Physiological performance of green soybean seeds: a systematic review

Performance fisiológica de sementes esverdeadas de soja: uma revisão sistemática

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RESUMO
A produção de sementes, principalmente de espécies oleaginosas, requer que as fases de maturação e colheita ocorram em condições ótimas, com temperaturas amenas e condições climáticas secas, evitando tensões bióticas ou abióticas, que resultam em morte prematura ou maturação forçada, produzindo sementes e grãos esverdeados, com qualidades fisiológicas e organolépticas reduzidas. As sementes esverdeadas são o resultado de uma translocação muito rápida das reservas e de taxas mais baixas de fotossíntese, evitando a degradação da clorofila. Estes níveis de clorofila presentes nas sementes podem ser afectados pelo genótipo e pelo clima, sendo um processo enzimático, evidenciado pelo aumento de clorilidas, resultando num aumento de feofitinas e outros derivados. As sementes verdes, quando destinadas à produção de óleo vegetal, resultaram num produto com maior acidez e custo de refinação mais elevado.

Palavras-chave: Clorofila; Qualidade de semente; Óleo vegetal; Degradação;

ABSTRACT
The production of seeds, mainly of oilseed species, requires that the stages of maturation and harvest occur under optimal conditions, with mild temperatures and dry climatic conditions, avoiding biotic or abiotic stresses, which result in premature death or forced maturation, producing seeds and grains, greenish, with reduced physiological and organoleptic qualities. Greenish seeds are the result of very rapid translocation of reserves and lower rates of photosynthesis, preventing chlorophyll degradation. These levels of chlorophyll present in the seeds can be affected by the genotype and the climate, being an enzymatic process, evidenced by the increase of chlorilides, resulting in an increase of pheophytins and other derivatives. Green seeds, when destined for the production of vegetable oil, resulted in a product with higher acidity and higher refining cost.

Keywords: Chlorophyll; Seed quality; Oilseed; Degradation;

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INTRODUCTION

In tropical countries, such as Brazil, where climatic conditions are characterized by large variations in temperatures and rainfall mainly in the physiological maturation period, the incidence of green seeds in soybean seed lots becomes a major problem. According to França-Neto et al., (2007) the production of quality seeds requires that the maturation and harvest phases occur under mild temperatures (around 22°C), associated with dry climatic conditions.

The chlorophyll content in soybeans is determined by the genotype and varies significantly between cultivars. This level can be affected by ripening stage, drying conditions, and weather conditions that can interfere with normal ripening in the field (SINNECKER, 2002). Uniformity of ripening, insect attacks, especially bed bugs that cause leaf retention, are all causes of green seeds in soybean crops. Other factors that predispose to the expression of green seeds are biotic and abiotic stresses, resulting in premature plant death or forced maturation. Among the biotic stresses we can cite root diseases and as abiotic stresses we can cite inadequate management, such as irregular distribution of fertilizers, application of desiccants before the ideal stage and water deficit associated with high temperatures (FRANÇA-NETO et al, 2005).

Factors such as lack of uniformity of ripening, insect attack, especially by bedbugs that cause leaf retention, are causative of green seeds in soybean crops.
(CÂMARA AND HEIFFIG, 2000). Other factors that predispose to the expression of seeds are biotic and abiotic stresses, which result in premature death of the plant or a forced maturation. Among the biotic stresses we can mention root diseases and as abiotic stresses we can mention inadequate management, such as irregular distribution of fertilizers, application of desiccants before the ideal stage and water deficit associated with high temperatures (FRANÇA-NETO et al., 2005).

Soybean seeds with intense green or even greenish coloration usually present high levels of deterioration that can result in reduced germination, vigor and viability in seed lots (FRANÇA-NETO et al., 2005). In this sense, there is the possibility that recently harvested greenish seeds may germinate and produce normal seedlings, but of low vigor, and when stored in non-climatizes conditions, they may lose their viability and tend to lose their germination potential (FRANÇA-NETO et al., 2012).

**METHODOLOGY**

The methodology if this review was conducted through a bibliometric and systematic review with analysis of articles produced in international databases in the Scopus repository from keywords related to the subject of the study. The search was carried out in October 2022.

The terms defined for the database search were "soybean" AND "green seeds" AND "physiology". The search, selecting material from the last 10 years, resulted in 51 articles, including 46 research articles, 4 review article, and 1 book chapter. These articles were chosen to build this literature review based on their main focus: green seed physiology in soybean. Those that did not present results related to the subject were eliminated. To better understand the occurrence of green seeds, their behavior and their physiology, articles were used in addition to this research, creating the context presented during the review.

Through the RStudio® (package bibliometix), software a word cloud was generated in order to identify clusters and to create a relationship between keywords that make up this research, as shown in Figure 1. For this purpose, the keywords used in the search were "soybean" AND "green seeds" AND "physiology" AND "chlorophyll", using the Scopus platform, in order to perform a robust and complete research on the subject. The word cloud provides an analysis of the most common terms.
Figure 1. Wordcloud from Scopus base with keywords "soybean" AND "green seeds" AND "physiology" AND "chlorophyll".

Source: Prepared by the authors.

CHLOROPHYLL ACCUMULATION AND DEGRADATION

Found naturally in plants, chlorophylls are light absorbers responsible for photosynthesis, and there may be accessory pigments such as carotenoids (beta-carotene and xanthophyll) and phycobilins (phycoerythrin and phycocyanin). These pigments are arranged in sets or functional bundles called photosystems (LEHNINGER et al., 1995).

The photosynthetic pigments present in plants vary according to the species, and in green plants, chlorophylls a and b are abundant. Chlorophyll a is the pigment used to carry out the photochemical step of photosynthesis, while the other pigments aid in light absorption and energy transfer, called accessory pigments, the main ones being type b chlorophylls and carotenoids (TAIZ & ZIEGER, 2004).

Chlorophyll a has a blue-green color, and chlorophyll b a yellow-green color, being found in an average ratio of 3:1, respectively. This proportion varies depending on the species, leaf age, and leaf location on the plant. Plants adapted to shady locations (umbrophyte plants) this proportion is lower than plants adapted to conditions with high irradiance (heliophilous plants) (TAIZ & ZIEGER, 2004).
All chlorophylls have a complex ring structure, being chemically related to the porphyrin-like groups found in hemoglobin and cytochromes. They have a long hydrocarbon tail that is almost always linked to the ring structure. The tail anchors the chlorophyll to the hydrophobic portion of its environment. The ring structure contains some electrons, being the part of the molecule involved in electronic transitions and redox reactions (reduction-oxidation) (TAIZ et al., 2017).

According to Heaton & Marangoni (1996) the first step of chlorophyll degradation corresponds to the cleavage of the phytol group by the action of the chlorophyllase enzyme, resulting in the formation of chlorophyll; the second stage involves cleavage of the porphyrin ring by the action of the oxygenase enzyme, forming fluorescent and colorless compounds. These compounds are exported from the chloroplasts to the vacuole, where they are converted into non-fluorescent compounds, also called “rusty pigments”, leading to a loss of fluorescence. The final product is colorless.

In most species, the amount of chlorophyll in the seed decreases during the maturation phase, and, at the same time, the seed color changes from green to the characteristic color, depending on the species and cultivar (FRANÇA-NETO, 2012). Chlorophyll degradation begins at senescence by endogenous factors and can be influenced by external factors such as water stress, reduced light, changes in temperature,
increased ethylene or internal factors such as increased membrane permeability and changes in pH (HEATON; MARANGONI, 1996).

Quickly dehydrated seeds do not have chlorophyll degraded at the same rate as slowly dehydrated seeds, which have 1µg chlorophyll seed-1. The seeds remain visibly green when rapidly dehydrated, reaching up to 29µg chlorophyll seed-1 (ADAMS et al., 1983). Corroborating this information, Sinnecker (2002) observed in the field, in the advance of maturation in soybean or in seeds harvested before the physiological maturity period, submitted to slow drying at room temperature (250 °C), an enzymatic system involving the oxygenase enzymes, which acted by degrading the green pigment to colorless compounds. However, he also observed seeds that had not yet completed their cycle when exposed to rapid drying in an oven at a temperature of 400 °C, the mechanism of chlorophyll degradation appearing to have occurred in two ways: enzymatic, mediated by chlorophyllases, forming pigments dephtylated (chlorophyllides and pheophorbids); and chemical route, evidenced by the accumulation of pheophytins (an accessory pigment of the chlorophyll type that does not have magnesium ion).

**PHYSIOLOGICAL MATURATION OF GREEN SEEDS**

The development of a seed is divided into three phases: embryogenesis, maturation and late maturation (BEWLEY et al., 2013). At maturation, grain filling occurs and physiological quality, germination capacity, acquisition of tolerance to desiccation and seed vigor are defined (GUTIERREZ et al., 2007). High temperatures during maturation accelerate the translocation of photosyntates to the seed, making it difficult to degrade chlorophyll, causing “forced maturation” and the formation of greater amounts of greenish seeds, with reduced physiological potential (MARCOS FILHO, 2005).

The last phase, characterized by late maturation and loss of water, maximum accumulation of dry matter and completely degraded chlorophylls. (MARCOS FILHO, 2005). This phase is important to keep the seed viable, occurring from the R8 stage (LIMA et al., 2017), which under favorable conditions, plants ripen and chlorophyll degradation occurs, resulting in soybean seeds of normal color (PÁDUA et al., 2009).

The process of degradation of chlorophyll in seeds remains incomplete in the final stages of maturation in adverse environmental conditions, such as high temperatures, drought, cease or decrease the activity of enzymes such as chlorophyll and magnesium.
chelatase, responsible for degrading chlorophyll (RANGEL, 2011). Factors such as uneven maturation, insect attack, application of desiccants, inadequate management, inadequate distribution of limestone and fertilizers can cause uneven maturation problems, which will consequently result in immature and greenish seeds (COSTA et al., 2001; ZORATO et al., 2001; ZORATO et al. al., 2007).

According to McGregor (1991) when working with rapeseed, the degradation of chlorophyll in the seed was affected by the genotype used and by the climatic conditions, corroborating the results obtained by França-Neto et al. (2005) for soybean seeds. In studies by Takamiya et al. (2000), soybean seeds submitted to conditions of water stress had high levels of chlorophyll b in comparison with those produced with adequate water supply.

According to França Neto et al. (2012), the early harvest of soybeans, with moisture between 17% and 20%, can also result in the harvest of immature and greenish seeds. However, since this seed is larger in size, due to the higher water content, it can be easily removed from the seed mass during processing in the pre-cleaning stage.

The incidence of green seeds is also affected by fungal infection. Microorganisms produce toxins above, and according to Halloin (1986), they can cause increased damage to membranes, increased leaching of solutes from seeds in the imbibition phases, inhibition of chlorophyll activity, granulation of the endoplasmic reticulum and, consequently, reduced germination of the seeds.

The fungus Phakopsora pachyrhizi, which causes Asian soybean rust, interferes with the functioning of the tissues, thus causing defoliation, resulting in smaller grain size and, consequently, greater loss of yield and quality, due to the occurrence of greenish seeds. Diseases such as fusariosis and stem canker also result in the premature death of the plant or formed maturation of the same (FRANÇA-NETO et. al., 2005).

A study carried out by Costa et al. (2001) with four levels of green seeds and four soybean cultivars, showed that percentages of green seeds greater than 10% in the lot could lead to serious physiological quality problems. They also verified that the incidence of green seeds is directly related to the process of deterioration by moisture, thus causing a marked reduction in germination, vigor and seed viability.

Soybean seeds harvested at six maturation stages and dried at three temperatures, 25°C, 40°C and 75°C, showed complete chlorophyll degradation only in seeds harvested from the R7 stage. At 25°C, chlorophyll was completely degraded. However, at
temperatures of 40°C and 75°C, there was an accumulation of chlorophyll and pheophytins, and at the highest temperature the rapid loss of moisture caused inactivation of the chlorophyllase enzyme (SINNECKER, 2002).

GREEN SEEDS AND THE EFFECT ON OIL PRODUCTION

Many vegetables synthesize and store large amounts of oil during the seed development process. Fats and oils are important forms of reduced carbon storage in many important seeds, such as soybeans, canola and sunflowers. These oils accumulate in organelles called oleosomes. These are unique among organelles as they are bounded by a monolayer of phospholipids (TAIZ & ZEIGER, 2017).

Protein concentration in soybean seeds is almost constant during seed development, but oil concentration is initially slow and increases during seed development, reaching a maximum level as the seed approaches physiological maturity (BLACKMAN et al., 1992). The phenomenon of green seed in soybean is of great importance because the green color affects the quality of grains on the market. The presence of chlorophyll and other green pigments in canola and soybeans gives a dark and undesirable color to the oils (GOMES et al., 2003).

Bohner (2002) reported two types of green soybean seeds in the United States, the first category being characterized by the green color of the outer part of the seed, while the inner part remains yellow. In the second category, the green color is found throughout the seed, which is classified as damaged seed at a discount to the producer because chlorophyll is transported to the oil during processing, having a negative effect on quality.

Research carried out by Mandarino (2005) shows that green soybeans have the same percentage of protein as mature beans, however, they present an average of 2% to 3% less oil, which oil will present greater acidity, in addition to having a higher refining cost, as the removal of chlorophyll from the oil requires specific processes. Because chlorophylls are potent oxidizing agents, the quality of oil contaminated with chlorophyll may be impaired if it is stored in the presence of light.

Also, green beans provide lower yields in the production of protein isolates, and it is essential that chlorophyll be removed from soy products and derivatives, or else, that the occurrence of green beans is avoided. The presence of pigments, even in small amounts, generates a higher refining cost and reduces the commercial value of the grain.
Lots that have green beans cannot be traded internationally, making it a relevant fact in view of the huge volume of exports (MANDARINO, 2005).

In the United States and Canada, the presence of green beans is observed in rapeseed, where the maximum chlorophyll content allowed for first quality seeds is 22 mg/kg (dry basis). In Europe, Sweden since 1970, has the price of canola established according to the chlorophyll content in the oil, and high quality seeds have an oil with a maximum content of 30 ppm of total chlorophyll (DAHLÉN, 1973).

CONCLUSIONS

Soybean seeds can be harvested from physiological maturity, although it is more usual to harvest when the plant reaches a humidity of around 14%. Since the presence of green seeds is a result of weather conditions, diseases and/or premature harvesting procedure, it is evident that greening impairs physiological quality and negatively interferes with batch quality and oil processing.

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