growth, and in every way handled as an entire seed or seedling would be. For sterile culture work we have followed with about equal success either of two methods. The whole seed may be sterilized by immersion for 5 minutes in 1:1000 bichloride of mercury

FIG. 1.



Photographs of seedlings after 48 hours growth. 1a and b: Normal intact control seeds; 2a and b: Seeds from which testae had been removed from the start.

solution, and the testa then removed as an aseptic surgical procedure. This technique is based on the assumption that in the intact seed the embryo is sterile. Or the testa may be removed and the naked shelled embryo soaked for one minute in 1:1000 bichloride, and then rinsed off in sterile distilled water and sown on the sterile agar plate.

The advantages of removing the testa from the dry seed before starting any experimental procedure are: (1) higher percentage of germination, amounting almost invariably to 100 per cent of all fertilized seeds, (2) prompter germination, (3) much more rapid and vigorous growth of the seedling per unit of time. These facts are illustrated by the following typical experiment.

From a stock lot 400 seeds of *Cucumis melo* were taken, and divided in two groups (1 and 2) of 200 seeds each. The weights of the dry seeds in the two groups were as follows.

Lot 1 = 5.0473 grams.
Lot 2 = 5.0440 grams.

From each seed in Lot 2 the testa was removed in the way described above. Each lot was then divided into 5 batches of 40 seeds each, and each such batch was soaked for exactly 3 hours in 100 cc. of sterile distilled water. They were then removed, lightly blotted with filter paper, and sown on agar plates, 100 seeds to a plate, and put in a dark incubator operating at 30° C.

At the end of exactly 48 hours the plates presented the appearance shown in Fig. 1.

It is evident from the photographs that the shelled seeds had grown much better than the controls. After the photographs were made each seedling was carefully removed from the agar plate, and with a sharp scalpel the cotyledons were cut off from the sprout in each case. The fresh weights of the cotyledons and of the sprouts (stem + root) were then immediately determined. The material was then put in an oven operating at 98° C. and dried to constant weight. The results are shown in Table I.

From this table it appears that the 48-hour growth of the shelled seeds (stems + roots) was nearly 153 per cent in excess in fresh weight, and 87 per cent in excess in dry weight, over that of the normal control seeds.

This excess growth of the shelled seeds is probably mainly due to more rapid and greater water absorption by the cotyledons and to better oxygen supply. The testa is a protective envelope for the embryo, but apparently the plant pays a rather high physiological price for the protection, chiefly in the form of reduced permeability

TABLE I.

Growth of seedlings—Cucumis melo.

Seeds.	No. of germinated seeds	Per cent germination	Fresh weights. Grams.		Dry weights. Grams.	
			Cotyledons	(Stem + roots)	Cotyledons	(Stem + roots)
Lot 1. (Intact controls)	191	95.5	4.6234	2.4543	2.6040	0.2330
Lot 2. (Testa removed)	200	100	5.9591	6.2010	2.6166	0.4352
Excess of Lot 2 over Lot 1 Absolute	+9	—	+1.3357	+3.7467	+.0126	+0.2022
Excess of Lot 2 over Lot 1 Per cent	+4.7	—	+28.9	+152.7	+0.48	+86.8

even to so physiologically desirable a substance as water. Table I shows that whereas the dry weight of the cotyledons was practically identical in the two series, the cotyledons of the seeds in Group 2 absorbed nearly 29 per cent more water during the experiment than was able to get through the seed coats in Lot 1.

The greater immediate water absorbing power of the shelled

seeds is demonstrated by the following typical experiment: From a stock of seeds of *Cucumis melo* two samples (A and B) of 100 seeds each were drawn. Lot A was the control. From each seed in Lot B the testa was removed. After weighing each lot was soaked for exactly 3 hours in 100 cc. of distilled water at room temperature. The seeds were then removed, lightly blotted with filter paper, and immediately weighed. The results are shown in Table II.

From Table II it is seen that the seeds in the B series absorb a higher percentage of water, in proportion to their initial dry weight.

TABLE II.
Water absorption by seeds. Cucumis melo.
(All weights in grams)

Seeds	Initial wgt. of dry seeds	Weight when put in H_2O	Weight after soaking 3 hrs.	Weight of water absorbed	Per cent wh. water absorbed is of weight when put in H_2O	Weight of cotyledons after soaking 3 hrs.
Lot A. (Intact control)	2.4920	2.4920	3.1597	0.6677	26.8	1.8064
Lot B. (Testa removed)	2.4918	1.5880	2.1068	0.5188	32.7	2.1068

If we suppose, as seems entirely justifiable, that the initial dry weight of the *cotyledons* alone was approximately the same in the two series, it then follows from the last column in Table II that at the end of the period of soaking the cotyledons of the intact seeds of Lot A had absorbed 0.2184 gr. of water, or 13.75 per cent of their assumed initial weight, while the shelled cotyledons of Lot B had absorbed 0.5188 gr., or 32.7 per cent of their initial weight. This is a complete paper.

[1] Pearl, R., and Allen, A. L., *J. Gen. Physiol.*, 1926, viii, 215-231; Pearl, R., *Am. Nat.*, in press.

[2] Terreine, E. F., Trautmann, S., Bonnet, R., and Jacquot, R., *Bull. Soc. Chim-Biol. T.*, 1925, vii, 461-473.

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Ocular Manifestations in Anaphylaxis.*

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In our experiments with guinea pigs, in which anaphylactic symptoms were induced either by subjecting sensitized animals to a dust-laden atmosphere or by parenteral injection, we have noted that there also occurs, in some cases, a suffusion of one or both eyes.

This suffusion at first is thin and watery, rapidly becoming thick and creamy, and in some cases eventuates in the eyelids becoming matted together. This suffusion lasts for half an hour and sometimes longer.

These symptoms were manifested more frequently in sensitized animals exposed to dust than in those animals injected parenterally. These manifestations were not observed in animals which died rapidly in anaphylactic shock, accounting for the greater percentage of positive results in those exposed to the dust.

Out of a total of 523 positive anaphylactic experiments, 53, or 10.1 per cent, showed these ocular symptoms. Symptoms were noted in 15.3 per cent of the dust inhalation experiments, 8.3 per cent of the experiments with intravenous injection, and 4.5 per cent of the experiments with intraperitoneal injection. (See table.)

* This work is being carried on under "The Crane Fund for the Study of Anaphylaxis."