

## Water Potential: The Key to Successful Seed Priming

An increasing number of investigators are becoming interested in seed biology with the objective of understanding and controlling the many aspects of seed germination and seedling establishment. The reported opportunity to increase germination rates as well as to improve seedling stands and yields has inspired investigation into the physiological principles controlling these reactions. In addition to the natural regimen established by nature in native plant seeds there have been other innovative procedures developed to augment nature's best efforts, such as wetting, drying, chilling, thermal shock, irradiation, aeration, hormone treatments, and others. For those interested in further details, there are numerous publications available in the literature, discussing the subject at all levels (Bradford, 1995).

Presence of water is known to be the most important physiological factor needed to bring about seed germination. But how much water, and under which physical constraints has always been a difficult problem. Two measures of water status are necessary to describe the state of water in seeds. One relates to the amount of water and the other to the energy status of the water. Water content as measured by loss on drying in an oven is a measure of the amount of water. Although widely used for this purpose in the past, is not an accurate expression of water available for germinating seeds. Water potential ( $\Psi$ ) is a measure of the energy status of the water in the seed. It measures the availability of the water to participate in chemical and physical processes.

To understand accurate approaches to seed "priming" it is necessary to review some basic principles related to measurement of physical states of water. Physicists have shown that water molecules exist in various states of "tension", or "negative gage pressure". Water molecules

exist in an energy relationship with each other, other molecules including salts in solution, and water molecules in surrounding soil substrate, cytoplasm, and plant root cells. The water potential is a measure of this relationship. Water molecules flow through substrate pores (in soil, seeds, etc.) from high water potential sites to low water potential sites to reach equilibrium within seed environments over time. Units of water potential are megapascals (MPa). Water potential is generally a negative number because water in plant and soil systems is almost always under suction rather than pressure. Water potential values range from zero (pure water) to large negative numbers such as -100 MPa for air dry seeds. Thus, "high"  $\Psi$  is found in pure water and "low"  $\Psi$  in dry sites.

The total water potential is the sum of several component potentials. Those most relevant to seed priming are the osmotic potential and the matric potential. The matric potential results from a reduction in the energy status of water due to adsorption on cell walls, proteins, and soil colloids. At high water potential it also comes from the retention of water by small pores and capillaries. Osmotic potential is the reduction in the energy of water caused by its dilution with solutes such as salts and sugars. In seed priming the water potential of seeds is controlled by controlling either the matric potential or the osmotic potential of the seed environment.

### Seed priming techniques

The purpose of priming is to reduce the germination time, make germination occur over a short period and improve stand and percentage germination. Priming materials include substances for both matric and osmotic priming, although some seed priming carriers include both osmotic and matric processes. Some are available commercially while others are held for private

competitive business use.

- **Osmotic** priming is accomplished using chemicals that lower osmotic potential in the seed environment. Polyethylene glycol is a commonly used osmotic priming material because it is readily available and has no physiological reaction with seed. Very large molecules of this substance do not pass through seed cell membranes. Other osmotic priming agents include glycerol; mannitol, and Agro Lig.
- **Matric** seed priming substances or materials control  $\Psi$  by reducing the matric potential of the water in them through adsorption on particle surfaces. Seeds are mixed with materials of high surface area, with variable particle size (including colloidal) and a non-soluble solid state with low chemical reactivity. Specialized vermiculite compounds (Zonolite), Celite, and Micro Cel are used. These have high matric potential and low osmotic potential.

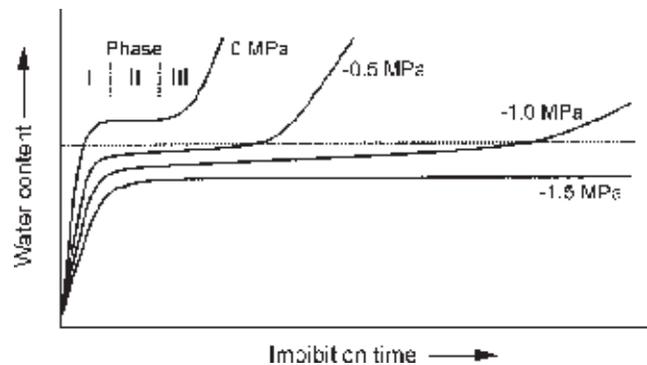
### Why is it so important to know $\Psi$ in seed priming?

Seeds germinate when  $\Psi$  reaches a critical physiological level in the seed. This varies within and between plant species, but in general occurs when the seed environment is between 0 and -2 MPa. Exceptions occur when seeds have impenetrable seed coats, or contain dormancy causing chemicals that must be removed before germination occurs.

Seeds having permeable seed coats usually go through three recognizable phases of germination: (1) imbibition;  $\Psi$  of the seed environment is higher than that in the seed, causing water molecules to flow through the seed epidermis into the embryo, leading to (2) the activation phase; in which stored seed hormones and enzymes stimulate physiological development leading to (3) growth of the radical; ending the germination phase. Dormant (dry) seeds are usually at very low  $\Psi$ , in

the range of -350 to -50 MPa. Some metabolism occurs even at these low  $\Psi$ 's. Water movement into dry seed during the imbibition phase is at first rapid but slows as  $\Psi$  of the seeds approaches  $\Psi$  of the environment. If imbibition is too rapid (from an environment where  $\Psi=0$ ) damage to hydrating cells often occurs.

It is only during the activation phase that seed priming can be successfully accomplished. A theoretical example of germination at a series of high  $\Psi$  levels is given in Figure 1, showing the effect of near-germination  $\Psi$  levels. Germination at high  $\Psi$ , as shown in the 0 MPa curve, occurs quickly, without allowing priming opportunity. By allowing the priming medium, and seeds, to come to equilibrium with each other at lower  $\Psi$  levels than the dotted line, time in phase II is lengthened, allowing priming activities to proceed. At low  $\Psi$  (-1.5 MPa) the water content is not sufficient for radical emergence to occur.



**Figure 1.** Time courses of seed  $\Psi$  during three phases of germination. Imbibition at reduced  $\Psi$  lowers seed water content, extends the length of phase II, and delays entry into phase III. Radical emergence and growth will occur if the water content exceeds a critical level indicated by the horizontal dotted line.

### Priming seeds

The priming process using matric priming requires that appropriate equipment be available to allow careful mixing of seed with a priming medium and

water, and an instrument such as Decagon's WP4 to accurately measure  $\Psi$  in seed and medium mixtures. In the beginning the seed will be dry and water is added to bring  $\Psi$  up to the value which starts the imbibition phase in the seed. The mixing process can be done using mechanical equipment or simple hand stirring in a bucket or bowl. Small cement mixers have been adapted where large amounts of seed are being processed, or a large drum with tight lid, and mixing vanes inside, has been rolled along the floor to thoroughly mix the seed together with the medium. There normally is a waiting period after the addition of water while diffusion within the mix brings equilibrium in  $\Psi$  throughout. Samples are taken and analyzed with the WP4 to reach desired activation phases.

The water potential required to keep the seed in activation phase is species dependent and must be determined by experimentation. Once this is known the approximate water content of the priming medium to reach that water potential can be established by making a moisture release curve (see Decagon's app note Generating a Soil Moisture Characteristic using the WP4 (AN 13380-00)).

**Measuring  $\Psi$  in seed priming programs**  
Equipment for measuring  $\Psi$  is available from Decagon Devices. WP4 Potentiometer provides accurate, rapid, and convenient water potential results. It provides sample analysis times of 5

minutes or less for most samples depending upon  $\Psi$  level. Because of its unusual speed, it can be used as an on-line monitor of  $\Psi$  during practical operations. Operation is automatic and only requires filling sample cups, placing the cup in the sample drawer, and turning the knob to start the analysis. Results appear directly in the display and can be downloaded to a computer. Measurements in the critical  $\Psi$  range of 0 to-2.5 MPa are accurate to within  $\pm 0.1$  MPa.

The future looks bright for innovative research and practice in this field, and the opportunity for success appears to be great. An important advantage is the availability of research and measurement instrumentation as a basis for immediate progress for those entering the program.

## References

- Bradford, Kent J. 1995. Water Relations in Seed Germination. Chapter 13, pp. 351 - 396. In "Seed Development and Germination". Ed. By Jaime Kigel and Gad Galili. Marcel Dekker, Inc, NY
- Wilson, A. M. and Grant A. Harris. 1966. Hexose, Inositol, and Nucleoside Phosphate Esters in Germinating Seed of Crested Wheatgrass. *Plant Phys.* 41: 1416 -1419.
- Decagon Devices Application Note: AN4101-10, Generating a Soil Moisture Characteristic using the WP4.