Using Hydrotime and ABA-time Models to Quantify Seed Quality of Brassicas during Development

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ABSTRACT. With many seed crops, the most difficult production decision is when to harvest. In indeterminate crops such as *Brassica* species, early harvests result in immature seed of low vigor while late harvests risk seed deterioration and seed loss due to shattering. To provide a biological basis on which to determine harvest timing, we have characterized seed development in rape seed (*Brassica napus* L. 'Weststar') and red cabbage (*Brassica oleracea* L. Group Capitata) using population-based hydrotime and ABA-time models. These models provide information relevant to assessing physiological maturity, and therefore, seed quality. The hydrotime and ABA-time models quantify germination rate, the uniformity of germination, viability, and the sensitivity of germination to water potential and ABA. Indices derived from these models, along with maximum germination and t_{50} values, were used to determine physiological maturity (maximum seed quality) of the seeds during development. The overall trends in seed development were similar in both species: as seeds matured, germination became more uniform and less sensitive to low ψ and externally applied ABA. The models accurately described germination time courses and final germination percentages except for seeds imbibed at very high concentrations of ABA. In rape seed, physiological maturity was attained several days after maximum seed dry mass, while in red cabbage physiological maturity occurred at or after maximum seed dry mass. Vigor indices were correlated with easily discerned traits such as moisture content and silique phenotypic characteristics. The results of these experiments suggest that hydrotime and ABA-time models can be successfully used to provide a biological basis on which to determine harvest in brassicas.

Distinct physiological events occur during seed development, and recognizable gene expression patterns have been associated with these events. During embryogenesis, seeds accumulate proteins, carbohydrates and lipids, resulting in an increase in seed dry mass. Ovule abscission marks the end of the accumulation phase when maximum seed dry mass is attained. Although there are $\approx 20,000$ diverse genes expressed at any one time during embryogenesis (Goldberg et al., 1989), gene expression appears to be organized into a small number of discrete patterns (Hughes and Galau, 1989). Based on the presence of specific mRNA species during embryogenesis, it has been demonstrated that distinct programs continue to be expressed after ovule abscission. Maturation programs occurring late in embryogenesis control the synthesis of storage products, prepare the seed for desiccation, prevent premature germination, and establish dormancy (Goldberg et al., 1994). Postabscission (PA), predesiccation (PD), and desiccation (D) programs all occur after ovule abscission and control the synthesis of putative desiccation protectants (Hughes and Galau, 1991). Most seeds are capable of germination shortly after cell division is complete, about one-third of the way through development. Hughes and Galau (1991) concluded that predesiccation-stage embryos are mature before desiccation occurs. Seed quality, however, continues to increase during development. Although not specifically tested, Hughes and Galau (1991) hypothesized that the PA program must be completed before maximum seed vigor could be attained. The PA program is enhanced by ABA and is hypothesized to be terminated by a critical concentration of one or more of its encoded proteins. In *Arabidopsis* (*Arabidopsis thaliana*, L.), the role of ABA in seed maturation was elucidated by creating double mutant seeds (*aba/abi3*) from ABA-deficient (*aba*) and ABA-insen-

sitive (*abi3*) crosses (Koornneef et al., 1989). Recombinant *aba/abi3* mutant seeds apparently fail to complete normal desiccation programs. These seeds display reduced water loss, are intolerant to desiccation, fail to accumulate storage proteins and lose viability upon drying (Koornneef et al., 1989; Meurs et al., 1992). Applying an ABA analog restored protein accumulation and desiccation tolerance in the *aba/abi3* seeds, indicating that ABA is required during the maturation process (Meurs et al., 1992). ABA accumulation patterns consist of either one or two peaks, and in either case, ABA levels decline as the seed matures. As ABA levels decline, accumulation and synthesis of storage reserves decline, and the sensitivity of germination to ABA decreases (Berry and Bewley, 1992; Finkelstein et al., 1985; Xu and Bewley, 1991). Since seed development is based on recognizable patterns of gene expression, it should be possible to correlate molecular processes that control seed development with physiological and/or morphological traits, which would serve as markers to indicate when these programs have been completed and physiological maturity attained. This would provide a biological foundation for scheduling harvests to obtain maximum seed quality.

It has generally been accepted that a physiologically mature seed has reached its maximum viability and vigor and subsequently begins to age and deteriorate (DeLouché, 1980; Powell and Matthews, 1984). Ideally, seeds are harvested after attaining physiological maturity but before deterioration begins. For many seed crops, however, it is difficult to determine when physiological maturity has occurred. Maximum seed dry mass has often served as a convenient measure of 'physiological maturity' or maximum seed quality (Harrington, 1972; Shaw and Loomis, 1950; TeKrony and Hunter, 1995). There is considerable evidence, however, that maximum seed dry mass does not always coincide with maximum viability and vigor. For example, physiological maturity was reached after maximum seed dry mass in bean (*Phaseolus vulgaris* L.) (Sanhewé and Ellis, 1996), broccoli (*Brassica oleracea* L. Group Italica) (Jett and Welbaum, 1996), carrot (*Daucus carota* L.) (Gray et al., 1984), muskmelon (*Cucumis melo* L.) (Welbaum and Bradford, 1988), mustard (*Sinapis alba* L.) (Fischer et al.,

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1988), pearl millet (*Pennisetum americanum* L.) (Kameswara Rao et al., 1991), pepper (*Capsicum annuum* L.) (Demir and Ellis, 1992), sweet corn (*Zea mays* L.) (Wilson and Trawatha, 1991), spring barley (*Hordeum vulgare* L.) (Pieta Filho and Ellis, 1991 a, 1991b), and tomato (*Lycopersicon esculentum* Mill.) (Berry and Bewley, 1991). Other easily identifiable criteria to characterize seed maturity include black layer formation in corn (Daynard and Duncan, 1969; Wilson and Trewatha, 1991) and sorghum [*Sorghum bicolor* (L.) Moench] (Eastin et al., 1973), outer integument color in cotton (*Gossypium hirsutum* L.) (Galau et al., 1987), pod color in soybean [*Glycine max* (L.) Merr.] (Gbikpi and Crookston, 1981), seed moisture content in pea (*Pisum sativum* L.) (Le Deunff and Rachidian, 1988), pod and seed moisture content in lima bean (*Phaseolus lunatus* L.) (Klein and Pollock, 1968) and white lupine (*Lupinus albus* L.) (Clapham and Barnes, 1990), and umbel or seed moisture content in onion (*Allium cepa* L.) (Steiner and Akintobi, 1986). In most cases, the criteria for seed quality have been yield (dry mass) or germination percentage rather than vigor.

Determination of physiological maturity is difficult in species that have indeterminate growth and an extended flowering period. This is true for *Brassica* species due to progressive development within a given raceme and variability among multiple racemes. Early harvest can result in poor seed quality due to immaturity, while delayed harvest may sacrifice yield due to shattering, which can result in seed losses as great as 50% under adverse weather conditions (Macleod, 1981). Assessment of seed quality includes the measurement of viability and vigor, with the latter category being the more sensitive indicator of seed quality (Powell and Matthews, 1984; Ram and Wiesner, 1988; Naylor and Gurm, 1990). In the experiments reported here, we applied hydrotime (Bradford, 1990, 1995; Dahal and Bradford, 1990) and ABA-time (Ni and Bradford, 1992, 1993) models to quantitatively characterize the sensitivity of germination to reduced or elevated concentrations of ABA. These models were used to quantify and describe seed germination patterns and assess seed quality during development in rape seed (*Brassica napus* L.) and red cabbage (*Brassica oleracea* L. Group Capitata). Germination rate and uniformity of germination are important components of seed vigor. The hydrotime and ABA-time models are driven by the rate of germination, which is related to the sensitivity to reduced ψ and increased exogenous ABA, respectively (Bradford, 1990, 1995; Ni and Bradford, 1992). In the case of the hydrotime model, each seed has a base or threshold water potential (ψ_b) which reflects its sensitivity to reduced ψ . Imbibition in solutions which are below (more negative) the ψ_b will prevent germination from occurring; imbibition in solutions above the threshold allows radicle emergence

to proceed, provided no dormancy is present. The ψ_b values are normally distributed, and the median ψ_b value ($\psi_b(50)$) characterizes the sensitivity of the seed lot to reduced ψ . The uniformity of germination is quantified by the variation in the ψ_b values (σ_{ψ_b}) (Bradford, 1990, 1995). High quality seed have low ($\psi_b(50)$) values, a low σ_{ψ_b} value, and high germination percentages at reduced ψ . A parallel explanation is made for the ABA-time model in the Materials and Methods section. ABA contents of the seeds were determined to assess the relationship between physiological maturity and ABA accumulation patterns. A phenotypic description of silique and seed characteristics is also included to provide a visual correlation with the physiological parameters derived from the hydrotime and ABA-time models.

Materials and Methods

PLANT MATERIAL. Seeds of rape (*Brassica napus* L. 'Weststar') were obtained from John Harada (Univ. of California, Davis) and sown in 7.6-L containers containing Redit-grow commercial medium. The plants were grown in a controlled environment at 20 °C and a 16-h photoperiod. The pedicel of each flower was marked with indelible ink on the day of anthesis. Each marking date was uniquely coded so that upon harvesting the siliques could be separated according to age [days after flowering (DAF)]. The seeds were dried in their siliques to 5% moisture content with forced air at 28 °C. Seed moisture content and seed dry mass were determined immediately after harvesting according to ISTA methods (International Seed Testing Association, 1993).

Seeds of red cabbage (*Brassica oleracea* L. Group Capitata) were obtained from hybrid seed production fields in Yolo County (Calif.). The central racemes of individual plants were harvested every 7 d beginning 32 DAF (days after full bloom, in this case) in 1992. There was a 35-d interval from the beginning of bloom to the end of bloom. Full bloom was estimated to have occurred 14 d after the beginning of bloom. For each harvesting date, siliques were counted sequentially into groups of twenty in a basipetal direction. Each group was numbered according to its original position on the raceme, with Group 1 comprising the youngest 20 siliques. Within the central raceme, there was an estimated 30 d difference in flowering dates between the proximal and distal ends at plant maturity. Germination experiments were performed on each age group of the central raceme. Because each group showed similar developmental patterns, only germination data from siliques 21 to 40 (Group 2) will be presented. Moisture content and seed dry mass were determined as above.

GERMINATION TESTS. To determine the sensitivity of germina-

Table 1. Moisture content, silique, and seed characteristics of rape and red cabbage during seed development.

Rape ^z	Red cabbage ^y	Moisture content (%)	Silique characteristics	Seed characteristics
	27	85	Waxy, dark green	Light green, liquid endosperm
21	33	80	Waxy, green moist inside silique	Light green
33	44	60	Light green-yellow, slightly moist	Dark green, shiny, still moist inside, fiber beginning to form along the inside length of silique
36	50	55	Light green-yellow, more fibrous	Dark dull green, not moist inside
42	54	50	Yellow-green, fibrous	Brown and black
	56	40	Yellow, fiber covering entire inside of silique	Black, beginning to wrinkle
	61	10	Yellow-white, brittle, dehiscent	All seeds black

^zDays after flowering.

^yDays after full bloom. See Materials and Methods for further details.

tion to water potential (ψ) or abscisic acid (ABA) during development, seeds were imbibed in distilled water or solutions of polyethylene glycol (PEG-8000) or ABA. Germination rates were averaged from three replications of 50 seeds per replication. Seeds were placed on blotter paper moistened with 4.0 mL distilled water, PEG (-0.25 or -0.5 MPa), or ABA (10 or $100 \mu\text{M}$) in covered 5.0-cm-diameter petri dishes and incubated at 20°C in the dark. Benlate fungicide was added to the solutions at a concentration of 0.2% (w/v). Germination was scored at frequent intervals to obtain complete germination time courses. Time to 50% germination was calculated from probit-transformed germination time courses. To maintain constant ψ and ABA concentrations, seeds, which had not germinated, were transferred to fresh solutions after the first 24 h of imbibition and every 48 h thereafter.

ABA ANALYSIS. ABA was extracted from three replicates of 25 seeds as described previously (Bradford and Chandler, 1992). Extracted ABA was measured by an indirect ELISA (Walker-Simmons, 1987) using monoclonal antibody to ABA (Idetek Inc., San Bruno, Calif.) and ($\pm$) ABA as a standard. Because the monoclonal antibody is specific to ($-$) ABA, the standard curve concentrations were multiplied by 0.5. The procedures to test for sample loss, false positives, and nonspecific interference were as reported by Still et al. (1994).

DATA ANALYSIS. Data were analyzed as described previously (Bradford, 1990, 1995; Dahal and Bradford, 1990; Ni and Bradford, 1992, 1993) by repeated probit analysis using the SAS statistical package. The ψ that just prevents a particular fraction of the seed population from germinating is known as the base water potential (ψ_b), or threshold water potential. Germination rate (inverse of time to radicle emergence) is linearly related to the difference between the ψ and ψ_b of a given seed. Times to germination of fraction g of the seed population (t_g) in solutions of different ψ can be related to a hydrotime constant (θ_H) with units of MPa-h:

$$\theta_H = (\psi - \psi_b(g))t_g \quad [1]$$

where ψ is the water potential of the imbibition medium, $\psi_b(g)$ is the base water potential allowing germination of percentage g , and t_g is the time to germination of percentage g . For a given $\psi_b(g)$, the value of θ_H indicates the progress toward germination (per unit of hydrotime); higher values indicate lower germination rates.

To estimate the distribution of $\psi_b(g)$ within the seed population, a probit regression method was used (Bradford 1990; 1995). This distribution was estimated by solving Eq. [1] for $\psi_b(g)$:

$$\psi_b(g) = \psi - (\theta_H/t_g) \quad [2]$$

The optimal θ_H value was obtained by varying the value of θ_H in repeated probit regressions of probit g versus $\psi - (\theta_H/t_g)$ until the optimal fit was obtained.

Using the repeated probit analysis technique, the median threshold ($\psi_b(50)$) and standard deviation (σ_{ψ_b}) of this distribution were estimated for each sampling date. These parameters indicate the median water potential threshold for germination and the uniformity of threshold values among individual seeds. Lower (more negative) values of $\psi_b(50)$ indicate a greater tolerance of stress and a more rapid germination rate. Smaller values of σ_{ψ_b} indicate a more uniform population, or a shorter time between the first and last seeds to germinate.

Similarly, the response of germination to exogenous ABA can be quantitatively characterized by substituting ABA concentration for ψ (Ni and Bradford, 1992):

$$\theta_{ABA} = (\log [ABA] - \log [ABA_b(g)])t_g \quad [3]$$

where θ_{ABA} is the ABA-time constant, $\log [ABA]$ is the exogenous

ABA concentration, $\log [ABA_b]$ is the log threshold or base ABA concentration inhibiting germination of percentage g . The threshold values ($\log [ABA_b(g)]$) differ among seeds and are normally distributed. The mean $ABA_b(50)$ and the standard deviation (σ_{ABA_b}) were estimated using repeated probit analysis. These parameters indicate the median ABA threshold for the seed population and the uniformity of threshold values among individual seeds. Higher values of $ABA_b(50)$ indicate less sensitivity to inhibition by ABA and a more rapid germination rate. Smaller values of σ_{ABA_b} indicate a more uniform population, or less variation in ABA sensitivity or germination times.

Because the ABA analysis is based on a logarithmic relationship with ABA concentration, data for germination in water ($0 \mu\text{M}$ ABA) cannot be used directly in the calculations (Ni and Bradford, 1992). Therefore, data for seeds germinated in water were modeled utilizing $ABA_b(50)$ and σ_{ABA_b} values generated by analyzing seeds imbibed in 10 and $100 \mu\text{M}$ ABA and inserting a value for $0 \mu\text{M}$ ABA, which fit the experimentally derived germination time course of seeds imbibed in water. This represents the highest ABA concentration that would produce a time course not significantly different from the one obtained in water. Coefficients of determination were calculated using only 10 and $100 \mu\text{M}$ ABA data.

Results

Phenotypic (agronomic) characteristics

Easily discernible phenotypic characteristics of seed and siliques and the representative seed moisture contents (fresh mass basis) of rape and cabbage are summarized in Table 1. The morphological features of the two species were very similar, the only difference being the time scale of development. There was an estimated 30 d difference between the youngest and oldest seeds within the central raceme in cabbage. The large disparity in age within the central raceme was reflected in the moisture contents of the seeds, which was most apparent at 54 DAF, coinciding with ovule abscission (Fig. 1). By 61 DAF, all but the youngest (Group 1) seed had undergone the rapid loss of water associated with post-maturation drying (Fig. 1).

RAPE. Maximum seed dry mass was attained by 33 DAF (Fig. 2A). Moisture content declined from 80% in 21 DAF seeds to about 55% at harvest (Fig. 2B). Seeds were harvested at high moisture content to prevent seed loss due to shattering. Viability increased and t_{50} values decreased after maximum dry mass was attained (Fig. 2C), indicating that seed quality continued to im-

Fig. 1. Moisture content (fresh mass basis) of red cabbage seeds during development. The siliques are numbered from the apex of the rachis at each harvest date (DAF = days after full bloom).

Fig. 2. Seed dry mass (A and D), moisture content, fresh mass basis (B and E), germination percentage, and time to 50% germination (t_{50}) (C and F) for rape (A, B, C) and red cabbage (D, E, F) seeds sampled various days after full flowering. Data points are mean values $\pm$ SE except where the latter are smaller than the symbol. Red cabbage data are from Group 2 siliques.

prove after mass maturity. The t_{50} values were not calculated when germination was $<50\%$.

RED CABBAGE. Each silique group showed similar trends in dry mass accumulation and moisture content during development; therefore, data will be presented from Group 2 siliques only (siliques 21 through 40). Maximum seed dry mass or mass maturity was reached by 54 DAF (Fig. 2D) which was followed by a sharp decrease in moisture content in Group 2 siliques (Fig. 2E). The plants were cut 61 DAF and placed in windrows for 10 d before being threshed. The 68 DAF sample was taken from windrowed plants, 3 d before threshing. Maximum germination percentage was achieved by 54 DAF, while the t_{50} declined from 127 h at 40 DAF to 17 h 54 DAF before increasing to 30 h in 68 DAF seed (Fig. 2F).

Sensitivity to reduced water potential

RAPE. Final germination of water-imbibed seed increased by 20% between 33 and 42 DAF, while t_{50} values decreased from 62 h to 24 h (Fig. 3 A–C). Seeds imbibed in PEG solutions were slower to germinate and final germination percentage was lower compared to water-imbibed seeds. Hydrotime analysis was performed on seed from all harvest dates where final germination in water exceeded 50%. The experimental data are shown as open symbols while the modeled germination time courses are the solid curves. The hydrotime model fit the data well with an average R^2 of 0.95 for experiments shown in Fig. 3 A–C (Table 2). The base water

potential allowing 50% germination ($\psi_b(50)$), is a measure of the sensitivity of germination to decreased water potential. Median ψ_b values decreased by 0.22 MPa between 33 and 37 DAF and remained essentially unchanged in the 42 DAF seed. The uniformity of ψ_b thresholds among seeds of different harvest dates was quantified by the standard deviation of the threshold distribution, or σ_{ψ_b} (Table 2). This value became smaller as seeds matured and can be seen by the progressive narrowing of the ψ_b threshold distribution (Fig. 4 A). The hydrotime constant (θ_H) also decreased during development, reflecting the increasing germination rates (Table 2).

RED CABBAGE. The hydrotime model accounted for about 88% of the variation observed in germination times of cabbage seeds during development (Fig. 3 D–F; Table 2). Seeds harvested 40 DAF were more sensitive to reduced ψ ($\psi_b(50) = -0.85$) compared to 54 DAF ($\psi_b(50) = -0.99$ MPa) or 61 DAF ($\psi_b(50) = -1.31$ MPa) (Fig. 3 D and F; Table 2). The variation in $\psi_b(g)$ values among seeds (σ_{ψ_b}) decreased between 40 and 48 DAF before increasing in 61 DAF seeds [Table 2; Fig. 4 B (for clarity, 61 DAF curve is not shown in this figure)]. This trend is illustrated by the wide $\psi_b(g)$ distribution of the 40 DAF curve and the comparatively narrow 48 and 54 DAF curves (Fig. 4 B). The hydrotime constant (θ_H) declined between 40 and 48 DAF but increased to 41 MPa·h in seed sampled 61 DAF (Table 2).

Fig. 3. Germination time courses of rape (A, B, C) and red cabbage (D, E, F) seeds in water or PEG solutions at different harvest dates. The symbols represent the experimental data, and the curves are the modeled time courses from the hydrotime model using the parameters given in Table 2.

Table 2. Parameters² from the hydrotime model characterizing the sensitivity of rape and red cabbage seeds to reduced water potential during development.

Species	DAF	ψ_b (MPa)	σ_{ψ_b} (MPa)	θ_H (MPa·h)	R^2
Rape	33	-0.84	0.61	41	0.96
	37	-1.06	0.51	38	0.96
	42	-1.04	0.40	32	0.92
Red cabbage	40	-0.85	0.47	58	0.88
	48	-0.84	0.17	23	0.89
	54	-0.99	0.16	24	0.87
	61	-1.31	0.31	41	0.92

²DAF = days after flowering (rape) or days after full bloom (red cabbage); $\psi_b(50)$ = the water potential that prevents 50% of the seed population from germinating; σ_{ψ_b} = the standard deviation of water potential sensitivity among individual seeds; θ_H = the hydrotime constant related to the rate of germination.

Sensitivity to ABA

RAPE. Imbibing seeds in 0, 10 and 100 μM ABA tested the sensitivity of germination to ABA. The ABA-time model accounted for an average of 94% of the variability over all harvest dates (Table 3). The reduction in t_{50} between 33 and 42 DAF was not reflected in the ABA-time constant (θ_{ABA}) (Table 3). As rape seeds matured, their sensitivity to ABA decreased (Fig. 5 A–C; Table 3). At 33 DAF a concentration of 16 μM ABA would prevent 50% of the seeds from germinating while seeds harvested 42 DAF required 178 μM ABA (Fig. 5 A and C; Table 3). Germination uniformity improved in seeds harvested later as indicated by the decrease in θ_{ABA} values, and is graphically illustrated by a narrower base of the 42 DAF curve versus the 33 DAF curve (Fig. 6 A; Table 3).

RED CABBAGE. The trends in sensitivity to ABA were similar to those observed for rape. ABA inhibited germination rates and final germination, especially at the highest concentration (Fig. 5 D–F). The model fit the data well for 48 and 54 DAF sampling dates with an average coefficient of determination of 0.92 (Table 3). The low R^2 value reported in seed harvested 40 DAF was due to the virtual inhibition of germination in 100 μM ABA (Table 3; Fig. 5A). Nonetheless, the modeled parameters matched the germination patterns well (Fig. 5 D). Sensitivity to ABA, as quantified by $ABA_b(50)$ values, decreased as the seeds matured and seeds harvested 54 DAF displayed the lowest sensitivity (Figs. 5 D–F and 6B; Table 3). The variation in ABA sensitivity within the population of seeds from each harvest declined as seeds matured (Fig. 6B; Table 3). Seed harvested 61 and 68 DAF had $ABA_b(50)$ and σ_{ABA_b} values similar to 54 DAF seed (data not shown).

ABA CONTENT. Although the developmental period was longer for cabbage than for rape, the pattern of increase in ABA content followed by a decrease was consistent for both species (Fig. 7). The seed ABA contents were essentially identical at similar developmental stages for both species.

Summary of developmental indices

The times of maximum seed quality, as determined by maximum germination percentage, germination rate and parameters derived from the hydrotime and ABA-time models, are summarized in Table 4. Physiological maturity, or maximum seed quality, was attained in rape four to nine days after maximum seed dry mass. In red cabbage, maximum seed quality was attained at, or seven days later than, maximum seed dry mass.

Discussion

The overall pattern of seed development was similar between

rape and red cabbage. In both species, germination capacity and rate increased progressively during development and was maximal after maximum seed dry mass was attained. Both species showed high $\psi_b(50)$ values early in development followed by low values at late maturation, indicating an increasing tolerance to reduced ψ during maturation. As seeds matured, a trend toward more rapid and uniform germination was observed in PEG- or ABA- imbibed seeds in both species. Under the controlled environment conditions employed during these studies, ‘Weststar’ had a seed-to-seed time of ≈ 90 d, while other rapid cycling Brassicas typically produce seed in 45 to 60 d under similar conditions. Red cabbage has a much longer life cycle, ≈ 270 d, because of the vernalization requirement needed for flowering. Results from these experiments demonstrate the similarity in seed development between rapid cycling Brassicas grown in controlled environment chambers with field grown red cabbage. This suggests that rapid cycling Brassicas may be used as a model system to answer fundamental questions concerning seed development and quality that are applicable to field-grown Brassicas with longer life cycles.

ABA regulates seed development by suppressing germination early in development (Finkelstein et al., 1985), stimulating accumulation of storage reserves (Finkelstein et al., 1985), imposing dormancy (Hilhorst and Karssen, 1992), and determining the sensitivity of germination to ψ (Ni and Bradford, 1993). Endogenous ABA levels in rape peaked before seed desiccation began as indicated by seed fresh mass loss (Figs. 1 and 2 B and E), in agreement with data of Finkelstein et al. (1985). Sensitivity of germination to exogenous ABA decreased in both rape and red cabbage as seeds matured (Figs. 5 and 6; Table 3). Reduction in

Fig. 4. Distribution of base water potentials (ψ_b) within rape (A) and red cabbage (B) seed populations at various days after flowering (rape) or full flowering (red cabbage).

Table 3. Parameters² from the ABA-time model characterizing the sensitivity of rape and red cabbage seeds to ABA during development.

Species	DAF	ABA ₀ log[M]	ABA _b (50) log [M]	σ _{ABA_b} log [M]	θ _{ABA} log [M]·h	R ²
Rape	33	-5.62	-4.80	-1.15	-45	0.90
	37	-5.58	-4.00	-0.63	-47	0.97
	42	-5.69	-3.75	-0.78	-49	0.94
Red cabbage	40	-5.77	-5.54	-0.58	-25	0.47
	48	-5.65	-4.49	-0.44	-41	0.89
	54	-5.80	-4.36	-0.43	-30	0.95

²DAF = days after flowering (rape) or after full bloom (red cabbage); ABA₀ = the highest ABA concentration not affecting germination (used to model time courses in water); ABA_b(50) = the median ABA concentration required to prevent radicle emergence of 50% of the population; σ_{ABA_b} = the standard deviation of ABA sensitivity among individual seeds; θ_{ABA} = the ABA-time constant related to the rate of germination.

Fig. 5. Germination time courses of rape (A, B, C) and red cabbage (D, E, F) seeds in water or ABA solutions at different harvest dates. The symbols represent the experimental data, and the curves are the modeled time courses from the ABA-time model using the parameters given in Table 3.

sensitivity of germination to ABA during maturation has been reported in alfalfa (*Medicago sativa* L.) (Xu and Bewley, 1991), muskmelon (Welbaum et al., 1990), rape (Finkelstein et al., 1985), sorghum (Benech Arnold et al., 1991), soybean (Eisenberg and Mascarenhas, 1985), tomato (Berry and Bewley, 1992) and wheat (*Triticum aestivum* L.) (Walker-Simmons, 1987). The ABA-time model measures the sensitivity of germination to exogenous ABA and the variation in sensitivity among seeds. The analysis was made with the assumption that all seeds had the propensity to germinate at all sampling dates. Using this analysis there was an 11-fold increase in ABA_b(50) from 33 DAF to 42 DAF in rape

versus a 15-fold increase from 40 to 54 DAF in red cabbage (Table 3). Alternatively, the data may be analyzed by including only the population of seeds that germinated, which at earlier harvest dates was <100%. Using this analysis, the difference in ABA_b(50) between rape harvested 33 and 42 DAF was 146 μM (5.6-fold difference) while a difference of only 37 μM (6-fold) was evident between 40 and 54 DAF in cabbage (data not shown). By either analysis, the data indicate seeds, which are able to withstand desiccation and germinate at early developmental stages, are more sensitive to ABA than seeds which are developmentally older. Endogenous ABA content varied 4.5-fold or 6.5-fold between minimum and maximum values in rape and red cabbage, respectively (Fig. 7). The magnitude of the sensitivity of germination to ABA as measured by ABA_b(50) values was similar to the magnitude of the difference in endogenous ABA levels, so the reduction in sensitivity of germination to exogenous ABA may be related to the decline in endogenous ABA levels.

In these experiments, maximum seed quality was defined by a number of indices that were derived from the hydrotimic and ABA-time models in addition to standard attributes such as maximum germination percentage and t₅₀ values (Table 4). Seed quality in both rape and red cabbage was attained after maximum seed dry mass (Fig. 2; Table 4). High quality seed have low t₅₀ values, uniform germination (low σ_{ψ_b} or low σ_{ABA_b}), and high tolerance to abiotic stress factors (low ψ_b(50) or high ABA_b(50) values). The vigor indices for rape were optimal four to nine days after maximum seed dry mass, while in red cabbage the optimum values were attained at or after maximum seed dry mass was attained for all indices (Table 4). These data support the notion that maximum seed quality continues to develop after ovule abscission, indirectly supporting the hypothesis of Galau et al. (1991) and Sanhewe and

Fig. 6. Distribution of log ABA_b values for rape (A) and red cabbage (B) seeds harvested at different harvest dates.

Table 4. Summary of developmental indices associated with maturation and quality of rape and red cabbage seeds.

Seed quality index	Rape ^z	Red cabbage ^y
Maximum dry mass	33	54
Maximum germination (%)	42	54
Most rapid germination	42	54
Lowest $\psi_b(50)$	37	61
Lowest σ_{ψ_b}	42	54
Highest ABA _b (50)	42	54
Lowest σ_{ABA_b}	37	54

^zDays after flowering.

^yDays after full bloom. See Materials and Methods for further details.

Ellis (1996) that postabscission programs must be completed before optimum seed quality is attained. Alternatively, the continued improvement in seed quality after ovule abscission could be due to a rapid loss of dormancy, or an after-ripening effect. When the $\psi_b(g)$ distribution overlaps 0 MPa, that fraction of the seed population for which $\psi_b > 0$ MPa will be prevented from germinating in water, which is a functional definition of dormancy (Bradford, 1995). Rape seed harvested 33 and 37 DAF have approximately 25 and 13%, respectively, of the population with a $\psi_b > 0$ MPa (Fig 4). It is likely the nongerminating fraction of rape seed were desiccation intolerant, rather than dormant, as this seed had approximately the same germination percentages and t_{50} values one year after harvesting. In red cabbage seed harvested 48 DAF or later, the fraction of seed that did not germinate in water was never greater than 2%, indicating that dormancy was not present. The magnitude of changes observed in germination rate (t_{50} values) were much greater than those observed in germination percentage of red cabbage seed harvested between 48 and 68 DAF, indicating that physiological changes occurred after ovule abscission. In these experiments, optimum seed quality was attained just after siliques started to turn yellow and the seeds turn from green to brown (Table 1). Physiological maturity developed at, or after, ovule abscission but well before harvest maturity, which occurred 17 d after physiological maturity. The high moisture content of the siliques may preclude mechanical harvesting at this time, but our data indicate that seed quality never improved thereafter and in fact declined slightly at later harvest dates.

In summary, we found that the hydrotime and ABA-time models accurately predicted germination time courses of seed

imbibed at a range of water potentials and ABA concentrations. Parameters derived from these models indicated that physiological maturity developed at, or after, ovule abscission in both species, and harvest maturity occurred after physiological maturity. During maturation, the $\psi_b(g)$ decreased while the ABA_b(g) distribution increased. The physiological and molecular basis for this developmental progression is not known, but future experiments will address these issues.

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Fig. 7. Endogenous ABA content of rape (A) and red cabbage (B) during seed development. Data are mean values $\pm$ SE except where the latter is smaller than the symbol.

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