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Seed development in *Camellia sinensis* (L.) O. Kuntze

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Abstract

Seed development of tea was studied to identify the maturity index and the optimal time of seed collection. After harvest, the moisture content (28–30% fresh weight basis) of mature seeds, which germinated 100%, declined progressively (19% moisture content) after shedding, with a decrease in seed germination and viability. However, this viability loss could be prevented to some extent by storing seeds within intact fruits. The maximum rate of seed dry matter accumulation coincided with the accumulation of starch in the embryos and seeds at stage 8, the embryo maturation phase. Although the embryo abscisic acid (ABA) content was highest at stage 8, free ABA declined in the tea embryos throughout the remainder of the seed maturation cycle.

Keywords: abscisic acid, *Camellia sinensis*, seed development, starch, sugars, tea

Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is an important commercial crop (Jankun *et al.*, 1997). Despite the availability of a number of good clones for vegetative propagation, the tea industry is still dependent on seeds for replanting and crop improvement (Barua, 1989). Programmes for crop improvement and conservation are greatly hindered by the recalcitrant nature of tea seeds (Berjak *et al.*, 1993). Any strategy for long-term storage of tea seeds would require identification of the maturity index for the collection of seeds at the proper developmental stage in order to avoid collection of immature or non-viable seeds. Although seed development is governed by many factors, the plant hormone abscisic acid (ABA) is especially important. ABA influences accumulation of storage proteins and lipid reserves (Kermode, 1990), as well as acquisition of desiccation tolerance and induction of seed dormancy (Marion-Poll, 1997). Generally, embryo development is associated with high levels of endogenous ABA coupled with high osmolarity in the surrounding tissues (Xu *et al.*, 1990; Farrant *et al.*, 1992). Carbohydrates also play a key role in seed or embryo development. Starch functions as the most important energy reserve, while high levels of sugars – particularly sucrose, raffinose and stachyose – may stabilize membranes in the maturing seeds (Farrant *et al.*, 1992).

Although some mention has been made about the prolonged development of tea seeds (Bezbaruah, 1975; Barua, 1989), the precise seed developmental stages have not been identified to date. Therefore, in the present paper, we (1) document the temporal details of tea seed development and also (2) report on a study of the associated changes in starch, sugars and the endogenous levels of ABA to identify their roles in tea seed development.

Materials and methods

Plant material

Twenty bushes of *Camellia sinensis* (L.) O. Kuntze, growing in India at the Institute of Himalayan Bioresource Technology Experimental Farm, Banuri, Palampur (36°N and 78.18°E and 1290 m above sea level), were selected randomly, and 10 branches of each bush were tagged. Just after their initiation, green floral buds of uniform size were counted and tagged to follow further development. For three consecutive years the same sets of bushes were selected and inflorescences were tagged randomly.

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Observations were recorded at one-month intervals for development of the green floral buds into white buds (stage 1, May–June) and finally into flowers (stage 2, July–August). Anthesis was noted daily, and the date of maximum anthesis was recorded in order to identify the day on which the seed development process started. Data were also recorded on flower abscission, pollination, fertilization, fruit set (stage 3, September–October) and fruit growth (stage 4, November–December). Seed development was followed from the first visible sign of seeds (stage 5, January–February), and observations were recorded every month with respect to: (1) seed weight and diameter, (2) testa or seed coat colour, (3) stage of endosperm, (4) embryo size and weight and (5) ratio of the space occupied by the embryo to that of the endosperm. The entire seed development period was divided into six stages (from stages 5 to 10). Mature seeds about to be shed from fruits were considered as stage 10.

Growth characteristics, moisture content and seed germination

Fruits were harvested from the full circumference of each bush, and samples were drawn from the combined bulk. The fresh weights of individual components were recorded on the day of harvest. Moisture contents (fresh weight basis, fwb) were determined on samples of whole fruits (seed and fruit wall), seeds (embryo, endosperm while present and testa) and embryos (cotyledons and axes) after drying at 103°C for 17 h in an oven (ISTA, 1985). Three replicates with 10 individuals in each set were taken for each component. The pooled dry weights were used to define the growth characteristics.

To characterize the natural loss of moisture from seeds at ambient temperature, mature seeds collected with or without fruit wall, prior to fruit dehiscence, immediately after dehiscence or stored for 1 month after dehiscence were tested for moisture content and germination at 25 ± 2°C. Five replicates of 10 seeds each were sown in soil in 10-cm pots in darkness. Radicle emergence was considered as the index of germination and observations were recorded at 7-d intervals after removing the seeds from the soil. All data were recorded for three consecutive years.

Viability determination

To find out whether the seeds that failed to germinate were dormant or non-viable, the seeds were subjected to the 2,3,5-triphenyl tetrazolium chloride (TTC) test. Randomly selected mature seeds prior to dehiscence, immediately after dehiscence and 1 month after dehiscence were de-coated and cut into longitudinal halves. These halves were then immersed in aqueous 0.1% TTC solution for 24 h in the dark at room temperature. Five replicates with 20 seeds in each dish were taken, and the number of embryos that had stained red was recorded.

Estimation of starch and total sugars

Starch and total sugars were separately estimated in the embryos and seeds at stages 5–10 for three consecutive years. Powders (1.0 g) of dried samples were extracted in 80% ethanol for 24 h at room temperature and were used for the estimation of total sugars after filtration using the anthrone method, as outlined by McCready *et al.* (1950). Starch was measured as liberated glucose using the anthrone reagent following hydrolysis of the extracted powders (Adams *et al*., 1980).

ABA estimation

Fruits from stages 5–10 were collected at regular intervals for three consecutive years and separated into fruit wall, seed coat and embryo (cotyledons + axes). These were then processed on the day of harvest for the estimation of endogenous ABA levels (Nagar, 1996). Each sample of 15 g fresh mass was separately homogenized in chilled 80% methanol (20 ml g^{-1}) containing butylated hydroxytoluene (BHT) at 100 mg l^{-1}. The homogenates were stored for 24 h in the dark at 4°C and then filtered *in vacuo*. The residues were re-extracted five times with chilled methanol, filtered and centrifuged at 10,000 rpm at 5°C. The supernatants were pooled, dried *in vacuo*, dissolved in 2 ml of 0.1 M potassium phosphate buffer (pH 8.8) and then applied to polyvinylpyrrolidone (PVP) columns. The columns (20 cm × 1.5 cm, internal diameter (ID)) were eluted with phosphate buffer (pH 8.0), and the resulting eluates were adjusted to pH 2.5 with 1 N HCl and partitioned against peroxide-free diethyl ether (×5) containing BHT (100 mg l^{-1}). The combined organic phases were evaporated to dryness *in vacuo*, taken up in methanol (HPLC grade) and used for free ABA estimation, with more than 75% recovery irrespective of the type of tissue used (Nagar, 1996).

The remaining aqueous fractions were hydrolysed at pH 11.0 for 1 h at 60°C, cooled, adjusted to pH 2.5 and partitioned against diethyl ether containing BHT (×5). The ether phases were evaporated *in vacuo* and taken up in methanol (HPLC grade) for the estimation of bound ABA (Harris and Dugger, 1986).

Reverse-phase HPLC was carried out on a HPLC Kontron system 400 (Kontron Institute Ltd., Switzerland) for ABA estimation (Ciha *et al*., 1977). The partially purified methanolic extracts were filtered through 0.45 µm Millipore filters and injected into a 20 µl injector loop of a Lichrosorb RP 18 (10 µm)

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column (250 × 4.6 mm, ID) protected by a guard column. Elution was carried out with 40% methanol (HPLC grade) in 30 mM acetic acid (HPLC grade) at a flow rate of 1 ml min^{-1}. The solvents were filtered through 0.45 µm pore-size Whatman membranes and degassed under vacuum prior to use. The column eluates were passed through an ultraviolet (UV) detector (Kontron 430) at 254 nm, and ABA was measured by referring to an authentic standard of (±) ABA (Sigma Chemical Co., St. Louis, USA).

Results

For tea plants in the Himalayan foothills, the entire development period from flower bud initiation to the mature seed comprises about 18 months. Sporadic flowers occur throughout the year, but peak flowering occurs during the summer months. Initiation of buds takes place during mid- to late-May, with subsequent enlargement into open white buds with a differentiated calyx during June (stage 1). About 85% of the flowers open during July–August (stage 2). Insect-mediated cross-pollination results in fertilization followed by zygote formation in 50% of the ovaries. Fruit set occurs during September–October (stage 3)(data not shown).

Fruit growth

Fruit, seed and embryo growth characteristics (stages 4–10) are summarized in Table 1. During the initial phases of growth, the fruits were light green and continued to grow throughout the winter months (stage 4). Faster growth with normal development was observed in fruits with 2–3 healthy seeds as compared to the fruits with only one seed (data not shown). Mature fruits of about 26.5 mm diameter and 6.7 g fresh weight (Table 1) contained 2–3 healthy seeds. Dry brown patches in the fruit wall generally appeared during stage 10 and were indicative of seed maturity and fruit dehiscence towards the end of stage 10.

Seed development

Visible embryos, bathed in transparent liquid endosperm, were observed during January–February (stage 5). The seeds had brittle white testae and tiny white embryos (less than 0.1 mm diameter). Thereafter, a progressive increase in the embryo growth rate occurred (Table 1). Rapid growth (fresh weight) was observed during the initial stages of seed and embryo development (up to stage 8), with a gradual increase thereafter (Table 1). While the dry weights of seeds and embryos continued to increase throughout the seed developmental phases, the maximum rate of dry matter accumulation was observed during stage 8 (Table 1).

Liquid endosperm occupied 98% of the entire volume of seeds during the initial phases of growth (stages 5 and 6), but decreased thereafter with a concomitant increase in embryo volume. At stage 8, the endosperm changed from a liquid to viscous consistency and occupied only 50% of the seed volume. Ninety per cent of the endosperm was consumed by the growing embryo at stage 9. The endosperm shrivelled into a thin, fine, papery layer that remained attached to the inner wall of the seed coat at stage 10.

Moisture content and seed viability

With progressive growth of the fruits, seeds and embryos, a concomitant decrease in moisture content and seed viability was observed. However, the moisture content decrease was more pronounced in the seeds and embryos than in the fruits (Fig. 1).

Irrespective of the presence or absence of the fruit wall, 100% seed viability and germination were observed in the seeds prior to shedding (Table 2), indicating the non-dormant nature of the seeds (MC 30.6%, fwb). The fruit wall helped to maintain seed MC at 28–28.6%, and seeds germinated 98% when tested immediately after shedding and after storage within the fruit for 1 month. When the seeds were stored for 1 month without their fruit walls, the moisture content was reduced to 19.3%, viability declined to 75% and germination was reduced (60%) (Table 2).

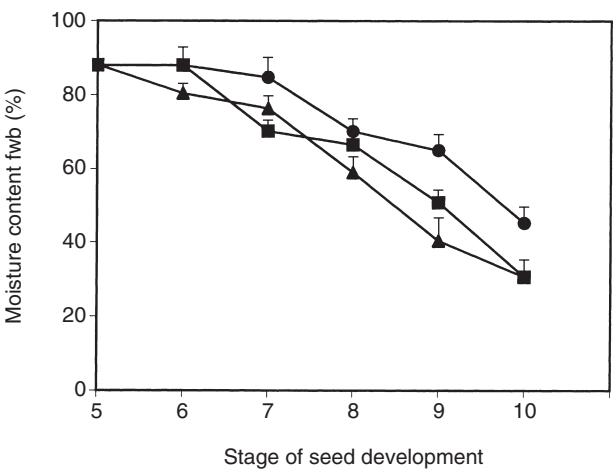


Figure 1. Pooled data from three consecutive years on the changes in moisture content (fwb) of fruit (seed and fruit wall: ●), seed (embryo and testa: ■) and embryo (cotyledon and axis: ▲) during seed development in tea from stage 5 to stage 10. Vertical bars represent standard errors of the mean, where $n = 3$.

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Table 1. Growth and other characteristics of tea fruits, seeds and embryos during developmental stages 4–10

Months/ stage[a]	Fruit				Seed				Embryo			
	F. wt (mg)	D. wt (mg)	Diam. (mm)	Fruit wall colour and texture	F. wt (mg)	D. wt (mg)	Diam. (mm)	Seed coat colour and texture	F. wt (mg)	D. wt (mg)	Diam. (mm)	% Space occupied by embryo[b]
Nov–Dec Stage-4	115.0 ± 0.1	10.0 ± 5.8	2.4 ± 0.04	Light green	Seeds not visible macroscopically			–	–	–	–	–
Jan–Feb Stage 5	178.4 ± 0.3	20.0 ± 1.2	6.8 ± 0.02	Light green, thin	Macroscopically visible seeds			White, brittle	White, visible embryos		–	–
Mar–Apr Stage 6	524.8 ± 1.2	102.0 ± 1.5	9.8 ± 0.9	Green, thin	42.0 ± 1.7	29	4.2 ± 0.2	White, brittle	0.9 ± 0.1	4.0 ± 2.2	–	2
May–Jun Stage 7	2132.7 ± 1.7	201.0 ± 2.1	16.4 ± 0.2	Dark green, fleshy	407.5 ± 0.3	120	8.6 ± 0.2	Light brown, rubbery	45.5 ± 0.6	68.0 ± 1.5	3.8 ± 0.2	20
July–Aug Stage 8	4901.3 ± 1.8	203.0 ± 0.0	34.3 ± 0.9	Dark green, fleshy	1185.0 ± 3.4	695	12.1 ± 0.1	Light brown, brittle	582.7 ± 2.6	172.0 ± 1.5	9.8 ± 0.5	50
Sep–Oct Stage 9	6694.2 ± 1.2	380.0 ± 0.2	26.5 ± 1.9	Dark green, dried patches	1564.9 ± 0.1	793	15.6 ± 0.3	Dark brown, brittle	997.4 ± 1.3	348.0 ± 1.2	12.7 ± 0.6	90
Nov–Dec Stage 10	6690.0 ± 0.6	400.0 ± 0.03	26.2 ± 0.2	Greenish brown, dried	1564.0 ± 2.1	1019	15.6 ± 0.2	Black, hard, brittle	997.9 ± 0.9	682.0 ± 1.8	12.6 ± 0.2	100

[a] Fertilization occurred during July–August (stage 2), and fruit initiation occurred during September–October (stage 3).
[b] The remaining seed volume was occupied by the endosperm.

Table 2. Pooled data of three consecutive years on moisture content (%) and germination (%) of mature seeds (stage 10) of tea, both freshly harvested and stored enclosed within and without the fruit wall in paper bags at room temperature. Values ± SE

Mature seeds	Moisture content (%)	Seeds stained red by TTC (%)	Germination (%)
Without fruit wall prior to shedding	30.6 ± 0.3	100	100
With fruit wall prior to shedding	30.6 ± 0.2	100	99.9 ± 0.03
Without fruit wall immediately after shedding	26.8 ± 0.5	99 ± 1	98.6 ± 0.6
With fruit wall immediately after shedding	28.6 ± 0.2	100	98.9 ± 0.6
Without fruit wall 1 month after shedding	19.3 ± 0.2	75 ± 5	59.8 ± 0.6
With fruit wall 1 month after shedding	28.0 ± 0.2	98 ± 1	97.9 ± 0.5

Total sugars and starch

A steady increase in the total soluble sugars (per seed/embryo basis) was observed in both the seed and the embryo during the initial phases of development, to a maximum at stage 9 (Fig. 2A), but declined thereafter. An increase in starch content was also observed in both the seed and the embryo up to stage 9 with a major increase between stage 7 and stage 8 (Fig. 2B).

Changes in endogenous levels of ABA

A progressive increase in free ABA content (per seed-part basis) was observed in the embryos from stage 5 onwards until ABA reached a peak at stage 8 (Fig. 3A). With decreasing free ABA content from stage 8 onwards, a concomitant increase in the content of embryo-bound ABA was observed. In the testa, free and bound ABA were low up to stage 7, but increased at stage 8 through stage 10. The level of testa-bound ABA was higher than free ABA, and a constant level was maintained throughout the rest of the development process (Fig. 3B). Appreciable amounts of both free and bound ABA were detected in the fruit walls. An increase in bound and free fruit wall ABA was observed up to stage 7 and 8, respectively, but declined thereafter (Fig. 3C).

Discussion

An overall period of 18 months from anthesis to seed maturation is generally required for tea seed development. As in other plants, the process of fertilization in tea triggers the initiation of fruit development (Gillaspy *et al.*, 1993). Growth was initiated only in 50% of fertilized ovaries while the rest aborted. Increases in fruit size and weight were dominated by increases in the developing seeds and continued until the seeds were mature. In tomato the number of fertilized ovules generally determines the initial growth rate of fruits (Varga and Bruinsma, 1986), and lopsided fruits are produced when the seeds abort or do not develop (Hobsons and Davis, 1970). This has also been found to be the case in tea, where fruits with 2–3 healthy seeds exhibit rapid growth and normal development, in comparison to those that are one-seeded. Tea fruits with four locules usually have one or two aborted ovules and are hence lopsided (data not shown).

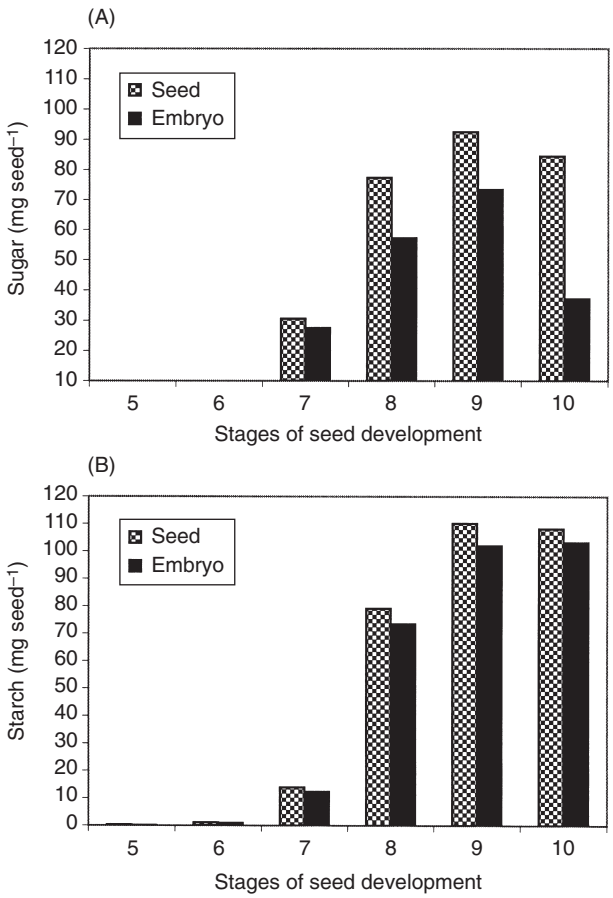


Figure 2. Pooled data from three consecutive years on the changes in the (A) total sugars and (B) total starch in seed (embryo and testa) and embryo (cotyledon and axis).

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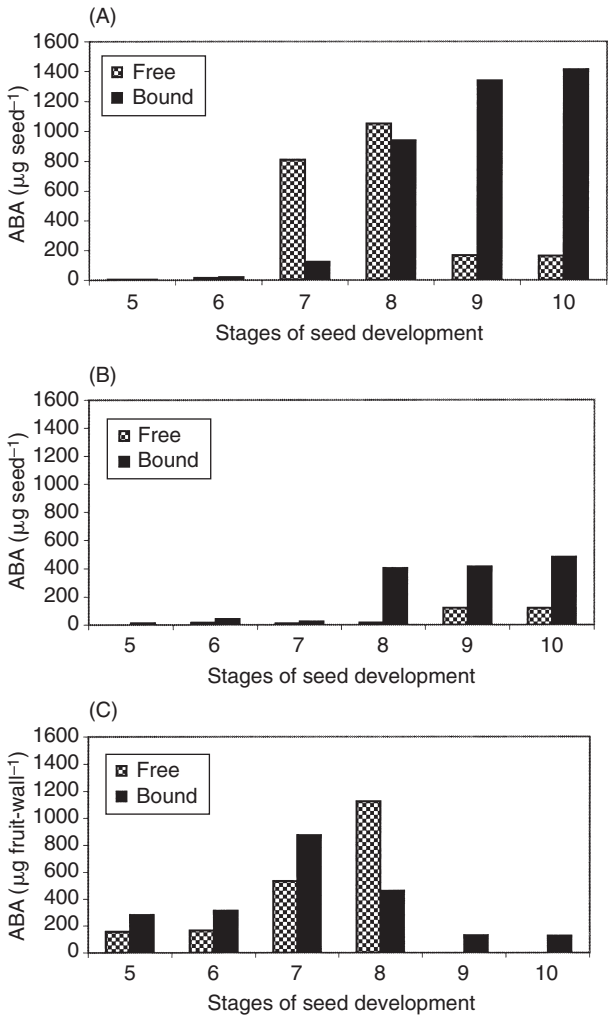


Figure 3. Pooled data from three consecutive years on the changes in free and bound ABA levels in (A) the embryo, (B) the testa and (C) the fruit wall during seed development in tea from stage 5 to stage 10.

During the initial phases of seed growth in tea (stages 5 and 6), the endosperm occupied almost the entire volume (98%) of seeds, as in all ex-endospermous seeds (Lopes and Larkins, 1993). Subsequently, the endosperm decreased due to its consumption by the growing embryo until a papery thin and transparent layer remained. This is probably due to the absorption of the liquid endosperm in the free nuclear state by the growing embryo, as in other species (Marinos, 1970). With progressive development of tea seeds, an increase in the embryo dry matter accompanied endosperm depletion. However, the maximum rate of dry matter accumulation occurred during stage 8, when an increase by about 25-fold and 6-fold are recorded in the embryos and the seeds, respectively (Table 1). Accumulation of dry matter, even in mature seeds prior to shedding, is consistent with the recalcitrant behaviour of tea seeds, which, unlike orthodox seeds, have no clear end point to development (Finch-Savage *et al*., 1992). This is further supported by the fact that total soluble sugars continue to increase up to stage 9 of embryo development. A considerable increase in the starch content during stages 8 and 9 coincided with the maximum rate of dry matter accumulation. Thus, it appears that stages 8–9 may represent the embryo maturation phase in tea.

At stage 10, mature tea seeds with moisture contents of 30.6% were capable of germination, even if they were collected prior to fruit dehiscence/seed shed. However, after removal of the fruit wall, a decline in moisture content to 19.3%, as well as in germination (59.8%), was observed in the seeds after 1 month of storage. This indicates that tea seeds cannot tolerate desiccation. However, loss of moisture and viability during storage could be minimized by collecting fruits at stage 10 and storing them intact for 1 month. Berjak *et al*. (1993) have shown that the relative desiccation sensitivity of tea embryonic axes at different stages of development was related to the degree of metabolic activity, and this may be more important than the physical properties of water in determining desiccation sensitivity.

ABA is known to play a vital role in the maturation of embryos in most orthodox species (Vertucci and Farrant, 1995) and is implicated in triggering the accumulation of storage reserves (Rock and Quatrano, 1995; Bonetta and McCourt, 1998) and in the acquisition of desiccation tolerance by the embryos (Merlot and Giraudat, 1997). In tea, the level of endogenous free embryonic ABA, which is high at stage 8, declined considerably during embryo maturation (stages 9 and 10).

With the decline in the level of free ABA during the embryo maturation phase, a concomitant increase in bound ABA was observed in the embryos, such that the level of bound ABA exceeded that of free ABA during the stage of full maturity prior to seed shed. This is suggestive of an interconversion of free ABA into its inactive storage form (Milborrow, 1983). The sharp decline in the ABA content of tea embryos may be associated with the rapid decline in the total sugar content after stage 9 in the present study. This may indicate that ABA plays some role in sugar metabolism (Tetteroo *et al*., 1995). Bianchi *et al*. (1992) found that ABA-treated callus of *Craterostigma plantiginum* accumulated sucrose and became desiccation tolerant. This tolerance to desiccation was attributed to the production of di- and trisaccharides (Hoekstra *et al*., 1997) and to the enhanced formation of a glassy state (Bruni and Leopold, 1992; Buitink *et. al*., 1998). A well-defined transient peak in ABA concentration was also associated with a rapid increase in the starch content of the cotyledons of

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maturing recalcitrant embryos of *Quercus robur* (Finch-Savage *et al*., 1992).

The sudden reduction in the embryonic ABA levels during the actual phase of embryo maturation of tea, and its relation to carbohydrate metabolism, therefore, seems to require a deeper understanding of the biochemical basis of the role of this plant hormone in tea seed development.

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References

Adams, C.A., Rinnie, R.W. and Fjerstad, M.C. (1980) Starch deposition and carbohydrase activities in developing and germinating soyabean seeds. *Annals of Botany* **45**, 577–582.

Barua, D.N. (1989) *Science and practice in tea culture*. Tea Research Association, Tocklai Experimental Station, Jorhat, Assam.

Berjak, P., Vertucci, C.W. and Pammenter, N.W. (1993) Effects of developmental status and dehydration rate on characteristics of water and desiccation sensitivity in recalcitrant seeds of *Camellia sinensis*. *Seed Science Research* **3**, 155–166.

Bezbaruah, H.P. (1975) Development of flower, pollination and seed set in tea in north east India. *Two and a Bud* **22**, 25–30.

Bianchi, G., Gamba, A., Murelli, C., Salamini, F. and Bartels, D. (1992) Low molecular weight solutes in desiccated and ABA-treated calli and leaves of *Craterostigma plantagineum*. *Phytochemistry* **31**, 1917–1922.

Bonetta, D. and McCourt, P. (1998) Genetic analysis of ABA signal transduction pathways. *Trends in Plant Science* **3**, 231–235.

Buitink, J., Claessens, M.M.A.E., Hemminga, M.A. and Hoekstra, F.A. (1998) Influence of water content and temperature on molecular mobility and intracellular glasses in seeds and pollen. *Plant Physiology* **118**, 531–541.

Bruni, F. and Leopold, A.C. (1992) Cytoplasmic glass formation in maize embryos. *Seed Science Research* **2**, 251–253.

Ciha, A.J., Brenner, M.L. and Brun, W.A. (1977) Rapid separation and quantification of abscisic acid from plant tissues using high performance liquid chromatography. *Plant Physiology* **59**, 821–826.

Farrant, J.M., Pammenter, N.W. and Berjak, P. (1992) Development of the recalcitrant (homoiohydrous) seeds of *Avicennia marina:* Anatomical, ultra-structural and biochemical events associated with development from histo-differentiation to maturation. *Annals of Botany* **70**, 75–86.

Finch-Savage, W.E., Clay, H.A., Blake, P.S. and Browning, G. (1992) Seed development in the recalcitrant species *Quercus robur* L. Water status and endogenous abscisic acid levels. *Journal of Experimental Botany* **43**, 671–679.

Gillaspy, G., Ben-David, H. and Gruissem, W. (1993) Fruits – A developmental perspective. *Plant Cell* **5**, 1439–1451.

Harris, M.J. and Dugger, W.M. (1986) Levels of free and conjugated abscisic acid in developing floral organs of the navel orange (*Citrus sinensis* [L.] Osbeck cv. Washington). *Plant Physiology* **82**, 1164–1166.

Hobsons, G. and Davis, J. (1970) The tomato. pp. 437–482 *in* Hulme, A.C. (Ed.) *The biochemistry of fruits and their products*. Volume 2. London, Academic Press.

Hoekstra, F.A., Wolkers, W.F., Buitink, J., Golovina, E.A., Crowe, J.H. and Crowe, L.M. (1997) Membrane stabilization in the dry state. *Comparative Biochemistry and Physiology* **117**, 335A–341A.

ISTA (International Seed Testing Association) (1985) Determination of moisture content. *Seed Science and Technology* **13**, 338–341.

Jankun, J., Selman, S.H., Swiercz, R. and Skrzypczak-Jankun, E. (1997) Why drinking green tea could prevent cancer. *Nature* **387**, 561.

Kermode, A.R. (1990) Regulatory mechanisms involved in the transition from seed development to germination. *Critical Reviews in Plant Science* **9**, 155–195.

Lopes, M.A. and Larkins, B.A. (1993) Endosperm origin, development and function. *Plant Cell* **5**, 1383–1389.

Marinos, N.G. (1970) Embryogenesis of the pea (*Pisum sativum* L.). I. The cytological environment of the developing embryo. *Protoplasma* **70**, 261–279.

Marion-Poll, A. (1997) ABA and seed development. *Trends in Plant Science* **2**, 447–448.

McCready, R.M., Guggolz, J., Silviera, V. and Owens, H.S. (1950) Determination of starch and amylose in vegetables. Application to peas. *Analytical Chemistry* **22**, 1156–1158.

Merlot, S. and Giraudat, J. (1997) Genetic analysis of abscisic acid signal transduction. *Plant Physiology* **114**, 751–757.

Milborrow, B.V. (1983) Pathways to and from abscisic acid. pp. 79–112 *in* Addicott, F.T. (Ed.) *Abscisic acid*. New York, Praeger.

Nagar, P.K. (1996) Changes in endogenous abscisic acid and phenols during winter dormancy in tea (*Camellia sinensis* (L.) O. Kuntze). *Acta Physiologiae Plantarum* **18**, 33–38.

Rock, C.D. and Quatrano, R.S. (1995) The role of hormones during seed development. pp. 671–697 *in* Davies P.J. (Ed.) *Plant hormones: Physiology, biochemistry and molecular biology* (2nd edition). Dordrecht, Kluwer Academic.

Tetteroo, F.A.A., Peters, A.H.L.J., Hoekstra, F.A., van der Plas, L.H.W. and Hagendoorn M.J.M. (1995) ABA reduces respiration and sugar metabolism in developing carrot (*Daucus carota* L.) embryoids. *Journal of Plant Physiology* **145**, 477–482.

Varga, A. and Bruinsma, J. (1986) Tomato. pp. 461–480 *in* Monselise, S.P. (Ed.) *Handbook of fruit set and development*. Boca Raton FL, CRC Press.

https://doi.org/10.1079/SSR200196 Published online by Cambridge University Press

Vertucci, C.W. and Farrant, J. (1995) Acquisition and loss of desiccation tolerance. pp. 237–271 *in* Kigel, J.; Galili, G. (Eds) *Seed development and germination*. New York, Marcel Dekker.

Xu, N., Coulter, K.M. and Bewley, J.D. (1990) Abscisic acid and osmoticum prevent germination of developing alfalfa embryos, but only osmoticum maintains the synthesis of developmental proteins. *Planta* **182**, 382–390.

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