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Effects of seed coloration, hydrochloric acid concentration and exposure time during seed extraction, on germination of tomato seed

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Summary

Seed was extracted from fruits of four cultivars of tomato (Strobelee, Q2, Floradel and Walter) by acid digestion. The effects on seed germination of digestion in 1, 2, 4, 8, and 16% HCl for 180 min and of 5% HCl for 22.5, 45, 90, 180 and 300 min were assessed. In addition, seed of the cv. Walter was exposed to the range of acid concentrations for 180 min to determine the effect of red brown coloration on treatment response.

Germination of seed of cv. Walter (mean 76.2%) was always less than Strobelee (98.4%), Q2 (96.2%) or Floradel (95.8%). As acid concentration increased from 1 to 16% w/v seed germinations decreased from 88.5 to 62.5%, 100 to 95%, 97.5 to 92.5%, 98.5 to 89.5% for cvv. Walter, Strobelee, Q2 and Floradel respectively.

As duration of acid digestion increased, there was no decrease in germination of Q2, Floradel or Strobelee, however cv. Walter decreased from 87.5 to 74.5% as duration increased from 45 to 90 min.

The cvv. Strobelee, Q2 and Floradel had less than 23% of seed showing red brown colour whereas 64% of cv. Walter seed showed colour.

Within seed of cv. Walter for any digesting acid concentration, the percentage germination decreased with increasing intensity of seed coloration.

1. Introduction

Germination of cv. Walter tomato seed produced under the seed certification scheme in Queensland was often less than the 80% prescribed in the seed certification rules. A 3-hour acid digestion using 3% v/v commercial concentrated hydrochloric acid was the prescribed extraction method.

Tomato seed germination can be influenced by an interaction of genotype and hydrochloric acid concentration. Richie (1971) found the germination of cv. Minibinger was reduced from 98% using 0.25% v/v HCl to 2% using 5% v/v

HCl applied for 24 hours, whereas germination in cv. Harbinger, which is isogenic with cv. Minibinger except at the Baby Lea Syndrome (bls) locus, reduced from 100 to 92% over the same range. Philouze (1974) showed the seed germination of some wholly brown seeded lines (genetically homozygous for bs or bs–2) was low (52 and 11% respectively) whereas germination of the parental line, not carrying the bs or bs2 gene, was good (89% for Heinz 1370). Further, germination of cv. Pieralbo (bs–2) was 0, 7 and 39% and cv. Sioux (bs) was 7, 48 and 0% when extracted with hydrochloric acid, fermentation or acetic acid respectively. The date of harvest and method of extraction also interacted and background genotype altered the effect of bs and bs–2 on colour intensity and seed germination.

Preliminary observations indicated that many seeds of the cv. Walter showed reddish brown coloured areas on the seed coat. These coloured areas have not been reported previously and in the remainder of the article I shall refer to them as RBC. Coloration was present before extraction and was restricted to the testa and the outside of the cuticle; the proportion of testa affected on seed of cv. Walter varied from zero to more than half, and even varied, apparently randomly, on seed within the same locule. The RBC condition was apparently distinct from the brown pigmentation described by Philouze (1974), which uniformly covered the seed surface.

A possible relationship between germination and the occurrence of RBC was investigated with a tomato cultivar (Walter). In the course of this study, the effects of acid concentration and duration of acid treatment were investigated in three other cultivars Floradel, Q2 and Strobelee.

2. Materials and methods

Tomato fruit of the cvv. Strobelee, Q2, Floradel and Walter were obtained from vines grown at Ormiston, Queensland. At extraction, fruit maturity ranged from pink to full ripe.

A sample of seed was extracted from each cultivar by hand pulping and washing through a 1.5 mm nylon gauze. An amount of the resultant mixture was suspended in hydrochloric acid solutions at the rate of 40 mL per 10 g of mixture. After treatment, the suspension was shaken vigorously for 15 s, rinsed twice, washed onto nylon gauze, dried for 3 days at 25°C and stored in plastic jars at room temperature.

A sample of seed from each cultivar was separated according to RBC characteristics by visually estimating the proportion of seed surface affected with RBC. The proportions used in grouping were 0,>0 to 6.2, 6.3 to 12.4, 12.5 to 24, 25 to 49, 50 to 100%.

To test for cultivar differences in the proportion of seeds in RBC classes, Chi square and analyses of variances were conducted. Chi square values were calculated on the RBC distributions of four cultivars ($\chi^{2}{}_{(9)}$), three cultivars (Strobelee, Q2, Floradel) ($\chi^{2}{}_{(6)}$) and Walter versus the other three combined ($\chi^{2}{}_{(3)}$). RBC classes 12.5 to 24, 25 to 49, 50 to 100% were pooled as 12.5 to 100% for Chi square tests. The analysis of variance within each RBC class used untransformed percentages, as well as the arc sine transformation.

3. Results

Less than 23% of the seeds of cvv. Strobelee, Q2 and Floradel exhibited any red brown coloration. In contrast, seed of cv. Walter was heavily coloured and only 35.3% of its seed were free of the disorder. Of the seed of cv. Walter, some 47% were slightly coloured (0 to 6.2% of testa) and 17.7% were affected to the extent of 6.3 to 100% of the testa surface (table 1).

Table 1. The percentage of tomato seeds in classes according to cultivar and the extent of RBC condition

Cultivar	Percent seeds in RBC class						
	N*	0	0–6.2	6.3–12.4	12.5–24	25–49	50–100
Walter ..	4	35.3	47.0	7.3	4.3	4.1	2.0
Strobelee ..	2	86.9	9.3	2.7	0.8	0.4	0.0
Q2	2	86.2	12.0	1.0	0.7	0.0	0.0
Floradel ..	2	77.8	19.2	3.0	0.0	0.0	0.0
	..	**	ns	ns	*	ns	ns†
LSD 5% ..	2,4	29.6	38.6	7.9	3.3	4.9	2.07
LSD 1% ..	2,4	44.9	58.6	12.0	4.9	7.4	3.0
SEM	2	9.88	12.90	2.64	1.09	1.6	0.7
	4	6.99	9.12	1.87	0.77	1.1	0.5

† Arc sine transformation gives * in class 50–100, other responses are as above.
* N = Number of replications.

The Chi square values below, and analysis of variance (table 1) indicate the distribution of RBC in cv. Walter is different from other cultivars and that Strobelee, Q2 and Floradel may be similar.

χ^2 $^{(9)}$ = 205.6 ** 4 cultivars compared
χ^2 $^{(6)}$ = 13.97 * 3 cultivars (Strobelee, Q2, Floradel) compared
χ^2 $^{(3)}$ = 197.5 ** cv. Walter compared to Strobelee, Q2 and Floradel combined

The initial germination percentages of seed of cvv. Strobelee, Q2 and Floradel were at least 97% whereas that of cv. Walter was 89%.

As the acid concentration increased from 1 to 16% v/v (table 2), the seed germinations decreased from 100 to 95%, 97.5 to 92.5% and 98.5 to 89.5% for cultivars Strobelee, Q2 and Floradel respectively. In contrast, the germination of seed of cv. Walter decreased from 88.5 to 62.5% over the same concentration range, with rapid decreases from 88.5 to 74% and from 78 to 62.5% as concentrations increased from 1 to 2% v/v and from 8 to 16% v/v respectively. This trend is apparent even though the F value for interaction was not significant ($P = 0.056$).

Table 2. The effect of concentration of 10.16 N hydrochloric acid applied for 180 min. during seed extraction on germination percentage in four tomato cultivars

Cultivar	Acid concentration (% v/v, 10.16 N HCl)					Mean
	1.0	2.0	4.0	8.0	16.0	
Strobelee	100.0	98.0	99.5	99.5	95.0	98.4
Q2	97.5	98.0	96.5	96.5	92.5	96.2
Floradel	98.5	98.0	96.5	96.5	89.5	95.8
Walter	88.5	74.0	78.0	78.0	62.5	76.2
Mean	96.1	92.0	92.6	92.6	84.9	91.6

L.S.D. Cultivar	5%=3.3	1%=4.5	SE=1.11**
Concentration	5%=3.7	1%=5.0	SE=1.24**
Interaction	5%=7.4	1%=10.1	SE=2.49 ns

As the duration of acid treatment increased (table 3) there was no significant decrease in germination percentage of 98%, 97.5% and 97% for seed of cvv. Q2, Floradel, or Strobelee respectively. In contrast seed germination of cv. Walter decreased rapidly from 87.5 to 74.5% as duration of treatment increased from 45 to 90 min with no further reduction in germination up to 300 min.

Table 3. The effect of the duration of hydrochloric acid (5% v/v, 10.16 N HCl) extraction on seed germination

Cultivar	Duration of acid extraction (min.)					Mean
	22.5	45	90	180	300	
Q2	98.0	96.5	96.0	96.0	93.0	95.9
Floradel	97.5	95.5	98.0	97.5	96.5	97.0
Strobelee	97.0	99.5	98.0	97.5	96.5	97.7
Walter	89.0	87.5	74.0	75.0	72.5	79.6
Mean	95.4	94.8	91.5	91.5	89.6	92.5

L.S.D. Cultivar	5%=1.7	1%=2.3	SE=0.58**
Duration	5%=1.9	1%=2.6	SE=0.65**
Interaction	5%=3.8	1%=5.2	SE=1.29**

Both the amount of red brown colour and acid concentration had an effect on seed germination in cv. Walter with an interaction between the two factors (table 4). As RBC increased from 0 to >0 to 6.2, to 6.3 to 12.4, to 12.5 to 24, to 25 to 49% of testa surface, mean germination decreased from 92.3 to 72.9, to 27.8 to 16.3, to 0% and maximum germination decreased from 95.5 to 89.4, to 66.1, to 39.2, to 0%, respectively. Where no red brown colour

was present, germination percentage was reduced appreciably although not significantly ($P < 0.05$) from 95.5, to 95.6, to 86.6% as acid concentration rose from 1, to 4, to 16% v/v, respectively. The overall mean germination reduced from 72.6 to 56.6 to 35.3% over the same range. Where slight red brown colour was present (0 to 6.2% of testa surface) seed germination was reduced from 89.4 to 72, to 54.6% as acid concentrations increased from 1, to 4, to 16% v/v, respectively. Where RBC was 6.3 to 12.4% of testa surface, germinations decreased more rapidly. No germination occurred with seed having RBC 25 to 100% of testa surface.

4. Discussion

My work suggests differences in susceptibility of cultivars Walter, Q2, Strobelee and Floradel to extraction with hydrochloric acid. Both duration of acid treatment up to 300 min and concentration of acid dip up to 16% v/v have an effect. My results support the work of Ritchie (1971) and Philouze (1974) who found genotypic differences in responses to hydrochloric acid extraction, but neither the bls nor bs–2 nor bs gene implicated by the respective authors is known to occur in cv. Walter.

The high incidence of red brown coloration in seed of cv. Walter (table 1) and the relationship between coloration and acid treatment (table 4) in my experiments suggest a possible causal relationship, particularly since seed without red brown coloration are unaffected by extraction with acid between 1 and 16%.

Table 4. The effect of acid concentration and degree of red brown coloration on the seed germination percentage of cv. Walter. Treatment duration was 180 min.

Concentration %v/v, 10.16 N HCl	Degree of red brown coloration (%)*						Mean†
	0	0–6.2	6.3–12.4	12.5–24	25–49	50–100	
1	95.5	89.4	66.1	39.2	0	0	72.6
2	90.3	79.6	40.2	7.1	0	0	54.3
4	95.6	72.0	23.8	34.9	0	0	56.6
8	93.5	69.0	8.0	0.0	0	0	42.8
16	86.6	54.6	0.0	0.0	0	0	35.3
Mean† ..	92.3	72.9	27.8	16.3	0.0	0.0	51.7

L.S.D. Degree of coloration 5%=7.2 1%=10.0 SE=2.89**
Concentration 5%=8.6 1%=11.8 SE=2.83**
Interaction 5%=16.8 1%=23.2 SE=5.58**

* degree of red brown coloration is given as percent of seed surface affected.

† degree of red brown coloration classes 25–29% and 50–100% were excluded from analyses.

Since all treatments cleaned seed equally well, a suggested method of acid extraction of seed in samples with a high incidence of red brown colour, such as in cv. Walter, would be to use a low concentration 1% v/v of hydrochloric acid for less than 1 hour and as short a time as possible.

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Modern seed extraction systems retain mostly the seed and surrounding mucilage from the initial pulping of fruit, and this constitutes 80 to 85% of the suspension. If acid is added directly to this mixture, extremely high effective concentrations would initially result, for example, a 1% v/v would effectively be a 6.7% v/v solution. Thus since the suggested extraction concentration of 1% v/v is based on a suspension of seed to water of 1 to 4, the suspension should be reconstituted to these proportions before adding acid. The method has been successfully used in commercial trials.

References

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