Seed Enhancements

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1) The objective of this module is to provide an overview of the physiology and techniques involved in seed enhancements. This umbrella term encompasses many presowing, ‘value-added’ techniques including priming, seed coatings, and pre-germination. These methods are not mutually exclusive and many techniques can be combined to obtain additive effects. Opportunities for future seed enhancement research and commercial development are also highlighted.
2) Seed enhancement is a broad term to describe the range of practical beneficial treatment techniques performed on seeds (after harvesting and conditioning) to improve their physical or physiological performance. By consensus, conventional crop protection chemical treatments (e.g. fungicides, insecticides) are considered to be a separate category.
3) Manipulating the vigor of seed can be accomplished using (i) various hydration treatments, (ii) chemicals to trigger SAR (systemic acquired resistance), or incorporation/induction of seed antioxidants. Other types of seed enhancements facilitate the handling and planting of seeds, through various methods of pelleting, coating or encrusting. Seed enhancements can also deliver materials (e.g. nutrient inoculants, beneficial microorganisms) needed at the time of sowing.
4) Seed enhancements can also be designed to remove weak or dead seeds from a seed lot using upgraded, non-traditional conditioning treatments such as color

sorting, or x-rays. Tagging seeds with visible markers for identity preservation and traceability programs is another recent type of seed enhancement. A future seed enhancement may involve the application of agents integral to Genetic Use Restriction Technologies (GURT) and patent-protection of GMO germplasm which limits seed viability to only one generation.

5) Seed enhancements are used most extensively in high-value, horticultural crops such as vegetables and flowers. Many agronomic species are now film coated to provide better delivery of high-value crop protection chemicals and higher cost, stacked genetic traits seed. The use of various seed coatings is also well established for small-seeded legumes and some turfgrass species. As seed value increases, priming and other commercial seed enhancements are more commonly available and requested.
6) Seed priming describes a broad group of hydration techniques employed to enhance seed performance in the field or in controlled environment production systems. The term, seed priming, is also used to describe the biological processes and changes that occur during seed hydration (and drying) treatments. Priming is of interest to seed researchers as a tool for understanding the germination process, and is of considerable interest to the seed industry as a vehicle for improved seed performance and quality.
7) Seeds are primed (imbibed) to a water content and/or time period less than that required for complete germination, and then (usually) dried. Primed seeds are essentially held in phase II of germination by these restrictions in water potential,

or because of insufficient time. Phase III water uptake and seed germination is achieved upon subsequent sowing and rehydration.

8) In addition to the improvements in germination speed and/or uniformity common with primed seed lots, primed seed often contributes to improved seedling establishment under sub-optimal conditions at sowing (e.g. temperature extremes, or excess moisture). Primed seed is also valuable for increasing the level of useable seedlings produced in greenhouse environments.
9) Because of the variability in response from one seed lot to another, the optimum priming protocols used in commercial seed enhancement operations are often determined on a case-by-case basis. This can be accomplished by conducting ‘pilot’ priming runs on small samples and testing the germination responses. Optimal priming procedures, and the resulting improvements in uniformity of emergence are especially valuable for high-value crops where multiple harvests are expensive or impractical (e.g. lettuce).
10) Seed priming is most extensively commercialized in the field seeding or plug transplant production of tomato, pepper, onion, carrots, leek - and the production of ornamental plants including begonia, *Viola*, cyclamen, primrose and many herbs. Priming is also commonly used with seeds of sugar beet, some turf species, and has been used for decades to circumvent seed thermodormancy in lettuce and other species.
11) Priming leads to better germination in a wider range of temperatures. For example, primed tomato seeds (Fig A; open circles) germinate to higher percentages at 35^{o}C than unprimed seeds, and are able to germinate well at 10^{o}C

whereas control seeds do not germinate. Similar results are seen for primed leek seeds -Fig. B; open circles- vs. control leek seeds, even though leek is a cool-season crop.

12) Our understanding of the biochemical, physiological and cytological processes involved in seed priming has developed from studies of (i) germination kinetics and modeling using hydrotime (and hydrothermal time) concepts, (ii) DNA replication and cell division plus cell cycle changes in seeds, and (iii) endosperm weakening by hydrolases in species where the embryo is mechanically restrained from expanding.

13) Hydrotime and hydrothermal time are concepts which model water potential and temperature in combination. Tb is the base temperature permitting radicle emergence, and the base water potential for a species or seed lot is that value which just prevents germination. Hydro(thermal) time can be adapted to explain the influence of external factors on germination, and to describe the basis and design of priming protocols. Accumulated hydrotime effects on priming effectiveness generally reaches a peak, followed by potential ‘overpriming’. The highest germination rate for broccoli seeds occurred after 218 MPa hrs, for example. The accumulation of hydropriming time greater then 252 MPa hrs reduced the germination rate for this broccoli cultivar.

14) When priming occurs at suboptimal temps, it is useful to add thermal time to the model. An ultimate goal of hydrotime modeling is to better predict optimal priming protocols, and minimize the need for small scale, pilot testing of seedlot

samples. These models should also be useful in predicting seedling emergence in a wide range of agro-ecological environments.

15) Linking the phases of water uptake in germinating seeds and associated physiological events to priming protocols is essential for success. The rapid initial water uptake of phase I occurs at the time of DNA and mitochondrial repair, plus protein synthesis using existing mRNA's. Seed water content increases slightly during phase II, while new processes – synthesis of mitochondria, creation of proteins from new mRNA's, completion of DNA repair – are occurring. Successfully primed seeds do not lose desiccation tolerance and can be redried for ease of storage and handling.

16) Several methods have been proposed to regulate water availability (as a liquid or in the vapor phase) to seeds. Three basic systems used to deliver and restrict H_2O, and supply adequate amounts of O_2 to seeds are (i) osmopriming, (ii) matrix priming, and (iii) hydropriming. All three systems can be modified for research use, or for commercial use as batch processes. Commercial priming systems can handle seed quantities from tens of grams up to several tons at a time.

17) Osmopriming places seeds in contact with aerated solutions of low water potential; seeds are then rinsed off upon completion of the priming step. Various inorganic salts – KNO_3, KCl, $Ca(NO_3)_2$ – have been used as osmotica, but their small molecular size permits uptake and potential seed damage. High molecular weight polyethylene-glycol (PEG 6,000 to 8,000) is a preferred osmoticum since its large molecular size prevents seed uptake. Continuous aeration is important in any osmopriming system.

18) For small quantities of seeds, they are either placed on the surface of blotter paper moistened with osmopriming solutions, or immersed in aerated columns of solution. A new variation – membrane priming – uses a rotating tube with an outer jacket, where the seed is separated from the osmoticum by a selectively permeable membrane.

19) Solid, or dry matrix priming layers or mixes seeds in a mixture of water and insoluble matrix particles – such as vermiculite, diatomaceous earth, clay beads – in predetermined proportions. Seeds in matrix priming slowly imbibe water, reaching an equilibrium hydration level determined by the reduced matrix water potential adsorbed on the particle surfaces. After equilibrium is achieved, the moist matrix material is removed by screening, or can be partially incorporated into the seed coating.

20) Solid particulate systems can be used to increase seed moisture in a wide range of species and seed sizes, and are generally compatible with the addition of biological controls. Early studies with matrix-priming (or ‘moisturized’ seed) and hydropriming (or ‘hardened’ seed) of sweet corn seed substantially improved early emergence and uniformity of stand in two field plantings. Osmopriming with PEG 8000 did not perform as well in these experiments, which reinforces the need for pilot runs to optimize priming protocols in commercial operations.

21) Matrix priming mimics the natural process of water uptake by seeds from soil particles or components of greenhouse soilless mixes. Matric potentials in this type of priming generally range from -0.4 to -1.5 MPa at 15-20^{o}C for 1 to 14 days. This technique has been successfully used in the priming of species

including carrot, celery, fescue, lettuce, onion, pepper, tomato, purple coneflower, common bean, soybean, and sweet corn.

22) Hydropriming can be used both in the sense of steeping – imbibition in water for a short time - and in the sense of the continuous or staged addition of a limited amount of water, with or without subsequent incubation in humid air. Hydropriming has the economic advantage of minimal waste materials being generated, compared to osmopriming and matrix priming methods. A disadvantage of hydropriming is that seeds are sometimes not evenly hydrated, which decreases the potential for uniform germination. Slow imbibition through hydropriming is also the basis of drum priming techniques.

23) Water availability is not limited in the simplest hydropriming (or steeping) methods, and some seeds will eventually complete germination unless the process is time limited to prevent the onset of phase III water uptake and radicle emergence. Steeping has been employed as an agricultural practice over many centuries. Overnight seed steeping without drying ('chitting') is still advocated in many parts of the world as a pragmatic, low cost and low risk on-farm priming method for improved seedling establishment of many crops. Steeping can also be designed to remove water-soluble germination inhibitors from the seed coats of many *Apiaceae* species (e.g. parsley, celery) and sugar beets.

24) Hydropriming is also used to infiltrate water soluble crop protection chemicals to control seedborne diseases. These treatments usually immerse or percolate seeds at up to 30^{o}C for several hours followed by drying to near the original seed moisture content. Thermotherapy, or hot water seed treatments when properly

used can kill most bacterial disease-causing organisms on or within seed. Hot water treatments of about 50°C for 10-30 minutes can be used along with exacting pre-warming and post-treatment cooling and drying steps. Note that seeds of cucurbits can be severely damaged by hot water and should not be hot-water treated. Differences in seed maturity and initial seed vigor can influence seed lot responses to hot water treatment, so pilot tests on small samples are recommended prior to large scale thermotherapy treatment.

25) Drum priming is used commercially to hydrate seeds evenly and slowly to the desired moisture content – typically 25-30% on a fresh weight basis – by precise misting, or dribbling with water. The duration of this hydropriming method depends on the absorptive characteristics of the species and seed lot and the target seed moisture content. In European models, the priming drum sits on a scale which determines seed weight increases. Seed lots are tumbled in the drum for uniform hydration, aeration and temperature control.

26) Another experimental drum priming system controls seed hydration by time intervals and volume of water applied. A preset water volume is injected during each cycle as regulated by a timer attached to a solenoid. Dividing the total water volume required by the preset water injection volume establishes the number of cycles. Seed absorptive patterns of a species and cultivar must also be known to minimize the amount of free water remaining after a given cycle. Once the seeds obtain the desired water content, they can be incubated for time periods which ensure uniform seed hydration and give optimal germination results.

27) Biopriming techniques involve the addition of beneficial rhizosphere microorganisms in the priming process, either as a method for efficient delivery to the crop or to control pathogen proliferation during priming itself. Matrix priming, osmopriming, and hydropriming methods have all been employed to increase beneficial microbial populations on the seed. Compatibility of these microbes with chemical seed treatments, inoculants, and other additives can vary. Microbial formulations, quality control, delivery systems and costs of registration have slowed commercial use of biopriming to date. Biological control organisms continue to present, however, a unique approach for alternative control of soil pathogens and managing soil borne diseases.

28) Benefits of seed priming can also be combined with enhanced germination effects from plant growth regulators or hormones, especially gibberellins and ethylene. Seed treatments or priming with growth retardants such as paclobutrazol – a GA inhibitor – can effectively dwarf the growth of bedding plant transplants, producing stocky, high-quality seedlings. Treated seedlings are also greener, more uniform with thicker stems and have a higher root:shoot ratio compared to controls.

29) Drying following priming can be accomplished using forced air, ambient conditions, or controlled atmospheres of specific relative humidity. Drying methods and rates of drying are important to subsequent seed performance. Slow drying at moderate temperatures is generally preferred. Controlled seed moisture loss after priming can extend longevity by 10% or more in studies with hydroprimed tomato seed lots. Heat shock treatments are also used to improve

the longevity of primed seeds. Heat shock methods include keeping seeds under a mild water and/or temperature stress for several hours (tomato) or days (*Impatiens*) before drying.

30) Pansy seeds were hydroprimed to a seed moisture content of 38%, then transferred to a rotating drum for 3-4 d at 20^{o}C. At the end of imbibition, seeds were desiccated rapidly using forced air at 20^{o}C and 40% relative humidity. Seed moisture contents decreased 5-10% per hour in this ‘fast drying’ treatment. Alternatively, seeds were ‘slow dried’ using still air of 20^{o}C and 75% relative humidity for 3 d – seed moisture contents in this treatment decreased 0.1 – 0.3% per hour. At the end of this slow drying treatment, seeds were transferred to ‘fast drying’ conditions to complete desiccation. In the left side panel, t_{50} values of 4 pansy varieties either dried slowly (black) or fast (grey) are shown. The middle panel shows survival of these seeds after 24 h of controlled deterioration (50^{o}C water bath treatment). The third panel indicates germination of pansy seeds after 23 months of storage.

31) Hydropriming and osmopriming have also been shown to change the development of free space in tomato seed, almost all at the cost of endosperm area. Dehydration prior to seed priming is required for this phenomenon to occur. The increased percentage of free space in primed tomato seeds may speed up germination by reducing endosperm restraint to radicle emergence, or by facilitating water uptake and availability

32) Since priming often leads to improved seed performance, it is suggested that priming reverses some of the detrimental events occurring during seed

deterioration. As seeds deteriorate, a cascade of disorganization steps occurs which ultimately leads to a complete loss of cell function. This figure highlights some proposed events associated with seed deterioration during storage, and seed repair potential during seed hydration that occurs in imbibition or priming. The current model of seed deterioration views lipid peroxidation as a key cause of cellular damage through free-radical attack. Priming is thought to increase enzyme activities and partially counteract the effects of lipid peroxidation.

33) Seed priming has been successfully employed for many crops and species where rapid, uniform and complete seedling emergence are essential. One practical drawback in many species is that primed seeds can often suffer from more rapid deterioration under normal storage conditions. Increased attention to drying protocols after priming, and storing primed seeds in conditions where ^{o}F + % RH is equal to or less than 80 will decrease storage concerns. Priming protocols combined with enhanced screening and removal of weak or dead seeds is needed to achieve near perfect seedling establishment targets.

34) Pre-germination technologies allow seeds to germinate prior to sowing for those interested in optimum and uniform seed performance. Fluid drilling (also known as fluid sowing or gel seeding) starts with the selection of germinated seeds using density separation. The sprouted seeds are then suspended in a viscous gel and sown by extrusion into the soil. Pre-germination techniques permit the separation of germinable seeds from dormant seeds, and can be used to salvage high-value seeds from within a seedlot where deterioration has occurred.

35) In a more recent type of pre-germination process, fully imbibed seeds are germinated to the point of radicle protrusion, sorted (by machine vision, floatation, etc.) to remove ungerminated seeds, and gradually dried to induce desiccation tolerance. This process can produce either damp pre-germinated seed (30-55% SMC) with a storage life of a few weeks, or dry seed that remains viable for a few months. Pre-germinated seeds are physically fragile and should be handled even more carefully than conventional seed.

36) Another area of seed enhancement involves a wide range of pelleting and coating technologies. These enhancements improve planter performance by making seeds rounder or larger, and can be used as a carrier for various seed additives such as nutrients, growth regulators, and crop protection materials. Pellets are made to precise size tolerances and are very useful in combination with precision seeding equipment.

37) In comparison to pellets, encrusting – also known as mini-pelleting or coating – applies less material so that the original seed shape is still apparent. Where the amount of material added is small, the resulting encrusted seed product may be hard to distinguish from film coated seed. Seed pelleting and coating enhancements have drawn heavily from techniques used in the production of candies and pharmaceutical tablets.

38) Seeds for pelleting are first placed in revolving pans or drums of various design and sizes, and blends of powdered materials, hydrophilic or hydrophobic additives, and binders are progressively added in a layering process along with water until the desired seed pellet weight or size increase is achieved. The wet-

coated seeds are then dried with heated air in a separate step. Although automated for some species and products, many pelleting processes involve skilled manual inputs. Batch size can range from about 250 g up to 100 kg of seed per batch.

39) The tumbling action of seeds, water and the pelleting materials distributes and uniformly molds the mixture to the desired size distribution. Tumbling in the rotating pans or drums also minimizes the formation of empty pellets, or seeds/pellets sticking together. If desired, special techniques can be employed to create multi-seeded pellets. Seed pelleting protocols can be designed to increase seed weight by about 2-fold up to 50-fold or more (in tobacco seed pellets, for example). This compares to increases of only 0.1 to 2X for coatings or encrusting, and less than 0.1X weight increase with film coating.

40) Coated and pelleted seeds are commonly colored for product identification and improved visibility in the soil in order to monitor planting depth and spacing efficiency. This photo illustrates (from left to right) film coated onion seeds, encrusted seeds, raw onion seeds, and two degrees of pelleted onion seeds. Conventional commercial pelleting and coating types are designed to impose minimal mechanical or physiological barriers to germination. Alternatively, temperature-activated polymers have been developed into seed coatings (such as Intellicoat™, from Landec Ag) that allow early corn or canola planting while reducing risks of seed injury and poor seedling establishment associated with cold, wet soils.

41) Commonly pelleted or coated species include a number of higher-value vegetable, flower and specialty agronomic crops. Encrusting is also employed in these crops to (1) pre-inoculate rhizobia on small-seeded legumes such as alfalfa, (2) reduce seed size variation in maize and sunflower, and (3) add weight to avoid seed drift during the sowing of chaffy, low-density turfgrass species. Pelleting and coating continues to rely heavily on the skills of experienced operators. New seed coating and pelleting equipment operators often practice with dead seeds for several months before actual runs with very valuable commercial seed lots are allowed.

42) For more advanced pelleting and coating options, enhancement modifications allow tailored performance to fit the season of crop production. Lettuce seed pellets, for example, can be modified to rapidly disintegrate, or split in two to expose the seed to adequate soil oxygen after initial water uptake. Water-attracting materials added to seed coats and pellets can aid in imbibition and seed-soil contact under low water conditions. Temperature-sensitive polymers and oxygen-generating materials can also be added to seed coats/pellets for better seed performance in specific cropping systems or environments.

43) Many of the newer filmcoating seed products rely on the spouted bed (left) and side-vented pan coater systems illustrated here. Typical seed capacities in these devices range from less than 1 kg up to 25 kg (spouted bed) and 250 kg (side-vented pan) of seed. Film coating is also widely used to apply insecticides, fungicides and pigments to dry pelleted seed.

44) Seed enhancements have also led to more extensive use of seed testing in assessing seed lots prior to and after treatments. Priming efficacy is monitored

using germination (especially early count) tests, thermogradient table results, plug tray performance and seed storability tests (e.g. AA, SSAA). Uniform test standards, development of new seed quality testing methods (e.g. image analysis) and global harmonization of enhanced seed testing protocols are essential for seed consumers and the seed industry.