Water loss during the development of tomato seeds

R. G. Thomas

Department of Plant Biology and Biotechnology, Massey University, Palmerston North, New Zealand.

Abstract

Previous investigators have shown an apparent decrease in water content of seeds of tomato (*Lycopersicon esculentum* Mill.) at a stage of development between 20 and 45 d after pollination (d.a.p.). This occurrence has been difficult to explain in a succulent fruit. The present study confirmed this apparent loss in cv. 'Yates Sweet 100' but demonstrated that the observation is an artefact arising from loss of water from the seed testa caused by blotting with absorbent paper before weighing. Over the period 30-40 d.a.p., while the water content of the testa fell from about 8 to about 2 mg/seed, the water content of the embryo and endosperm in each seed showed a slight (but statistically not significant) increase.

Additional key words: *embryo, endosperm, testa*

Introduction

Two fundamental questions led to the investigation reported here: firstly, "do seeds inside succulent fruits lose moisture as they mature?", and, secondly, if so, "what causes them to do so?" A survey of literature suggested that the answer to the first question was "yes", at least in tomato (McIlrath *et al.*, 1963), pumpkin and cucumber (Prokof'ev and Kholodora, 1968), but left unanswered the question of the cause of the previously reported water loss. The present study was therefore undertaken in an attempt to provide an answer to that question with reference to tomato seed.

Tomato seeds within mature fruit contain about 50-60% of moisture (McIlrath *et al.*, 1963) and are enclosed within a mucilaginous sheath of placental tissue which makes the extracted 'seeds' slippery to the touch. Removal of this placental sheath reveals that the seeds themselves are coated by a layer of fine hairs (Smith, 1935; Fahn, 1967). These hairs are not epidermal trichomes but are formed instead by the breakdown of the epidermal cells of the testa and represent fragments of their irregularly thickened walls. The testa cells grow relatively very large during seed development and their inner and radial walls become irregularly thickened by alternating depositions of cellulose and pectic laminations. The outer tangential wall and the areas of the radial walls which are not thickened remain very thin and break down at maturity (Czaja, 1963).

The study of water loss carried out by McIlrath *et al.* (1963) showed that in the cultivar 'Marglobe' the total water content fell from over 10 mg per seed in young green fruit 22 days after pollination (d.a.p) to about 3.5 mg per seed when the fruit was mature at 55 d.a.p. even though the moisture content of surrounding placental and pericarp tissue remained constant at over 95%. Additionally, these authors reported the seemingly anomalous observation that some young seeds placed in distilled water before blotting and weighing appeared to lose water by exosmosis.

It was the last mentioned observation in particular which led to the present reassessment of the mechanism of water loss.

Materials and Methods

All experiments were carried out using cherry-type tomatoes (*Lycopersicon esculentum* Mill. cv. Yates Sweet 100). Plants were grown in a heated glasshouse at Massey University in PB 18 bags of potting mix consisting of one part peat to one part sand to which were added 170 cm^3 of dolomite lime, 35 cm^3 of slow release fertiliser (Osmocote) and 25 cm^3 of superphosphate for every 1000 cm^3 of mix. Experiments were carried out in two separate series in different years and at different times of the year.

Series I experiments

Seeds were sown in February 1984 and plants grown in natural light until June. Over that time the maximum daily temperature dropped from 32°C in February to 22°C in June but the minimum night temperature was maintained at 15°C throughout. Anthesis began on 2 April. Each flower was pollinated by hand at anthesis and labelled with the date at that time. Experiments were carried out from 23 May to 8 June.

Series II experiments

Seeds were sown in November 1989 and plants grown in natural light until February 1990 with daily maxima reaching 33°C and night temperatures not falling below 15°. Anthesis began in December 1989 and individual flowers were pollinated and labelled as in the series I experiments.

Estimation of seed water content

In all experiments seeds of all ages extracted from fruit were removed from their surrounding placental sheaths under a dissection microscope, blotted gently with filter paper to remove free surface moisture, and weighed to determine fresh weights. Moisture content was then determined by subtracting dry weights (obtained after 24 h drying in an oven at 95°C) from fresh weights.

In the series II experiment the determination of the fresh weight of embryos extracted from young moist seeds posed a problem. With practice it became possible to remove embryos from seeds in about 15 seconds using a dissecting microscope. In the process, however, most embryos became broken into at least two pieces, and because the surface area: volume ratios of the embryos was high and the total volume of tissue small, the rate of moisture loss per unit volume was very high. To minimise this loss by minimising the time they were exposed to a drying atmosphere, embryos were transferred into weighed amounts of water in thin vials (internal diameter 5 mm) immediately they had been isolated.

Before the embryos were placed in them, the vials, half-filled with water and foil-capped, were kept in still laboratory air for 30 min by which time the rate of water loss from them was only about 10µg per minute.

Embryos were weighed in groups of ten within each vial, together with the weighed amount of water in each vial, immediately after the tenth had been transferred. That the lowest water content measured for 10 embryos was 31 mg shows that the error in fresh weight determination resulting from evaporative water loss from the vials was relatively very small. Dry weights of these groups of embryos were determined after evaporation of water from the vials containing them without their removal from the vials.

In younger seeds (30-40 d.a.p.) it was possible to remove the embryos relatively easily from the surrounding semi-liquid endosperm but in seeds from 45-55 d.a.p. this proved to be very difficult. In the latter case, therefore, embryos were weighed together with their surrounding endosperm and combined weights were recorded. Conversely the process of removal of embryos from endosperm in the younger seeds prevented the determination of endosperm weights in these.

Results

Figure 1 shows the relationship between seed water content and seed dry weight in seeds of different ages in the plants grown for the series I experiments. Up to the

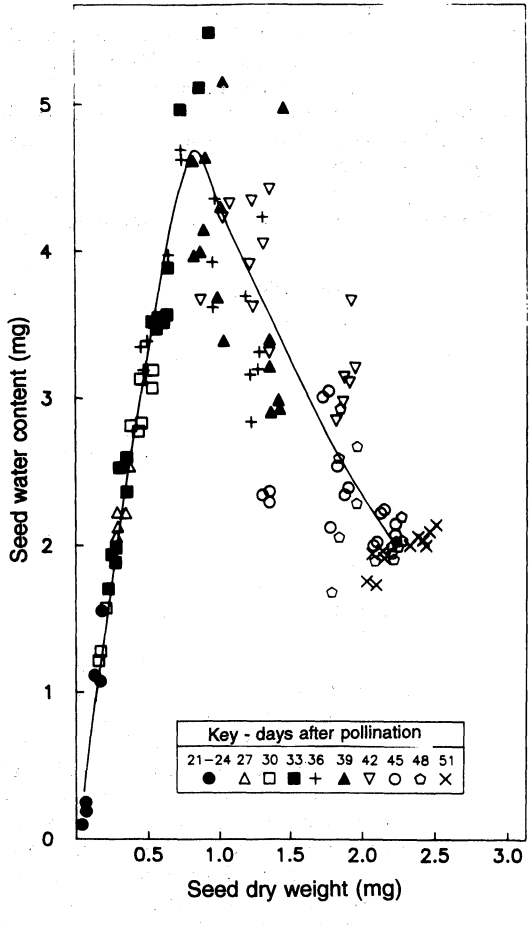

Figure 1. Relationship between seed water content and seed dry weight in seeds aged 21 to 51 d after pollination. Five fruits were sampled at each age except 21 and 24 d.a.p. Each point on the graph represents the mean of one replicate of 10 seeds.

age of 33 d.a.p. the relationship was almost linear, with seed water content increasing up to about 5 mg while dry weight increased to about 0.9 mg. After that age there was a clear decrease in water content as the dry weight continued to rise, but whereas there was extremely little variation in water content relative to dry weight up to 33 d.a.p., moisture contents at any given dry weight became very variable from 36 d.a.p. onwards.

Two sets of observations were made that gave some insight into the causes of the change that took place from 36 d.a.p. onwards. The first concerned the effect that blotting the seed surfaces to remove excess moisture had on their appearance under a dissecting microscope. It was found that seeds aged 42 d.a.p. or less looked 'normal' after blotting, with all their large testa cells appearing to be turgid; but the testa cells on blotted seeds 45 d.a.p. or older appeared to be collapsed and extended into a 'crest' at the distal end.

The second set of observations arose from a reinvestigation of the finding by McIlrath *et al.* (1963) that seeds lost water when placed in distilled water. Table 1 shows the percentage change in weight of seeds of various ages subjected to such treatment for 2 h. Seeds of 41 d.a.p. or younger clearly did lose water but the water content of seeds 45 d.a.p. or older increased markedly.

To understand this water loss better, seeds of ages 36 and 39 d.a.p. were placed in distilled water and observed under a dissecting microscope. After a few minutes, localised bursting of the relatively large epidermal cells released small clouds of cell contents into the water and neighbouring cells in the vicinity collapsed. In one 36 d.a.p seed the distal cells appeared to 'unzip' and the tear spread sideways down the flanks. Similar, but less severe, damage was found to occur in 0.2M sucrose solutions also.

These observations led to the hypothesis that the loss of seed moisture which began at 36 d.a.p. might have been caused mainly as a loss from the testa cells, in which case embryo and endosperm tissues might not lose water to the same extent. The series II experiment, the results of which are shown in Figure 2, was designed to test this hypothesis. This figure shows very clearly that while the water content of intact seeds decreased from about 9 to about 3.5 mg per seed over the period from 30 to 40 d.a.p., the water content of the embryos together with the surrounding endosperm tissue remained virtually unchanged. Over this same period, it is obvious that the loss of water from the whole seed was brought about solely by loss from the testa (calculated by subtraction of embryo and endosperm water from the whole seed water). This loss was accompanied by an apparent slight (though statistically not significant) rise in water content of embryo and endosperm tissue.

Table 1. Effect of distilled water on change of fresh weight of tomato seeds. Each figure is the mean of 6 replicates of 10 seeds.

Age (d.a.p)	36	39	41	45	48
Change in fresh weight (%)	-13.4	-6.6	-3.6	+23.7	+60.3

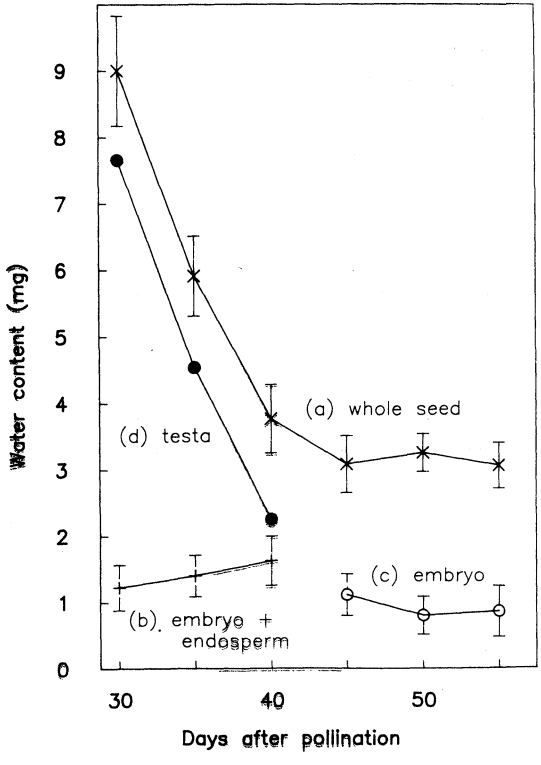

Figure 2. Change in water content in tomato seed components (a. whole seed; b. embryo + endosperm; c. embryo only; d. testa (calculated by subtraction of b. from a.)). Each point represents the mean of 10 replicates of 10 seeds. Vertical bars represent ± twice the standard error.

Discussion

The results of the present investigation confirm those obtained by McIlrath *et al.* (1963) who found that there was a marked decrease in the water content of tomato seeds from 30 to 40 days after anthesis. In the series II experiment the decline for cv. Yates Sweet 100 occurred over exactly the same developmental time period (30-40 d.a.p.) as that reported for cv. Marglobe by McIlrath *et al.* (1963). In both these investigations the experiments were performed in summer. The decline in the present series I experiments with cv. Yates Sweet 100 began later, probably because these experiments were carried out in the cooler shorter days of late autumn/winter when growth would have been slower.

These observed decreases in water content, however, must either wholly or in part have been an artefact of the blotting procedure used to remove excess surface moisture from the seeds before weighing. Clearly, by 45 d.a.p., blotting caused the collapse of the testa cells and removed water from them. It is not known whether the thin walls of these cells actually break down within the fruit, but it is certain that they become weakened to the extent that gentle pressure from blotting forces liquid out of them into absorbent paper. It is also clear from the experiments in which seeds were placed in distilled water that the difference in water potential between the cell contents and the surrounding water is sufficient to cause the bursting of the cells. Thus, even in younger seeds, the cell walls are not strong enough to resist the wall pressure brought about by this water potential difference. As there was no sign of cell wall breakage in young seeds when newly removed from fruit, it is possible that the testa epidermal cell walls are also still intact in 45 d.a.p. seeds when they are contained within the fruit. Further investigations are required to confirm this.

The most important finding of the present investigation is not so much that the apparent loss of moisture from tomato seeds during development is possibly caused by blotting the seeds before weighing them, but rather that the water content of the embryo and endosperm does not decrease during development. The impression that whole seeds of tomato lose water during development is false, and points to the obvious need to make comparable studies of seed development in other succulent fruits.

Acknowledgements

Thanks are due to Wendy Ross for carrying out the series I experiments as part of a B.Sc. Honours project; to Suzanne Cash for developing the technique for determining fresh weights of embryos as part of an undergraduate research project; to Lynda Dixon for carrying out the series II experiment; and to Massey University for providing the facilities necessary to carry out these investigations.

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