Development and maturation of sesame seeds and capsules

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Abstract

Seed loss is a major problem for sesame producers. If growers could accurately assess seed and capsule maturity, then harvest times could be adjusted to maximise yield of mature seed. The development of seeds and capsules of three sesame cultivars was investigated. Maximum seed dry weights were reached about 35 days after seeds began developing. Maximum germination rates were reached when seeds were 35–53 days old. Changes in seed appearance clearly signalled that mass maturity and germination maturity had been reached. Capsule senescence commenced more than 53 days after capsules began developing. Senescence resulted in shrinkage of mesocarp cells in the capsule wall, creating tension that forced capsules open. All sesame cultivars had a window of at least 18 days between seed mass maturity and capsule senescence, suggesting that mature seed could be harvested before seed loss from a sesame crop as long as the cultivar sown has a flowering period of less than 25 days. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

It is difficult to decide when to harvest a sesame (Sesamum indicum L.) crop to maximise yield, because plant growth is indeterminate and capsules dehisce when mature. Plants may flower over a period of 50 days (or as long as conditions permit; Ashri, 1998). This generates a succession of capsules of different developmental stages in leaf axils along the main stem. New capsules may be produced at the top of the plant, while mature capsules near the base of the plant dry, split and lose seed (Ashri, 1998). If plants are harvested too early, then seed quality of the whole crop is reduced by the inclusion of immature seed from near the top of the plant. If plants are harvested too late, then yield may be reduced by loss of seed from the earliest maturing capsules. To maximise yield of high quality seed, growers must be able to accurately assess seed maturity in relation to capsule maturity.

Seeds may be considered mature when they attain maximum dry weight (mass maturity) or maximum germination rate (germination maturity) (Hilhorst and Toorop, 1997). Sesame seeds reach mass maturity 24–45 days after seeds begin to develop (days after flowering, DAF); most commonly 35–40 DAF (Sheelavant et al., 1978; Kang, 1985; Jankam, 1989; Narayanan et al., 1990). Seeds reach germination maturity 35–45 DAF (Sheelavantar et al., 1978; Kang, 1985; Jankam, 1989; Narayanan et al., 1990). Germination maturity is always reached a few days later than mass maturity.

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Seed merchants grade sesame seed by colour, size, and oil and moisture concentration. Australian premium grade sesame has bright uniform white colour, 1000 seed weight greater than 3 g, greater than 50% oil, and less than 6% moisture (Bennett et al., 1998). Thus commercial seed maturity is based on mass maturity, oil concentration and dry seed appearance; yet growers must use fresh seed appearance to decide when to harvest a crop. Growers are advised that fresh seeds are mature when they have final colour and a dark tip (hilum) (Ray Langham, pers. comm.). Mature fresh seeds may also have a visible seed line running from the hilum along one of the broad faces of the seed (Ray Langham, pers. comm.).

An yellow capsule is the only non-destructive feature that growers can use to indicate that the seed within may be mature. A change from green to yellow may indicate that capsule senescence has begun; yet capsules are only considered mature when they begin drying. As capsules dry, they split and loose seed. Capsule dehiscence is thought to result from tension created when endocarp and mesocarp cells shrink by different amounts during drying of the capsule wall (Ashri and Ladijinski, 1964; Day, 2000). The tension forces the capsule open, back from the capsule tip and from the seam along the capsule sides.

This paper examines the development and maturation of sesame seeds and capsules to:

1. understand the relationship between mass maturity, germination maturity, and mature appearance of fresh and dry seed;
2. understand the relationship between seed maturity and capsule maturity;
3. identify a window of opportunity: a period of time during which a sesame crop can be harvested with maximum yield of mature seed.

2. Methods

2.1. Growth conditions

Three sesame cultivars, Aussie Gold, Magwe Brown, and UCR82-6NS, were grown under field conditions. Aussie Gold was developed by Beech at CSIRO Tropical Agriculture. Magwe Brown is a selection from the Burmese cultivar Hnani 25/160. Aussie Gold and Magwe Brown are branched cultivars with average plant maturity. UCR82-6NS is a semi-indehiscent cultivar developed by Yermanos at the University of California, Riverside. This cultivar remains unbranched, has late plant maturity, and displays most of the pleiotropic features associated with the idid genotype (see Langham, 1946; Day, 2000). All three cultivars can produce three capsules per leaf axil.

Plants were grown from January to April (Summer–Autumn) under periodic irrigation in alluvial clay-loam at the CSIRO Cooper Laboratory, south-east Queensland. Each cultivar was sown in three, 6 m plots with an interplant distance of 10 cm and row spacing of 1 m. Flowering of the cultivars on a plot basis commenced about 29 days after sowing. One week later, open flowers were tagged. If there were three flowers per leaf axil, only central flowers were tagged. Irrigation ceased when Aussie Gold and Magwe Brown plants ceased flowering, about 67 days after sowing (about 31 days after flowers were tagged).

2.2. Measurement of capsule anatomy

Five tagged capsules per cultivar were collected at each harvest time. Transverse sections (1 mm thick) were cut from the mid-capsule region, and trimmed to just greater than quarter size. Sections were vacuum infiltrated with 2% glutaraldehyde and 2% formaldehyde in 0.05 M PO₄ buffer (pH 7) for 48 h, then dehydrated using an ethanol series. Sections were infiltrated with Spurr’s resin (Spurr, 1969) in a desiccating chamber in three, 24 h stages (50% resin: 50% ethanol, 100% resin). Sections set in the resin overnight at 70°C. Thin sections (1–2 μm) were cut with a glass knife using a Reichert Jung Ultracut microtome, stained with Toluidine Blue O (0.5% in 1% boric acid), then photographed through a Wild dissecting microscope with Kodak Tmax 100 film.

Anatomical dimensions (Fig. 1) were measured directly from photographic prints of four sections per cultivar. Endocarp thickness (ET) and mesocarp thickness (MT) were measured mid-way between the carpel septum and the false septum. The maximum capsule radius (RM) and the minimum distance from the median vascular bundle to the external wall of the epicarp (VBE) were measured. From the maximum locule length (LL) and locule width (LW), an estimation of locule area in cross-section was calculated (LA = \[1/2 LL \times 1/2 LW \times \pi\]).
2.3. Measurement of capsule fracture force

Fracture force was measured with a Lloyd LRX 2K5 fitted with a 500 N load cell from 10 capsules per cultivar at each harvest time. Only Aussie Gold and UCR82-6NS capsules were measured. Fresh capsules were placed on their side with the plane of the epicarp sutures vertical. An 8 mm probe moving at a rate of 30 mm min$^{-1}$ compressed the capsule adjacent to the most distal seeds. The Lloyd R Control (Version 3.0) software illustrated the change in resistance to movement of the probe during compression of the capsule (Fig. 2). The fracture force could be calculated by subtracting the force at the point of contact between the probe and capsule (zero point), from the force at the fracture point. The fracture point was identified as the first break in the curve (Fig. 2). This point indicated a change in resistance to movement of the probe, signalling that the capsule had split.

2.4. Seed and capsule development

Seeds were removed from 10 capsules per cultivar at each harvest time. A section of capsule wall from the mid-capsule region (approximately 100 mg fresh weight) was finely chopped and placed in a 1.5 ml micro-centrifuge tube. The fresh weight (FW) was recorded before 1 ml 100% methanol was added. After 24 h at 4°C, the sample was centrifuged (13 000 rpm, 2 min). The absorbance ($\lambda$=652 and 665.2) of 500 µl of the supernatant was measured with a Beckman DU 640 spectrophotometer. Chlorophyll concentration was calculated using the equations of Porra et al. (1989). Methanol was allowed to evaporate and the dry weight (DW) of the capsule wall sample measured.

The seeds from each capsule (above) were assessed separately as replicates for the seed appearance and germination measurements. The appearance of fresh seeds was recorded: seed colour (1: opaque white, 2: mixed opaque white and final colour, 3: final colour); seed tip (hilum) colour (1: light, same colour as seed, 2: dark); seed line (1: absent, 2: visible). Seed length and width was measured from two seeds per capsule. Total seed number and fresh weight were recorded. Seeds were dried at room temperature for at least 3 months to allow dormancy to dissipate. Appearance was re-assessed and seed DW was recorded. Seeds were placed on double-thickness filter paper in 9 cm petri dishes and soaked with 0.36 g l$^{-1}$ Thiram 800 fungicide (Amalgamated Chemicals, Australia). After 5 days at 30/20°C for 16/8 h (ISTA/AOSA standard germination conditions; Ellis et al., 1985), the percentage of germinated seed was calculated.

The data were grouped by the number of days after individual flowers opened (DAF). The results could have been grouped by day degrees after sowing (DD) to remove some of the influence of seasonal effects (DD = $\sum$[mean daily temperature], assuming a base temperature of 0°C). To equate these measurements under the growth conditions described here: 0
DAF=906 DD; 14 DAF=1276 DD; 24 DAF=1523 DD; 35 DAF=1809 DD; 53 DAF=2277 DD, 64 DAF=2537 DD; 76 DAF=2782 DD.

3. Results

3.1. Seed development

The rate of seed development was similar in the three cultivars. Fresh seeds reached maximum length and width between 10 and 14 days after they commenced development (DAF) (data not shown). Seeds consisted nearly totally of water at this stage of development (Fig. 3), but had the ability to retain their shape when air dried. Seeds accumulated dry matter during 14–35 DAF; nearly 2.5 mg was added to seed DW during this period (Fig. 3b). Seed DW did not increase after 35 DAF (Aussie Gold, F_{(2,27)}=1.87, NS; Magwe Brown, F_{(2,27)}=0.41, NS; UCR82-6NS, F_{(2,27)}=0.22, NS).

Fig. 3. Seed development of Aussie Gold (square), Magwe Brown (diamond), and UCR82-6NS (circle): (a) average individual seed fresh weight at harvest; (b) average individual seed DW after storage; (c) average individual seed water content. Data are mean±standard error of seeds from 10 capsules.

Fig. 4. Development of fresh (filled symbols, dashed line) and dry (open symbols, solid line) seeds from Aussie Gold (square), Magwe Brown (diamond), and UCR82-6NS (circle) plants: (a) percentage germination; (b) seed colour; (c) seed tip (hilum) colour; (d) visibility of seed line. Data are mean±standard error of seeds from 10 capsules.
Mass maturity (maximum DW) and mature appearance of dry seeds were reached at a similar developmental stage, about 35 DAF (Figs. 3 and 4). Germination maturity (maximum germination rate) and mature appearance of fresh seeds were reached at a later developmental stage, between 35 and 53 DAF (Fig. 4). Thus, seeds continued to mature in appearance as they dried.

Seeds of UCR82-6NS appeared to mature slightly later than the other two cultivars (Fig. 4), and the decrease in seed water content was delayed toward the end of development (Fig. 3c). Seed maturity of all cultivars was attained before capsules began to dry.

### 3.2. Capsule development

All capsule tissues were present 7 DAF (Figs. 5a and 6a). Between 7 and 10 DAF, capsules of Aussie Gold and Magwe Brown plants reached maximum radius, and locule area (data not shown), and the mesocarp reached maximum thickness (Fig. 7b). UCR82-6NS capsules reached maximum size by 14 DAF (capsule radius and locule area). During 10–14 DAF, deep blue staining with Toluidine Blue O indicated thickening of endocarp cell walls of all varieties (Figs. 5b, 6b and c). Endocarp tissue only reached maximum thickness after all anatomical measurements had reached maximum values (Fig. 7).

For the greater part of capsule development (14–53 DAF) there was no change in capsule anatomy measurements, fracture force, or capsule wall percentage moisture (Fig. 7). The chlorophyll concentration of capsule walls declined slowly throughout this period, but the ratio of chlorophyll \( a/b \) increased (Fig. 8). Some separation and lysis of the thin-walled cells of the false septa was apparent by 14 DAF, and

![Images of capsule development](image-url)
became severe before capsules commenced drying (Figs. 5 and 6).

Drying of capsules occurred rapidly in all cultivars (Fig. 7e). Mesocarp cells shrank (Figs. 5d and 6e) causing a decrease in mesocarp thickness (Fig. 7b), but the endocarp did not shrink (Figs. 5d, 6e and 7a). The mesocarp broke apart and disassociated from the endocarp in Aussie Gold and Magwe Brown capsules (Fig. 5d and not shown).

Aussie Gold and Magwe Brown capsules were generally similar in anatomy, and in the sequence of capsule development. UCR82-6NS capsules had a thicker mesocarp and lower capsule-wall chlorophyll concentration than the other cultivars (Figs. 7b and 8a).

Fig. 6. Mid-capsule transverse sections of UCR82-6NS capsules during development: (a) 6 DAF; (b, c) 14 DAF, sections from either side of the same capsule showing different numbers of mesocarp cell layers between the median vascular bundle and the epicarp; (d) 64 DAF; (e) 76 DAF. Tissues can be identified using Fig. 1. Bar=1 mm.
Capsules of this cultivar were slower to develop, and dried down later than the other varieties (Fig. 7).

UCR82-6NS capsules had more mesocarp cell layers between the median vascular bundle and the epicarp (large VBE) than Aussie Gold capsules (Fig. 7c). This feature afforded greater resistance to a fracture force (Fig. 7d) and prevented capsules splitting when dry (Fig. 6e). There was asymmetry in the VBE measurements from single UCR82-6NS capsules (paired t-test, \( t_{(9)} = 6.11, p < 0.001 \)) (Fig. 6b and c). This difference in strength either side of capsules resulted in the Lloyd LRX 2KS recording two fracture points (Fig. 2).

### 4. Discussion

Sesame seeds and capsules expanded together during early development. Maximum seed and capsules size, and maximum thickness of capsule tissues were recorded 10–14 DAF (data not shown and Fig. 7). By 14 DAF, endocarp cell walls had thickened (Figs. 5b, 6b, 6c and 7a); the endocarp forming a rigid, protective container for the seed.

Following the initial expansion period, sesame seed and capsule development showed little coordination. No change occurred in capsule tissue thickness, capsule-wall percentage moisture, or fracture force while seeds accumulated dry matter and matured. During seed filling, the maximum chlorophyll concentration was reached in the capsule-wall (Fig. 8a). This supports a hypothesis proposed for other species, that...
photosynthesis occurring in the capsule wall assists with the provision of assimilates for the seed (King et al., 1998). Because the subsequent decline in capsule-wall chlorophyll concentration was gradual (Fig. 8a); yellowing of capsules, which has been used to indicate when seeds are mature, is an imprecise measure of seed maturity.

Sesame seed colour, seed tip colour, and seed line visibility clearly indicated that seeds were mature. Seeds of all three cultivars reached mass maturity and dry seeds appeared mature, about 35 days after seed development began (Figs. 3 and 4). Seeds oil concentration was probably near maximum levels at this time, because Kang (1985) found that maximum sesame seed DW and maximum oil concentration were reached at the same developmental stage. Thus sesame seeds were commercially mature (maximum DW, maximum oil concentration, mature dry seed appearance) 35 DAF. A week or two later, seeds achieved germination maturity and fresh seeds had mature appearance (Fig. 4).

The decrease in chlorophyll concentration in the capsule wall (Fig. 8a) may indicate the commencement of capsule senescence. But most often during senescence, both chlorophyll concentration and the chlorophyll a/b ratio will decrease (Siffel et al., 1991; King et al., 1998). The decrease in chlorophyll concentration suggests there are fewer chloroplasts. The increase in chlorophyll a/b ratio throughout most of capsule development (Fig. 8b) suggests that the structure of the remaining chloroplasts is not disrupted (Siffel et al., 1991). These results suggest a controlled decrease in chloroplast number, probably because of shading rather than capsule senescence.

Capsule senescence was signalled by rapid water loss from the capsule wall 53 DAF (Aussie Gold and Magwe Brown) or 64 DAF (UCR82-6NS). The water loss caused shrivelling of mesocarp cells but not endocarp cells (Figs. 5d, 6c, 7a and b). This probably created tension within the capsule wall; forcing separation of the mesocarp from the endocarp, separation of the septa from the placenta, and eventually forcing the capsule to split (Fig. 5d). Capsule halves separated along the false septa. This region was weakened by separation and lysis of thin-walled cells, which began as early as 14 DAF and extended from the false septum to the median vascular bundle before capsule senescence began (Figs. 5b and 6d).

Many UCR82-6NS capsules split along one seam only. This is probably related to the different numbers of mesocarp cell layers between the median vascular bundle and epicarp on either side of capsules (Fig. 6b and c). Only on the seam that had many mesocarp cell layers was separation of the capsule halves prevented (Fig. 6e).

Seeds of the three sesame cultivars were commercially mature 35 DAF, and capsule senescence commenced at least 53 DAF. There is at least an 18 day window during which mature seeds could be harvested before capsules begin to split. Thus sesame cultivars where the number of days between flowering at the base of plants and at the top of plants (flowering period) is less than 25 days (individual flowers were tagged 7 days after the crop commenced flowering, +18 day window), could be harvested after seeds at the top of plants are mature and before capsules low on plants have dried. Kang (1985) found that seed mass maturity took longer in capsules from low on plants (40–45 DAF) than in capsules higher on plants (35–40 DAF). Therefore, it may be possible to harvest sesame cultivars with a flowering period greater than 25 days without sacrificing either seed quality or seed yield. Only one of 17 cultivars (including the three cultivars here) grown in the conditions described here, had a flowering period less than 25 days (Day, 2000). Although the flowering period of these cultivars may be shorter in more tropical areas, more effort is required to develop determinate sesame cultivars with shorter flowering periods. These new cultivars may be suitable for Australian conditions.

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References


