INVESTIGATIONS OF GERMINATION AND BENCHING IN SWEET ORANGE SEED

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Abstract. In an attempt to improve seed germination and reduce benching in sweet orange and citrange seedlings, seeds were peeled, abraded, or treated with acid, gibberellin, or cellulytic enzymes. Peeled or acid-treated seeds germinated rapidly. Only peeled seeds grown with bottom-heat and mist-watering showed reduced benching. Thus the testa affects germination, but additional factors contribute to benching.

INTRODUCTION

There are two aspects of plant growth which cause concern in the commercial production of citrus seedlings. First, the amount and rate of seed germination and, second, the relative straightness of the stem.

Germination performance has many obvious and important effects on the management of the citrus nursery. Good seed quality (of high specific gravity and stored under favourable conditions) and freedom from disease are necessary prerequisites. Furthermore, germination and early seedling growth are sensitive to temperature and water conditions; the large effects of temperature and the value of bottom-heat were stressed by Kumar (5). Also, the value of minimal water stress is well demonstrated by the success of misting techniques. Despite the application of this knowledge, the germination of sweet orange (*Citrus sinensis*) seeds and those of *C. sinensis* × *Poncirus trifoliata* hybrids remains unreliable.

The second aspect, stem straightness, relates to the phenomenon called "benching" whereby a proportion of seedlings develop with bends, twists, and loops in the stem near the seed (3,6,7). These defects are not serious when the seedlings are grown in a field nursery for a year or more before budding since, at lifting, only looped seedlings have to be discarded. But when seedlings are to be budded at a young age, in a nursery system based on rapid turnover of container-grown plants, benching may lead to a large degree of culling at the first transplanting stage.

It is possible that variable germination and benching are both associated with the properties of the testas (seed coats) of seed derived from C. *sinensis*. The effects of the testas could by physical (due to impeded gas or water exchange, or to mechanical interference with elongation of the radicle and stem) or chemical (in particular due to water-soluble growth inhibitors present in the testa). On the assumption that treatments which weaken the testa should lessen these problems, a variety of acid, abrasion, and enzyme treatments were tested in this study.

MATERIALS AND METHODS

Four randomised block experiments were carried out: two were commenced in water (Exp. 1 on July 20, Exp. 2 on August 20) and two in summer (Exps. 3 and 4 on December 11). The first two experiments were conducted at Renmark, South Australia using 'Troyer' citrange seed. After treatment the seed was planted uniformly in flats at 25 mm spacings using plots of 42 (Exp. 1) or 28 (Exp. 2) seeds. These were held in a polythene-sheet house under mist with bottom-heat.

Exps. 3 and 4 were performed at the Waite Agricultural Research Institute using sweet orange seed planted in pots, one plot of 36 seeds (25 mm spacing) per pot. These were held in a glasshouse with evaporative cooling, and watered daily. For all experiments three replicates were used. Both seed lots were 100% reactive to tetrazolium (8), indicating that the seeds were viable.

As controls, untreated seeds were planted dry (control-dry) or presoaked in water for 1 day (control-presoaked). In Experiments 1, 3 and 4 one treatment consisted of peeled seeds; the testas were removed by hand after soaking for 2 hours in water.

Acid digestion of the testa was tested preliminarily in Exp. 1 and extensively in Exps. 2 and 3. Seeds were immersed in 50% sulfuric acid for the times specified (¼ to 4 hours), then rinsed extensively in water before planting. In Experiment 1 a second acid treatment was used consisting of a 4 hour dip in 25% hydrochloric acid plus 0.25M zinc chloride.

Abrasion of the testa was tested extensively in Experiments 1, 2 and 3 using a variety of abrasion methods. In Exp. 1 the dry seed was tumbled at 50 rpm in a 2 l. lidded glass jar lined with carborundum paper on horizontal rollers for 12 hours. In Exp. 2 a tougher paper was used (cc-280-cw silicon carbide wet-and-dry sandpaper) lining a closed cylindrical tin and run for longer times (48, 72 and 96 hours). In Exp. 3 wet seed was tumbled for varying periods to achieve a specified percentage of abraded testas, sufficient to reveal the cotyledons; in the most severe treatment seeds were rotated to the 90% abrasion stage, then for an additional 12 hours. Dry seed abraded to 50% was also included.

As used by Burns and Coggins (2), gibberellin was applied by soaking the seed in aqueous GA_3, 1000 ppm in Exp. 1 and 100 ppm in Exp. 2. This treatment was combined factorially with other treatments in Exp. 2. After the acid or abrasion treatments the seed lots were halved and soaked in either water or GA_3 solution.

Enzymatic digestion was attempted in Exps. 1 and 4, using cellulase (Onozuka R-10) and pectinase (ex *Aspergillus niger)* both at 2% w/v in 50 mm citrate buffer pH 4.5 at 30°C. The digestion was continued for 24 hours (Exp. 1) or for a variety of times (Exp. 4).

At intervals after planting emerged seedlings were counted. After about 4 months the seedlings were uprooted and classified into six categories of severity of benching as illustrated in Figure 1; grades 1 to 3 are regarded as acceptable for propagation. The tabulated "per cent benching" is the sum of grades 4, 5 and 6 per 1000 seedlings assessed. Many seeds produced multiple seedlings and the majority of seedlings were nucellar; the incidence of benching appeared similar in nucellar and zygotic seedlings so no record was kept of the separate populations. The data was analysed by Anova, and where the Variance Ratio was significant at $p = 0.05$, an LSD was calculated.

RESULTS AND DISCUSSION

The results of the four experiments are presented in Tables 1 to 4 and Figure 2. Comparisons may be made between Exps. 1 and 2, and between 3 and 4, as similar conditions and seed were used in each pair. In all experiments, the control seeds, with or without presoaking, germinated slowly but eventually produced more than one seedling per seed planted; about half of these seedlings were benched and judged unacceptable for budding.

Peeling. Hand peeling of citrange seeds produced very rapid germination and a low percentage of benching, yielding a high number of acceptable seedlings (Table 1). The germination of peeled sweet orange seed was also rapid but there was no increase in the final number nor any reduction in the percentage benched (Tables 3 and 4). It is now apparent that each species will require a separate program and that this information can not be extrapolated across species.

Table 1. Effects of several treatments on the germination and development of Troyer citrange seed (Exp. 1).

Treatment	Number of seedlings emerged (per 100 seeds planted) on: Day 25	Day 70	Day 120	Number benched per 100 seedlings	Final number of acceptable seedlings (per 100 seeds planted)
Control-dry	1	62	128	37	79
Control-presoaked	5	43	127	36	83
Peeled	160	160	160	11	143
H_2SO_4 4h	1	3	6	11	5
HCl + $ZnCl_2$ 4h	3	56	85	38	52
Abraded	8	74	129	35	84
GA_3 1000 ppm	8	69	126	36	81
Pectinase + cellulase	8	79	99	48	57
LDS p = 0.05 (excluding peeled)	n.s.	6.8	13.4	n.s.	10.5

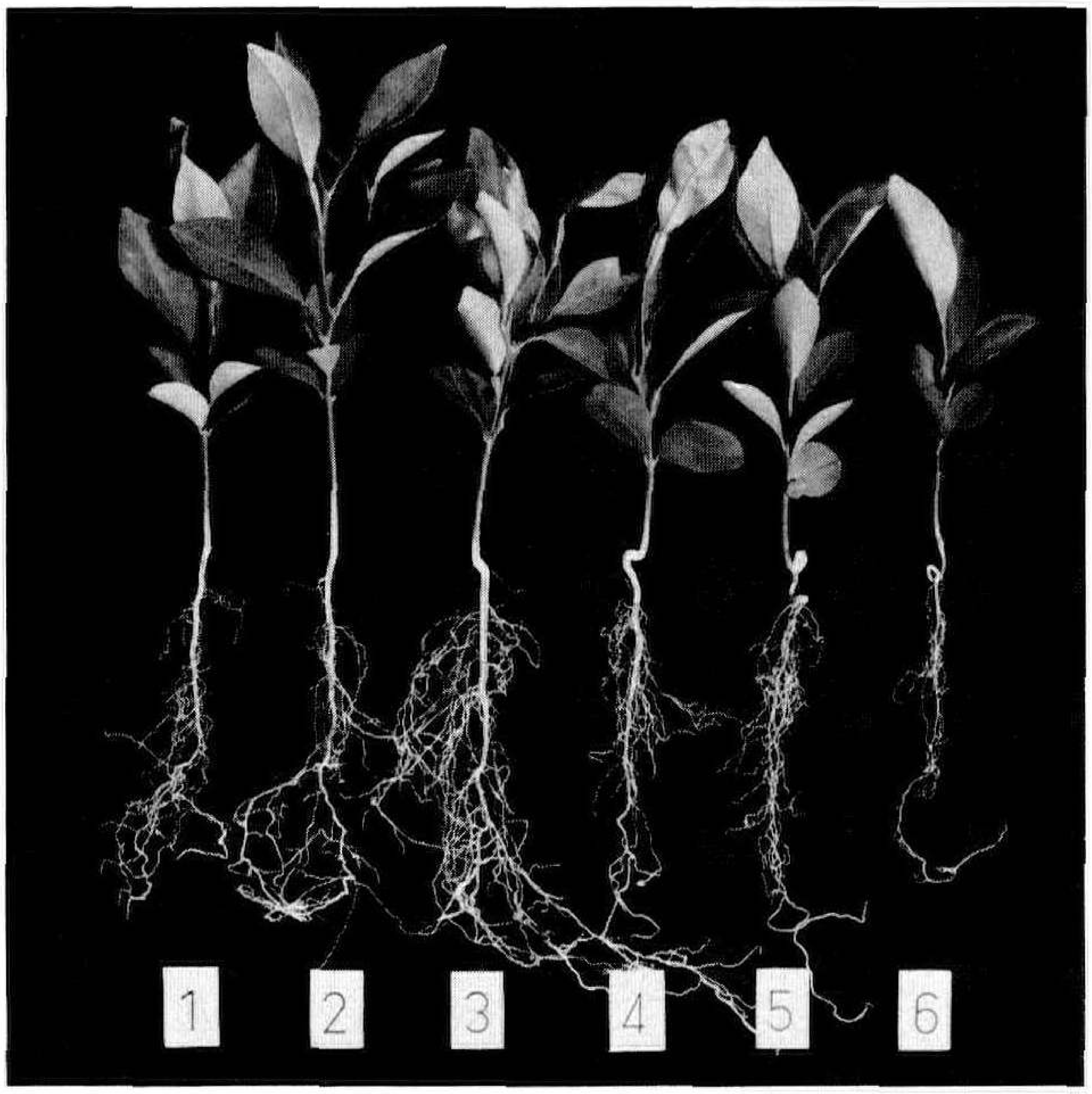

Figure 1. Sweet orange seedlings (Exps. 3 and 4) showing the six grades of severity of benching used for classification. Grades 1 to 3 are acceptable for propagation.

Acid Treatment. Clearly, 4 hours in 50% sulfuric acid (the treatment used routinely in our laboratory for dehusking barley) severely damaged citrus seed (Tables 1 and 2). Four hours in 25% hydrochloric with zinc chloride was less harmful.

Shortening the time of acid treatment caused less damage (Tables 2 and 3): one hour in 50% sulfuric acid resulted in significantly faster seed germination than in the control, though with no increase in the number of acceptable seedlings (Table 3 and Fig. 2); treatment for less than an hour had little effect.

Table 2.- Effects of acid and scarification on germination and development of Troyer citrange seed (Exp.2)

Treatment		Number of seedlings emerged (per 100 seeds planted) on: Day 25	Day 120	Number benched per 100 seedlings	Final number of acceptable seedlings (per 100 seeds planted)
Control-presoaked		2	102	54	51
H_2SO_4	2h	15	32	37	20
	3h	5	19	37	12
	4h	1	2	*	*
H_2SO_4, then GA_3 100 ppm	2h	32	58	65	20
	3h	18	19	47	8
	4h	5	6	17	5
Abraded	48h	13	142	40	85
	72h	10	114	24	87
	96h	62	131	34	86
Abraded, then GA_3 100 ppm	48h	18	137	39	85
	72h	11	102	54	48
	96h	73	108	59	46
LSD $p = 0.05$		16.4	10.5	32.8	9.9

* 100% of the seedlings were benched but the population was small and therefore atypical

Abrasion. In some instances seed abrasion hastened seedling emergence. The dry abrasion used in Exp. 2 (Table 2) resulted in an increased number of acceptable seedlings. However, the treatments used in Exp. 3 (Table 3) caused no increase. Abrasion did not significantly affect benching.

Gibberellin. GA_3 at 1000 ppm had no effect by itself but, applied to abraded seed (Table 2), GA_3 at 100 ppm negated the improved emergence and acceptability deriving from abrasion. GA_3-treated seedlings were soft and spindly and some were chlorotic. The significant decline of benching recorded in the treatment with GA_3 + sulfuric acid for 4 hr (Table 2) is discounted because of the paucity of emerged seedlings.

Pectinase and Cellulase. A pectolytic enzyme has been used to recover seed from a seed-pulp mixture (1) so it was of interest to test the effect of enzymes under these conditions. Pectinase caused a marginal and temporary increase in the rate of seed germination but enzymes had no other recorded effect (Table 4). In Exp. 1 the initial incubation softened the citrange seed testas but no modification of sweet orange seed was noted in Exps. 3 and 4. This highlights a general problem in exploiting enzymes: they may tolerate very little variation in conditions and material.

Table 3. Effects of acid and scarification on germination and development of sweet orange seed (Exp. 3).

Treatment		Number of seedlings emerged (per 100 seeds planted) on: Day 34	Day 51	Day 110	Number benched per 100 seedlings	Final number of acceptable seedlings (per 100 seeds planted)
Control-dry		5	46	152	77	34
Control-presoaked		4	46	153	66	52
Peeled		76	91	138	77	33
H_2SO_4	0.25 h	8	58	153	66	53
	0.50 h	7	68	145	67	45
	1.00 h	19	75	147	61	57
	2.00 h	7	63	117	73	31
Wet abrasion	5%	4	48	151	61	58
	50%	7	67	115	67	35
	90%	13	66	122	69	38
	>90%	14	43	69	56	30
Dry abrasion	50%	32	68	139	65	49
LSD p = 0.05		2.7	4.3	9.4	n.s.	n.s.

Table 4. Effects of enzyme digestion on the germination and development of sweet orange seed (Exp. 4).

Treatment		Number of seedlings emerged (per 100 seeds planted) on: Day 34	Day 51	Day 110	Number benched per 100 seedlings	Final number of acceptable seedlings (per 100 seeds planted)
Control-presoaked		5	44	142	50	71
Peeled		85	94	151	60	60
Cellulase & Pectinase						
	18 h	2	44	143	49	73
	24 h	7	40	129	45	72
	30 h	2	40	144	58	59
	36 h	6	44	149	55	68
Pectinase	24 h	4	52	144	49	73
	36 h	1	54	137	45	75
Cellulase	24 h	1	44	140	45	77
	36 h	7	42	134	45	73
LSD p = 0.05		0.7	5.6	n.s.	n.s.	n.s.

Figure 2. Plots of sweet orange seedlings from Exps. 3 and 4. The faster development of treated seeds relative to the presoaked controls (Water, 24 h.) is apparent, especially that from peeled seeds.

CONCLUSIONS

The treatment given had a variety of effects on seed germination and seedling growth of sweet orange and citrange, some detrimental and some helpful. Abrasion of the testa and treatment with enzymes or gibberellin gave variable results and did not increase the number of acceptable seedlings. Treatment with sulfuric acid gave a significantly faster germination though only when applied for a period of intermediate length; 1 hr was better than shorter or longer times.

At its best, acid treatment did not approach the high germination rate found with peeled seed. The beneficial effect of peeling was accompanied by a lowered percentage of benching in the case of Exp. 1 with Troyer citrange seed germinated with bottom-heat and under mist. Peeling caused no reduction in the percentage of benching in Exps. 3 and 4 despite its large significant effects in hastening germination.

It would appear, therefore, that benching is associated with more than the testa effect. Despite the lessened benching found in Exp. 1, compared with Exps. 3 and 4, it is not possible to say whether the lack of benching was associated with one or more of the following factors: seed type (sweet orange versus citrange), bottom-heat, misting, or the more rapid growth rate obtained in this experiment.

It is possible that initial growth rate is an important determinant of the likelihood of benching. Correction of a bend in the stem near the seed, caused initially by the chance aposition of that seed, is a geotropic response. Firn and Digby (4) consider that the tissues which perceive a geotropic signal are the outer layers of the stem and, further, that these are the tissues which effect the response. In any case the amount of the response varies with the growth rate of these tissues. It might be profitable therefore to compare early growth of sweet orange seedlings under temperature and water conditions that would influence stem growth rate.

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