



Water uptake dynamics in soybean seeds: influence in seeds performance and DNA integrity

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ABSTRACT: Relationships among water uptake rate and temperatures were investigated in five soybean seeds lots cv. M 6410 IPRO. Germination, field emergence and electrical conductivity tests were applied to determine seed performance after fast (complete immersion in water), control (moist substrate) and slow (moist atmosphere) imbibitions both at 20 °C and 30 °C. The fresh weight increment over time in soybean seeds during imbibition was recorded. In parallel, analysis of the DNA integrity of seedlings obtained from such seeds was performed in gel electrophoresis. Absorption pattern of soybean seeds were similar among temperatures, but absorption characteristics vary with time progress according to the way of water entrance into the seeds. Upon fast imbibition seeds germinated lower than non-fast-imbibed seeds, besides loss of seed performance. In contrast, slow-imbibed seeds showed high germination, low abnormal seedlings and maintenance of seeds performance. DNA isolation from fast imbibed seeds was highly degraded; although, some considerably degraded samples were reported in controlled imbibed seeds and the best preserved DNA was found in slow-imbibed seeds. The pattern of DNA degradation is typically passive or non programmed cell death. Our results showed it is important to consider the water uptake rate during germination test, since DNA integrity plays a critical role during seed imbibition, preserving soybean seed performance.

Key words: DNA disassembly, early seed imbibition, germination, passive cell death.

Dinâmica de absorção de água em sementes de soja: influência no desempenho de sementes e integridade do DNA

RESUMO: As relações entre a taxa de embebição em água e temperaturas foram estudadas em cinco lotes de sementes de soja cv. M 6410 IPRO. Testes de germinação, emergência a campo e condutividade elétrica foram aplicados para avaliar o desempenho das sementes após rápida embebição (imersão direta em água), controlada (substrato úmido) e lenta (atmosfera úmida), ambos a 20 °C e 30 °C. O incremento no peso fresco das sementes durante a embebição foi registrado. Paralelamente, a integridade do DNA de plântulas oriundas das sementes foi avaliado em gel por eletroforese. O padrão de embebição das sementes de soja foi similar entre as temperaturas, mas houve variações com o tempo de acordo com o modo de entrada de água nas sementes. Após a embebição rápida, as sementes apresentam menor germinação e desempenho que as sementes submetidas aos demais métodos de embebição. O DNA isolado a partir de sementes que embeberam rapidamente encontrava-se degradado, embora em sementes que foram hidratadas de forma controlada também fosse encontrada degradação do DNA. O DNA mais preservado foi encontrado em sementes que embebem lentamente. O padrão de degradação do DNA é típico de morte passiva ou não programada de células. Os resultados indicam que é importante considerar a taxa de embebição em água durante o teste de germinação, pois a integridade do DNA desempenha uma função importante durante a embebição, preservando o desempenho das sementes de soja.

Palavras-chave: desintegração do DNA, embebição inicial de sementes, germinação, morte passiva de células.

INTRODUCTION

Seed germination is a complex and tightly regulated process which starts with water absorption by the dry seed and ends when radicle elongates. Its achievement requires a synchronized attainment of many cellular processes including DNA repair, protein synthesis or membrane reorganization (WEITBRECHT et al., 2011; BEWLEY et al., 2013). Since membrane reorganization is one of the major events occurring during seed imbibition, and since cold temperatures are known to modify membrane

phospholipids, it relies on the accumulation of saturated or poorly unsaturated fatty acids, whatever the phospholipid class (NOBLET et al., 2017).

It is worth noting that seed imbibition is an important process in the plant life cycle and determines whether seed germination and plant growth will be successful or not (RIBEIRO et al., 2015). Even DNA does not escape the severity of drying and rehydration without some damage, and repair is an early priority. Thus, it is anticipated that following imbibition the synthesis of enzymes and compounds to limit and repair cellular damage is prevalent (NONOGAKI et al., 2006).

It is; therefore, surprising that the integrity of nucleic acids during seed imbibition, and, perhaps more importantly upon different imbibition rate has received relatively little attention in the recent literature, especially until the complete seedling establishment. The complex trait of soybean seed imbibition prior to germination has been demonstrated especially due to the importance of seed quality to the crop establishment and sustainable agricultural productivity. Rapid seed imbibition leads to the leaching of more electrolytes before the cell membranes have time to reorganize, even under slower hydration conditions (ROSSETTO et al., 1997) and in different physiological quality and contrasting coats of soybean seeds (CAVARIANI et al., 2009; GRIS et al., 2010, BAHRY et al., 2017).

In most cases, loss of seed vigor neither the safeguard genome, as being important markers of quality was not recorded even of great importance to analysis of soybean seed lots. Besides, DNA repair plays a critical role during seed imbibition, preserving seed vigor (BALESTRAZZI et al., 2011) mainly during the early imbibition phase. In agreement with this hypothesis, effective DNA repair takes place only when repair effectors are accessible to the damaged sites (BALESTRAZZI et al., 2015).

Furthermore, it is important to consider water uptake prior seed performance for breeding purposes, seed analysis and crop field. Although, this attempt has already been made by some seed companies, germination characteristics are seldom included in breeding programs (NONOGAKI, 2006) and could help analyzing the real physiological potential of seeds lots. We hypothesized soybean seed performance behaves differently according to water absorption rate and to temperature, since slow rehydration if not beneficial, at least is not partially damaging to the seed, even at lower temperature. We evaluated germination and seeds performance of soybean seeds cv. M 6410 IPRO, which is largely cultivated in tropical areas of Brazil central, upon different water uptake rate at two temperatures and its relationship to nucleic acid integrity, especially DNA.

MATERIALS AND METHODS

Plant material, germination test and seeds performance

Five soybean seeds lots cv. M 6410 IPRO were produced in Dourados, MS, Brazil (22° 11' 44" S, and 54° 56' 08" W and at an altitude of 430 m) from 2017-2018 crop and freshly harvested (85% germination, 61% accelerated test, 70%

field emergence and 52,23 $\mu\text{S cm}^{-1} \text{g}^{-1}$ electrical conductivity, in average – no differences at Tukey's test, $p>0.05$). The seeds lots were kept in a cold room (10 °C and 50% air relative humidity) for the duration of the study. Initially, seed water content was determined with two replications of 20 seeds per lot were subjected to the oven-drying method, which uses 105 ± 3 °C for 24 h (BRASIL, 2009).

The Rules for seed analysis (BRASIL, 2009) prescribe that soybean seed germination testing should be conducted at the temperatures of 20-30, 25 or 30 °C. Here, we used the temperature of 30 °C as control and constant 20 °C to assess the influence of low constant temperature during seeds water uptake. For imbibition experiments, seeds were transferred to beakers filled with distilled water (100 ml) (fast imbibition); or paper roll (two filter papers moistened with 2.5 mL, as being control) or moist atmosphere (slow imbibition) - seeds were spread in a single layer on stainless-steel screens and placed in plastic germination boxes (11 × 11 × 3.5 cm) containing 40 ml of distilled water at the bottom. The beakers, paper rolls and boxes with the seeds were maintained in chambers at 20 °C and 30 °C, at constant white light photon flux density of $150 \mu\text{mol}^{-1}\text{m}^2\text{s}^{-1}$ with a relative humidity (RH) of 70–80%. After 24 h the seeds lots were evaluated for germination, seedling emergence in field and electrical conductivity tests, according to the procedures detailed below.

Germination: four replications of 50 seeds per lot were distributed in rolls of paper (substrate) moistened with deionized water equivalent to 2.5 times the dry substrate mass and kept in a germination chamber at 30 °C in the constant light. Seedling evaluations were performed 8 days after sowing; results were expressed as percentage of normal and abnormal seedlings (BRASIL, 2009).

Seedling emergence in the field: four replications of 50 seeds were manually distributed in 2.5 m rows at 3 cm depth and 0.05 m spacing between rows. Seedling emergence was recorded 14 days after sowing and the results were expressed as percentages (NAKAGAWA, 1999).

Electrical conductivity: four replications of 50 seeds per lot were individually weighed (0.01 g precision) and soaked in 75 ml deionized water for 24 h. Electrical conductivity was then measured on a MS Tecnonon mCA 150; results were expressed in $\mu\text{S cm}^{-1} \text{g}^{-1}$ (International Seed Testing Association, 2016). The electrical conductivity of the fast-imbibed seeds was determined considering also the conductivity performed immediately after the first 24 h.

A completely randomized design was used in a factorial scheme 3 x 2 (imbibitions methods and temperatures). The results analysis considered the average of the five soybean seeds lots for each treatment. Data were submitted to analysis of variance (ANOVA) at 5% probability. When statistically significant differences were reported ($P \leq 0.05$), means were compared using the Tukey test at 5% probability (Sisvar).

DNA extraction and analysis

Briefly, frozen cells were ground in liquid nitrogen, and genomic DNA was extracted from radicles (after each water uptake rate, 24 h, 20 °C, white constant light) according to the Kit DNA extraction mini spin 50 (KASVI[®]). DNA electrophoresis was performed to assess DNA fragmentation. DNA samples (5 mg per lane) were loaded on a 2.0 % agarose gel and stained with 0.2 mg ml⁻¹ ethidium bromide.

RESULTS

Seeds showed 13.4%, in average (fresh weight basis). The overall uptake trend was similar for soybean seeds at 20 and 30 °C (Figure 1). In contrast, the dynamics of water entrance into the seeds showed differences according to water sorption isotherms (Figure 1). As hydration progressed, this phenomenon became more pronounced with the fast imbibition than by the other techniques, regardless the temperature. The seed water content on this treatment gradually increased about thirty percentage points during the first 8 hours (Figure 1).

At the same time, the seeds imbibed according to the control treatment (wet substrate) increased from 13% to about 35%; and slow hydration gradually increased from 13% to about 24%, as this technique expose the seed tissues to intermediate moisture contents for the whole imbibition period.

A similar effect was observed with the progress of time; fast imbibed seeds gained water

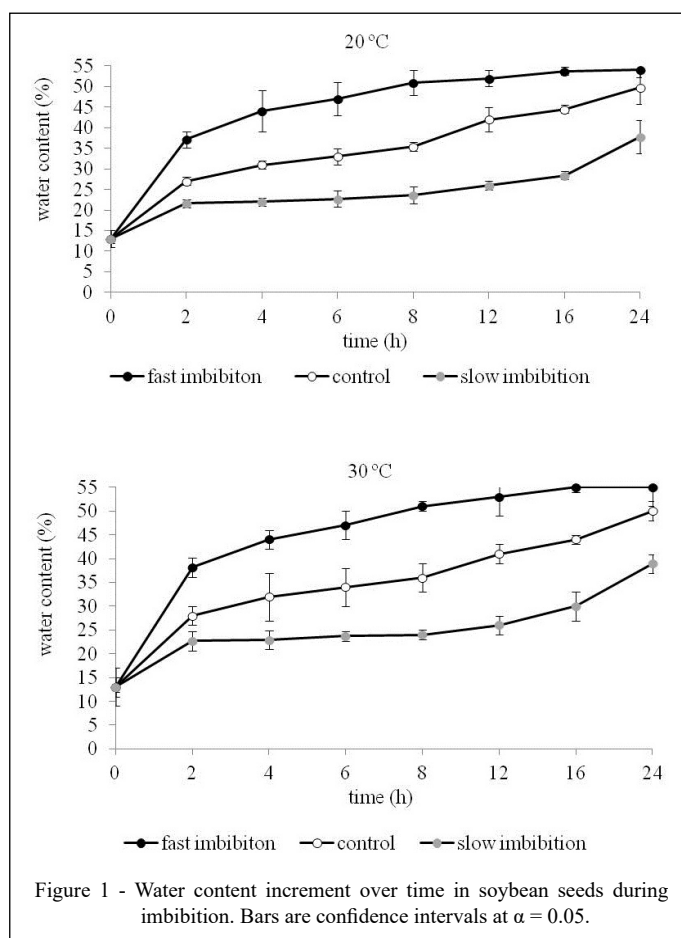


Figure 1 - Water content increment over time in soybean seeds during imbibition. Bars are confidence intervals at $\alpha = 0.05$.

continuously (Figure 1), at the end of 24 h the fresh weight was about 55% at the both temperatures. Generally, such condition make difficult to evaluate whether the sorption experiment has reached equilibrium. In seeds from the control (wet substrate) a similar pattern was apparent; although, absolute values of water content were lower (49%) (Figure 1). Slow hydration slightly increased after initial changes and seeds reached water contents almost constant for at least 24 h at both temperatures (Figure 1).

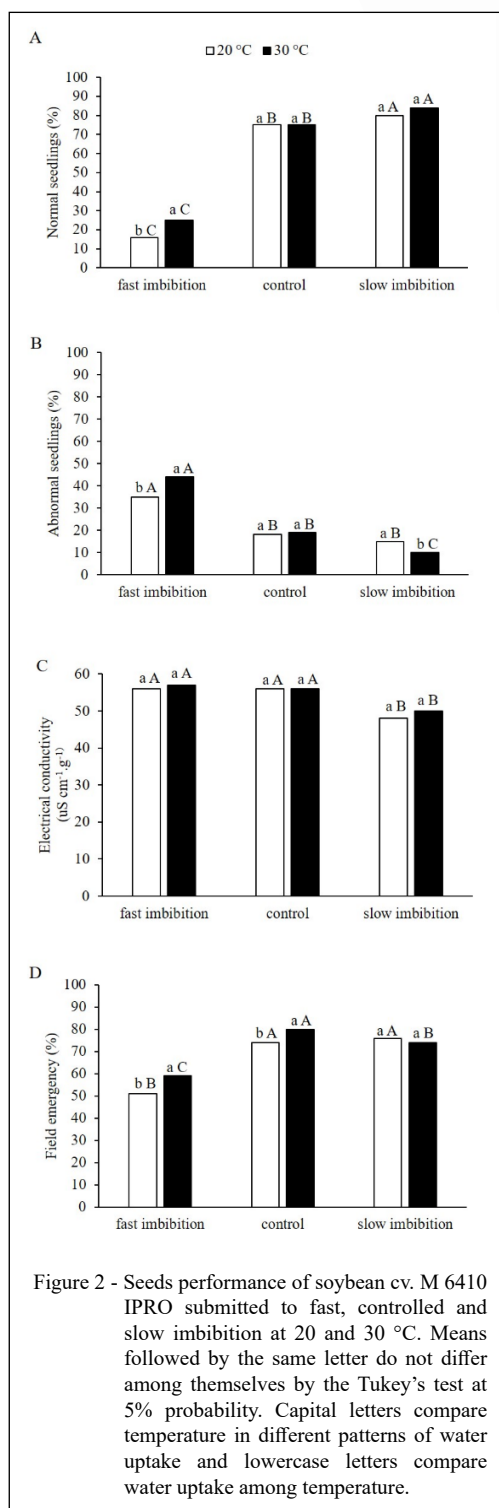
In order to evaluate if the course of water uptake by the seeds could affect the physiological seed quality, we evaluated characteristics tightly connected to the capacity of seeds to properly develop. In this way, the soybean seeds performance is affected by either water uptake or temperature (Figure 2).

Overall, the physiological response of seeds was negatively influenced by the fast water uptake rate. As a consequence of the course of seeds water uptake, seeds submerged in water (fast imbibition) showed low normal seedlings (16%) than the other imbibition methods, regardless the temperature (Figure 2A). Although, the seeds submitted to slow imbibition (moist atmosphere) showed higher normal seedlings (80%) than the control (75%). Interestingly, during the fast imbibition at 20 °C, the seeds became more sensitive to low temperature and the normal seedlings further decreased (Figure 2A).

When seeds rapidly imbibed, the capacity of germination decreased and the abnormal seedlings appeared, especially at high temperature (Figure 2B) whilst the control did not differ among the temperatures (Figure 2B). However, after slow imbibition the percentage of abnormal seedlings was lower than the other methods, but at 20 °C the results of abnormal seedlings increased (35%) (Figure 2B).

There were discernable differences in electrical conductivity between the slow water uptake and the faster imbibition methods, but no differences between temperatures (Figure 2C) and highlights the negative effect of fast water uptake on the ability to maintain the cell membranes integrity (Figure 2C). A similar pattern was observed with the field emergency; although, during slow imbibition no differences was apparent among the temperatures (on average 75%) (Figure 2D). However, fast imbibition and the control at 20 °C showed lower field seeds performance (Figure 2D).

The water content – time of imbibition relationships observed at 20 °C were similar at 30 °C: there appeared to be no differences in sorption characteristics at the both temperatures. But we cannot deny the sensitivity in the performance of the



seeds exposed at the lowest temperature (Figure 2). To analyze further the change in the nucleic acid, we focused on genomic DNA extraction from the seeds submitted to each imbibitions method at 20 °C and

we examined the gel electrophoresis (Figure 3). Seeds submitted to fast imbibition showed intense DNA degradation followed by the control (moist substrate) (Figure 3). Upon fast imbibition seeds germinated lower than non fast-imbibed seeds, besides field performance and cell membrane integrity loss (Figure 2).

The fast imbibition resulted in a marked DNA degradation, whereas only a slight decrease of DNA integrity was detected in control (Figure 3), since the control-seeds still presented 75% normal seedlings. Nevertheless, it is worth noting that maintenance of DNA integrity was observed in slow-imbibed seeds (Figure 3).

After rapid imbibition, seeds with only 16% of germination (normal seedlings) (Figure 2A) are henceforth referred to as 'non-viable'. Throughout

imbibition, the intensities of DNA degradation coinciding with loss of seed performance (Figure 3); further fast imbibition caused progressive viability loss. Instead, as slow imbibition occurs, there is maintenance of high seed germination ability (80%) (Figure 2A) correlated to the maintenance of DNA integrity (Figure 3). However, seeds imbibed in wet substrate showed decrease of normal seedlings (75%) and increased DNA degradation (Figure 3).

DISCUSSION

Water sorption describe the relationship between the water content and the time progress and were constructed according to the way of water uptake at 20 and 30 °C to characterize the effect of the water uptake course into the seeds on that relationship. Sorption pattern of soybean seeds were similar among temperatures, but sorption characteristics vary with time progress according to the way of water entrance into the seeds (Figure 1).

Collectively, the uptake characteristics of soybean seeds were not related systemic uptake patterns according to the all of the imbibition methods. This could be attributed to the way of water entrance related to the time of seed imbibition. The changes in sorption characteristics of soybean seeds imply negative alterations of some useful biomarkers: seed germination and ability to develop normal seedlings in the field and cell membrane integrity. Other reports also indicated that environmental factors as temperature related to the rate of seed imbibition at the first ~24 h in distilled water tend to cause loss of seed viability, as seen in soybean (SILVA & VILLELA, 2011), maize (ZUCARELI et al., 2011), mung beans (SHUKLA et al., 2018) and rice (CHENG et al., 2017; XU et al., 2017).

Besides, temperature plays an important role in water uptake rate, reserve mobilization and reactivation of metabolism during early seed imbibition as demonstrated for wheat (KASHIWAKURA et al., 2016) and *Ricinus communis* (RIBEIRO et al., 2015). Rapid imbibition can induce stress during the transition of membranes from a rigid gel phase to a liquid crystalline phase, resulting in solute leakage (KRANNER et al., 2010), as seen in soybean seeds (Figure 2C). Furthermore, low temperature dramatically affected different metabolic pathways and reduced some physiological and biochemical processes of soybean seed before germination (CHENG et al., 2010).

Indeed, as rapidly water uptake occurs damages are concomitantly, so these changes can

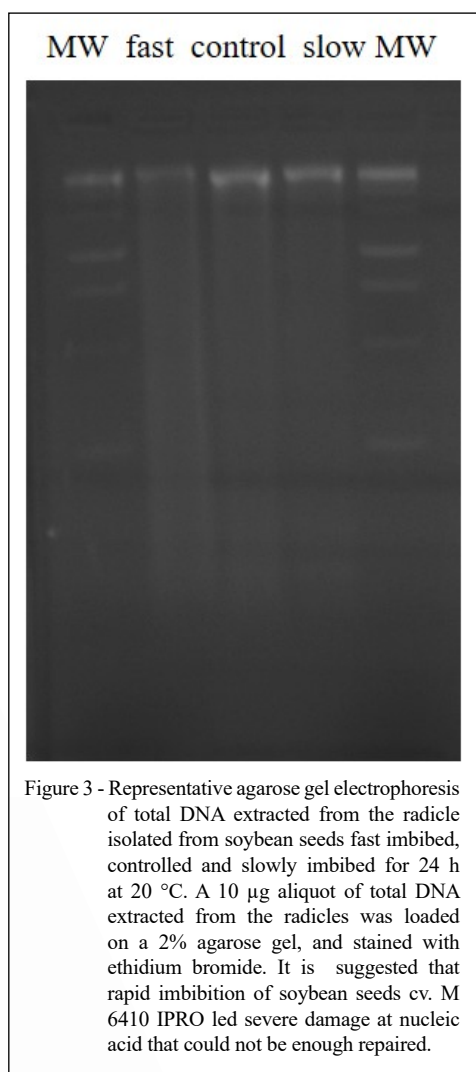


Figure 3 - Representative agarose gel electrophoresis of total DNA extracted from the radicle isolated from soybean seeds fast imbibed, controlled and slowly imbibed for 24 h at 20 °C. A 10 µg aliquot of total DNA extracted from the radicles was loaded on a 2% agarose gel, and stained with ethidium bromide. It is suggested that rapid imbibition of soybean seeds cv. M 6410 IPRO led severe damage at nucleic acid that could not be enough repaired.

affect the natural process of DNA replication and transcription which in turn results in loss of cell viability (WATERWORTH et al., 2011). In this sense, we demonstrated that inability of normal seedlings formation and loss of field performance maintenance might be an indicator about DNA stability loss due to the rapid water uptake. Besides, the lower temperature exposure during imbibitions (20 °C) may have accentuated the sensitivity of the seeds to the entry of rapid water culminating in normal seedlings reduced either in optimal (Figure 2A) or field conditions (Figure 2D). The primary event of injury by low temperature perhaps is the impaired reorganization of cell membrane in soybean seeds, since most of soybean cultivars are either chilling-sensitive or moderately chilling-resistant, which results in severe economic loss due to low temperature before germination (CHENG et al., 2010).

DNA analysis enabled us to conclude firstly that the seeds water uptake dynamics resulted in DNA changes. Secondly, the abrupt water entrance into the seeds resulted in DNA degradation and gradually seeds imbibition allows the maintenance of DNA integrity (Figure 3). Nonetheless, there is a tight relationship between the maintenance of DNA integrity, capacity of germination and seed vigor maintenance and the water absorption dynamics in soybean seeds. Therefore, the increased DNA degradation could be attributed to the fast imbibition of the seeds as decrease in germination and seeds performance, besides the increase in the abnormal seedlings (Figure 2) most likely resulted from the disassembly of DNA structure.

Although, we do not have direct evidence that DNA degradation precedes and is causal for the losses in normal seedlings formation, the complete DNA degradation implies that these processes happen before cell death and not after. However, the changes in the pattern of DNA degradation of soybean seeds were clear according to the way of water entrance (Figure 1 and 3). In summary, DNA degradation may have triggered loss of soybean seed performance.

Lesions in the DNA contributed by various damaging agents, may result in changes in both the chemical and physical structures of DNA and thus generate both cytotoxic and genotoxic effects after/ or during seeds ageing (EL-MAAROUF-BOU TEAU et al., 2011), storage (MICHALAK et al., 2013) and imbibition (BALESTRAZZI et al., 2011; WATERWORTH et al., 2015) adversely affecting the seeds performance. To survive, organisms have evolved effective DNA damage detection, signaling and repair mechanisms (WATERWORTH et al.,

2011); following detection of DNA damage, repair is initiated by the recognition and excision of the damaged region and completed by DNA synthesis and ligation (MACOVEI et al., 2010; WATERWORTH et al., 2015). The complexity of the networks of DNA damage control/repair functions has been only partially elucidated in plants and concerns nucleotide and base excision repair, non-homologous end joining and homologous recombination which play specific roles, all of them being critical to ensure genome stability (VENTURA et al., 2012; WATERWORTH et al., 2015).

Nevertheless, the complete submersion of seeds in water - due to the rapid entry of water in seeds - promoted DNA damage (Figure 3) that, at least, was not sufficiently repaired to re-establish the normal seedling development and seed performance (Figure 2). This phenomenon have already been observed in seeds submitted to other abiotic stress, as shown in aged seeds from *Triticum aestivum* and *Avena sativa* (LEINO et al., 2009) and in desiccated and pre-moistened seeds of *Campomanesia adamantium* (DRESCH et al., 2015) and *Sesbania virgata* (MASETTO et al., 2015).

The pattern of DNA degradation (Figure 3) is considered as being a hallmark of passive or non-programmed cell death, thus suggesting that is not purely an accidental form of cell death as once thought. It is involved in mitochondrial dysfunction itself and a further exacerbation of the injury events and culmination in necrotic cell death. Because of the rapid decline of ATP generation and the rupture of mitochondria, the cell loses the ability to regulate plasma membrane integrity and necrosis fully ensues (KARCH & MOLKENTIN, 2015).

The present research shows that loss of seed viability and performance after imbibition at the constant temperatures (20 °C or 30 °C) strongly depends on the rate of water uptake. Interestingly it is shown that the extent of DNA damage also depends on the rate of water entrance into the seeds, since DNA degradation varies with the way of water uptake. Based on these premises, our results showed that seed water uptake regulates the rate of DNA alteration in soybean seeds and may severely affect seeds performance.

CONCLUSION

Two important factors are related to imbibitional injury: the temperature and the rate at which water enters the soybean seed. Fast imbibition decrease soybean seeds performance and trigger DNA

degradation, especially at 20 °C. Seeds are better prepared for germination and have best preserved DNA if submitted early to slow imbibition.

DECLARATION OF CONFLICTS OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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