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ANA CAROLINA PICININI PETRONILIO

INNOVATIVE TOOLS TO PREDICT AND IMPROVE SEED PHYSIOLOGICAL QUALITY: CASE STUDIES IN TOMATO AND SOYBEAN

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Aos meus amados pais,

Sandra e Rosival,

dedico.

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“[1]Filho meu, se aceitares as minhas palavras e esconderes contigo os meus
mandamentos, [2]para fazeres atento à sabedoria o teu ouvido e para inclinares o
coração ao entendimento, [3]e, se clamares por inteligência, e por entendimento alçares
a voz, [4]se buscares a sabedoria como a prata e como a tesouros escondidos a
procurares, [5]então, entenderás o temor do Senhor e acharás o conhecimento de
Deus. [6]Porque o Senhor dá a sabedoria, e da sua boca vem a inteligência e o
entendimento." (Provérbios 2:1-6).

BÍBLIA. Bíblia Sagrada. Traduzida por João Ferreira de Almeida. Barueri, SP: Sociedade Bíblica do Brasil, 1999.

ABSTRACT

The seed quality is made up of physical, sanitary, genetic, and physiological attributes. Seed physiological quality refers to factors that allow rapid and uniform germination and emergence under broad climatic conditions, which ensures vigorous initial plant establishment and produces an increased yield. This research sought to elucidate how the physiological quality of seeds can be predicted and improved. To this end, this research used soybean and tomato species as models. 1 – the physiological quality of soybean seeds produced under water and heat stress during the maturation phase was determined, and seed multispectral images were acquired. Machine learning models associated with multispectral imaging technology were developed to verify the possibility of separating seed groups autonomously. The quality of stressed seeds was lower compared to non-stressed ones. The multispectral images produced markers that made it possible the segmentation between seed groups. The ML models showed high performance in recognizing stressed seeds. This work opens up the possibility of using this technology as a results manager, as it provides information about the environment of seed production and its consequences on the physiological quality of the seeds. 2 – we performed soybean genetic transformations for further study of the molecular factors involved in seed chlorophyll degrading/retention. For this, the GmABI5 gene was overexpressed and silenced (RNAi) using the glycinin promoter in a hygromycin-resistant expression cassette. Somatic embryos were induced from the cotyledons of immature seeds and when they reached the globular stage they were transformed via bioballistics. Transformed embryos were selected for resistance to hygromycin. The presence of the expression cassette in the resistant embryos was confirmed via PCR and they were regenerated into seedlings. This work will allow the functional study of the ABI5 gene and also the investigation into the possibility of reproducing a genotype tolerant to chlorophyll retention in soybean seeds. 3 – we performed gene expression studies in tomato seeds during osmo-priming to investigate mechanisms involved in the reduction of longevity. For this, we collected a total of seven samples: four samples during the 60 hours of priming, one control group (without priming), one sample after post-priming drying, and one after post-priming thermal shock and drying. We investigated transcripts associated with stress response. The seed vigor and longevity after treatment were also determined. Primed seeds had their longevity impaired compared to unprimed ones. Genes from the heat-shock protein family were down-regulated during the priming process. This research brings new insights into the mechanisms involved in the reduction of longevity of primed seeds and allows the use of these transcripts to monitor longevity in primed tomato seeds.

Keywords: seed quality; multispectral imaging; environmental stress; chlorophyll fluorescence; primed seeds.

RESUMO

A qualidade de sementes é composta por atributos físicos, sanitários, genéticos e fisiológicos. A qualidade fisiológica de sementes refere-se a fatores que permitem que a germinação e a emergência ocorram de forma rápida e uniforme sob amplas condições climáticas, o que garante um estabelecimento inicial vigoroso das plantas e resulta em aumento de produtividade. Esta pesquisa buscou investigar como a qualidade fisiológica das sementes pode ser estimada e aprimorada. Para tanto, esta pesquisa teve como modelo as espécies de soja e tomate. 1 – foi determinada a qualidade fisiológica de sementes de soja produzidas sob estresse hídrico e térmico durante a fase de maturação e adquiridas imagens multiespectrais das sementes. Modelos de aprendizado de máquina associados à tecnologia de imagem multiespectral foram desenvolvidos para verificar a possibilidade de separar os grupos de sementes de forma autônoma. A qualidade das sementes estressadas foi inferior em comparação às não estressadas. As imagens multiespectrais produziram marcadores que possibilitaram a segmentação dos grupos de sementes. Os modelos de aprendizado de máquina apresentaram alto desempenho no reconhecimento de sementes estressadas. Este trabalho possibilita a utilização desta tecnologia como gestora de resultados, pois fornece informações sobre o ambiente de produção de sementes e suas consequências na qualidade fisiológica das sementes. 2 – Foram realizadas transformações genéticas em soja para futuramente investigar os fatores moleculares envolvidos na degradação/retenção da clorofila nas sementes. Para isso, o gene *Gm*ABI5 foi superexpresso e silenciado (RNAi) utilizando o promotor glicinina em um cassete de expressão resistente à higromicina. Embriões somáticos foram induzidos a partir de cotilédones de sementes imaturas e quando os mesmos atingiram o estágio globular foram transformados via biobalística. Os embriões transformados foram selecionados quanto à resistência à higromicina. A presença do cassete de expressão nos embriões resistentes foi confirmada via PCR e eles foram regenerados em plântulas. Este trabalho permitirá o estudo funcional do gene ABI5 e a investigação da possibilidade de reproduzir um genótipo tolerante à retenção de clorofila em sementes de soja. 3 – foram realizados estudos de expressão gênica em sementes de tomate durante o condicionamento osmótico (*osmo-priming*) para investigar mecanismos envolvidos na redução da longevidade. Para isso, foram coletados um total de sete amostras: quatro amostras durante as 60 horas de priming, uma amostra do grupo controle (sem priming), uma amostra após secagem pós-priming e uma após choque térmico pós-priming e secagem. Foi investigada a expressão gênica de moléculas associadas à resposta ao estresse. O vigor e a longevidade das sementes após o tratamento também foram determinados. As sementes condicionadas tiveram sua longevidade reduzida em comparação às não condicionadas. Os genes da família das proteínas de choque térmico foram regulados negativamente durante o processo de *priming*. Esta pesquisa traz novas percepções sobre os mecanismos envolvidos na redução da longevidade de sementes condicionadas e permite o uso destes genes para monitorar a longevidade em sementes de tomate submetidas ao *priming*.

Palavras-chave: qualidade de sementes; imagem multiespectral; estresse ambiental; fluorescência da clorofila; sementes condicionadas.

SUMMARY

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GENERAL INTRODUCTION

In agriculture, seeds are one of the main inputs (Carvalho; Nakagawa, 2012). A new generation of plants begins through seeds, assuring plant life and food security worldwide. Much attention is given to producing high-quality seeds because it supports crop production. Seed quality consists of genetic, physical, sanitary, and physiological attributes. Physiological quality refers to viability, desiccation tolerance, vigor, and longevity, which ensure proper seedling establishment (Bewley et al., 2013).

Seed physiological quality can impact crop yield by affecting the speed and uniformity of germination and emergence and plant density (Tekrony et al., 1991). Low-quality seed lots result in a higher rate of abnormal seedlings, reducing population density (considering density recommended limits) and impacting productivity. On the other hand, high-quality seeds produce seedlings that emerge earlier and uniformly during a suitable sowing time. Seedling emergence during the suitable sowing time ensures that environmental conditions will be more favorable for growth than if the seedling emerges later, when the climate conditions might be less advantageous. Fast and uniform emergence also avoids competition between crop plants and weeds (Finch-Savage, 1995). Seed physiology studies have contributed to technology development and crop yield increase. High-quality seeds improved crop yield by more than 4% in corn production (Graven; Carter, 1991). According to Rajala et al. (2011), barley seeds with superior quality improved grain yield. High-quality seeds improved rice yield up to 19% in the Philippines (Diaz et al., 1998). The progressive increase in the level of vigor in soybean seeds led to an increase of up to 28 $kg.ha^{-1}$ in grain productivity (Bagateli et al., 2019). In this regard, molecular-level seed physiology research, seed priming, and imaging techniques are ways to predict and improve seed quality and will be addressed in this research.

In recent years, imaging analysis has been proposed as an alternative to traditional seed tests to assess seed quality. The traditional tests depend mainly on a visual inspection by the analyst, which can lead to a subjective interpretation of the results. Furthermore, these methods are destructive and mostly time-consuming. Therefore, there is a need to advance the use of more assertive tools, which promote time savings and eliminate subjectivity, and which additionally allow the integration of machine learning and process automation. Several imaging technologies are available nowadays, such as X-ray, X-ray microtomography, X-ray fluorescence, near-infrared spectroscopy, and magnetic resonance imaging that can assess seeds (Bianchini et al., 2021; Cotrim et al., 2019; Gomes-Junior et al., 2019) and multispectral imaging that can assess both seeds and seedlings (Elmasry et al., 2019; Galletti et al., 2020). X-rays were used to detect mechanical damage in several species, such as beans (Gomes-Junior et al., 2019; Mondo et al., 2009), and were associated with seed physiological quality. Autofluorescence multispectral images associated with artificial intelligence tools have been used to recognize soybean maturation stages and predict seed quality (Barboza Da Silva et al., 2021; Batista et al., 2022). Multispectral imaging and machine learning methods can also be used to provide information regarding pigments. Chl fluorescence and anthocyanin in peanut seedlings were reported as efficient in segregating seeds with superior quality (Fonseca De Oliveira et al., 2022).

Seed physiological quality is a multigenic trait and is largely influenced by the environment (Tripathi; Khare, 2016). Over the years, in plant breeding programs, much attention has been given to improving grain quality traits such as oil and protein quality and yield (Rajcan; Hou; Weir, 2005), grain yield, and insect and disease resistance (Krzyzanowski, 1998). However, little attention is given to associating the selection of the aforementioned traits with seed physiological quality traits (Kalaji; Pietkiewicz, 2004). There is a need to further research seed physiological quality since it is essential to enable the expression of the productive potential of cultivars. Some researchers have studied the genetic gains regarding seed physiological quality in breeding research. In soybean, Monteiro et al. (2021) showed that the heritability of physiological quality traits was moderately high, which confirms the possibility of breeding to improve seed physiological quality traits. Mello Filho et al. (2004) highlighted the possibility of breeding to improve protein quality while keeping high-quality seeds and satisfactory yield. Hatzig et al. (2018) suggest the use of molecular markers of mean germination time for selection of germination capacity in oilseed rape cultivars.

Molecular-level seed physiology studies aim to investigate the molecular factors involved in the seed physiological quality attributes. This approach has been applied in the soybean “greenish-seed problem” research. Soybean greenish seeds are mature seeds with chlorophyll (chl) retention. This usually happens in response to abiotic stresses during the soybean maturation phase. Chl retention has been a significant problem in these last decades in Brazil because it impacts seed and oil quality (Pádua et al., 2007; Luccas, 2018). Besides the environmental circumstances, genetic factors are also involved in the susceptibility of chl retention in soybean seeds (Ajala-Luccas et al., 2023; Pádua et al., 2009; Teixeira et al., 2016). Teixeira et al. (2016) suggested that in susceptible genotypes, the impaired expression of genes related to photosynthesis (D1, D2. PPH2, NYC1) might be involved in chlorophyll retention. Batista (2022) showed that the acquisition of longevity is impacted in stressed seeds with higher chl content. In the same study, the expression of genes related to chl degradation (D2, NYC1) is impacted in immature stressed seeds. Moreover, seeds that suffered stresses during the maturation phase had a higher expression of the HSP21 gene, which is a protection mechanism against stresses, compared to non-stressed seeds (Batista, 2022). Another approach for molecular-level seed physiology studies was presented by Ducatti et al. (2022) who reported transcripts associated with vigor in soybean seeds.

Thus, the above-mentioned transcripts can be used to monitor the quality attributes and provide knowledge that can be applied in plant breeding programs aiming at marker-assisted selection of superior genetic materials for seed quality. In addition, the genes discovered through molecular-level seed physiology studies and plant breeding might also be applied in genetic transformation and gene editing, which are additional tools that might improve seed quality. Genetic transformation and gene editing can be applied in gene functional studies to understand the behavior of a candidate gene, and furthermore, it can also provide genetically modified varieties for high-quality seed production.

Seed priming is a technology used to increase the speed and uniformity of germination and improve seedling emergence and stress tolerance through controlled seed hydration. The germination process starts during the treatment, but radicle protrusion is impeded. Thus, seeds are dried after a determined priming time. Therefore, germination and seedling emergence are faster when the seeds are sown because the metabolism was previously activated during priming treatment. Priming is particularly helpful in seeds with slow and uneven germination (Marcos-Filho, 2015). Besides the increase in seed physiological quality by priming (Brocklehurst; Dearman, 1983; Forti et al., 2020; Silveira et al., 2023), studies show crop yield improvement. Salicylic acid priming promoted a fruit yield increase of 33% and improved plant resilience to water stress in tomatoes and also improved yield and high-temperature germination tolerance in carrots (Chakma et al., 2021; Mahmood-Ur-Rehman et al., 2020). In wheat seeds, osmo-priming increased grain productivity by 1.10 tons per hectare (Farooq et al., 2020). Hussain and collaborators (2006) obtained increases in the productivity of sunflower achenes through NaCl and KNO_3 osmo-priming.

Thus, studies of imaging analysis, molecular-physiology level studies, and seed priming, are tools that can predict and improve seed quality (Paparella et al., 2015; Fonseca De Oliveira et al., 2022; Ducatti, et al., 2022). Therefore, this research aims to study how seed physiological quality can be predicted and improved through innovative tools using two economically important crops as models, tomato, and soybean.

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FINAL CONSIDERATIONS

This research contributed to the advancement of knowledge and technology in the area of seed physiology. The maternal environment directly impacts seed quality, and due to climate change creating challenging environments, reductions in seed quality have been noticed season after season. In this regard, the multispectral imaging technology associated with machine learning proposed here is a robust way of classifying seeds that have suffered stress during their formation. Besides this, since it is an autonomous system, it can be incorporated into the seed companies' daily routine. The greatest advantage however, is that this information can help seed companies better understand the causes of reduced quality in a seed lot. Alternatively, the overexpression and silencing of the ABI5 gene in soybean will enable, in further studies, a better understanding of the role this gene plays in the quality and retention of chlorophyll in soybean seeds. On the other hand, priming is a technique broadly used by companies for tomato seeds. Nevertheless, it impairs seed shelf life. Here we elucidated one of the mechanisms involved in reducing the longevity of primed tomato seeds and suggested the use of this knowledge as a marker to monitor the longevity of primed seeds. Thus, concepts generated here can be used at industrial and academic levels to solve classic problems regarding seed physiological quality.

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