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Changes in Metabolites and Embryo Growth during Seeds Priming in Tobacco

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ABSTRACT

Some metabolites and embryo growth of primed tobacco (*Nicotiana tabacum* L. cv. 'KF109') seeds were observed during priming. The seeds were primed at 15 and 25℃ for 1, 2, 3, 5, 10 and 15 days in a −0.8 MPa polyethylene glycol 6000 (PEG) solution. The time to 50% seed germination (T_{50}) was greatly reduced when the seeds were primed at 25℃ when compared with 15℃. The α-amylase activity and sugars and amino acid contents in the seeds primed at 25℃ greatly increased, while α-amylase activity was similar, and sugar and amino acid contents increased slightly in the seeds primed at 15℃. When the seeds were primed at 25℃, growth of the embryo which was enclosed by endosperm was detected, while the endosperm became thinner as the priming duration was extended.

Keywords : **priming, polyethylene glycol 6000, tobacco, T_{50}, α-amylase activity, sugar, amino acid, embryo, endosperm.**

The priming of seeds in the low water potential solutions, e.g., polyethylene glycol (PEG) solution, has been widely used as a preplant seed treatment to enhance germination time, to synchronize germination, and to increase germination rate (Bradford, 1986; Heydecker & Coolbear, 1977; Khan, 1992). Heydecker et al (1978) reported that during priming, seeds absorbed enough water to be physiologically so active that they could mobilise their reserves and initiate their germination sequence. Although the mechanism of the priming is not fully understood, it has been observed that the physiological and biochemical changes occurred during priming could allow for seeds to develop a germination sequence immediately before germination. This would be a basis for germination improvement or better emergence potentials of the primed seeds. Early studies with tomato and lettuce seeds indicated that DNA synthesis was associated with priming (Coolbear & Grierson, 1979). Tomato seeds had also been shown to accumulate a much higher level of RNA than normally germinated ones and onion seeds solubilised some of their reserved lipids, translocated, and redeposited them as starch in the radicle tip during seed priming (Khan, 1992).

Several workers have shown an enhancement of protein synthesis during osmoconditioning and/or subsequent germination in lettuce, tomato, pepper, leek, and wheat seeds (Khan et al., 1978, 1980, & 1981; Mazor et al., 1984). When pepper seeds were imbibed in PEG solution, nearly twice as much more amino acid was incorporated to protein during the first 24 hours compared with water soaking (Mazor et. al., 1984).

Some effects of PEG priming were explained by an increase in enzyme activity and inhibition of cell elongation during the treatment (Ells, 1963; Heydecker et al., 1975). In contrast to these opinions, Austin et al. (1969) showed that each cycle of hardening led to an increase in embryo length of carrots. Wiebe and Tissen (1979) indicated that the pretreatments of seeds were successful in the vegetable crop seeds of which embryos are relatively small compared with the total seed size such as carrots, celery, onions, beets, tomatoes, and peppers. The hypothesis for this priming effectiveness was that presowing seed treatments led only to early emergence if the embryo grows during the treatment.

This study was conducted to observe some metabolites and anatomical changes in primed tobacco seeds to understand some mechanisms of priming effects by detecting; 1) α-amylase activity and the contents of sugars and amino acids in the tobacco seeds during the priming at suboptimal (15℃) and optimal (25℃) temperatures. and 2) the morphological changes, especially embryo, in the seeds during priming.

MATERIALS AND METHODS

The test tobacco (*Nicotiana tabacum* L. 'KF109') seeds, flue-cured type, were stored for one year at 4℃ after harvest in 1995. The germination rate and T_{50} (time to 50% germination) of the control seeds were 85% and 9.0 days, respectively, when germinated at 15℃. The seeds were primed in the temperature controlled incubators at 15 and 25℃ for 1, 2, 3, 5, 10 and 15 days in the −0.8 MPa polyethylene glycol 6000 solution. The priming and the osmotic level of

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-0.8 MPa followed the method of Min and Seo (1999). After priming, the seeds were rinsed in distilled water, and then dried at room temperature.

Germination test was conducted depending on the AOSA method (1993) with three replicates of 100 seeds at constant 25 and 15℃ incubators. Time to 50% germination (T_{50}) was calculated according to the formula (Coolbear et al., 1984),

$T_{50} = t1+\{(N+1)/2 - n1/(nj-ni)\} \times (tj - ti)$, where N is the final number of seeds germinating and ni, nj, total number of seeds germinated by adjacent counts at time ti, tj, where $ni < (N+1)/2 < nj$.

One fifth gram (0.2 g) of the primed seeds were ground in a mortar with 10 ml of 0.2M phosphate buffer (pH 7.5) and centrifuged at 15,000×g for 30 minutes at 4℃. The supernatant solutions were used for assaying α-amylase activity, sugars, and amino acids.

The activity of α-amylase was determined by a method of Shuster and Gifford (1962). To make starch solution, 67 mg of soluble starch was added to 100 ml of 0.06M KH_2PO_4, heated to boiling, and cooled to room temperature. One milliliter of the starch solution was added to 0.2 ml of seed extraction solution for assaying α-amylase activity and made a final volume of 2.0 ml with distilled water. The mixture of starch and enzyme solutions were incubated for 5 minutes at 25℃, then the reaction was stopped by the addition of 1.0 ml of iodine-HCl solution (60 mg of KI and 6 mg I_2 in 100 ml of 0.05 N HCl). Then 5 ml water was added and mixed well. The optical density (OD) of the resulting solution was measured at 620 nm. The activity of α-amylase was defined as a unit of $A \times V'/T \times V$. (A: OD of the mixture of starch and enzyme solutions), V': the volume of original enzyme solution in ml, V: the volume of OD measured enzyme solution in ml, T: reaction time in minutes).

Total sugars were quantified by the phenolsulfuric acid method with glucose as a standard (Dubois et al., 1956). Total amino acids were measured by the ninhydrin method with glycine as a standard (Yemm et al., 1955).

To observe morphological changes, tobacco seeds were primed in the -0.8 Mpa PEG 6000 solution for 5, 10 and 15 days at 25℃. The seeds were fixed in the mixture solution of ethanol : acetic acid (3 : 1) for 12 hours. The seeds were then washed by soaking in distilled water for 12 hours, dehydrated through a graded ethanol series, and embedded in paraffin. Sections were cut with a microtom (Nippon Optical Works, Japan), stained with 1% safranin for 24 hours, and then restained with 0.5% fast green for 30-40 seconds. The embryos of the seeds were examined and photographed with a light microscope (Olympus BHS, Japan).

RESULTS AND DISCUSSION

In our previous study, -0.8 MPa was found to be the optimum water potential of PEG 6000 solution for the highest water potential just preventing germination of tobacco seeds and this osmotic level was used for priming in this study (Min and Seo, 1999). However, the effects of priming duration and temperature were not studied for tobacco seeds. Thus, seeds were primed at two temperatures and five different durations to know the optimum priming duration and temperature. Germination speed, α-amylase activity and total sugar and amino acid contents were examined.

The time from planting to 50% germination (T_{50}) of primed tobacco seeds is shown in Fig. 1. As the duration of priming was extended, T_{50} of seeds primed at 25℃ was reduced at a faster rate compared with seeds primed at 15℃; the priming effect of tobacco seeds was greater at 25℃ than primed at 15℃.

The α-amylase activities of the seeds primed at 15℃ and 25℃ with different priming durations is shown in Fig. 2A. When seeds were primed at 25℃, α-amylase activity increased gradually up to 10 days priming and decreased thereafter. However, at 15℃ the α-amylase activity of the seeds was similar and fluctuated over the priming periods. This would indicate that priming temperature greatly affected metabolic activity of the tobacco seeds during priming. The marked increase in the α-amylase activity of the seeds primed at 25℃ compared with 15℃

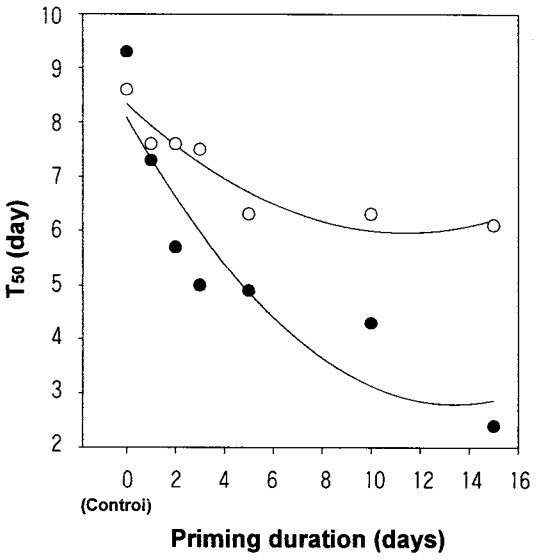

Fig. 1. Time to 50% germination (T50) of tobacco seeds primed at 15℃ (○, $y=8.3-0.4x+0.02x^2$, $r=0.95$) and 25℃ (●, $y=8.1-0.8x+0.03x^2$, $r=0.91$) depending on the various priming durations when germinated at 15℃.

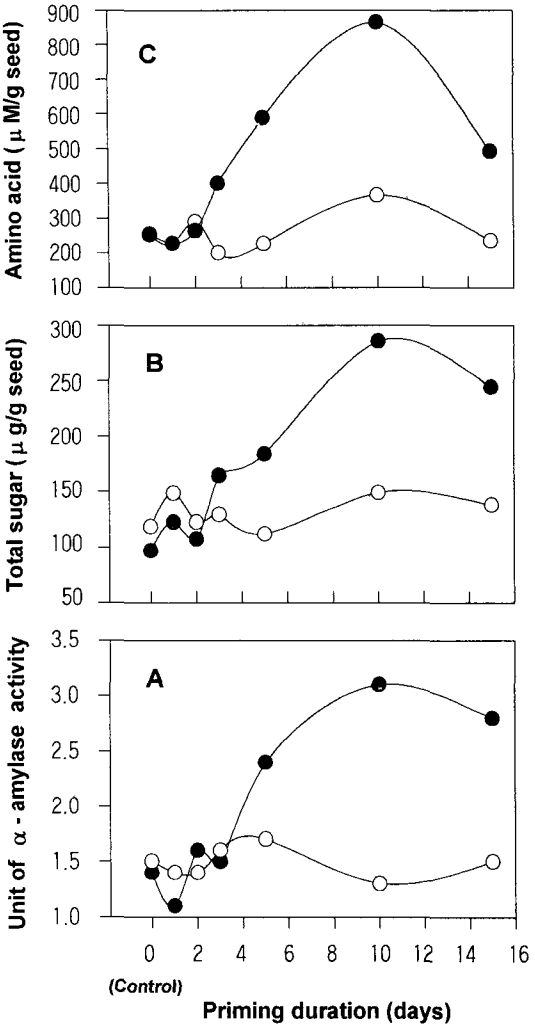

Fig. 2. Changes of α-amlase activity (A), total sugar content (B), and total amino acid content(C) of tobacco seeds primed at 15℃ (○) and 25℃ (●) for different priming durations.

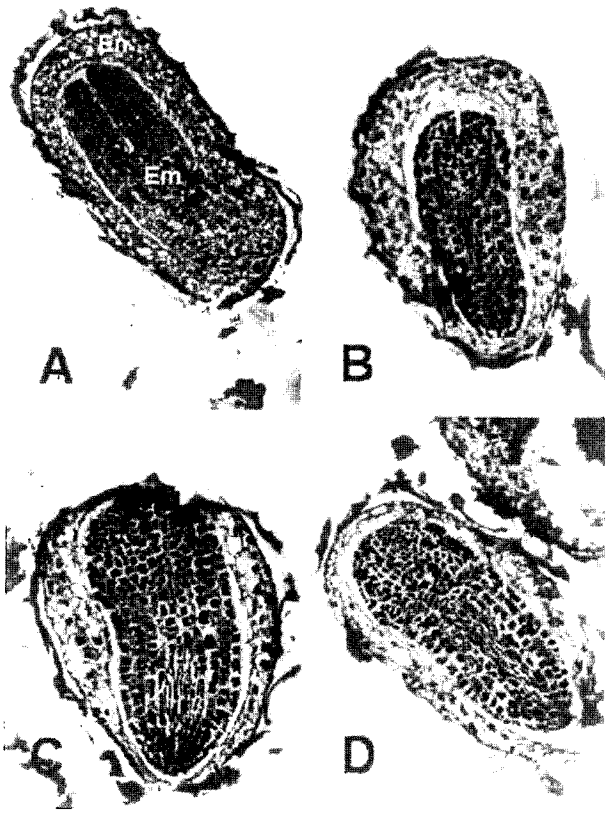

Fig. 3. Micrographs of cross sectioned tobacco seeds (x200). (A: Non-primed, En: endosperm, Em: embryo. B, C, and D: primed for 5, 10, and 15 days at 25℃).

corresponded well to the reduction in T_{50} as shown in Fig. 1.

The changes in the total sugars and amino acid content in the seeds primed at 15 and 25℃ during the priming are shown in Fig. 2, B and C. Like α-amylase activity, at the priming temperature of 25℃ the content of both total sugars and amino acids increased to 10 days priming, and then decreased. However, at 15℃ the content of both total sugars and amino acids were similar or fluctuated over the priming periods.

Bewley and Black (1978) explained the kinetics of water uptake by seeds as a triphasic pattern; the phase I was an initial uptake of water and a consequence of the matric forces of the cell walls and cell contents of the seed, and this uptake occurred irrespective of whether seeds were viable or nonviable. The phase II was the lag period of water uptake, when the matric potential was high, as was the solute or osmotic potential and the phase III was associated with visible germination. During phase II, the seed underwent many biochemical processes essential for germination, such as an increase in respiration, synthesis of hydrolytic enzymes, breaking down stored nutrients, and the synthesis of new materials needs for embryo development. Seed priming was generally referred to a hydration technique of seeds, but preventing the completion of germination, that would be to keep only in phase II by soaking seeds in a solution of low osmotic potential. Therefore, it could be expected that the release of hydrolytic enzymes including α-amylase would result in a degradation of endosperm in the phase II, which would be a duration of priming, and release sugars from starch and amino acids from protein breakdown.

In this study, the results indicated that maximum effectiveness of the priming could be obtained from the seeds which had maximum metabolic activity during priming. When the seeds were primed at suboptimal temperature of 15℃, little changes in α-

amylase activity and contents of total sugars and amino acids were observed and the effectiveness of priming were considerably low compared with the seeds primed at 25℃. While at 25℃, the markedly reduced T_{50} was corresponded to the increased α-amylase activity and content of total sugars and amino acids. This implies that the vigorous metabolic activities, such as breaking down and mobilization of the reserved food would provide substrates to growing embryos when primed under the appropriate conditions. The α-amylase activity and content of total sugars and amino acids started to decline when primed for 15 days. Although the reason of the decline was not verified in this study, the stored starch and protein started to deplete and the hydrolyzed sugars and amino acids could be utilized for the synthesis of new materials.

The micrographs of cross sectioned both non-primed and primed tobacco seeds are showed in Fig. 3. The embryo growth was observed in the seeds primed for 10 and 15 days at 25℃ (Fig. 3, C & D). The endosperm of the properly primed seeds surrounding the embryo became thinner compared with non-primed seeds. This indicates that the degradation of the endosperm occurred during the priming to provide the substrates for embryo growth. The germination of tobacco seeds occurred in the following sequence; imbibition, enzyme activation, initiation of embryo growth, rupture of the seed coat, and radicle emergence. Thus, in this study, it was clear that fast germination of primed tobacco seeds would be attributed to the advanced germination sequence such as enzyme activation and embryo growth during priming. Therefore, the primed seeds were ready to germinate immediately after imbibition of water.

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