Embryo Drying Rates during the Acquisition of Desiccation Tolerance in Maize Seed

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ABSTRACT

Maize (Zea mays L.) seed quality is often reduced because of drying injury, although the causes and impairment mechanisms are poorly understood. In this study, we investigated changes in embryo drying rates and their effect on the acquisition of desiccation tolerance in maize seed. Ears of hybrid maize [B73 × (H99 × H95)] were harvested at about 550, 500, 400, and 320 g H2O kg⁻¹ fresh weight (fw) and subjected to preconditioning (PC) (ear drying at 35°C and 0.47 m s⁻¹ airflow rate) for 0, 12, 24, 36, and 48 h before fluidized bed (FB) drying (shelled seed, 35°C and 5.10 m s⁻¹ airflow rate) treatments to decrease moisture content (MC) to about 130 g H2O kg⁻¹ fw. Additionally, ears were entirely dried under PC (35°C) and unheated-air (NH) conditions. At the four harvests, different drying rate phases were evident in embryos of seed dried entirely at PC (35°C) conditions. A slower drying phase coincided with the PC period, which increased with increasing maturation. Under FB drying, embryo MC declined at a faster rate down to about 400 g H2O kg⁻¹ fw, followed by an intermediate drying rate down to about 200 g H2O kg⁻¹ fw, and a slower drying rate below this point. As embryo MC declined to 400 g H2O kg⁻¹ fw at slower drying rates, either with PC or field drying, the ability to withstand the faster drying rates of the FB progressively increased. This effect was illustrated by lower cell solute leakage and better performance in germination and vigor tests. We conclude that slow embryo drying rates to threshold levels may be crucial to acquire their ability to withstand higher drying rates without detrimental effect on seed germination and vigor.

Maize seed in the Midwest is often harvested at moisture contents >400 g H2O kg⁻¹ fw and subsequently dried mechanically to storage levels (120 g H2O kg⁻¹ fw). In seed dryers, heated air is the energy source and transport vehicle to remove moisture from the seed mass. Although drying damage often occurs, it is not clear whether high temperature or high drying rates cause the injury. Heat injury during seed drying has been poorly defined. Herter and Burris (1989b) suggested that membrane injury is one of several expressions of drying damage in seed. This kind of injury has been inferred by measuring the electrical conductivity of seed exudates during rehydration (Simon and Raja Harun, 1972; Abdul-Baki and Baker, 1973). Electrolyte and sugar leakage were higher in seed dried at temperatures >45°C as compared with those dried at temperatures lower than 35°C (Seyedin et al., 1984; Herter and Burris, 1989b; Peterson, 1997).

It is well documented that orthodox seed types (tolerant to desiccation) acquire the ability to tolerate higher drying temperatures without detrimental effects on seed germination and vigor as maturation progresses. Maize ears harvested from 400 to 500 g H2O kg⁻¹ fw could be safely dried down to 120 g H2O kg⁻¹ fw with temperatures around 40°C (Kiesselbach, 1939; Washko, 1949; McRostie, 1949; Navratil and Burris, 1984), whereas ears harvested at MC < 250 g H2O kg⁻¹ fw could be dried at 50°C (Navratil and Burris, 1984). Nevertheless, maize ears harvested as early as 30 d after silking and dried slowly are able to withstand desiccation (Knittle and Burris, 1976; Peterson, 1997). This may illustrate the importance of drying rate.

Maize seed is composed of different tissues, which appear to dry at different rates because of their position. Under field drying conditions, embryo moisture remains about 150 g H2O kg⁻¹ fw higher than whole seed as it drops from 400 to 300 g H2O kg⁻¹ fw. Then, the embryo MC decreases rapidly to equilibrium with whole seed at about 150 g H2O kg⁻¹ fw (Struve, 1958). This phenomenon has also been reported under artificial drying and preconditioning (slow drying before fast drying) treatments (Loeffler and Burris, 1982; Herter and Burris, 1989a; Peterson, 1997). The present study was conducted to increase our understanding of the drying rates of maize embryo during seed development and maturation and its implication in acquisition of desiccation tolerance. Our specific objectives were to study the changes in maize embryo drying rates under preconditioning and fast drying environmental conditions and their implications in the acquisition of desiccation tolerance as quantified by standard germination, seed leakage, and vigor tests.

MATERIALS AND METHODS

Plant Material

The inbred line B73 and the sister line H99 × H95 (female and male, respectively) were grown in 1998, 1999, and 2000 at the Curtis Farm, Iowa State University, Ames, IA. Each year, hybrid ears were bulk harvested at about 550, 500, 400, and 320 g H2O kg⁻¹ fw, as measured by the oven method (Lawrence, 1961) but at 105°C for 24 h. At each harvest after dehusking, ears with similar maturation characteristics (color, milk line, black layer) were selected to reduce moisture variability. These ears were placed in mesh plastic bags and assigned to the respective drying treatment.

Drying Treatments

Ears were subjected to a preconditioning (PC) environment (about 35°C, airflow rate of 0.47 m s⁻¹, and about 25% RH) with thin-layer dryers (Navratil and Burris, 1982) for 0, 12, 24, 36, and 48 h. After PC, the mid-ear portion was hand shelled and transferred to a fluidized bed (FB) dryer or fast fluidized bed (FFB) dryer at 35°C and 0.47 m s⁻¹ airflow rate.

Abbreviations: PC, preconditioning; FB, fluidized bed; MC, moisture content; fw, fresh weight; RH, relative humidity; NH, unheated-air; COND, electrical conductivity; SG, standard germination test; AA, accelerated aging.
drying system [35°C, 5.10 m s⁻¹ airflow rate, and about 25% relative humidity (RH)] to dry the seed to about 130 g H₂O kg⁻¹ fw. Additionally, as negative controls, ears were dried entirely under PC (35°C) and with unheated air (NH). For all the treatments, three replications of five ears were used. The experimental layout by harvest was a randomized complete block design considering dryers as blocks. Seed was stored at 10°C and 50% RH until seed quality evaluation was performed.

Moisture Content

Moisture content (MC) was measured by the oven method (105°C for 24 h). In 1998 and 1999, MC was determined after each PC time in three replications of 10 intact seeds and five excised embryos. In 2000, embryos were excised in a humidified chamber at about 75% RH, and three replications of five seeds and five embryos were used instead. In addition to determining MC before fast drying, measurements were continued every 12 h until full drying was achieved at the PC conditions. Furthermore, at each harvest, intact seed and embryo moisture were monitored at intervals of 30 min during the fast drying treatment in seed that had been PC for 0, 24, and 48 h. Additionally, MC was determined at irregular intervals as seed maturation and drying occurred under field conditions. Moisture contents are reported as g H₂O g⁻¹ fw, and drying rates are calculated at particular drying periods.

Seed Quality Evaluations

Standard germination in rolled towel (7 d at 25°C) and accelerated aging (AA) tests (Delouche, 1996) were conducted on 100 untreated seeds per lot divided into four replicates of 25 seeds. Seedlings were evaluated according to Association of Official Seed Analysts Rules (1992). Electrical conductivity was measured with the Individual Seed Analyzer, Genesis-2000 (Wavefront, Inc., Ann Arbor, MI), on 100 seeds per lot divided in four replicates of 25 seeds. In each cell, 3.75 mL of distilled H₂O were added, and readings were taken after 24 h soaking at room temperature with values reported in microsiemens per milligram of seed tissue at about 120 g H₂O kg⁻¹ MC.

Trends of moisture contents and seed quality parameters were analyzed by harvest in all three years. Since trends were similar in all three years, we only present data obtained in 2000 for moisture content and data obtained in 1998 for seed quality parameters. The standard error of the mean is reported in all cases.

RESULTS

Changes in Moisture Content

During the PC drying phase (0–48 h), intact seed harvested at 550 g H₂O kg⁻¹ fw (Fig. 1A) exhibited the fastest drying rate (4.9 g H₂O kg⁻¹ fw h⁻¹). Drying rates decreased slightly to 3.6, 3.0, and 2.5 g H₂O kg⁻¹ fw h⁻¹ for seed harvested at 500, 400, and 320 g H₂O kg⁻¹ fw, respectively (Fig. 1B–D). This trend was reflected throughout the entire drying period at the PC conditions (35°C). Embryo drying rates, on the other hand, decreased at about 0.6 g H₂O kg⁻¹ fw h⁻¹ during the PC phase for seed harvested at 550 and 500 g H₂O kg⁻¹ fw. Then, embryo-drying rates increased to about 1.0 and 2.0 g H₂O kg⁻¹ fw h⁻¹ during the first 36 h of PC for seed harvested at 400 and 320 g H₂O kg⁻¹ fw; followed by about 5.0 and 6.0 g H₂O kg⁻¹ fw h⁻¹ from 36 to 48 h.
Fig. 2. Decreases in intact seed and embryo moisture contents under field maturation-drying conditions. Pollination occurred about July 21 and samples were taken at irregular intervals from August to September. MC = moisture content; Bars = standard error of the mean.

PC, respectively (Fig. 1C, D). Drying entirely at the PC conditions, three drying rate phases could be distinguished in embryos of seed harvested at 550 and 500 g H₂O kg⁻¹ fw (Fig. 1A, B). A slow drying rate (0.6 g H₂O kg⁻¹ fw h⁻¹) during the PC phase (0–48 h), an intermediate drying rate (about 4.0 g H₂O kg⁻¹ fw h⁻¹) from 48 to 72 h, and a fast drying rate (8.0 g H₂O kg⁻¹ fw h⁻¹) in the remaining 36 h of drying. On the other hand, only a slow drying rate (1.0 g H₂O kg⁻¹ fw h⁻¹) and (2.0 g H₂O kg⁻¹ fw h⁻¹) during the first 36 h and a fast drying rate (about 5.0 g H₂O kg⁻¹ fw h⁻¹, both harvests) were evident for seed harvested at 400 and 320 g H₂O kg⁻¹ fw, respectively. In all harvests, embryo attained equilibrium MC with intact seed below 140 g H₂O kg⁻¹ fw.

Under field conditions, embryo MC is very similar to intact seed at MC higher than 500 g H₂O kg⁻¹ fw (Fig. 2). Then, intact seed MC decreased at faster rates than the embryo exhibiting a difference of about 100 to 120 g H₂O kg⁻¹ fw as intact seed dry from about 400 to 250 g H₂O kg⁻¹ fw; subsequently, the embryo moisture declined rapidly and reached equilibrium with intact seed at about 150 g H₂O kg⁻¹ fw. Similar trends in embryo-moisture loss were observed under PC drying (35°C), but a difference in MC >150 g H₂O kg⁻¹ fw is evident between intact seed and embryo as MC declined from about 400 to 250 g H₂O kg⁻¹ fw for seed harvested at 550 and 500 g H₂O kg⁻¹ fw (Fig. 1A, B). This difference became <150 g H₂O kg⁻¹ fw for seed harvested at 400 and 320 g H₂O kg⁻¹ fw (Fig. 1C, D).

In the fluidized bed, seed harvested at 550 and 500 g H₂O kg⁻¹ fw and dried without PC exhibited an intact seed drying rate about 100 g H₂O kg⁻¹ fw h⁻¹ as it dried to about 400 g H₂O kg⁻¹ fw (Fig. 3A, B). Once 400 g H₂O kg⁻¹ fw is achieved either with PC or maturation, there is a slight decrease in drying rates, and the drying trends are comparable within and across harvests (Fig. 3, 4). On the average, intact seed drying rates ranged from 40 to 60 g H₂O kg⁻¹ fw h⁻¹ as moisture declined from about 400 to 230 g H₂O kg⁻¹ fw and below 230 g H₂O kg⁻¹ fw drying rates slowed to less than 20 g H₂O kg⁻¹ fw h⁻¹.
Embryos of seed harvested at 550 and 500 g H$_2$O kg$^{-1}$ fw and dried without PC in the FB shared similar trends (Fig. 3A, B). The drying rates are fast (about 75.0 g H$_2$O kg$^{-1}$ fw h$^{-1}$) as embryo MC declines down to about 420 g H$_2$O kg$^{-1}$ fw, which seems to be a breaking point for a slight change in the embryo-drying rate (about 50 g H$_2$O kg$^{-1}$ fw h$^{-1}$). Another slight decrease in embryo drying rates (about 24.0 g H$_2$O kg$^{-1}$ fw h$^{-1}$) occurs below about 200 g H$_2$O kg$^{-1}$ fw. Below 400 g H$_2$O kg$^{-1}$ fw, embryo-drying rates seem to follow a steadier trend for seed harvested at 500 g H$_2$O kg$^{-1}$ fw than seed harvested at 550 g H$_2$O kg$^{-1}$ fw. In advanced harvests (400 and 320 g H$_2$O kg$^{-1}$ fw), embryo drying occurs at slightly lower rates as MC declined to about 400 g H$_2$O kg$^{-1}$ fw in comparison with previous harvests (Fig. 3C, D). Consequently, the break point at about 400 g H$_2$O kg$^{-1}$ fw became less evident. PC treatments before fast drying appeared to simulate the effect of seed maturation but to a lesser degree in seed harvested at MC > 500 g H$_2$O kg$^{-1}$ fw. Seed harvested at 400 g H$_2$O kg$^{-1}$ exhibited steadier decreases in embryo drying rates after 24 h PC than seed harvested at 550 g H$_2$O kg$^{-1}$ and been preconditioned for 48 h before fast drying (Fig. 4A, B).

**Seed Quality**

Seed harvested at 550 and 500 g H$_2$O kg$^{-1}$ fw, subjected to FB drying without PC (0 h) and 12 h PC, exhibited high electrical conductivity (COND) and 0% germination in the standard germination test (SGT) (Fig. 5A, B). This coincided with faster embryo drying rates as MC declined to about 400 g H$_2$O kg$^{-1}$ fw. Conductivity decreased while standard germination increased with longer PC times before FB drying. However, even 48 h PC was insufficient to decrease drying injury, such that COND and SGT values were compara-
ble to those observed in seed dried entirely at the PC conditions (35°C) or with unheated air (NH) (Fig. 5A, B). In both 550 and 500 g H₂O kg⁻¹ fw harvests, the only change evident in embryo MC before FB drying (PC phase) was a decrease of about 30 g H₂O kg⁻¹ fw at very slow drying rates. This result suggests that even a very small embryo water loss triggers desiccation tolerance mechanisms but are insufficient to manifest complete desiccation tolerance. Nevertheless, even though drying rates slightly increased in the FB for following 48 h of PC (Fig. 4A), conductivity decreased and germination increased compared with less PC time.

Electrical conductivity of seed harvested at 400 g H₂O kg⁻¹ fw and dried in the FB without PC was lower than previous harvests (Fig. 5C). Although low, some germination in SGT was observed in this treatment. The effect of PC, again, was illustrated in reduced conductivity and enhanced germination. Furthermore, less PC time was required to reach COND and SGT values similar to the 35°C and NH treatments (Fig. 5C). The effect of maturation on COND and SGT was also observed in seed harvested at 320 g H₂O kg⁻¹ fw, 0 h PC (Fig. 5D). The PC effect on these quality variables, however, was almost nullified. Thus, the embryo ability to withstand the dehydration rates of the FB may increase as embryo MC declines to about 400 g H₂O kg⁻¹ fw at slow drying either with PC or field maturation. The shorter PC times, in late harvests, required for attaining lower conductivity and better germination suggests that desiccation tolerance components may be closer to their “threshold levels”.

Accelerated aging (AA) test before germination resulted in further reduction in germination. Seed harvested at 550 g H₂O kg⁻¹ fw MC exhibited 0% germination with no improvement with PC (Fig 5A). A slight increase in AA after 36 h PC was observed for seed harvested at 500 g H₂O kg⁻¹ fw, and a major increase was noted with 48 h PC for seed harvested at 400 g H₂O kg⁻¹ fw (Fig. 5B, C, respectively). On the other hand, AA increased linearly with PC time for seed harvested at 320 g H₂O kg⁻¹ fw (Fig. 5D). After 36 h PC, AA percentages were comparable with percentages obtained under 35°C and NH drying conditions. Increases in AA germination are associated with low initial embryo moisture contents before fast drying. It appears that an initial MC < 400 g H₂O kg⁻¹ fw is required to achieve >60% germination in AA and a MC < 300 g H₂O kg⁻¹ fw to reach percentages >90%. These results support our previous statement that a slow embryo-drying rate during PC as well as field maturation might be crucial to acquiring desiccation tolerance.

**DISCUSSION**

Intact seed moisture decreased 230 and 170 g H₂O kg⁻¹ fw during the preconditioning phase for seed harvested at 550 and 500 g H₂O kg⁻¹ fw while embryo moisture decreased only about 30 g H₂O kg⁻¹ fw. For seed harvested at 400 and 320 g H₂O kg⁻¹ fw, intact seed drying rates decreased slightly and embryo-drying rates exhibited a remarkable increase, particularly from 36 to 48 h PC. Drying entirely at PC (35°C) conditions, intact seed drying rates decreased linearly whereas embryos exhibited distinctive drying rate phases. Overall, the embryo-drying pattern coincided with that observed by Herter and Burris (1989a) who used preconditioning drying temperature of 50°C before ears were dried at 35°C, and the observation by Peterson (1997) who reported that moisture content of embryo axes was always higher than intact seed dried at different drying conditions.

Struwe (1958) reported that under field drying embryo moisture remained about 150 g H₂O kg⁻¹ fw higher than whole seed in the range of 300 to 400 g H₂O kg⁻¹ fw. Below 300 g H₂O kg⁻¹ fw seed moisture, the embryo MC dropped rapidly and reached the whole seed moisture about 150 g H₂O kg⁻¹ fw. Our results are in agreement with this finding. Furthermore, the embryo-drying trend observed under PC drying at each individual harvest was similar with the trend observed under field drying conditions. However, the difference in moisture content observed between intact seed and the embryo is higher under PC drying than field drying, particularly for seed harvested at 550 and 500 g H₂O kg⁻¹ fw. This may be due to the fact that under artificial drying, water loss may depend mainly on environmental conditions, while at this maturation stage water loss in the field may depend on environmental conditions as well as rate of storage compound deposition.

There is no completely logical or accepted explanation for the pattern of embryo moisture loss. Herter and Burris (1989a) stated that the slow embryo-drying rate during the early drying phase could be associated with its position on the ear. At high moisture content, seeds may be closely oppressed to each other restricting dry-air circulation to the seed surfaces (mainly endosperm and pericarp tissues), from where water may be removed (Fig. 6A). As intact seed moisture content decreases during artificial drying as well as with field maturation, seed shrunk and the space between seeds opens, facilitating dry-air circulation to the lower portion of the seed and evaporation of water from embryo tissue (Fig. 6B). Additionally, water migration from embryo tissue (wetter) to dryer tissues may be possible. Cob drying patterns were not evaluated in this study, but we do not discard the possibility that they may also influence embryo drying. These drying characteristics, among others, may also be associated with the linear decrease in moisture content exhibited by intact seed.

Under the FB drying conditions, intact seed water loss is characterized by a sharp decrease during the first drying hours followed by a gradual decrease as drying time progresses. These characteristics are more evident at very high MC (>400 g H₂O kg⁻¹ fw) and to a lesser extent down to about 230 g H₂O kg⁻¹ fw. The embryo also began losing water as soon as drying was started and in general follows a trend similar to the intact seed. In seed harvest at high MC (550 and 500 g H₂O kg⁻¹ fw), the embryo exhibited a very rapid drying rate down to about 400 g H₂O kg⁻¹ fw; followed by a slight decrease as it declines to about 200 g H₂O kg⁻¹ fw, and below this MC another slight decrease in drying rate is evident.
With advancing maturation (400 and 320 g H₂O kg⁻¹ fw), the first two drying rates exhibited slight decreases concomitant with slightly lower and more uniform drying rates, and consequently a less perceptible break in the drying rate at about 400 g H₂O kg⁻¹ fw. PC before fast drying seems to have an effect similar to maturation, but to a lesser degree in seed harvested at MC > 500 g H₂O kg⁻¹ fw. Changes in embryo drying rates under fluidized bed conditions seem to coincide with the types of water in the seed distinguished by calorimetric and motional properties (Vertucci and Farrant, 1995). The fast drying rate (down to about 400 g H₂O kg⁻¹ fw) coincides with Type 4 water, which is believed to be a concentrated solution or capillary water and is detected between 700 and 450 g H₂O kg⁻¹ dry matter (DM). The slightly lower drying rate (from about 400 to 230 g H₂O kg⁻¹ fw) coincides with Type 3 water, which is suggested to form bridges over hydrophobic moieties on macromolecules and is detected between 450 and 250 g H₂O kg⁻¹ DM. The low drying rate (below 200 g H₂O kg⁻¹ fw) coincides with Type 2 water, which has glass characteristics and is believed to have strong interactions with polar surfaces of macromolecules of hydroxyl groups or solutes and is detected between 250 and 80 g H₂O kg⁻¹ DM. In addition, the drying characteristics of an individual seed may also contribute to the changes in embryo drying rates observed under FB conditions. In shelled seed, the entire surface is exposed to air circulation, which allows rapid evaporation of water vapor from the surface (Fig. 7A). As a result, water from internal seed tissue will move to the periphery of the seed to compensate the moisture gradient (Fig. 7A, B). The first water to be removed will be the closest to the periphery and weakly tied to macromolecular surfaces. Consequently, the farther the water needs to travel from the internal tissue, the lower the water available to be removed from the periphery. This may explain the higher drying rates observed at high moisture content and lower rates as moisture content decreases not only for embryo tissue but also intact seed. Nevertheless, since the embryo is composed of living tissue, other intracellular changes may also be associated with the drying pattern. The alignment of lipid bodies along the plasma membrane (Fig. 7C, D) appears to be associated with decreases in embryo drying rates and we are currently analyzing this relationship.

The slight increase in embryo drying rates exhibited after some PC treatments (Fig. 4A) may be associated with the FB drying characteristics. Since air velocity was fixed, lower seed moisture contents at the transfer point to FB may increase the drying potential by increasing the movement of seed within the dryer due to light seed and decreasing air relative humidity. These changes may also take place during the FB as drying progresses. We observed an increase in seed movement as seed MC decreased, however, we did not quantify this drying parameter.

It is evident that high embryo drying rates during the early drying phase resulted in a severe negative effect on seed quality as illustrated by high electrical conductivity.
and poor performance in SGT and AA in almost all treatments subjected to the FB conditions. Nonetheless, in embryos with MC > 500 g H₂O kg⁻¹ fw (550 and 500 g H₂O kg⁻¹ fw harvests) even a very small amount of water loss was associated with lower cell solute leakage and higher germination performance. Thus, increases in drying tolerance to the FB drying rates appears to be associated with slow embryo drying rates during the early drying phase and before the embryo moisture losses under FB drying. However, moisture threshold levels may vary with the seed quality parameter used. An initial embryo moisture content of about 450 g H₂O kg⁻¹ fw before fast drying is associated with germination >80% (SGT) and low electrical conductivity. This germination percentage in AA was obtained only in those embryos with initial moisture content <400 g H₂O kg⁻¹ fw before fast drying. Decreases in electrical conductivity are associated with increases in SGT but not with AA. This suggests that the plasma membrane impairment caused by rapid drying rates may be overcome under favorable germination conditions but not if seed is exposed to deleterious conditions (AA) before germination. Additionally, it may suggest that plasma membrane impairment is not the only damage that takes place under fast drying conditions.

The significance of the slow embryo drying rates down to about 400 g H₂O kg⁻¹ fw either with PC or field drying may be associated with maintaining moisture levels such that ultrastructural and chemical changes associated with desiccation tolerance can occur. Using similar preconditioning treatments, Chen and Burris (1990) reported increases in the ratio of raffinose/sucrose in maize excised embryos, and Chen and Burris (1991) reported increases in the ratio phosphatidylcholine to phosphatidyethanolamine. Perdomo and Burris (1998) observed that lipid bodies in the radicle meristem of seed harvested at MC > 400 g H₂O kg⁻¹ fw, first observed throughout cell cytoplasm, were aligned along the plasma membrane with preconditioning drying as well as field maturation. Taking samples at different intervals under different drying treatments, Peterson (1997) reported an increase in the proportion of heat soluble proteins to total proteins as well as changes in sugar compositions similar to the previous reported by (Chen and Burris, 1990). All these changes were associated with an increase in drying tolerance at high temperatures and maintenance of high seed quality.

We conclude that changes in embryo drying rates at moisture contents >400 g H₂O kg⁻¹ fw are associated with changes in seed quality and that slow embryo drying rates to threshold levels may be crucial to acquire the ability to withstand higher drying rates without detrimental effects on seed quality.

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