1 Seed Storage, Germination, Quality, and Enhancements

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Before vegetables are harvested, before their growth and development, even before the seedling becomes photosynthetically competent, the source of the vegetable—the seed—holds major keys to final product yield. To fully comprehend the complexities of vegetable crop growth and development, we must understand seed physiology related to storage, germination, quality, and enhancements. The challenge is to present an in-depth knowledge of vegetable seeds that covers considerable diversity with respect to botanical classification, seed size, and composition. This chapter and book encompasses 33 common vegetable crop seeds from ten plant families (Table 1.1). This diverse group of plants makes a comprehensive overview of vegetable seeds difficult to achieve, especially since there is little scientific literature on seed physiology of many small-seeded crops of minor economic importance. Due to these limitations, we enlist several approaches to develop a coherent picture of vegetable seeds. Relative differences between seeds are shown with regard to seed size and composition, storage longevity, seed and seedling morphology, temperature requirements for germination, and the use of reserve materials during emergence. More generalized information is presented on factors and events associated with storage, germination, and aging. Finally, the process of aging and other aspects of seed physiology and technology are illustrated using specific crop examples.

The focus of this chapter is on postharvest aspects of seeds; the period of seed development and production stages are not addressed. We start with storage of seeds with low moisture content as most vegetable crop seeds have unique characteristics that permit them to withstand desiccation (Leopold and Vertucci, 1986). Desiccation tolerance is essential for long-term survival and allows for a time interval between seed production and crop production. Most vegetable seeds imbibe water readily and, provided with a suitable environment, will germinate and resume active growth. The seed uses its reserve materials following germination and then becomes an active photosynthetic seedling, fixing its own carbon and producing energy.

Seed quality is a broad term and encompasses several attributes of seeds including the germination and seedling performance. In this chapter, certain physiological and biochemical processes associated with loss of seed quality are described, and symptoms of seed aging are presented at physiological and whole-plant levels. The sowing environment has a direct effect on germination and stand establishment, and under severe stress, seedling performance is seriously impaired. To ensure stand establishment, high quality seeds are needed for transplant production and for direct seeding. Seed performance is improved by seed enhancements to achieve maximum emergence when sown in suboptimal conditions. In addition, seed-coating technologies are used to improve

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Table 1.1. Botanical and common names (Maynard and Hochmuth, 2007). Thousand seed weight (TSW), percent oil and protein content from representative seed samples (Royal Botanic Gardens Kew, 2017, except where noted).

Class, family, *genus*, *species*	Common name	TSW (g)	Oil (%)	Protein (%)
Monocotyledons				
Alliaceae (onion family)				
Allium ampeloprasum L. *Porrum group*	Leek	2.26	15‡	27‡
Allium cepa L. *Cepa group*	Onion	3.32	19	–
Liliaceae (lily family)				
Asparagus officinalis L.	Asparagus	24.1	15‡	16‡
Poaceae (grass family)				
Zea mays L. subsp. *Mays*	Corn, sweet	227	6‡	12‡
Diocotyledons				
Apiaceae (carrot family)				
Apium graveolens L. var. *dulce* (Mil.) Pers.	Celery	0.30	29	19
Daucus carota L. subsp. *Sativus* (Hoffm.) Arcang.	Carrot	1.00	19	24
Pastinaca sativa L.	Parsnip	3.00	33	19
Petroselinum crispum (Mill.) Nym. Var. *crispum*	Parsley	1.70	27	20
Asteraceae (sunflower family)				
Lactuca sativa L. *var. capitala* L.	Lettuce	1.00	38	29
Brassicaceae (mustard family)				
Brassica napus L. var. *napobrassica* (L.) Reichb.	Rutabaga	3.30	42	25
Brassica oleracea L. var. *acephala* DC.	Kale, collards	2.30	26	34
Brassica oleracea L. var. *botrytis* L.	Cauliflower	3.15	32**	–
Brassica oleracea L. var. *capitata* L.	Cabbage	3.15	35**	28‡
Brassica oleracea L. var. *gemmifera* Zenk.	Brussels sprouts	3.15	34**	–
Brassica oleracea L. var. *italica* Plenck.	Broccoli	3.15	31**	–
Brassica rapa L. var. (DC.) Metzg. *Rapa*	Turnip	2.10	39	25
Raphanus sativus L. *Radicula group*	Radish	19.0	41	31
Chenopodiaceae (goosefoot family)				
Beta vulgaris L. *Crassa group*	Beet, garden	12.9	5.1	13
Spinacia oleracea L.	Spinach	6.80	5.3	20
Cucurbitaceae (gourd family)				
Citrullus lanatus (Thunb.) Matsum & Nakai	Watermelon	83.0	20	18
Cucumis melo L. Reticulatus *group*	Muskmelon	28.8	36	36
Cucumis sativus L.	Cucumber	16.3	32	28
Cucurbita maxima Duchesne	Pumpkin, w. squash	241	48	39
Cucurbita moschata Duchesne	Squash, butternut	88	48	40
Cucurbita pepo L.	Squash, summer	145	47	39
Fabaceae (pea family)				
Phaseolus coccineus L.	Bean, runner	1066	2.0	24
Phaseolus lunatus L.	Bean, lima	456	1.0	24
Phaseolus vulgaris L.	Bean, snap bean	307	1.1	28
Pisum sativum L. ssp. *Sativum*	Pea, garden	168	1.2	24
Solanaceae (nightshade family)				
Capsicum annuum L. *Grossum group*	Pepper, bell	7.09	28†	–
Capsicum frutescens L.	Pepper, tabasco	5.00	20	18
Solanum lycopersicon Mill. *(formerly Lycopersicon esculentum* Mill.*)*	Tomato	1.97	20	–
Solanum melongena L.	Eggplant	3.50	27*	–

*Kaymak, 2014.
†Jarret *et al*., 2013.
‡Taylor, 1997.
**West *et al*., 2004.

precision placement of seeds for sowing, and to provide a delivery system for compounds and agents to both protect and enhance seed and plant performance.

Composition and Water Status in Seeds

The composition of seeds is important to many aspects of seed physiology, and the percent oil or lipid content, and percent protein, are provided for the 33 common vegetable crop seeds in Table 1.1. Seed size expressed as thousand seed weight (TSW) was calculated; small-seeded vegetables have a TSW of < 10 g, while large-seeded vegetable crops have TSW > 100 g. Intermediate or medium-sized seeds range from 10 to 100 g TSW. The relationship of TSW and percent seed oil content is shown in Fig. 1.1.

There are two groups of seeds based on their storage reserves: starch and lipid storing. Seeds with less than 10% oil are starch-storing seeds, and the starch content of snap bean, pea and sweet corn is 42%, 48%, and 53%, respectively (cited by Taylor, 1997). Table beet seeds contain starch, which is stored in the perisperm (see "Seed and seedling morphology" section). All small-seeded crops (TSW < 10g) are oil-storing seeds with > 18% oil. Though large-sized seeds generally store starch, the large-seeded cucurbits are exceptions with nearly half of their weight as lipids. All seeds contain proteins, and values range between 12% and 40% (Table 1.1).

Seeds in storage are said to be in a dry condition; however, "dry" is a relative term and does not mean that water is absent from seeds. Water is present in seed tissue, and the status of water is related to many aspects of seed physiology, including seed longevity. First, the concentration of water is measured and expressed in meaningful units.

Standardized gravimetric methods to determine the moisture content of seeds were published by the Association of Official Seed Analysts (Elias *et al.*, 2018). Other primary and secondary methods were described and reviewed by Grabe (1989). Seed moisture content calculated on a wet or fresh weight (fw) basis is commonly used in seed testing and in commerce. The following formula is used to determine the percentage of seed moisture content on a fresh weight basis:

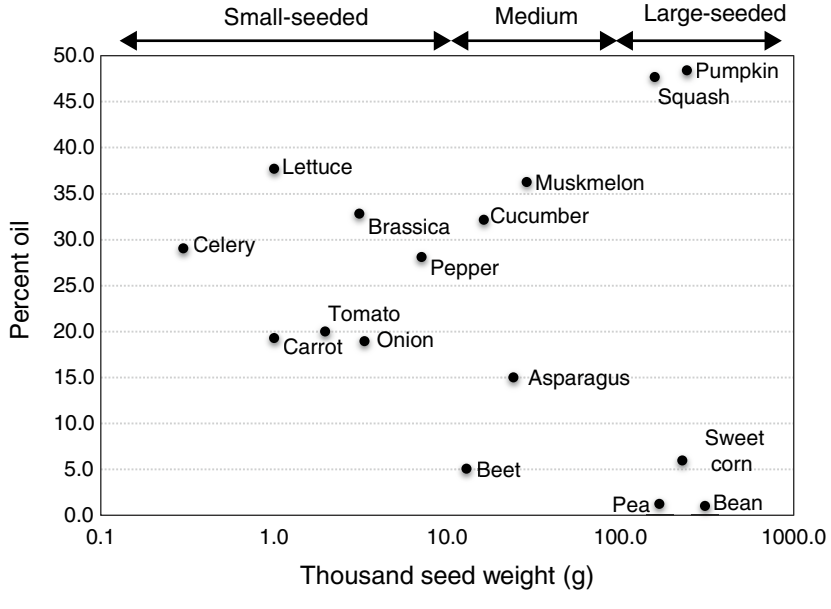

Fig. 1.1. The relationship of seed size expressed as Thousand Seed Weight (TSW) in grams in relationship to percent seed oil content. Seeds are grouped as small-seeded, medium and large-seeded with <10, 10–100 and >100 g TSW, respectively. Data from Table 1.1

$$\% \text{ moisture, fw basis} = [\text{weight of water (dry weight of seed + weight of water)}^{-1}] \times 100$$

The seed moisture content expressed on a fresh weight basis provides a direct assessment of the concentration of water in any quantity of seed. For example, 100 grams of seeds with 10% moisture content contains 10 grams of water and 90 grams of seed dry weight. Another unit to express seed moisture content is g H_2O g^{-1} fresh or dry weight.

The seed moisture content comes into equilibrium with the relative humidity of the air. The relationship between the equilibrium moisture content and relative humidity reveals a negative sigmoidal-shaped curve known as a moisture isotherm (Iglesias and Chirife, 1982). This relationship is determined by placing seeds in a range of known relative humidity conditions produced in closed containers with the use of saturated salt solutions (Taylor *et al.*, 1992). We prepared a number of saturated salt solutions and desiccants to achieve a range of humidity levels from *c.* 0% to 89% in equilibrated snap beans (*Phaseolus vulgaris*) and broccoli (*Brassica oleracea* italica group) seeds. Three regions or zones of water binding are observed: Zone I, < 20% RH; Zone II, 25–65% RH; and Zone III, > 70% RH (Fig. 1.2).

The curves are similar for both kinds of seeds; however, the equilibrium moisture content at a given relative humidity is always greater for snap beans compared to broccoli. Differences between these two species are attributed to seed composition, in particular lipid content, as lipids have little affinity for water. Thus, seeds with high lipid content have lower equilibrium moisture content at a given relative humidity than seeds with low lipid content. In our isotherm, the lipid content for the snap bean and broccoli seeds was 1% and 31%, respectively (Table 1.1).

Another method used to quantify the water status of seeds, which is not affected by seed composition, is to measure the water activity (a_w). Water activity is defined as the ratio of the vapor pressure of water in a seed to the vapor pressure of pure water at the same temperature (Bourne, 1991). Water activity is measured by determining the equilibrium relative humidity of the seed's headspace in a closed container and is expressed as a decimal. For example, a seed (any seed) equilibrated to 50% relative humidity has a water activity of 0.50. Measuring water activity of pelleted seeds (described in the "Seed enhancements" section) was the only nondestructive method to accurately measure the water status of coated seeds (Taylor *et al.*, 1997).

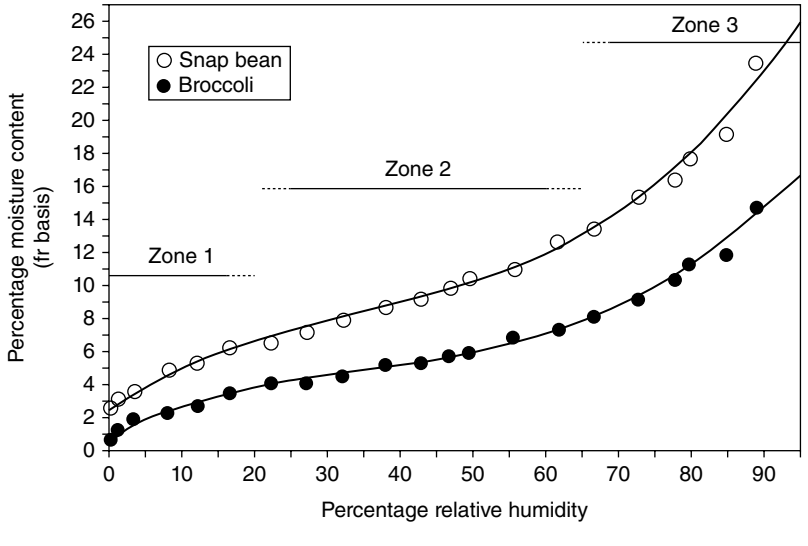

Fig. 1.2. Moisture isotherms for snap bean and broccoli seeds with oil contents of 1.0 and 31 percent, respectively. Figure from Taylor, 1997.

Storage: Moisture Content and Temperature

Two major environmental factors that influence seed storage are seed moisture content and temperature. Moisture content, as previously shown, is determined by the storage relative humidity and by seed characteristics, largely lipid content. The concentration of water in the seed tissue directly affects the rate of aging at a given temperature. The moisture isotherm reveals three zones or types (see Fig. 1.2) in which the water binding to seed tissues differ. Type I water is bound tightly and water interacts very strongly with charged groups of proteins (Vertucci, 1993). Type II water is less tightly bound and condenses over the hydrophilic sites of macromolecules (Leopold and Vertucci, 1989). Type III water is bound with negligible energy and forms bridges over hydrophobic moieties (Vertucci, 1993). The status of water in the seed tissue governs the kind of reactions (enzymatic or non-enzymatic) that occurs in storage (Vertucci, 1993). In practice, vegetable seeds are stored in Zone II (Fig. 1.2), and the recommended seed moisture content for vegetable crop seeds stored in hermetically sealed containers is listed in Table 1.2.

Temperature has a direct influence on longevity, and the rate of deterioration increases as temperature increases at a given relative humidity. Seeds are stored over a wide range of temperatures depending on the particular needs and available conditions. Seeds for most applications are stored above 0°C; however, for long-term preservation, seeds are stored below 0°C. Seeds with water contents in Types I or II are not injured since the water is non-freezable in the seed tissue. Long-term germplasm preservation has taken advantage of this ability to withstand freezing injury as seeds are stored above liquid nitrogen in the vapor phase at −150 to −180°C (Roos, 1989).

The need to keep seeds cool and dry for long-term preservation has been known for centuries. In the 1960s, "Harrington's Rules of Thumb" were developed as guidelines for storage (cited by Justice and Bass, 1978). The first two rules relate the influence of moisture content and temperature independently on longevity. The life of the seed is halved by a 1% increase in seed moisture, and this rule applies when seed moisture content ranges from 5% to 14%. The life of the seed is halved by a 5°C increase in storage temperature, and this applies to storage between 0 and 50°C. The most widely quoted rule combines both temperature and relative humidity to storage: "The sum of the temperature in °F and the percentage of relative humidity should not exceed 100."

Since the 1960s, mathematical equations have been developed to model seed aging for a

Table 1.2. Recommended seed moisture content for vegetable crop seeds in hermetically sealed containers. Minimum percentage germination standards for seeds in interstate commerce in the United States. Data from Federal Seed Act (USDA, 2017).

Vegetable	Seed moisture (%)	Germination (%)	Vegetable	Seed moisture (%)	Germination (%)
Asparagus	—	70	Lettuce	5.5	80
Bean, garden	7.0	70	Melon	6.0	75
Bean, lima	7.0	70	Onion	6.5	70
Beet	7.5	65	Parsley	6.5	60
Broccoli	5.0	75	Parsnip	6.0	60
Brussel sprouts	5.0	70	Pea	7.0	80
Cabbage	5.0	75	Pepper	4.5	55
Carrot	7.0	55	Pumpkin	6.0	75
Cauliflower	5.0	75	Radish	5.0	75
Celery	7.0	55	Rutabaga	5.0	75
Corn, sweet	8.0	75	Spinach	8.0	60
Cucumber	6.0	80	Squash	6.0	75
Eggplant	6.0	60	Tomato	5.5	75
Kale	5.0	75	Turnip	5.0	80
Leek	6.5	60	Watermelon	6.5	70

particular species maintained at a given condition of temperature and moisture content (fw basis), known as the Ellis and Roberts' equations (cited by Priestley, 1986). These equations take into consideration the initial percent germination of the seed lot and will predict the germination after a specified period of time in storage. The general equation for modeling the loss of germination in storage is as follows (Ellis *et al.* 1982):

$$v = K_i - \frac{p}{10^{[K_E - (C_w \times \log m) - (C_H \times t) - (C_q \times t^2)]}}$$

where v represents the probit of the percentage germination after a storage period of p days; K_i is the probit of the initial germination for the seed lot; K_E, C_w, C_H and C_q are species-specific constants; m is the seed moisture content (expressed on a fresh weight basis); and t is the storage temperature (°C). The species-specific constants for onion are: K_E = 6.975; C_w = 3.470; C_H = 0.040; and C_q = 0.000428 (Ellis and Roberts, 1981). We used this equation to plot onion seed aging curves (Taylor, 1997). For illustration, the influence of temperature, moisture content and initial viability were studied as variables for aging of onion seeds. Increasing temperature or moisture content independently had a profound effect on aging rates (Figs 1.3a,b).

The curves generally reveal a sigmoidal shape, especially when the initial germination is high, while the initial plateau phase is not observed with seed lots with low initial germination (Fig. 1.3c). Additional information on the seed viability equation and viability constants is available at the Royal Botanic Gardens, Kew (2017).

Species Differences in Storability

Although the storage environment is important, differences exist among species held under the same conditions. Results from a number of earlier storage studies on different species of seeds including vegetable seeds were summarized (Justice and Bass, 1978; Priestley, 1986). Attempts were made to relate seed longevity to other aspects of seeds such as composition. Many seeds with high lipid content are short-lived; however, tomato seed with 20% lipid content is a notable exception (Priestley, 1986). At this time, the physiological basis for differences in longevity between species is not well understood.

Long-term seed storage experiments were initiated in the past, and data from these studies were compiled and analyzed. As shown earlier, a sigmoidal relationship is revealed with a loss of germination in time. The sigmoidal curve is then transformed by probit analysis to reveal a linear relationship and from these data the P50 is calculated, defined as the period of time in years for viability reduced by half. The first set of P50 values were derived from tests of seeds held in open storage conditions in the temperate zone throughout the world (Priestley *et al.*, 1985). The second set of P50 values were obtained from seeds stored in semi-controlled conditions in the United States, and the initial samples were obtained from the US Department of Agriculture's Horticultural Field Station in Cheyenne, Wyoming (Roos and Davidson, 1992). This collection was moved to the National Seed Storage Laboratory in Fort Collins, Colorado, in 1962 and stored at 5°C and <40% relative humidity. In about 1977, most of the samples were transferred to sealed moisture-proof bags at −18°C and maintained under those conditions.

The P50 for the study conducted under open storage conditions ranged from 3 to 25 years, and seeds of asparagus, celery, parsley and parsnip were short lived, while tomato and pea were long lived (Table 1.3).

The P50 for the second study, in which the latter portion of the study was performed under controlled conditions, ranged from 29 to 130 years. In the second study, onion and pepper were short lived and okra was long lived. A significant linear relationship (r = 0.68*) was found between the P50s for the first and second study in which data was available for the same species. The regression equation revealed that the P50 values were approximately four times greater for the second study, compared to the first study. For example, the P50 of onion, the shortest-lived seed in the second study, was greater than tomato, the longest-lived seed in the first study. In conclusion, even though differences exist in the longevity among species, the major factor influencing storage life is the storage environment of temperature and relative humidity, which directly affects seed moisture content.

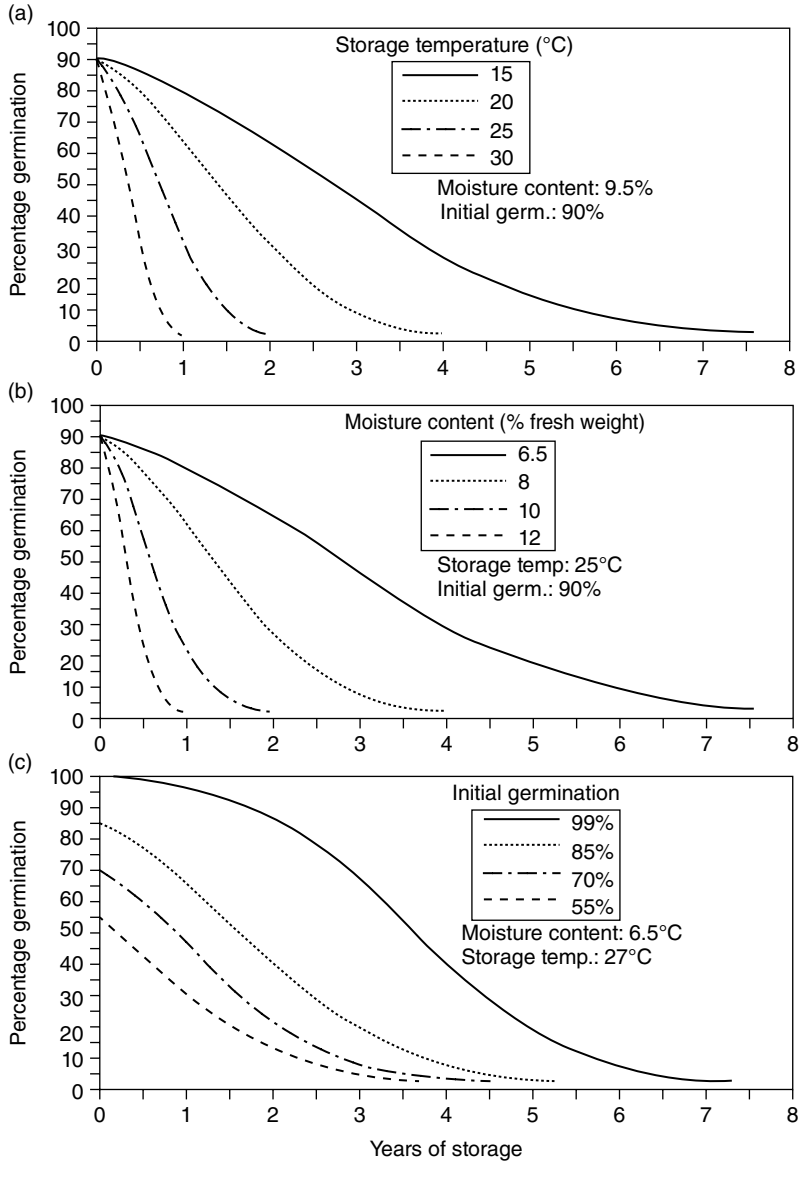

Fig. 1.3. The influence of temperature (a), moisture content (b) and initial germination (c) on onion seed ageing. Ageing curves were developed from the equation and constants of Ellis and Roberts (1981). Figures from Taylor, 1997

Germination and Seedling Growth

Germination is the transition period between the resting and the growth stages of the plant, and germination is considered completed at the time of visible radicle emergence (Bewley *et al.*, 2013). In this discussion, post-germination events are included to the point when the seed becomes a functional seedling. Definitions are needed to describe the resting seed condition with respect to environmental conditions favorable to support growth. Seeds in storage with low moisture content are in a state of quiescence, defined as the absence of growth because of environmental

Table 1.3. The half-viability periods (P50) in years for different vegetable seeds.

Seed	Genus species	P50*	P50†
Asparagus	*Asparagus officinalis*	3.92	—
Bean, lima	*Phaseolus lunatus*	13.12	—
Bean, runner	*Phaseolus coccineus*	7.99	—
Bean, snap	*Phaseolus vulgaris*	15.97	46
Beet	*Beta vulgaris*	16.51	43
Cabbage	*Brassica oleracea*	7.15	—
Carrot	*Daucus carota*	6.63	35
Celery	*Apium graveolens*	4.11	—
Corn, sweet	*Zea mays*	9.6	65
Cucumber	*Cucumis sativus*	4.92	45
Eggplant	*Solanum melongena*	—	54
Leek	*Allium porrum*	5.30	—
Lettuce	*Lactuca sativa*	6.42	—
Muskmelon	*Cucumis melo*	—	61
Okra	*Abelmoschus esculentus*	—	125
Onion	*Allium cepa*	5.43	29
Parsley	*Petroselinum crispum*	3.41	—
Parsnip	*Pastinaco sativa*	4.04	—
Pea	*Pisum sativum*	15.86	130
Pepper	*Capsicum annuum*	—	27
Radish	*Raphanus sativus*	13.82	—
Spinach	*Spinacia oleracea*	12.76	37
Tomato	*Lycopersicon esculentum*	24.52	124
Watermelon	*Citrullus lanatus*	—	43

*P50 values obtained from open storage conditions (adapted from Priestley *et al*., 1985).
†P50 values obtained from semi-controlled storage conditions (adapted from Roos and Davidson, 1992). Original samples from open storage in Cheyenne, Wyoming, were later transferred to the National Seed Storage Laboratory in 1962 and stored at 5°C and < 40% RH. In *c.* 1977, most samples were then stored at −18°C.

conditions that do not favor growth (Copeland and McDonald, 2001). The environmental factors needed to overcome this state of arrested development are water, oxygen, and a suitable temperature. Seed dormancy, in contrast, is a physical or physiological condition of a living seed that prevents germination even in the presence of otherwise favorable environmental conditions (Copeland and McDonald, 2001). For additional information on germination and dormancy, the reader is referred to books devoted to the subject area of seed physiology (e.g. Khan, 1977, 1982; Benech-Arnold and Sanchez, 2004; Bradford and Nonogaki, 2007).

Seed and seedling morphology

The botanical term *seed* refers to the mature ovule from the mother plant that contains the embryonic plant or embryo, and the integuments that become the seed coat and additional storage tissue such as the endosperm (Copeland and McDonald, 2001). Many *seeds* are enclosed in remnants of the fruit and are not technically true seeds. We will take a broader interpretation and consider the dispersal units or propagules from the mother plant including dry indehiscent fruits as seeds. The embryo morphology is generally similar within a plant family; however, vegetable seeds include both monocots and dicots, and each class contains several plant families (Table 1.1). Seeds that contain reserve materials in a well-developed endosperm are called *endospermic*. If they contain most of their reserve materials in the cotyledons (cotyledons are embryonic tissue), they are called *non-endospermic* (Black *et al*., 2006). After the completion of germination, the embryo develops the root and shoot system of the seedling. Seedlings are categorized into two groups depending on the orientation of the cotyledons with regards to the soil or growing media. Seedlings in which the cotyledons are raised above the soil by the expansion of the

hypocotyl are termed *epigeal*. Those seedlings, in which the hypocotyl does not elongate appreciably, and results in the cotyledons remaining in the soil, are termed *hypogeal* (Black *et al.*, 2006). The cotyledons often become photosynthetic in epigeous seedlings, while expansion of the epicotyl or mesocotyl is responsible for shoot development in hypogeous seedlings.

The following discussion will group selected vegetable seeds by their seed and seedling morphology (Table 1.4 and Fig. 1.4).

Sweet corn and asparagus have reserves in the endosperm and exhibit hypogeal seedling growth. The sweet corn seed is a caryopsis in which the pericarp is tightly fused to the seed coat (Copeland and McDonald, 2001), and the embryo is oriented in a lateral position as is typical of the grasses (Martin, 1946). The endosperm is non-living in sweet corn, and the scutellum, which is a part of the embryo, is considered analogous to the cotyledon. Many endospermic seeds, such as asparagus, have live endosperm tissue. The endosperm is considered living if there is a positive test with the vital stain 2,3,5-triphenyl tetrazolium chloride (AOSA, SCST, 2010). The solanaceous and umbelliferous crops and genus *Allium* seeds all contain linear embryos embedded in a living endosperm with epigeal germination. Though the embryo is considered linear, it is commonly found coiled, as in the case of tomato and pepper (Martin, 1946). In this group, carrot and celery are examples of schizocarps that have two fused carpels separating at maturity to form one-seeded mericarps (Copeland and McDonald, 2001). Beet and spinach have embryos that surround non-living nutritive tissue, and the nutritive tissue is a well-developed perisperm rather than endosperm (Hayward, 1938; Heydecker and Orphanos, 1968). Beet seed is a fruit and in many cultivars a *seed ball* is formed by the aggregation of two or three flowers to produce a multigermed propagule (Hayward, 1938). Non-endospermic seeds may contain remnants of endosperm that is adjacent to the testa; however, little reserve material is present. The endosperm is specialized in lettuce with a two-layer envelope surrounding the embryo that serves as a semi-permeable barrier to solute diffusion (Hill and Taylor, 1989). Non-endospermic seeds can exhibit hypogeal germination (found in pea and runner bean) or epigeal germination (represented by seeds from four different families). The bent embryo orientation is found in the large-seeded legumes and brassicas and is termed the *jackknife* position of the embryonic axis with the cotyledons. Lettuce and the cucurbits have spatulate embryos, and lettuce is an achene where the ribs on the seed surface are formed by the pericarp (Hayward, 1938). Definitions of anatomical parts of seeds and other seed terms throughout this chapter are found in *The Encyclopedia of Seeds* (Black *et al.*, 2006).

Water

Germination begins with water uptake. This essential process is explained by classical water relations, and this section is summarized from Bewley *et al.*, (2013). A more detailed explanation of water relations in seeds is found in

Table 1.4. The embryo orientation, the presence of a well-developed endosperm for storage of reserve materials and cotyledon orientation after germination of different vegetable seeds (Taylor 1997).

Seed kind	Embryo orientation	Location of reserves	Cotyledon orientation
Sweet corn	Lateral*	Endospermic	Hypogeal
Asparagus	Linear†	Endospermic	Hypogeal
Tomato, pepper, eggplant, onion, carrot, celery	Linear†	Endospermic	Epigeal
Beet and spinach	Peripheral‡	Endospermic	Epigeal
Pea and runner bean	Bent	Non-endospermic**	Hypogeal
Snap bean and *Brassicas*	Bent	Non-endospermic	Epigeal
Lettuce and cucurbits	Spatulate	Non-endospermic	Epigeal

*Embryo partially surrounded by non-living tissue.
†Embryo surrounded by living nutritive tissue.
‡Embryo surrounds non-living nutritive tissue.
**Non-endospermic seeds have little or no nutritive tissue as endosperm.

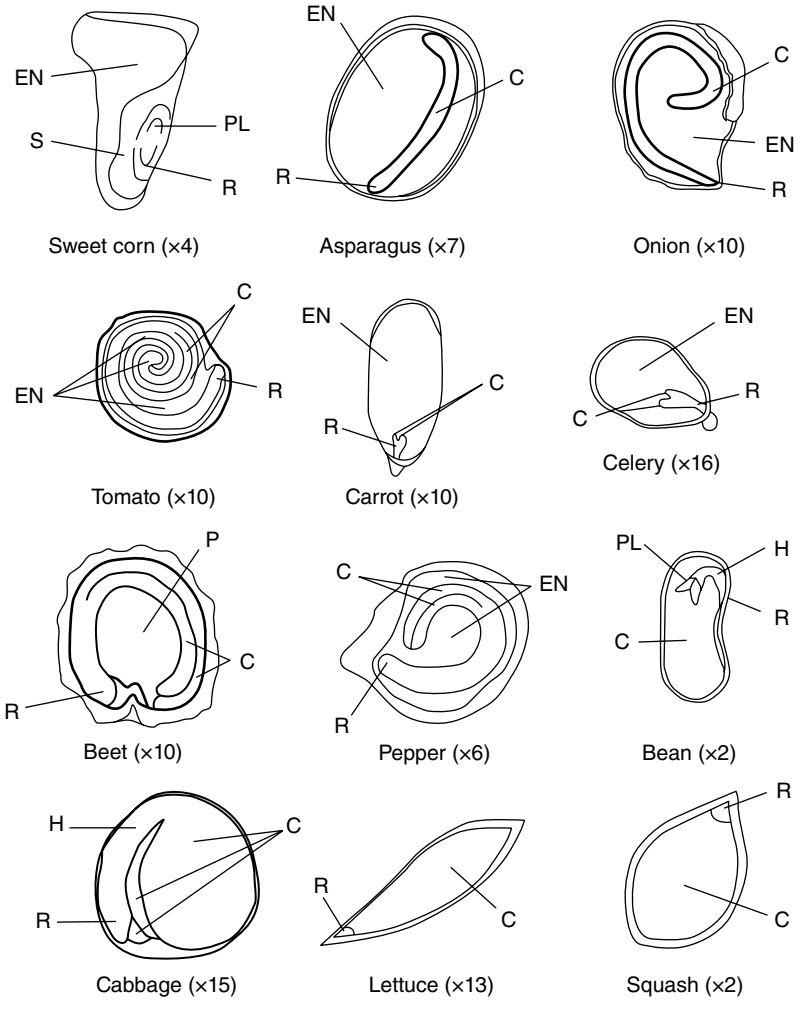

Fig. 1.4. Internal morphology of selected vegetable seeds (courtesy of D.H. Paine). EN, endosperm; S, scutellum; PL, plumule; R, radicle; C, cotyledon; P, perisperm; H, hypocotyl, (Figure from Taylor, 1997).

Koller and Hadas (1982). Water potential is an expression of the energy status of water. Water moves in a passive manner from a high to a low potential. The water potential of pure water is 0, and a decrease in water potential (less water available) is denoted by more negative values. The components of the water potential are the algebraic sum of the matric, osmotic, and pressure potentials. The matric or suction potential refers to the ability of the matrices in cells to bind water and is a negative value. The osmotic potential refers to the contribution of dissolved solutes to decrease water potential and is also a negative value. The pressure potential is a positive value and occurs when water enters the cells and creates an internal positive force on the cell walls. Water potential measurements can also be made on seeds in storage, and the water activity, described previously, is related to the water potential in a log-linear relationship (Taylor *et al.*, 1992).

To illustrate the process of water uptake in vegetable seeds, we conducted water uptake investigations by imbibing cabbage and tomato seeds on moistened blotters maintained at 25°C in the dark (Fig. 1.5).

Water uptake in seeds reveals a triphasic pattern with an initial rapid uptake phase, followed by a lag phase and then a second increase in moisture content. The generalized illustration of the triphasic pattern of water uptake is shown in the later section on "Seed Enhancements" (Fig. 1.9). Phase I is known as imbibition and is a physical process that occurs in both living and dead seeds (Bewley *et al.*, 2013). Seeds of most vegetable crops take up water readily, and, in our study, seeds were fully imbibed in a period of 4–8 h (Fig. 1.5). The rapid water uptake is attributed to the negative matric potential of the seed, which is caused by cell wall components and protein (Leopold, 1983). Swelling occurs during imbibition due to the expansion of hydrophilic compounds such as proteins, cellulose, pectic substances, and mucilage (Mayer and Poljakoff-Mayber, 1982). The rate of water uptake is influenced by a number of factors including temperature, initial moisture content, seed composition, and morphology. Seed–soil contact is another important factor; however, water vapor and not liquid water may be the principle source of water for seeds imbibed in unsaturated soils (Wuest, 2007). Some vegetable seeds, such as okra, do not imbibe water readily due to impermeable seed coverings (Anderson *et al.*, 1953). A lag in the imbibition time was shown in snap bean seeds with the semi-hard seed characteristic only when the initial seed moisture content was low (Taylor and Dickson, 1987). During the second phase (lag phase), there is little uptake of water. The matric potential is negligible during this period, and the osmotic and pressure potentials regulate the total water potential. During this phase, enzymes and membranes are functional in the fully hydrated cells as the seed advances to the completion of germination. The duration of Phase II is dependent on species and is influenced by environmental conditions (see "Water and temperature stress" sections to follow), and in our example, tomato has a longer lag period than cabbage even though tomato had greater moisture content after imbibition than cabbage (Fig. 1.5). Phase III of water uptake commences with visible germination (see arrows on Fig. 1.5) in which the seed coat is ruptured by the emerging radicle that forms the root system of the plant. Radicle growth is caused by cell elongation, and is then followed by shoot growth. Greater uptake of water is caused by a further decrease in osmotic potential caused by degradation of reserve materials into osmotically active smaller molecules (discussed under "Mobilization of reserves") and elongation of seedling tissue. The increase in the moisture content is much slower in Phase III (seedling growth) than

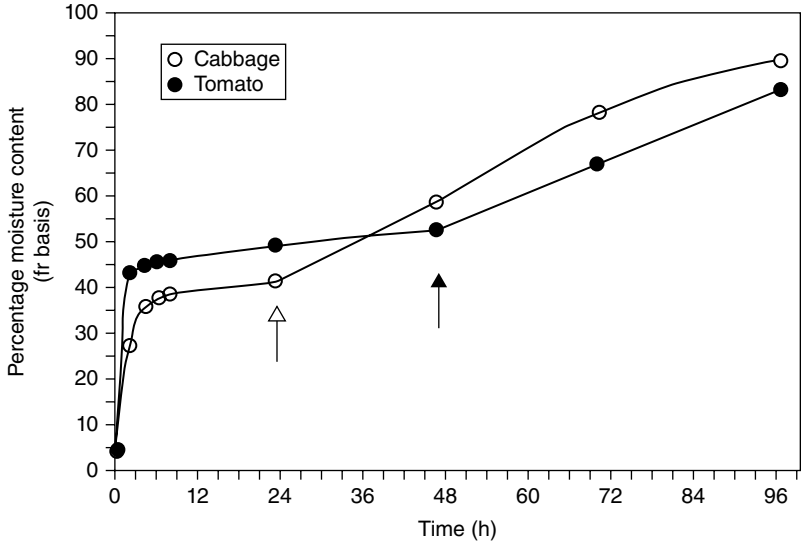

Fig. 1.5. Triphasic water uptake curves for imbibing cabbage and tomato seeds (Figure from Taylor, 1997). Phase I (imbibition) occurs from 0–6 h, Phase II (lag phase) from *c*. 6 to 24 h in cabbage and 6 to 48 h in tomato, and Phase III (seedling growth) occurs after visible germination as indicated by the arrow for each species.

the physical process of water uptake in Phase I (Fig. 1.5). In Phase III, the seed becomes a seedling and also loses its ability to withstand desiccation. Therefore, drying seeds after visible germination will result in death of the radicle, while drying seeds during Phase I or II is not injurious to the viability of the seed.

Oxygen

Oxygen is necessary for germination and is the substrate required for respiration to produce energy in the form of adenosine triphosphate (ATP). Seeds with low moisture content exhibit negligible gas exchange, and under a static environment, respiration is not appreciable until Type III water binding in which the water activity is greater than 0.9 (Vertucci and Roos, 1990). Germination is a dynamic process, and gas exchange increases rapidly as seeds imbibe water. The different phases of water uptake (previously described) are used to describe respiration patterns. In Phase I, there is a rapid increase in respiration which is attributed to the activation of existing enzymes. For example, in peas the respiration rate was shown to increase linearly with the degree of swelling (Kolloffel, 1967). In Phase II, a lag phase in oxygen uptake is observed in many large-seeded species and was attributed to the relative impermeability of the seed coat or seed coverings to gas diffusion since removal of the seed coat reduced this lag (Mayer and Poljakoff-Mayber, 1982). In Phase III, visible germination results in piercing of the seed coat, allowing ample oxygen for a second increase in the respiration rate. Also, there is an increase in the number of mitochondria resulting in greater total respiratory activity. Finally, Phase IV was described only for storage tissue, such as the cotyledons, in which there is a decline in respiration rate attributed to the exhaustion of reserve materials.

Mobilization of reserves

Germination was previously described in three phases with respect to water uptake and gas exchange. The source of substrates for respiration and/or growth is different before and after radicle emergence. Readily available substrates are needed for the early phases of germination since mobilization of reserve materials to produce smaller molecules does not occur until Phase III. Dry seeds store sugars that are a source of soluble carbohydrates needed for respiration during Phases I and II. Sucrose is commonly found in dry seeds, and other oligosaccharides such as raffinose and stachyose may also be present (Amuti and Pollard, 1977).

Mobilization of reserves is a post-radicle emergence event and was studied at the subcellular as well as the biochemical levels. Early studies were performed with germinating seeds in time-course experiments by removing samples, dissecting seed and seedling parts and weighing each component. There was a general loss of weight in storage tissue such as the cotyledons or endosperm with an increase in weight of roots and shoots (Mayer and Poljakoff-Mayber, 1982). These studies illustrated trends for utilization of reserves in germinating seed and provided the foundation for more detailed biochemical studies. Unfortunately, our understanding of biochemical events associated with mobilization of reserves in vegetable seeds is fragmentary. Most information is available on agronomic crops and especially those seeds that are also used for human consumption. The following section will briefly outline biochemical events associated with the catabolism of the reserve compounds: starch, lipids, proteins and phosphorus (Bewley *et al.* 2013). An expanded description of starch, lipids, and proteins is found in Black and Bewley (2000).

Starch is the common form of storage carbohydrate and is found in the cotyledons of beans and endosperm of sweet corn. Starch occurs in starch grains and is found as a straight chain polymer of glucose called *amylose* or the branched polymer, *amylopectin* (Copeland and McDonald, 2001). Starch is enzymatically degraded by amylase and other enzymes to form monomeric units of glucose. Glucose is respired or converted to the disaccharide sucrose since sucrose is the form that is transported to the growing regions of the seedling.

Seeds have unique biochemistry to produce and utilize lipids and to store oil as a reserve material in oil bodies within the cells. Noteworthy, no other part of the vegetable plant produces and stores lipids as a reserve material. Many small-seeded crops store lipids (Fig. 1.1) since

lipids are more chemically reduced than starch, and have higher energy content. The lipid is converted to a form that is transported from the storage tissue to the growing points. Lipid degradation occurs by unique biochemical pathways and also utilizes a specialized organelle, the glyoxysome (Bewley *et al*., 2013). Glyoxysomes are either present in dry seeds and enlarge during germination or are formed by *de novo* synthesis. The integration of the biochemical pathways with the organelles results in a process known as *gluconeogenesis* or *making new sugar*. Briefly, lipids are degraded by lipases to produce three free fatty acids and glycerol. Breakdown products from fatty acids are utilized ultimately to form sucrose and transported as described for starch.

Proteins are ubiquitous in seeds (see Table 1.1) and occur as enzymes or storage proteins. The storage proteins are found in protein bodies and degraded by proteinases to form different amino acids. The fate of the released amino acids is complex, and amino acids can be converted to other amino acids or transported to the growing points. Organic acids can also be formed from amino acids and later respired. Phosphorus is stored in seeds in an organic form called *phytic acid* or *phytin*. Phytic acid or myo-inositol hexaphosphate occurs as a salt and can contain potassium, magnesium, and calcium as well as the minor elements iron, manganese, and copper. Phytase releases phosphate, which is used for synthesis of nucleic acids, ATP, and phospholipids for membrane synthesis. Macro- and micronutrients are also released and are used for cell growth and development.

In conclusion, reserve materials in seeds provide a source of carbon in the form of sugars, nitrogen in the form of amino acids, and phosphorus in the form of phosphate along with other elements. Seeds are dependent on these stored reserve materials to support initial growth and development of the seedling. After seedling emergence and subsequent growth, the seedling becomes self-supporting by producing its own carbon in photosynthesis and through the uptake of other nutrients via the root system.

Seed Quality

Seed quality encompasses many parameters of a seed lot, but this discussion will focus on the robustness of germination and seedling growth potential. One method used to estimate seed quality is the standard germination test that is conducted under ideal environmental conditions in the laboratory (AOSA, 2017a; ISTA, 2017). The criterion for germination used by physiologists is radicle emergence; however, the seed analyst extends this interpretation by classification of seedlings as either normal or abnormal. Abnormal seedlings are those seedlings with an impaired root and/or shoot development or other seedling defects (AOSA, 2017b). Only normal seedlings are considered when reporting the actual germination of a seed lot and the percent normal seedlings is determined following standardized time and test conditions (AOSA, 2017a; ISTA, 2017). The standard germination test is the only test accepted for commercial labeling, and the minimum germination standards are presented for vegetable seeds in the United States (Table 1.2). However, in commerce, the germination is generally much higher to ensure high quality seeds for growers.

The term *seed vigor* is one component of seed quality, and the following definition was adopted by the AOSA (Baalbaki *et al*., 2009): "Seed vigor comprises those properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions." Seed vigor differs from germination in that vigor emphasizes the germination rate (rapid and uniform) and the application of the results to forecast field emergence rather than laboratory performance. Seed vigor is generally associated with a higher level of plant performance, and during the early phases of seed aging, a reduction in seed vigor occurs prior to a reduction in germination (Baalbaki *et al*., 2009).

The effect of seed aging is considered on a population or sample basis as well as on a single seed basis. The decline in the germination of a seed lot maintained under the same environmental conditions reveals a negative sigmoidal pattern with time (Fig. 1.3). This curve indicates that all seeds do not die at the same time and that, in general, most seed lots are composed of a mixture of viable and non-viable seeds. The sigmoidal pattern is most evident from lots with an initial high germination (Fig. 1.3c), and these lots are most desirable from a horticultural perspective since

they can withstand stress or aging before showing a marked reduction in germination.

On a single seed basis, seeds placed in a favorable environment will either germinate or fail to germinate. This categorical judgment is inadequate since a number of events occur to a viable seed before it is rendered non-viable. These events during aging also support the concept of a loss of vigor prior to a loss in germination. Different schemes or models were developed to illustrate changes associated with the loss of viability, and a proposed sequence of changes in seeds during aging was illustrated by Taylor (2003a) and is shown in Fig. 1.6.

Merits of this scheme are that both whole plant and physiological (biochemical and biophysical) responses are presented and the model does provide a frame that ranks the relative sensitivity of whole plant to physiological aging. In addition, events are ranked as early stage, or most sensitive to aging, to late stage events until the seed is rendered nonviable with the loss of cell viability. A review of other events associated with seed aging and another model of seed aging based only on biochemical and physiological changes associated with the loss of viability was described by Priestley (1986).

Whole plant responses

A primary interest in horticulture is to determine the consequence of seed aging on the whole-seed and whole-plant levels. The germination rate is the most sensitive index of seed quality at the whole-seed level (Fig. 1.6). However, germination rate is not recorded in a standard germination test; only the total or final germination is recorded. Actually, the only step of the germination process that is accurately measured is the onset of Phase III (visible germination). Therefore, the time to radicle emergence provides quantitative information on the relative vigor of a seed lot. To illustrate this effect, lettuce seed was aged for a short period by first increasing the moisture content to 20% (fw basis) and then incubating the seeds at 40°C for 24 hours (Tomas, 1990). This procedure was adapted from the controlled deterioration test developed as a method to assess seed vigor (Powell *et al.*, 1984). The time to radicle emergence was recorded using time-sequence photography and recording germination at two-hour intervals. Aged seeds germinated approximately six hours later than non-aged seeds and produced a greater percentage of necrotic seedlings (Fig. 1.7).

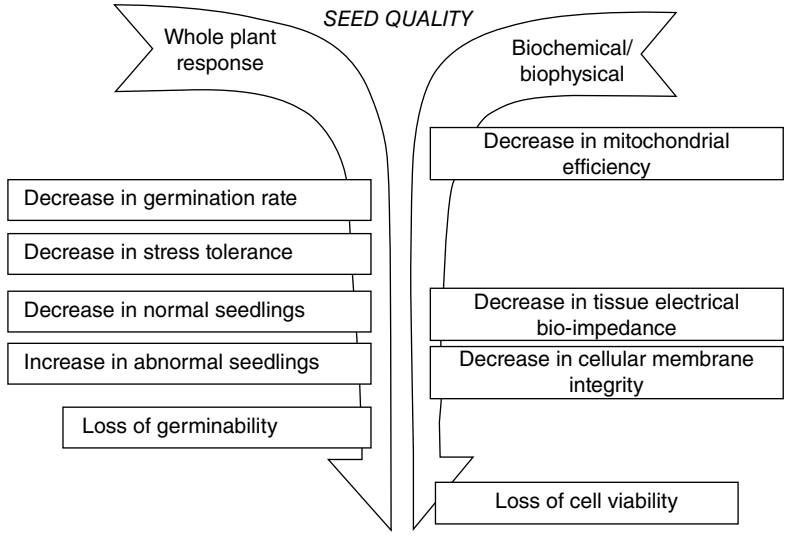

Fig. 1.6. The relative sensitivity of selected whole-plant and physiological events during ageing in storage. Reprinted with permission from Taylor (2003a).

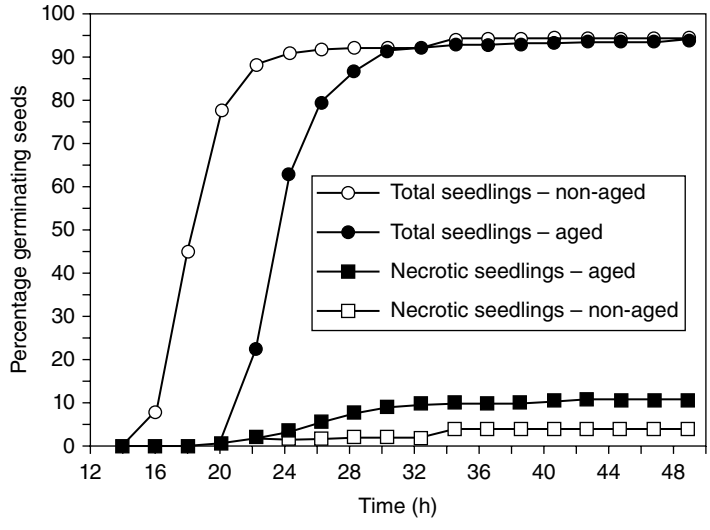

Fig. 1.7. The influence of ageing on germination rate of lettuce seeds including the presence of necrotic seedlings (adapted from Tomas, 1990, Figure from Taylor, 1997).

Necrotic seedlings are symptomatic of a disorder associated with aging in lettuce seeds called *physiological necrosis* (Tomas *et al.*, 1992) and are classified as abnormal seedlings (AOSA, 2017b). These data reveal that a mild aging treatment decreases the germination rate and increases the incidence of abnormal seedlings; however, the total percentage of seeds to germinate was not changed.

A decrease in the stress tolerance of a seed lot is a more sensitive indicator of seed quality than the standard germination test (Fig. 1.6). Stress tests are the most common type of vigor tests used by seed testing laboratories and by the seed industry. One of the most common tests is rapid aging under controlled conditions followed by a standard germination test. Methods used for rapid aging are termed "accelerated aging," "controlled deterioration," and "saturated salt aging," and the test methods are described in detail in Baalbaki *et al.* (2009). The basis of all three rapid aging techniques is to impose a controlled harsh storage environment for a specified short duration (generally several days), and then a standard germination is conducted after the aging regime. The test results are used to rank seed lot performance and estimate seed storage potential.

Another commonly used method to assess stress tolerance (Fig 1.6) imposes cold, wet soil conditions for a given period of time and then the seeds are transferred to warmer conditions for the completion of germination. This test is commonly referred to as the "cold test" (Baalbaki *et al.*, 2009), and is routinely used on sweet corn and adopted for other vegetable crops. The cold test imposes two environmental stresses: low temperature and wet soil conditions. The soil media used depends on the test conditions, and a commercially available sand is commonly used as a reproducible medium. Another test medium is a field soil from the same geographic region as the seed testing laboratory. Other variables to consider are the presence of indigenous soil pathogens; for example, using non-sterilized field soil. Despite these many factors, the test variables are standardized for a reproducible procedure.

Field emergence is of major horticultural importance for direct seeded crops since seeds are sown to achieve a desired plant population for optimal harvest efficiency. Seed vigor was related to field emergence (Roberts, 1972; Heydecker and Coolbear, 1977) with poor seed quality resulting in poor stand and reduced yield. Although the effect of plant population on yield was frequently studied and reviewed (Wiley and Heath, 1969), another more interesting question arises: does a reduction in seed vigor affect yield, if plant population is not a factor? Data from many separate

studies were summarized to address this question, and the following information was adapted from the review by Tekrony and Egli (1991). Crops were grouped based on the time of harvest of the harvestable product, either as vegetative, early reproductive or fully reproductive stages. Sources of seed quality differences were obtained by aging. In general, crops harvested in the vegetative stage showed the greatest response to aging with loss of vigor resulting in reduced uniformity of seedling emergence. Fairly consistent beneficial responses from sowing high quality seeds were also measured on crops that were harvested in the early reproductive stage. Crops harvested at full maturity, including most agronomic crops, generally did not have a positive yield response to seed vigor (Tekrony and Egli, 1991). Dry beans had a variable response to vigor. In conclusion, seed vigor is important for yield potential in many vegetable crops since most of these crops are harvested in the vegetative or early reproductive stages.

Physiological aspects

Respiration and energy synthesis pathways are vital to high vigor seed, and both processes are associated with early events during seed aging (Fig. 1.6). The mitochondrial membrane is required for respiration and electron transport (Goodwin and Mercer, 1983). Cytochrome C dissociates from the inner mitochondrial membrane during normal seed maturation drying, and seed aging impairs the re-association of cytochrome C (cited by Rutzke *et al.*, 2008). The production of ethanol, a by-product of respiration, was shown to increase under aerobic conditions as seeds age, while under anaerobic conditions, ethanol production decreases. A test was developed that employs the ratio of these two measurements, that provided a sensitive index of seed quality (Taylor *et al.*, 1999). The ratio of anaerobic to aerobic (ANA) ethanol production was more sensitive than a loss in either the percent germination or germination rate (Rutzke *et al.*, 2008).

Measuring electrical bio-impedance is a biophysical method adapted from medical diagnostics for investigating seed quality (Repo *et al.*, 2002). Snap bean seeds were first hydrated to desired seed moisture content and then small electrodes were inserted into the cotyledons. Seeds were exposed to a small alternating current, and the capacitive and resistive components of the seed tissue were measured to reveal an electrical impedance spectrum. Changes in the spectra were observed as seeds aged, and the sensitivity of the test results was comparable to a decrease in normal seedlings in a standard germination (Fig 1.6). The advantage is that the entire bio-impedance test was conducted in 24 hours compared to seven days for the standard germination.

Cell membrane integrity is tested directly or indirectly as a means to predict seed quality (Fig. 1.6). Certain vital stains are used in seed testing and provides a direct method to assess cellular integrity (Overaa, 1984). Indigo carmine, a cell membrane permeability stain, is excluded from living cells and is not injurious to subsequent embryo growth. Measuring leakage of compounds during the early stages of germination provides an indirect test of cellular integrity. Leakage tests are commonly performed on intact seeds and compounds such as electrolytes, sugars, amino acids, phenolic compounds, and others are measured from the imbibing solution (Priestley, 1986). Leakage tests have found application in seed quality testing, and methods to assess seed vigor by measuring conductivity are described (Baalbaki *et al.*, 2009). The major advantage of this method is that it is performed in a relatively short period of time and the data is objective. Unfortunately, there are serious limitations for the wide-scale use of leakage tests since seeds of many species possess an inner semi-permeable seed coat layer that restricts leakage of solutes and electrolytes (Beresniewicz *et al.*, 1995b). For example, seeds of onion and leek contain a semi-permeable layer composed of cutin, while tomato and pepper have a suberized layer (Beresniewicz *et al.*, 1995a). Therefore, measuring leakage of vegetable crop seeds is limited to large-seeded vegetable crops including pea and *Phaseolus* spp.

The loss of cell viability is the final step for the loss of seed viability (Fig. 1.6). All biochemical pathways require enzymes to catalyze reactions within the cell. The relationship of enzyme activity with seed aging was studied, and many enzymes remained active after all viability is lost; however, the dehydrogenases are one group of enzymes that were directly related with a loss of

cell viability (MacLeod, 1952). Dehydrogenase enzymes are found in several steps in respiratory pathways and catalyze oxidation-reduction reactions (Goodwin and Mercer, 1983). As previously discussed, respiration rate increases rapidly during imbibition, and maximal respiration is needed for the completion of germination. Therefore, dehydrogenase activity is determined during Phase II germination, a period of active metabolism. The most common method to measure dehydrogenase activity is with the vital stain 2,3,5-triphenyl tetrazolium chloride (TTC or TZ). Tetrazolium salts in the oxidized form are colorless and water-soluble and are reduced by dehydrogenase enzymes to the water insoluble red stain, formazan (Copeland and McDonald, 2001). Tetrazolium salts were first used in the 1940s, and the TZ test is still the most widely used test for seed viability (AOSA, SCST, 2010). Limitations are that the test is subjective and staining patterns are difficult to interpret, resulting in inaccurate assessment of viability. Other vital stains are used in seed testing (Overaa, 1984) and provides an alternative to the TZ method.

Sowing Environment

The soil environment is finally considered as it can greatly influence germination and seedling establishment of vegetable crops. In practice, direct seeding is often performed early in the growing season in soil conditions that are less than optimal for a particular species. Various abiotic stresses include water, temperature and physical impedance, and biotic stresses such as soil pathogens, insects, and other predators, are present from the time of sowing to seedling establishment. The following discussion will briefly review the role of the three major abiotic stresses and describe the sensitive phase for each.

Water stress

Water is essential for germination; however, water stress may occur as either an excess or deficit in the field for direct seeded crops or in the greenhouse for transplant production. In the case of water excess, oxygen is limiting for the completion of germination or seedling growth, because Phases II and III in germination are sensitive to oxygen deficiencies. Seeds of different species were subjected to anoxia by soaking in water, and germination was recorded after 72 hours (Crawford, 1977). Of the vegetable seeds tested, lettuce was tolerant to soaking, while pea was most sensitive, and it was shown that sensitivity to soaking was associated with a large production of the fermentation product, ethanol. Seeds are generally sown initially under favorable soil moisture conditions and then rain or irrigation can create a flooding condition. A period of low oxygen (hypoxia) can condition germinating seedlings so that seedling roots can survive for a longer period of time in a subsequent anaerobic condition compared to those that were not conditioned (Hole *et al.*, 1992).

Seeds are commonly sown at a shallow depth, and the soil may dry to a water potential below that necessary for the completion of germination. Due to their tremendous matric potential, seeds can imbibe or at least partially imbibe water even in dry soils and may enter Phase II germination (Bewley *et al.*, 2013). However, due to the dry conditions the seed cannot achieve sufficient moisture content to complete germination. Phase III is associated with cell elongation and later cell division, and these processes are most sensitive to water stress in growing tissue (Hsiao, 1973). Higher water potential (more water available) is needed for the initiation of cell elongation than for the maintenance of radicle growth after visible germination (reviewed by Hegarty, 1978). This indicates that a threshold moisture level must be achieved before the seed will complete germination (Phase III). Since seeds are desiccation-tolerant in Phases I and II, the inability to complete germination under water deficits would help to ensure survival under these stressful environments.

Temperature stress

Temperature regulates all aspects of biology including the germination of seeds. The cardinal temperatures of germination (minimum, optimum, and maximum) were summarized for vegetable crops seeds (Maynard and Hochmuth, 2007). Though temperature does affect the time for full imbibition, temperature primarily influences the germination rate by regulating the

duration of the lag phase or Phase II. Germination is predicted by incorporating a heat sum in degree days (S) and the minimum temperature for germination (T_{min}) (Bierhuizen and Wagenvoort, 1974). Seeds were germinated in a range of temperatures from 3–17 or 3–25˚C for fruit vegetables, and daily counts recorded. The S and T_{min} were calculated, and shown for selected vegetable crop seeds (Table 1.5) and the predictive equations were highly correlated over the temperature range tested. In general, the warm season crops have a higher T_{min} than the cool season crops, while the value for S varies with species.

Germination at low temperatures was investigated on sugary (su), sugary enhancer (se), and shrunken-2 (sh_2) sweet corn genotypes over a range of temperatures from 11.1–30.0˚C (Hassell *et al.*, 2003). Achieving 75% germination within seven days was the criterion for successful germination at each temperature for nine varieties of each genotype. The nine varieties examined were commercially released over a period of 89, 16 and 10 years for su, se and sh_2 genotypes, respectively. The mean number of days for 75% germination averaged over all temperatures tested was 3.6, 3.9 and 4.4 days for su, se and sh_2 genotypes, respectively. Varietal differences were measured within each genotype for low-temperature germination; however, there were no trends for improved cold tolerance with release date. Therefore, based on the scope of this study, plant breeding objectives have not included low temperature germination.

Temperature stress may occur, and temperatures may be suboptimal or supraoptimal for a particular species. The effect of elevated temperatures on lettuce seed germination is presented in Chapter 14. Seeds of many warm season crops are negatively influenced by low temperature, and this physiological disorder is known as chilling injury (reviewed by Herner, 1990; Bedi and Basra, 1993). There are two groups of chilling sensitive seeds:

> (i) those that are injured during Phase III germination such as the solanaceous crops and the cucurbits; and (ii) those seeds that are susceptible during Phase I and lima beans are very sensitive, while snap bean and sweet corn are sensitive (Bedi and Basra, 1993). In the second group, damage occurs during hydration of the seed tissue and is referred to as imbibitional chilling injury. Seeds become more susceptible to this type of injury as the initial seed moisture content decreases. Seed coat permeability and integrity are also important factors as seeds that imbibe water rapidly; especially seeds with cracked seed coats are more prone to imbibitional chilling injury.
>
> (Taylor *et al.*, 1992)

Table 1.5. Minimum germination temperature (T_{min}) and heat sum (S) in degree days for seedling emergence, and the applicable temperature (T) range for germination of various vegetables. Crops are ranked within groups by heat sum (S) in degree days (adapted with permission from Bierhuizen and Wagenvoort 1974).

Group	Crop	Genus species	T_{min} (°C)	S (degree days)	T (°C)
Leaf vegetables and *Brassica* crops	Lettuce	*Lactuca sativa*	3.5	71	6–21
	Turnip	*B. campestris var. rapa*	1.4	97	3–17
	Kale	*B. oleracea var. acephala*	1.2	103	3–17
	Red cabbage	*B. oleracea var. purpurea*	1.3	104	3–17
	White cabbage	*B. oleracea var. capitata*	1.0	106	3–17
	Brussels sprouts	*B. oleracea var. gemmitera*	1.1	108	3–17
	Spinach	*Spinacea oleracea*	0.1	111	3–17
	Cauliflower	*B. oleracea var. botrytis*	1.3	112	3–17
	Leek	*Allium porrum*	1.7	222	3–17
	Celery	*Apium graveolens*	4.6	237	9–17
	Parsley	*Petroselinum crispum*	0.0	268	3–17
Fruit vegetables	Tomato	*Solanum lycopersicon*	8.7	88	13–25
	Eggplant	*Solanum melongena*	12.1	93	15–25
	Gherkin (cucumber)	*Cucumis sativus*	12.1	108	15–25
	Melon	*Cucumis melo*	12.2	108	15–25

To better understand the role of seed moisture content and temperature on imbibitional chilling injury, two cultivars (chilling sensitive and tolerant) of snap bean seeds were adjusted to a range of moisture contents from 5% to 25% (dw basis) and germinated at 20°C or 5°C (Wolk *et al.*, 1989). Those seeds that were germinated at 5°C were transferred to 20°C after 24 hours. Critical seed moisture content was determined for each treatment that marked the onset of imbibitional chilling injury and was termed the breakpoint. Only seeds with moisture contents below the breakpoint showed a reduction in germination. At 20°C, the moisture content breakpoints for the chilling sensitive and tolerant cultivars were 15% and 11%, respectively. When seeds were tested at 5°C, the breakpoints were 19% and 16% for the sensitive and tolerant cultivar, respectively. Thus, at either temperature, the breakpoint moisture content was always greater for the sensitive cultivar, compared to the tolerant cultivar. Decreasing the temperature from 20°C to 5°C shifted the breakpoint to a higher level for each cultivar; however, the deleterious effects of imbibing seeds at 5°C were totally overcome by the elevated moisture content. Below the breakpoint for all treatments, there was an average 4.6% decrease in germination for each 1% decrease in moisture content. In conclusion, imbibitional chilling injury is influenced by the interaction of environmental and seed factors. The initial seed moisture content is the primary factor that determines the incidence of imbibitional chilling for a particular seed lot, while temperature has a moderating effect.

Physical impedance

The soil can act as a physical barrier to seedling emergence and may decrease or even prevent seedling establishment especially under conditions of soil crusting (Goyal *et al.*, 1980). Germinating seedlings must produce sufficient force to overcome this barrier. The sensitive period to this type of stress is late in Phase III when these seeds have already completed radicle emergence. There are several factors that influence the emergence ability of a seedling including the speed of germination and morphological characteristics (Inouye *et al.*, 1979). A faster emerging seedling has a better chance of escaping the physical barrier of a soil crust since it may emerge before soil crusting occurs. Two primary factors that determine the rate of emergence are soil temperature and species (Table 1.6). Considering a particular crop, seed quality may play a role because the rate of germination is influenced by vigor. Seedling morphology is also important (Table 1.4), since seeds with hypogeal germination have a smaller cross-sectional area to penetrate the soil than those with epigeal germination that must also pull the cotyledons through the soil.

Seedling emergence forces were quantified for a number of vegetable seeds and are shown in Table 1.6 (Taylor and Ten Broeck, 1988).

A positive relationship ($r = 0.98^{**}$) was determined between seed weight and force generated for eight species tested, and among small, medium, and large snap bean seeds of the same lot. The pressure (force per unit cross-sectional area of emerging hypocotyl or cotyledon in onion) was positively correlated ($r = 0.85^{*}$) with time to achieve maximum force. Seedlings with the ability to continue to generate forces may have a better chance of establishment than those that produced pressure for a short time. The energy content is related to seed composition as seeds generally store either starch or lipid (Table 1.1, Fig. 1.1). The energy content of the starch storing snap beans yielded 17 kJ g^{-1} in comparison to the lipid containing seeds that ranged from 22 to 26 kJ g^{-1} of seed. The use of reserves varied by seed type, but does provide some relative information on the efficiency of reserve mobilization. The study on seed size in snap bean revealed that small seeds contained fewer reserves than large seeds; however, the small seeds were more efficient in utilization of their reserves in seedling emergence forces (Taylor and Ten Broeck, 1988). A subsequent study measured emergence forces from snap bean seeds subjected to imbibitional chilling injury (Taylor *et al.*, 1992). The seeds subjected to chilling conditions produced less force per seedling and required a longer period of time to generate the maximum force, indicating that the injury sustained during imbibition reduced subsequent seedling growth potential.

Seed Enhancements

The last section of this chapter turns from the theme of seed biology to seed technology. *Seed enhancements* are defined as post-harvest methods

Table 1.6. Seedling emergence force characteristics and energy contents of vegetable crop seeds. Crops are ranked by maximum seedling emergence forces generated (reprinted with permission from Taylor and Ten Broek 1988).

Crop	Seed wt. (mg)	Maximum force (mN)*	Time to achieve maximum force (h)	Pressure exerted (kPa)†	Energy content (J seed^{-1})‡	Use of reserves (N kJ^{-1})***
Snap bean	268.0	3400 ± 360**	21 ± 1	234	4554 ± 12	0.75
Radish	10.0	558 ± 88	19 ± 8	317	231 ± 1	2.40
Cucumber	31.4	241 ± 49	9 ± 3	63	801 ± 1	0.30
Cabbage	4.20	157 ± 24	11 ± 2	241	111 ± 1	1.41
Onion	4.04	83 ± 11	19 ± 4	259	90.4 ±0.2	0.92
Tomato	2.90	44 ± 5	10 ± 2	96	71.8 ± 0.1	0.61
Carrot	1.00	35 ± 9	5 ± 1	117	23.8 ± 0.1	1.47
Lettuce	1.07	29 ± 6	7 ± 2	89	27.3 ± 0.1	1.06
Beets	–	26 ± 6	4 ± 2	62	–	–

*Maximum force achieved per seedling in millinewtons.
†Pressure exerted in kilopascals.
‡Energy content per seed in joules.
**Mean ± standard error.
***The ability of a seed to produce seedling emergence forces with respect to stored energy reserves

that improve germination or seedling growth, or facilitate the delivery of seeds and other materials required at the time of sowing (Taylor *et al.*, 1998). Therefore, single or multiple technologies are used on a particular vegetable crop seed from the time of harvest to sowing (Halmer, 2003). An overview of these technologies performed on commercial seed lots was adapted from Halmer (2000) (Fig. 1.8).

Seed conditioning is a physical method employed to remove contaminants from the seed lot including weed seed, other crop seeds, and inert matter, and seed conditioning principles and practices are described by Harmond *et al.* (1968) and Copeland and McDonald (2001). *Functional Treatment—Enhancement* includes a number of hydration methods under the heading of priming, such as *steep*, *soak*, and *pre-germinate*, and our discussion will focus on moisturization and priming. Active ingredients and materials are applied by seed treatment and coating technologies that are plant protectants, biostimulants, micronutrients, and inoculants. Finally, the need for high quality seeds is a prerequisite for the application of value-added seed enhancements (Kaufman, 1991), and seed testing is needed before and after an enhancement procedure to insure that high seed quality is preserved (Halmer, 2000). To place seed enhancements in context with seed testing (Fig. 1.8), the cornerstone of seed labeling in commerce is the standard germination and purity tests. A purity test includes the examination for weed seed, other crop seeds, and inert matter (AOSA, 2017a). One goal of the seed quality assurance by the seed industry is to provide a seed lot of pure seed. Seed health is testing for the presence of seed-borne pathogens. The importance of vigor was discussed in the earlier "Seed quality" section. Finally, storability is important as seed priming can negatively impact the storage life of seeds.

Hydration treatments

The importance of water in seeds is described previously in relation to storage, germination, and seedling establishment. Two hydration methods to improve germination and seedling establishment especially under stressful conditions, namely moisturization and priming, are presented. Moisturization was developed for large-seeded legumes, in particular snap beans; however, the technique has potential for other leguminous vegetable seeds. Priming is performed on many small-seeded vegetable seeds, and this method was adapted for a wide range of species.

The importance of seed moisture content on the resistance of bean seeds to imbibitional chilling injury was discussed (see "Temperature stress" section). Moisturization improved field

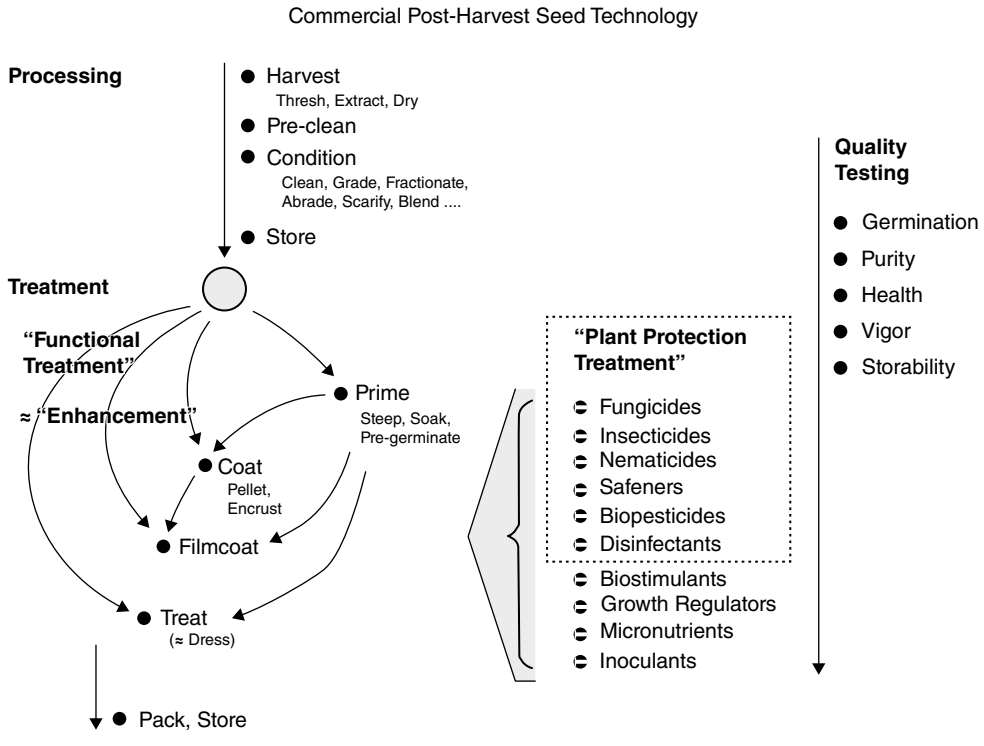

Fig. 1.8. The interrelationships of seed processing technologies used in commercial practice. Processing involves various techniques according to seed type. Optional treatments are used singly or in permutation according to seed type and market needs. Reprinted with permission and adapted from Halmer (2000).

emergence of early plantings, especially if the soil was wetted immediately after sowing (Wilson and Trawatha, 1991; Taylor *et al.*, 1992). The percentage of initial moisture content can affect germination of the same bean seed lot in the laboratory, and low moisture content samples had lower germination results than high moisture samples (Pollock and Manalo, 1970). Another benefit of moisturization is increased resistance to mechanical damage as moist seeds are less brittle (Bay *et al.*, 1995). However, moisturization at too high a level is deleterious, as the rate of aging in storage will increase dramatically. The moisture content of bean seeds is adjusted prior to packaging, and seeds are then stored in this condition. In practice, seed moisture content is adjusted to the upper level of Region 2, which corresponds to a seed moisture content of 12–13% (fw basis) or an a_w from 0.6 to 0.65 (Fig. 1.2). Seeds are moisturized by passing humidified air through the seed mass to increase the seed moisture content or by incubation with a moist solid media (Wilson and Trawatha, 1991).

Seed priming is a general term that refers to several different techniques used to hydrate seeds under controlled conditions, but preventing the completion of germination (Phase III). During priming, seeds are able to imbibe or partially imbibe water and achieve elevated seed moisture content usually in Phase II (lag phase) germination (Fig. 1.9).

Seeds are kept in this condition for a period of time that may range from less than one day to several weeks (Taylor and Harman, 1990). Priming temperatures range from 10°C to 35°C, but 15°C to 20°C is most commonly used (Bradford, 1986). Since seeds have not completed germination, they remain desiccation-tolerant and are dried for long-term storage. All priming techniques rely on the controlled uptake of water to achieve a critical

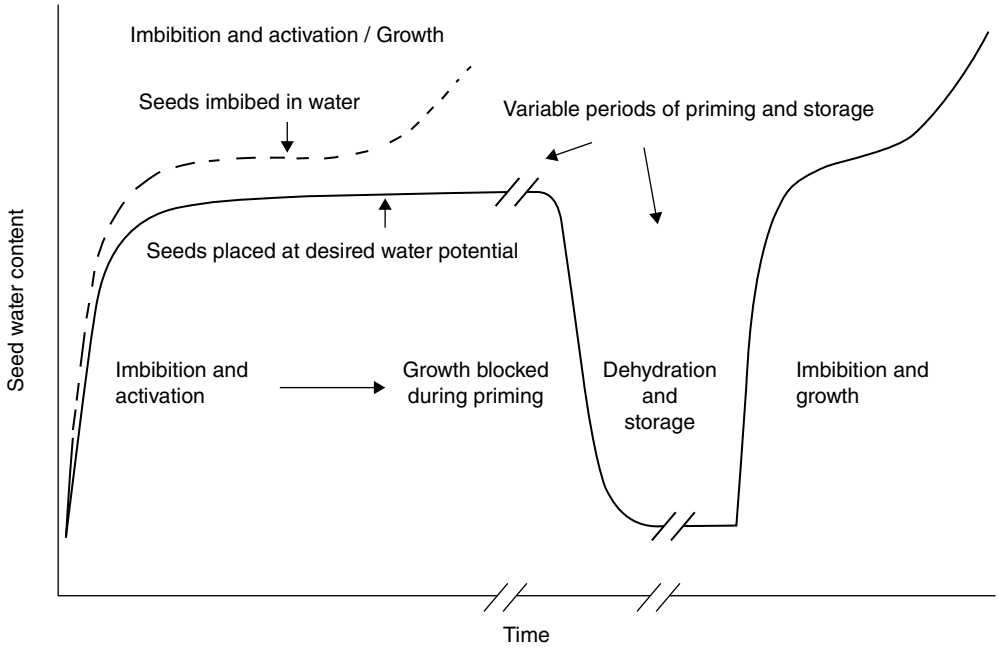

Fig. 1.9. Seed priming with respect to seed water content in comparison to imbibition in water. After priming period, seeds may be dried, stored and the transported to the grower. When seeds are sown, the lag period is reduced improving the germination rate and uniformity. Reprinted with permission and adapted from Bradford and Bewley (2003).

moisture content that will activate metabolic activity in a controlled environment. There are a considerable number of terms used in literature and in the industry to describe these methods, and definitions of *enhancement method* are found in *The Encyclopedia of Seeds* (Black *et al.*, 2006).

There are three priming techniques employed to advance the germination of seeds. The most studied technique utilizes aqueous solutions as the priming medium. In large-scale priming, a ratio of approximately ten parts priming solution to one part seeds is used (Nienow *et al.*, 1991). Therefore, due to the large reservoir of priming solution, the water uptake by seeds is regulated by the water potential of the solution, which varies by species and ranges from −0.5 to −2.0 MPa (Khan *et al.*, 1980/81). Many compounds were used to achieve a solution of known water potential and include inorganic salts such as NaCl, KNO_3, K_3PO_4, KH_2PO_4, and $MgSO_4$, low molecular weight organic compounds such as glycerol and mannitol and large molecular weight polymers such as polyethylene glycol (PEG) (Khan, 1992). The 8000 molecular weight PEG is widely used to regulate water potential, and formulas were developed to calculate the water potential of a solution of known concentration and temperature (Michel, 1983). Since gas diffusion is limited in solution, aeration is needed during the priming process. A number of terms used to describe this technique include *liquid priming*, *osmotic conditioning*, and *osmoconditioning*. The terms *osmo-* or *osmotic* are misleading when PEG is used since the water potential of PEG solutions is controlled primarily by matric forces (Steuter *et al.*, 1981).

Two other proprietary priming techniques, solid matrix priming (Eastin *et al.*, 1993) and drum priming (Rouse cited by Gray, 1994), have also gained attention as an alternative to liquid priming. In solid matrix priming (SMP), seeds are mixed with a solid particulate material and water (Taylor *et al.*, 1988). Seeds, due to their negative matric potentials, are able to imbibe

water from the solid material. Several materials was used in this process including leondardite shale, diatomaceous silica, exfoliated vermiculite, and expanded calcined clay (Khan, 1992). The amount of solid carrier required for a particular species depends on its water-holding capacity, and was reported from 2 to 0.2 times the weight of the seed (Taylor *et al.*, 1988; Khan, 1992). The amount of water needed to achieve equilibrium water potential conducive for priming is determined on an empirical basis as described for liquid priming. Leondardite shale is a material that regulates water potential by its osmotic potential (Taylor *et al.*, 1988). Diatomaceous silica materials regulate water potential by their matric properties, and their use in priming was termed *matriconditioning* (Khan, 1992). Drum priming was developed in the UK and involves hydration by misting seeds with water during a one- or two-day period in a revolving drum (Gray, 1994). The level of hydration is controlled for each species, and tumbling ensures uniformity of moisture distribution.

The benefits of priming by different techniques were documented for many vegetable seeds and were reviewed by Heydecker and Coolbear (1977), Bradford (1986), Khan (1992) and Parera and Cantliffe (1994). The subcellular basis for priming was reviewed by Varier *et al.* (2010). In general, priming hastens the rate of germination and seedling emergence, especially under suboptimal temperatures for germination. In the case of lettuce, priming ameliorates the deleterious effect of high temperatures causing thermoinhibition and thermodormancy. Priming may predispose the seed to aging, and primed lettuce aged faster than nonprimed seeds, especially those aged with a high relative humidity as used in the saturated salt aging test (Hill *et al.*, 2007).

Coating technologies

Seed coating technologies have evolved with time and are used for a different purposes to improve agricultural productivity and reduce environmental hazards. Early emphasis was placed on the development of coatings for small and irregularly shaped seeds to facilitate precision placement during sowing (Tonkin, 1979). Coatings were later developed to act as a delivery system for a number of materials required at time of sowing (Scott, 1989; Taylor and Harman, 1990). Coating technologies were further refined to reduce worker exposure to seed treatments during handling (Robani, 1994). Halmer (2000) and Taylor (2003b) reviewed seed coating technologies.

Several coating technologies are commercially used on different vegetable crops including seed treatment or dressing, film coating, encrusting, and pelleting (Fig. 1.8). The amount of material applied or weight gain differs for each coating technology (Taylor, 2003b). A conventional liquid treatment or dressing uses a small amount of water to uniformly apply the active ingredient over the seed surface and from seed-to-seed. Generally, less than 1% of the seed weight is applied by this method. The weight increase from film coating ranges from 0.5% to 5.0%. The weight increase varies with vegetable crop seeds, or more importantly seed shape to make a spherical pellet, and ranges from 2 to +50 fold (200 to +5,000% increase). An intermediate or mini pellet is termed *encrusting*, and ranges from 0.2 to 2.0 fold (20 to 200%).

Seed pelleting consists of the application of solid particles that act as a filler with a binder or adhesive to form a more or less spherically shaped dispersal unit (Fig. 1.10).

Pelleting is routinely performed by the seed industry on high-value, small-seeded vegetable seeds. In this process, seeds are generally pelleted on a batch basis in a coating pan or tumbling drum. Pellets are sized during and at the end of the process and then dried. The materials and techniques used are proprietary; however, a number of ingredients were listed in the literature (Halmer, 1988; Scott, 1989; Taylor and Harman, 1990). Seeds are tumbled with repeated applications of the coating filler material followed by intermittent spraying of seeds with water to activate the binder and result in the formation of the pellet around each seed. Encrusting, like pelleting, employs an application of a finely ground, solid particulate material and water or aqueous binder solution.

Film coating of seeds is a more recent development than pelleting and is derived from techniques originally developed for the pharmaceutical industry (Porter and Bruno, 1991). Film coating consists of spraying a solution or suspension of film-forming polymer onto a mass of

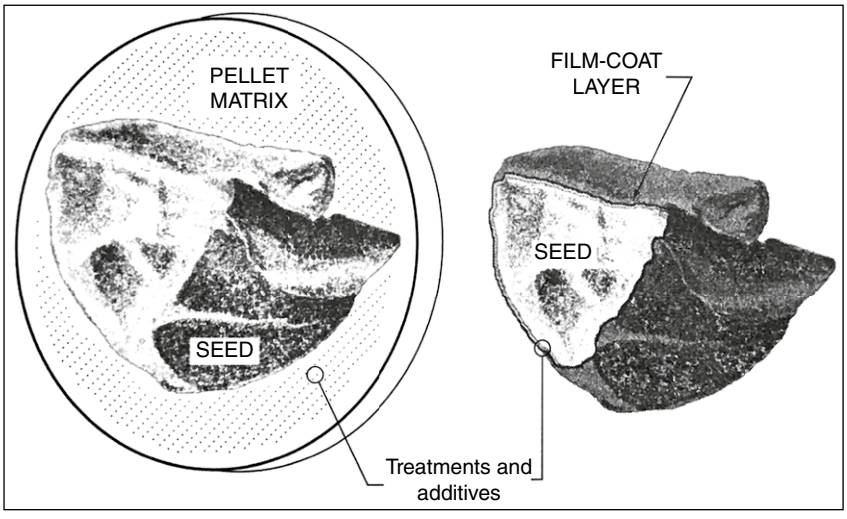

Fig. 1.10. Illustration of a pelleted (left) ad film coated (right) onion seed. Both pellet matrix and film coating layer can serve as a delivery system for active ingredients or other materials. Reprinted with permission from Taylor (2003b).

seeds to achieve a uniform deposition of materials (Fig. 1.10). A number of film-forming polymers and pigments are used (Halmer, 1988; Robani, 1994). Coating pans described for pelleting are used; however, in contrast to the wet operation of pelleting, the aqueous film-forming formulation is dried immediately after spraying to avoid agglomeration. Perforated pans are used to allow for rapid drying, and continuous flow methods were developed (Robani, 1994; Halmer, 2000). The benefits of film coating include uniform placement of seed treatment chemicals onto seeds, essentially a dust-free environment, and enhanced appearance due to the addition of pigments (Robani, 1994).

Seed treatments are categorized into different groups based on their mode of action or properties including plant protection, growth enhancement (Fig. 1.8) or environmental stress reduction. Plant protection by chemical seed treatments is performed on a global scale on vegetable and field crops. Seed treatment insecticide usage has increased dramatically over the past 20 years for below-ground insect pests (Nault *et al.*, 2006), and systemic seed treatment insecticides for above ground insects (Kuhar *et al.*, 2009). Seed treatments eliminated the need for in-furrow treatments, and resulted in a 90% reduction in pesticide usage (Taylor *et al.*, 2001).

Seed treatments are used to eradicate internal seed-borne pathogens, but to be effective the organic compound must first diffuse through the seed coat to the embryo. The physical/chemical nature of systemic compounds was investigated on uptake into the embryo of several vegetable crop seeds (Salanenka and Taylor, 2011). Seed coat permeability to systemic compounds was grouped into three categories: (i) permeable: snap bean; (ii) selective permeable: tomato, pepper and onion; and (iii) non-permeable: cucumber and lettuce. Selective permeable allowed nonionic compounds to diffuse, while ionic compounds were blocked. The semipermeable layer as the innermost layer of the seed coat restricted solute leakage from tomato, pepper, and onion seeds (Beresniewicz *et al.*, 1995b) and are also responsible for blocking ionic (charged) molecules from entering seeds. Information on seed coat permeability is used as a criterion for selecting effective compounds to target pathogens in the embryo.

There is a growing need for organic seed treatments, as synthetic chemical seed treatments are

not permitted for certified organic vegetable production. An organically approved insecticide, Spinosad, was effective in managing below-ground insects in onion (Nault *et al.*, 2006). Selected beneficial fungi and bacteria are effective as biological control agents for management of soilborne pathogens. The coating environment can be tailored to enhance the biocontrol organism by adjustment of the pH and addition of food bases (Taylor and Harman, 1990). Selected biological treatments can ameliorate abiotic stress in the soil environment. Biological treatments partially negated the environmental stress of soil salinity, and the most effective biological treatments increased the K^+/Na^+ ratio, which was positively correlated with plant growth (Yildirim *et al.*, 2006). Seed treatments can enhance plant growth and serve as *Biostimulants* (du Jardin, 2015). Plant proteins were investigated as biostimulants, and seed coatings containing soy-flour enhanced broccoli seedling and plant growth (Amirkhani *et al.*, 2016). Collectively, film coating, encrusting and pelleting can apply high loading rates of active materials onto seeds compared to conventional liquid treatments (Taylor, 2003b), and the coating technologies are delivery systems for single or multiple active agents that may serve as a protectant, enhancement or stress alleviator.

Concluding Remarks

An understanding of vegetable seeds is an important first step for the subsequent study of vegetable physiology and culture. Vegetable seeds are a diverse group of edible plants with respect to botanical classification, morphology, and composition, and this diversity impedes rapid progress in our understanding of vegetable seed physiology. This diversity, including different market needs and seed costs, also dictates the choice of seed enhancements employed for each crop, variety, and seed lot.

The high value of the harvested vegetable product increases the demand for seeds of high quality and maximum performance. The goal is for each seed sown to develop into a usable transplant or productive plant in the field. To achieve this goal, a seed lot must have complete, uniform and rapid germination and seedling emergence. This goal is seldom achieved due to one or a combination of factors including small seed size, slow germination rate, low inherent seed quality, and sensitivity to environmental stresses at the time of sowing. Unlike most agronomic crops, most vegetable seeds are not directly consumed, and have not been selected for large seed size. In the case of sweet corn—a crop selected for its high sugar content for consumption—the increased sugar content in mature seed can negatively impact seed quality potential and increases susceptibility to seed- and soil-borne pathogens.

To overcome some of the seed quality and slow germination challenges, seed companies routinely conduct seed conditioning to clean and upgrade seed quality. Seed enhancements, such as priming, can have a beneficial effect on seedling emergence of small-seeded vegetable crop seeds under environmental stress, but may accelerate aging and thus decrease seed quality after storage. Therefore, the potential risk/benefit of each seed enhancement requires consideration. Seed coating technologies provide a delivery system of active ingredients and agents required at time of sowing. Seed treatment and coating technologies are used on both small- and large-seeded crops.

Continued effort is needed by seed biologists to develop the knowledge of physiological mechanisms associated with seed aging, and the factors limiting seed performance. Some of these basic studies are performed with vegetable species, and others are adapted from agronomic crops and model plant species. Seed quality criteria should be incorporated into breeding and selection programs in the development of improved cultivars. In conclusion, by integrating several approaches and disciplines, seed quality of vegetable crops can be improved and will ultimately benefit vegetable growers.

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