The Development of Seed Quality in Brassicas

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ADDITIONAL INDEX WORDS. *Brassica napus*, rapeseed, *Brassica oleracea*, red cabbage, brussels sprouts, broccoli, hydrotime, controlled deterioration, seed aging

SUMMARY. Brassica crops have indeterminate growth and flower over an extended period of time. Harvested seed is therefore comprised of seed of varying degrees of physiological maturity and quality. Using population-based threshold models, broccoli (*Brassica oleracea* L. Group Italica), brussels sprouts (*B. oleracea* L. Group Gemmifera), red cabbage (*B. oleracea* L. Group Capitata), and rapeseed (*B. napus* L.) were characterized during seed development with respect to sensitivity to abiotic stress (reduced water potential) and shelf life. Using these models our data suggests that the physiological patterns of seed development are the same in all brassica crops we have tested to date. These population-based models can be used to provide a biological basis in which to evaluate cultural, postharvest and storage practices to ensure the production and maintenance of seed vigor.

The production of high quality seed and the maintenance of seed vigor are goals of every seed producer. Seed quality is a term with many possible definitions and numerous techniques have been used to measure it. A high quality seed lot is characterized by the ability to germinate rapidly and uniformly, to resist biotic and abiotic stress and deterioration. Other attributes of seed quality may be considered, but this definition is sufficiently generic as to apply to all seed used for agronomic and horticultural purposes.

Seed quality increases during development, reaches a maximum, and then declines while in storage. Seed development of orthodox species is well defined and can be broken into three distinct phases: 1) cell differentiation and division followed by expansion; 2) accumulation of dry weight by starch, lipid and protein synthesis; and 3) maturation, including development of desiccation tolerance. Biologically, an orthodox seed may be considered physiologically mature once the seed has undergone maturation drying and can withstand desiccation. Although seeds are capable of germination following cell division, the attainment of the characteristics associated with seed quality occur during the second and third phases (Finkelstein and Crouch, 1984; Hughes and Galau, 1991).

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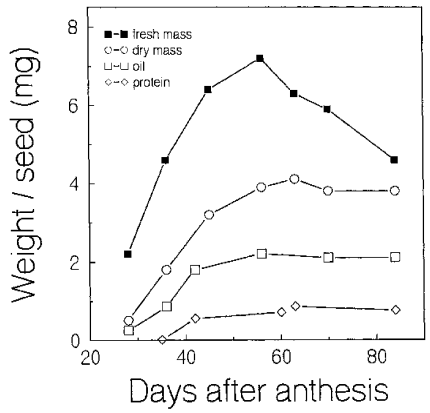


Fig. 1. Generalized pattern of brassica seed development. Reserve accumulation patterns are similar for most species and cultivars of brassicas although the length of development will differ as will the relative composition of the storage reserves. Soluble carbohydrates (not shown) are generally <2% of the seed dry mass. Abscisic acid accumulation patterns for rapeseed and red cabbage are shown in Still and Bradford, 1998. (Curves are based on data from Murphy and Cummins, 1989).

With agronomic and horticultural seeds, physiological maturity must also encompass vigor as well as viability. Thus, a seed that has reached its maximum viability and vigor is defined as being physiologically mature. Mass maturity is the point of maximum seed dry weight; physiological maturity occurs at or after mass maturity in brassicas and many other species (Still and Bradford, 1998). Harvest maturity can be defined as the point at which seed moisture content is sufficiently low so as not to incur mechanical damage during harvest.

The environment in which a seed matures while on the mother plant has a significant effect on seed germination characteristics and environmental aftereffects can be manifested in the progeny (Stoehr et al., 1998; Wulff, 1995). Surprisingly little is known about the physiological, biochemical, genetic and molecular mechanisms contributing to seed quality traits. This is due to the strong environmental influence on seed development and the fact that these traits are likely quantitative. By quantifying specific components of seed quality it is possible to investigate its underlying biological basis. Brassicas are an ideal model system in which to study seed quality because seed at several different stages of maturity can be harvested at any given time. Seed developmental patterns are similar among brassicas making it possible to utilize rapid cycling brassicas as a model system (*B. napus* cultivars; Still and Bradford, 1998). Although *Arabidopsis* (*A. thaliana* L.) is the model of choice for molecular studies, their small seed size hampers seed quality experiments whereas the relatively large seed size of *B. napus* facilitates experimental procedures. In this paper data is presented in which seed quality of several *Brassica* species is quantified in terms of germination rate, sensitivity to reduced water potential using hydrotime models (Bradford, 1990, 1995) and controlled deterioration studies. Using specific examples it is shown that brassica seeds have similar physiological developmental stages that can be quantified using population based models.

General pattern of seed development in brassica species

As brassica seeds develop, a rapid increase in fresh mass is observed concomitant with an increase in dry mass (Fig. 1). Dry weight accumulation is typically represented by a sigmoid pattern in *Brassica* species (Fischer et al., 1988; Murphy and Cummins, 1989; Norton and Harris, 1975; Still and Bradford, 1998). In rapeseed and cabbage, storage reserves are mainly in the form of oil (54% and 70% of total seed dry weight, respectively) and protein (20% and 30% of the total dry weight, respectively) (Murphy and Cummins, 1989; Qouta et al., 1991). Protein is the major seed reserve utilized after radicle emergence in cabbage and mustard (*Sinapis alba* L.) followed by lipids after proteins have been depleted (Gould and

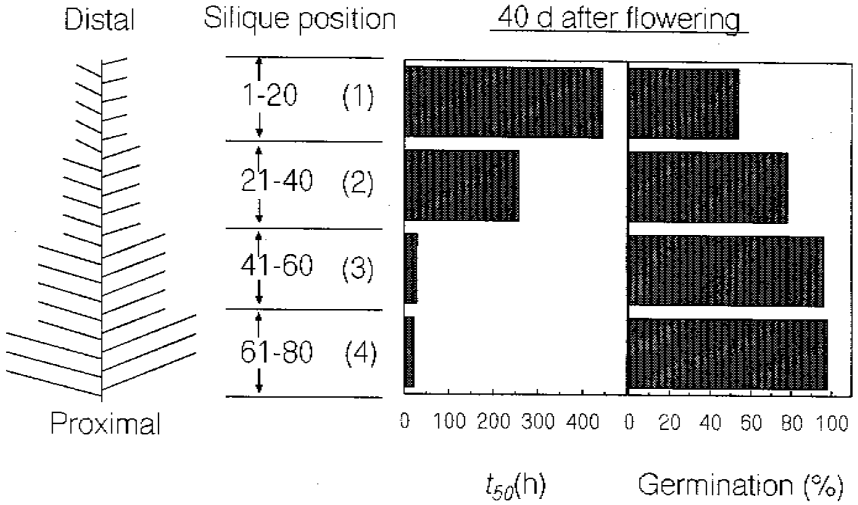


Fig. 2. Seed vigor characteristics of brussels sprouts harvested from central racemes 40 d after full flowering. Siliques were counted sequentially into groups of twenty in a basipetal direction. Each group was numbered according to its original position on the raceme, with Group 1 comprising the youngest seed. Time to 50% germination (t50) and final germination in water are given for each silique group (D.W. Still and K.J. Bradford, unpublished data).

Rees, 1965; Qouta et al., 1991). Ovule abscission marks the end of reserve deposition, which typically occurs at ≈50% seed moisture content (Murphy and Cummins, 1989; Still and Bradford, 1998). Abscisic acid is required for normal seed development and apparently has a role in desiccation tolerance and stimulating storage reserve accumulation (Finkelstein et al., 1985; Koornneef et al., 1989; Meurs et al., 1992). ABA accumulates during the dry matter accumulation phase and declines as the water content of the seed drops (Finkelstein et al., 1985; Still and Bradford, 1988). The seed then enters the postabscission phase during which time further physiological development occurs.

Brassicas have indeterminate growth and flower over an extended period. In broccoli, red cabbage and brussels sprouts there is a 20 to 30 d difference in flowering within the central raceme (Still and Bradford, 1998). The numerous lateral racemes are shorter in length than the central raceme and therefore flowering occurs over a briefer time span. The result is a plant comprised of seed at various stages of physiological maturity. A central raceme in brussels sprouts may contain up to 80 siliques (Fig. 2). Seeds harvested from the distal end have higher moisture contents compared to seed located on the proximal end of the central raceme (Still and Bradford, 1998). The range in physiological development in seed may be observed on single racemes taken from brussels sprouts plants harvested 40 d after flowering (DAF) from hybrid seed production fields in Yolo County, California in 1992 (Fig. 2). In immature seed (Group 1, siliques 1 to 20), the time to 50% germination (t_{50}) was >400 h and only 54% of the seed were able to withstand desiccation at this stage (Fig. 2). Seed from the proximal end of the raceme (Groups 3 and 4) were 20 to 30 d older than Group 1 seed; germination was near 100% and t_{50} values were ≈15 h (Fig. 2). The same general patterns of development were observed in Group 2 seed (siliques 21 to 40) harvested from field-grown brussels sprouts harvested 33, 40, 48, and 54 DAF (Fig. 3). Germination rate and viability increased as physiological maturity was attained. A decrease in germination rate and viability was noted in 54 DAF seed and is indicative of seed deterioration (Fig. 3). Physiological maturity occurred at 48 DAF and seed quality began to decline thereafter, as suggested by the slower germination rate and viability of the seed harvested 54 DAF (Fig. 3). A general pattern of increased physiological maturity followed by a slight decline by harvest maturity has been observed in red cabbage and broccoli seed (Still and Bradford, 1998; D.W. Still, unpublished data). The data shown in Figs. 2 and 3 are typical of the compromises brassica seed producers make when harvesting plants comprised of mixed maturity. Brassica seeds develop in siliques that upon maturity dehisce along the length of the silique due to the degradation of the middle lamella. Delaying harvest is not a viable alternative because of the risk of losing the seed due to shattering. It may be possible to manipulate, via molecular bioengineering, the timing or capacity of dehiscence to delay harvest until all seeds are physiologically mature (Coupe et al., 1993, 1994; Jenkins et al., 1996).

Measurement of seed quality

The objective of any measurement of seed quality is to determine the planting value of the seed. The standard germination test for viability is the most widely used test of seed quality, but it does not give any indication of seed vigor. Vigor has been defined as the sum of those properties that determine the potential for rapid, uniform emergence and the development of normal seedlings under a wide range of field or greenhouse conditions (Association of Official Seed Analysts, 1983). It has long been known that the standard germination test is sometimes a poor indicator of field emergence (Johnson and Wax, 1978; Perry, 1969; TeKrony and Egli, 1977; Yaklich and Kulik, 1979). Laboratory tests cannot accurately predict field emergence because it is impossible to mimic the edaphic,

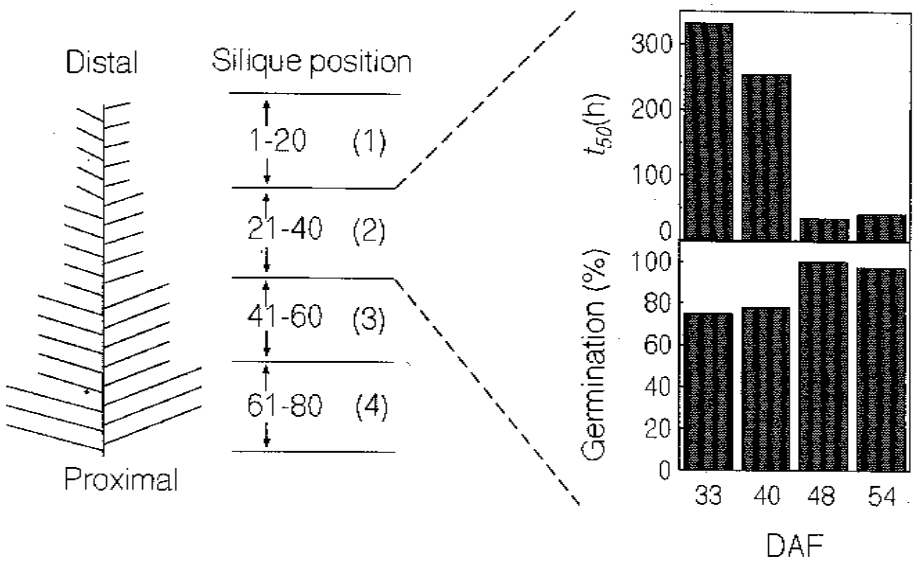


Fig. 3. Development of seed quality during maturation of brussels sprouts harvested 33, 40, 48, and 54 d after flowering (DAF). Seed vigor was measured by the time to 50% germination (t_{50}) and final germination in water of seed harvested from Group 2 siliques as described in Fig. 2 (D.W. Still and K.J. Bradford, unpublished data).

environmental and biotic conditions of the field. Instead, most growers and seed producers utilize field emergence tests to assess the planting value of the seed. Aside from being time-consuming, costly, and labor-intensive, field emergence tests often give conflicting results because different planting dates and fields create different environmental conditions at each planting. In other words, field emergence tests are not, and cannot be standardized and as such cannot unequivocally assess the physiological status of the seed. A more reasonable approach is to concede that all the variables that influence germination behavior in the field cannot be accounted for and recreated in the laboratory and no laboratory test can absolutely predict field emergence. Instead, we should focus on developing methods that quantify the physiological status of the seed. Methods that accurately measure a particular component of seed vigor should be developed. Because germination is a complex physiological process it is unlikely any one test can be developed that will accurately assess all aspects of seed vigor.

Clearly, seed vigor is a quantitative trait and germination requires the coordinated expression of many genes and their gene products. It is during the second phase of water uptake that the coordinated events that lead to radicle emergence occur. Upon rehydration one could envision that compared to a low vigor seed, a highly vigorous seed would have more of the necessary biomechanical and biochemical components intact. Compared to a nonvigorous seed, a highly vigorous seed would be expected to have a higher degree of tolerance to abiotic stress. Seed lots are composed of individual seeds, each of which has a unique threshold sensitivity to stress factors such as a reduced osmotic potential and growth regulators. Any assessment of seed quality should quantify the variation of the measured variable among individuals within a seed lot. Because the sensitivity to stress manifests itself through the germination rate, the sensitivity to that stress can be quantified. Germination kinetics in which an extended lag phase is observed before radicle emergence occurs could be evidence of extensive repair processes in a low vigor seed. Thus, it is not necessary to know which process (or processes) has (have) been affected by the stress, but the germination kinetics provided by such a test is evidence that this has occurred. Because the expression of seed vigor components such as seed longevity and sensitivity to abiotic factors is normally distributed within a seed population (Ellis and Roberts, 1980a; Bradford, 1990; Dahal and Bradford, 1990; Gummerson, 1986), it is possible to characterize that natural variation using parametric statistics. This approach can provide the foundation in which to study the biological basis of seed vigor while its immediate application provides a method to quantify seed quality (Still and Bradford, 1998).

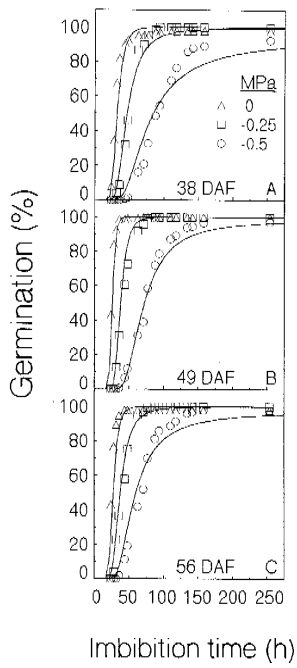


Fig. 4. Germination time courses of broccoli seeds in water or polyethylene glycol solutions from broccoli seeds harvested 38 (A), 49 (B), and 56 (C) days after full flowering (DAF). The symbols represent the experimental data, and the curves are the modeled time courses from the hydrotime model (D.W. Still, unpublished data).

Hydrotime tests

By imbibing seeds in a series of progressively lower water potential solutions and obtaining germination time courses, the threshold sensitivity distribution to reduced water potential (ψ_b) may be obtained (Bradford, 1990, 1995; Dahal and Bradford, 1990; Still and Bradford, 1998). Every seed differs in its sensitivity to stress, which in this case is an osmotic stress. The sensitivity is due to natural physiological variation and is likely to have a genetic component as well. All seeds within a population do not germinate simultaneously in water and under an osmotic stress the differences in time to germination are exacerbated. The relative difference in germination times under stress is the basis by which discrimination is made between seed populations of different physiological maturity or quality using hydrotime analysis. The hydrotime characteristics of a seed lot are characterized by three variables: θ_H, the hydrotime constant that is related to the speed of germination, $\psi_b(50)$, the water potential that prevents 50% of the seed population from germinating and $\sigma\psi_b$, the standard deviation of the $\psi_b(g)$ distribution. The reader is referred to Bradford (1990) for the theoretical basis of hydrotime, Bradford (1995) for further development and discussion of hydrotime, and Still and Bradford (1998) for the application of hydrotime and other population-based models to quantify seed quality in brassicas during development.

We have used hydrotime to evaluate the sensitivity of germination to reduced water potential. Broccoli seeds were harvested 38, 49, and 56 DAF from a hybrid seed production field in Yuma County, Ariz. The seeds were imbibed in polyethylene glycol (PEG 8000) solutions at 0, –0.25, and –0.5 MPa (–2.5 and –5.0 bars) at 20 °C (68 °F) in the dark and germination time courses were obtained (Fig. 4, D.W. Still, unpublished data). The experimental data are represented by symbols while the predicted time courses from the hydrotime model are represented by the solid lines. Osmotic stress reduced the germination rate of seeds from all three harvests (Fig. 4). Germina-

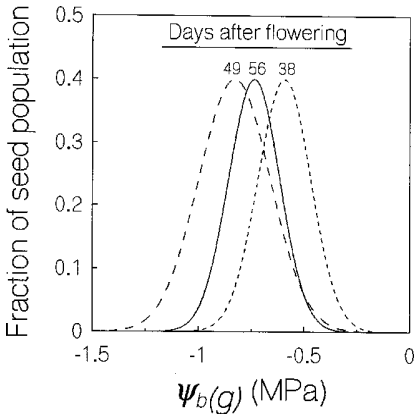


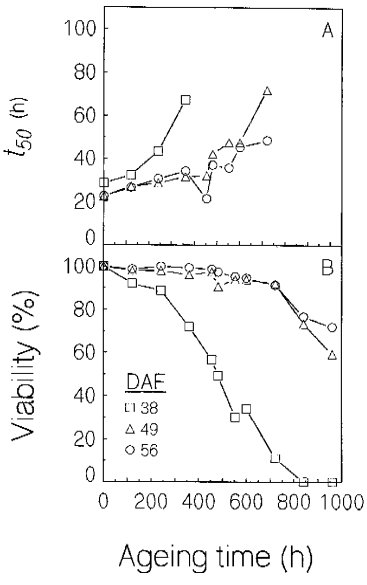
Fig. 5. Distribution of base water potentials $\psi_b(g)$ of broccoli seed populations harvested at various days after full flowering. (1 MPa = 10 bars) (D.W. Still, unpublished data).

tion percentage was slightly reduced at the lowest water potential in the youngest seed (38 DAF, Fig. 4A), but was not affected in seed harvested 49 and 56 DAF (Fig. 4B and C). As broccoli seeds matured, germination sensitivity to osmotic stress decreased and this is graphically illustrated by the distribution of $\psi_b(g)$ values (Fig. 5). Seeds harvested 38 DAF had a $\psi_b(50)$ value of –0.59 Mpa (–5.9 bars), decreased to –0.83 MPa (–8.3 bars) at 49 DAF and shifted up to –0.74 MPa (–7.4 bars) by 56 DAF (Fig. 5). This pattern of decreased osmotic sensitivity followed by a slight increase in sensitivity has also been observed in rapeseed and red cabbage (Still and Bradford, 1998) and brussels sprouts (D.W. Still and K.J. Bradford, unpublished data). Generally, as brassica seeds mature, $\sigma\psi_b$ values decline, which is another indication of physiological maturity (Still and Bradford, 1998). Conversely, as seeds deteriorate, germination uniformity may worsen ($\sigma\psi_b$ values increase) and germination rates decrease. This has been observed to occur while the seed is still attached to the mother plant as moisture content declines and harvest maturity is attained (Still and Bradford, 1998).

A key component of seed vigor is longevity, or the potential storage life of a seed. Vigor has been shown to decline before viability in a number of species (Dell'Aquila and Tritto, 1990; Ferguson et al., 1990; Naylor and Gurmu, 1990; Powell and Matthews, 1984; Ram and Wiesner, 1988). Unfortunately it is not possible to detect *a priori* a dead seed from a live seed, nor a vigorous seed from a low vigor seed. Inferences, therefore, must be made based upon population sampling. Ellis and Roberts (1980b) stated that "seed lots that meet industry standards for percent germination might vary widely in seed quality because a difference in just a few percentage points translates to a very large difference in seed deterioration." Our data from broccoli seeds harvested at different stages of maturity concur with this principle (Fig. 6, D.W. Still, unpublished data). Broccoli seeds were subjected to controlled deterioration by incubation at an elevated temperature [40 °C (104 °F)] and seed moisture content (11%, fresh weight basis). The seeds were withdrawn periodically from the controlled deterioration test and germination time courses in water were obtained. Seed harvested 38 DAF had slightly higher t_{50} values at 0 ageing hours when compared to seed harvested 49 and 56 DAF (Fig. 6A). The more rapid ageing of 38 DAF compared to 49 and 56 DAF seed was demonstrated by the higher t_{50} values of the 38 DAF at each ageing period (Fig. 6A) and after 450 h of ageing the t_{50} values were greater than 100 h (data not shown). Similarly, 38 DAF seed lost viability more rapidly compared to 49 and 56 DAF seed (Fig. 6B). A comparison between vigor, as measured by t_{50} values, and viability shows that vigor was a more sensitive indicator of the physiological status of the seed than was viability. Viability of 49 and 56 DAF seed remained essentially unchanged after 360 h of ageing (Fig. 6B) while t_{50} values increased by 33% during this same time frame (Fig. 6A). The seed harvested 38 DAF was capable of withstanding desiccation but it is clear that continued physiological development occurred between 38 and 49 DAF. After further ageing a difference was observed between the 49 and 56 DAF seed in t_{50} values (720 h, Fig. 6A) and viability (960 h, Fig. 6B), another indication of continued development after attaining mass maturity and desiccation tolerance.

Brassica seeds have similar physiological development patterns as demonstrated by their hydrotime characteristics during development. Because of the extended flowering time the harvested seeds will be comprised of seeds of different maturity. Immature seeds that are capable of withstanding desiccation and have full viability, seed size and mass, make it difficult to discriminate from those that are physiological mature. Under such a circumstance, population-based vigor testing is the only method available to detect these seeds. It is clear from hydrotime analysis and controlled deterioration studies that physiological development continues after ovule abscission (Figs. 4–6). To ensure maximum vigor, environmental conditions should be conducive to successfully complete postabscission programs that appear to contribute to seed vigor. Hydrotime and other population-based models can be used to quantify specific components of seed quality as well as provide insight into its biological basis.

Fig. 6. Time to 50% germination (t_{50}) (A) and viability (B) of broccoli seed harvested various days after full flowering (DAF). Ageing time refers to the number of hours seeds were subjected to controlled deterioration conditions of 40 °C (104 °F) at a seed moisture content of 11%. The seeds were removed and germination time courses performed for each ageing duration (D.W. Still, unpublished data).



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