Korean J. Crop Sci. 48(4): 322~325(2003)

Effect of Controlled Hydration on Germination of Tobacco Seeds

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ABSTRACT : Controlled hydration treatment of tobacco seeds enhanced seed performance greatly without additional materials associated with conventional osmotic or solid matrix priming technique. The seeds were hydrated by adding water to a level from 10 to 60% by 5% increments and incubated for 8 days at 25°C. After the treatment, the seeds were dried to the original seed moisture content under 20°C for 72 hours. The moisture content of tobacco seeds equivalent to 35% by the hydration treatment gave the greatest improvement in germination rate and speed compared to untreated or polyethylene glycol (PEG) primed seeds, especially at low temperature of 15°C.

Keywords: seed priming, polyethylene glycol, hydration treatment, moisture content

Seed priming has been used as a valuable tool in improving the rate, speed and uniformity of germination (Heydecker *et al.*, 1973). Priming technique does not permit radicle emergence but rely on the controlled uptake of water to achieve a critical moisture content for activation of metabolic activity (Taylor, 1997). There are several methods to control water uptake for seeds: liquid priming, solid matrix priming (SMP) and controlled hydration. The liquid priming has been accomplished using osmotica, such as salts or polyethylene glycol (PEG) regulating water potential of seeds. The benefits from the liquid primed vegetable seeds were reported by several researchers (Brockhurst & Dearman, 1983a, b). However, it was thought that large quantity of the salt or PEG solution would be needed for commercial large-scale priming system and required subsequent disposal (Buljalski *et al.*, 1989). As an alternative to use osmotic solution, a water-absorbing carrier such as peat or vermiculite is used, that is called solid matrix priming (Taylor *et al.*, 1988). When priming large amounts of seeds the SMP method would be also inconvenient because the solid matrix material should be separated after priming.

If the seeds could be hydrated only by water with controlled system to get the desired seed moisture content, it would be the ideal seed hydration method. Several controlled hydration techniques have been reported. Fujikura *et al.* (1993) reported a hydropri-ming as seeds were presoaked in water, then exposed to 100% relative humidity. However, it might result in nonuniform seed hydration as well as microbial growth. A developed technique of a controlled hydration methods is drum priming. The drum priming technique hydrates seeds to the desirable moisture level by using limited amounts of water rather than osmotic solutions or solid matrix materials (Gray *et al.*, 1990; Rowse, 1996; Warren *et al.*, 1997). Rowse (1996) developed a drum priming system. The seeds were hydrated in the rotating drum with a predetermined water content by releasing water vapour inside of the drum. A previous studies indicated that PEG priming of tobacco seeds had a great priming effect on their germination performance (Min, 1999).

This study was done to evaluate controlled hydration effect on tobacco seed germination compared to PEG priming and to find optimal treatment condition of controlled hydration using a simple hydration device.

MATERIALS AND METHODS

Tobacco (*Nicotiana tabacum* L.) seeds cv. Burley21 were used in this experiment. As a controlled hydration, water was added with 1 g seed into 16 m glass bottle with lid. The quantity of water to add was calculated to 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60% as final moisture content calculated by the following equation (Taylor *et al.*, 1998).

$$WtH_2O = Wti(MCf - MCi)(100 - MCf)^{-1}$$

Where WtH_2O is the water weight adding to the initial dry seed weight (Wti), MCi is the moisture content of initial seed sample, and MCf is the final moisture content. Seed moisture contents were expressed as a percentage on a fresh weight basis. After adding water to seeds, the bottles were closed with lids and laid them on the roller mixer(Fig. 1). The roller mixer was placed in 25°C incubator for 8 days and the bottles were kept rotating by the roller during the incubation. After 8 days incubation, the seeds were dried to the original moisture content for 72 hrs in 20°C air and then stored at 4°C. Moisture contents of the seeds were checked from the samples of the same hydration treatments as described above by a moisture balance (FD-240, Kett, USA).

For PEG priming, seeds were placed on the blotters that

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<Received July 8, 2003>

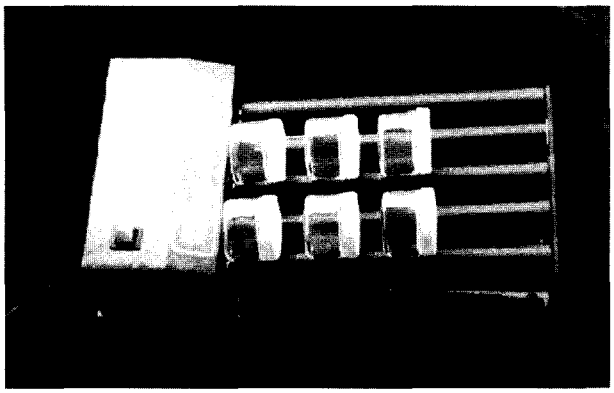

Fig. 1. The roller mixer used for hydration treatment of tobacco seeds.

were continuously moistened with PEG6000 solution in a plastic container (11×11×4 cm). Inside the container, 9 cm petri dish plate put down to support the blotter and two edges of the blotter were submerged and kept absorbing the 40 m PEG solution from the bottom of the container. The PEG6000 solution equivalent to -0.8 MPa (262 g/kg water) was used and the seeds were incubated at 25°C for 8 days, adapting the best results of the previous study (Min & Seo, 1999). After priming in PEG, the primed seeds were rinsed in tap water and dried to the original moisture content using the above drying method before stored at 4°C. Germination test was performed with the seeds equivalent to 10~40 % of moisture contents adjusted by adding water, as the seeds of 45% moisture content were germinated in the bottle during the hydration.

For germination test, seeds were placed on the blotters moistened with 40 m*l* water in the plastic container, the same method as the PEG priming described above. The germination test was done for 12 days in 15, 20 and 25°C incubators with 24 hours lighting a day. Germination was assessed using three replications of 50 seeds. All seeds with a visible radicle were counted and removed daily.

RESULTS AND DISCUSSION

After 8 days hydration treatment, seeds moisture contents were reached to almost same percentage as amount of water added from 10 to 35%, but no more than increased over 35%. It was considered that the maximum moisture content of the tobacco seeds was about 37%, because more weight increment was not shown in the seeds even added with water equivalent to 45%(Fig. 2). The seeds were germinated at 45 and 50% moisture contents during the incubation, but never done at the seeds of 55 and 60% moisture ones.

The germination speed and rate were greatly improved by hydration treatment compared to untreated and PEG primed

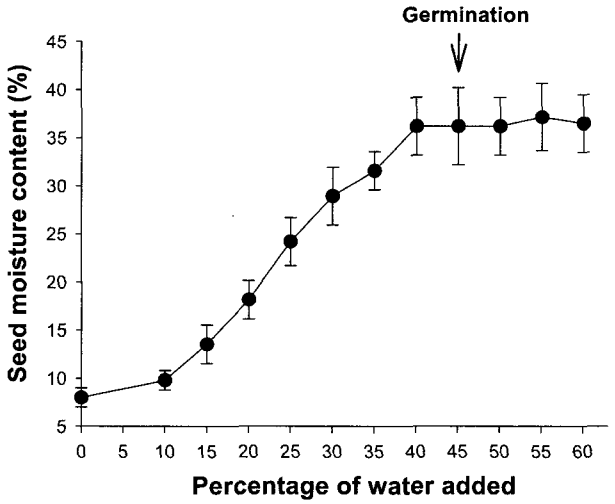

Fig. 2. Seeds moisture contents affected by hydration treatments through adding water. The seeds hydrated at 45% of water content (arrow point) were germinated during the treatment. Vertical bars mean standard error.

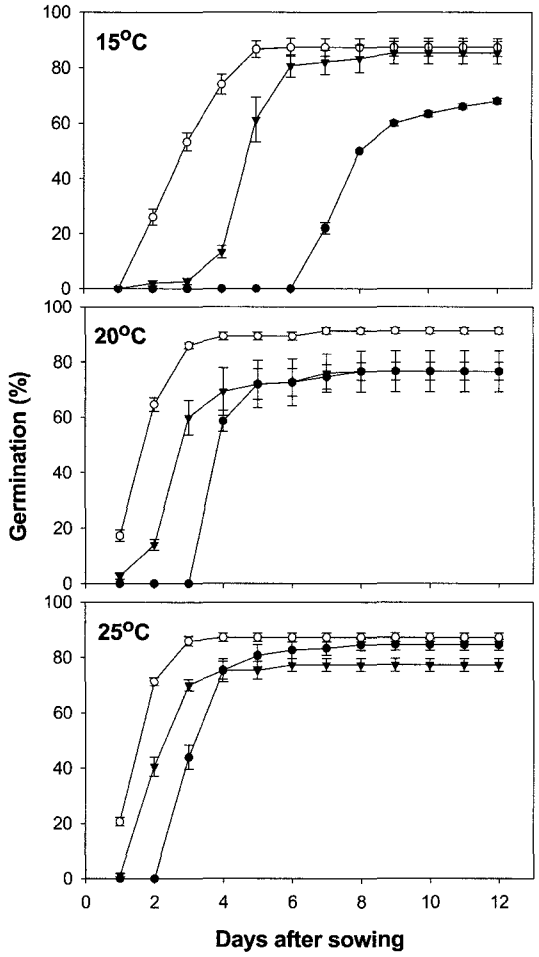

Fig. 3. Effect of hydration treatment and temperatures on the germination of tobacco seeds. Vertical bars mean standard error. Non-treated, ●; hydrated to 35% moisture content, ○; and PEG primed, ▼.

Table 1. Effect of seed hydration treatment and germination temperatures on T_{50} T_{10-90} and final germination of tobacco seeds.

Hydration treatment	T_{50} (day)†			T_{10-90} (day)‡			Final germination (%)		
	15°C	20°C	25°C	15°C	20°C	25°C	15°C	20°C	25°C
Control	7.4(0)§	3.7(0)	3.0(0)	3.1(0)	1.8(0)	2.0(0)	68d¶	77b	85ab
10%	7.4(0)	3.7(0)	2.6(0.4)	2.2(0.9)	1.7(0.1)	1.3(0.7)	90a	93a	83ab
15%	7.2(0.2)	3.7(0)	2.7(0.3)	2.5(0.6)	1.7(0.1)	1.6(0.4)	83bc	79ab	79b
20%	6.6(0.8)	3.6(0.1)	2.7(0.3)	1.5(1.6)	1.5(0.3)	1.7(0.3)	91a	92a	89a
25%	4.6(2.8)	2.7(1)	1.8(1.2)	2.0(1.1)	1.6(0.2)	1.6(0.4)	89a	86ab	94a
30%	4.3(3.1)	2.4(1.3)	1.6(1.4)	2.2(0.9)	1.6(0.2)	1.5(0.2)	76cd	81ab	89ab
35%	2.6(4.8)	1.6(2.1)	1.5(1.5)	3.1(0)	2.3(-0.5)	2.1(-0.1)	87a	91a	87ab
40%	7.3((0.1)	3.4(0.3)	2.5(0.5)	4.6(-1.5)	1.4(0.4)	1.1(0.9)	73cd	85ab	90a
PEG6000	4.6(2.8)	2.5(1.2)	1.9(1.1)	2.2(0.9)	2.6(-0.8)	1.1(0.9)	85ab	77b	77b

†T_{50}: Time(day) to 50% germination.
‡T_{10-90}: Time(day) from 10% to 90% germination.
§( ): Day differences between control and hydration treatments.
¶The same letters within column mean no significant different at 5% probability level by DMRT.

seeds especially at 15°C(Fig. 3). By the hydration treatments, the time to 50% germination (T_{50}) was gradually reduced in all three germination temperatures until hydration up to 35% moisture content, but increased at 40%. The most rapid germination (the smallest T_{50}) was revealed at 35% moisture content at 15°C, when the T_{50} of control was 7.4 days, that of 35% moisture content was reduced to 2.6 days; 4.8 and 2.8 days faster in germination than untreated (control) and PEG primed seeds, respectively (Table 1). Rowse (1996) reported that hydrated and drum primed leek seeds resulted in faster and more uniform germination than seeds primed by PEG. The time from 10 to 90% (T_{10-90}), indication of uniform germination was also slightly reduced but not much more improved than control. T_{10-90} at the seeds of 20% moisture content was reduced to 1.6 days (that was most improved T_{10-90} compared to control), but not much improved their T_{50} (it was 4 days higher than at the seeds of 35% moisture content) when germinated at 15°C. At 15 and 20°C germination temperature, final germination rate was significantly improved compared to control.

It could be concluded that the greatest priming effect was resulted in the controlled hydration treatment equivalent to 35% moisture content of tobacco seeds and incubated for 8 days at 25°C compared to untreated or PEG primed seeds, especially at low temperature. As Rowse (1996) mentioned about drum priming of leek seeds, it could be concluded that hydration treatment of tobacco seeds would not always more effective than PEG priming, but it could be the same or more effect than PEG priming with the advantage of no using PEG. In the germination test, the hydration effect was lost at 40% seed moisture content; T50 in 35% and 40% seed moisture contents were 2.6 days and 7.3 days, respectively (Table 1). It was considered that the detrimental hydration effect at 40% moisture content and no germination at 55 and 60% moisture contents might be caused by inhibition of respiration due to the excessive free water in the bottle. As the bottles kept closed during the hydration treatment in this experiment, the tobacco seeds might be damaged from preventing respiration due to reduction of oxygen. Therefore, it would be thought that experiments done in aerobic and anaerobic conditions should be needed to get more informations about seed respiration seed respiration. The simple device used for hydration treatment in this study was not expensive and a convenient tool for studying on the optimum hydration conditions in the lab.

ACKNOWLEDGEMENT

This work was supported by the Daegu University research grant, 2002.

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