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Systematic Review - State of the Art: strategies for vigor evaluation and production of high-vigor soybean seeds

Andreza Cerioni Belniaki[1]*, Mayla Daiane Correa Molinari[2], Fernando Augusto Henning[2], Maristela Panobianco[1]

ABSTRACT: A systematic approach was employed with the objective of compiling updated and more promising information on the quality of soybean seeds, traits, treatments and analysis techniques. Among the 6,899 academic materials retrieved between 2017 and 2022, 57 of them were included in the study, separated into three groups: Group 1 - Theoretical materials addressing traits related to seed vigor, which can be used in biotechnological strategies and improvement of different species; Group 2 - Practical materials that apply techniques of treatment of soybean seeds; and Group 3 - Practical materials that apply techniques to evaluate vigor in soybean seeds. From the approach used, it was possible to observe that several genes, proteins and QTLs are associated with seed vigor. Seed treatment techniques focus on the use of biological and physical products, but there is a lack of studies validating these benefits in the field. Among the methods for evaluating the vigor of soybean seeds, the techniques of image analysis (visible light, LIBS, NIR, FTIR, FT-NIR, HSI-NIR, FHSI, Hyperspectral, Fluorescence spectral) and the use of software (Vigor-S®, SVIS®, SAPL®, IJCropSeed, Ilastik, VideometerLab®, MATLAB, ENVI®) stand out.

Index terms: *Glycine max*, germination, nondestructive methods, seed classification, seed quality.

RESUMO: Foi realizada uma abordagem sistemática com o objetivo de compilar informações atualizadas e mais promissoras sobre a qualidade de sementes de soja, traits, tratamentos e técnicas de análise. Dentre 6.899 materiais acadêmicos recuperados, entre os anos de 2017 e 2022, 57 deles foram incluídos no estudo, sendo separados em três grupos: Grupo 1 - Materiais teóricos abordando traits relacionados ao vigor de sementes, que podem ser utilizados em estratégias biotecnológicas e de melhoramento de diferentes espécies; Grupo 2 - Materiais práticos que aplicam técnicas de tratamento de sementes de soja; e Grupo 3 - Materiais práticos que aplicam técnicas para avaliar o vigor em sementes de soja. A partir da abordagem realizada, foi possível observar que vários genes, proteínas e QTLs estão associados ao vigor das sementes. As técnicas de tratamento de sementes concentram-se no uso de produtos biológicos e físicos; porém, há carência de estudos validando esses benefícios em campo. Dentre os métodos para avaliação do vigor de sementes de soja, destacam-se as técnicas de análise de imagem (luz visível, LIBS, NIR, FTIR, FT-NIR, HSI-NIR, FHSI, Hiperespectral, Espectral de fluorescência) e o emprego de softwares (Vigor-S®, SVIS®, SAPL®, IJCropSeed, Ilastik, VideometerLab® MATLAB, ENVI®).

Termos para indexação: *Glycine max*, germinação, métodos não destrutivos, classificação de semente, qualidade de semente.

***Corresponding author**
E-mail: andrezacerioni@gmail.com

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[1]Federal University of Paraná, Caixa postal 19.061, 80035-050 - Curitiba, Paraná, Brasil.
[2]EMBRAPA Soja, Caixa Postal 231, 86001-970 - Londrina, Paraná, Brasil.

INTRODUCTION

The production of high-vigor soybean seeds is increasingly technified, requiring efforts and constant updating of techniques involving all stages of the process, i.e., genetics, management, treatment and seed quality analysis. This challenge increases, since vigor is a complex physiological characteristic, controlled by several loci and highly influenced by the environment (Wu et al., 2017).

In this context, strategies in the field of biotechnology, such as transgenics, gene editing, location of gene hubs via '-omics' methodologies (Smolikova et al., 2020), micro-RNAs (Dhaka and Sharma, 2021) and strategies in the field of classical breeding (Rajani et al., 2017), have been evaluated in conjunction with the consolidated strategies of good practices for seed production in the field (Rao et al., 2017). Additionally, there is an increasing number of studies and management techniques for seed treatment at different times of planting and post-harvest, which have also shown benefits for the maintenance of vigor and increase with "priming effect" (Bareke, 2018; Rifna et al., 2019).

However, although adequate management practices contribute significantly to the production of high-quality seeds during production and storage (Rao et al., 2017), vigor is highly influenced by biological and genetic factors and depends on the amount of reserve transferred from the parent plant to the seed (Arora, 2018; Bareke, 2018). Soybean, for example, for having high protein and oil contents in the seed, is quite vulnerable to biochemical degradation, which makes it difficult to maintain its physiological quality in the off-season (Louwaars and Jonge, 2021).

With new techniques for the production of high-quality seeds, the forms of evaluation should accompany the production process and research, to bring more assertive, fast and less subjective responses. The traditional evaluation of soybean seed lots with conventional tests, such as tetrazolium, germination, accelerated aging, field emergence, cold test, electrical conductivity and seedling length, is not automated, being destructive and requiring specialized training. Thus, these tests should be complemented with new techniques of image analysis (Kapadia et al., 2017).

Over the last decade, advances in computational resources and the development of new algorithms have allowed the analysis of large multidimensional datasets to implement increasingly accurate decision-making support systems (Baek et al., 2019; Xia et al., 2021; Xiao et al., 2022). This revolution of the big data era has impacted the value chain, as the use of data analysis systems makes it possible to employ unstructured seed datasets to gain insights for decisions on crop breeding (Daniel, 2020). New computer vision solutions, combined with artificial intelligence algorithms, can help recognize patterns in biological images, reducing subjectivity and optimizing the analysis process (Medeiros et al., 2020b). Similarly, the integration of seed phenotype image datasets with data from genomic and environmental domains can be used to gain insights for intelligent reproduction (Sharma et al., 2021).

High-throughput phenotyping and the use of software to monitor the variation in seed characteristics, applicable in quality control in seed production, can also be highlighted (ElMasry et al., 2019; Mortensen et al., 2021). Genomic understanding and molecular interactions in the dynamics of formation of quality seeds are indispensable for the advancement of genetic improvement and production (Dwivedi et al., 2021).

When it comes to soybean seeds, there are many novelties; however, the information available needs to be compiled for seed technology to advance globally. In this context, the present review aimed to survey: a) advances in genetics and related traits; b) the current trend for seed treatment and its results in yield components and seed quality; (c) new technologies in quality evaluation. All this context is aimed at directing new studies on soybean seed technology, presenting integrated ways to evaluate seed quality.

MATERIAL AND METHODS

The search for academic materials was carried out through a systematic review (Cogo, 2020), and the bibliography was selected through Harzing's Publish or Perish automation software on Google Scholar platform (Harzing, 2010).

The eligibility criterion consisted of selecting materials from keywords contextualized to the proposed theme (Cogo, 2020). The keywords used to search the database can be seen in Figure 1. Only academic materials published between 2017 and 2022 were retrieved.

The flowchart containing information on data collection and screening was delineated according to the Page et al. (2021) guide for systematic review. The materials were divided into three groups: Group 1 - Theoretical materials addressing analysis, improvement and characteristics involved in the vigor of seeds of different species, a group used for the theoretical basis of the manuscript; Group 2 - Practical materials that apply soybean seed treatment techniques; and Group 3 - Practical materials that apply techniques to evaluate the vigor of soybean seeds.

RESULTS AND DISCUSSION

A total of 6,899 academic publications were identified, of which 6,842 were excluded due to their title or abstract, based on the presence of search terms decontextualized to the proposed theme. Titles not relevant to the subject addressed were removed directly, and relevant titles had their abstracts analyzed to confirm exclusion or inclusion. A total of 57 materials had the search terms contextualized to the proposed theme, 22 included in the theoretical construction and 35 included in the practical construction.

Among the practical materials focusing on soybean, only articles published in journals indexed in Scopus and classified in Quartiles Q1 and Q2 in SCImago were evaluated as a selection criterion, given the scientific rigor of the journals and the impact of the publications. Of these, 23 articles dealt with techniques in seed treatment, 12 dealt with techniques of analysis of soybean seed vigor (Figure 2).

Traits related to seed vigor, which can be used in biotechnological and breeding strategies

In recent decades, several genes, proteins and QTLs associated with seed vigor have been identified in several species (Wu et al., 2017; Kofsky et al., 2020; Rasheed et al., 2021; Reed et al., 2022; Arif et al., 2022).

Tocopherol (vitamin E), ascorbic acid (vitamin C) and PER proteins are regulators of seed longevity, through their inhibiting effect on the accumulation of reactive oxygen species (ROS), thus maintaining the stability of the plant genome (Zhou et al., 2020). The LIG and OGG1 genes and the BER pathway are also involved in maintaining genome stability and seed longevity in various crops, as well as the ASPG1-1 gene, which participates in the protection of protein structures to maintain seed longevity (Zhou et al., 2020).

The oligosaccharides of the raffinose family (RFOs) have also been shown to be important for maintaining seed longevity, while the RS and GOLS genes are positive regulators of longevity via RFO (Zhou et al., 2020).

In soybean, it was observed that the increased expression of genes encoding protective chaperones, including HSPs, is involved in the increase in seed longevity (Arif et al., 2022). Twenty seven transcription factors that showed

Search terms	Source	Papers
✓ improve seed vigor or increase seed vigor and soybean and yield from 2017 to 2022	Google Scholar	1000
✓ improve seed yield or increase seed yield and soybean and vigor from 2017 to 2022	Google Scholar	1000
✓ soy* and seed production or seed quality and vigor and yield and crispr from 2017 to 2021	Google Scholar	994
✓ soy* and seed production or seed quality and vigor and yield and crispr from 2022 to 2022	Google Scholar	87
✓ soy* and seed production or seed quality and vigor and yield and plant breeding from 2017 to 2021	Google Scholar	995
✓ soy* and seed production or seed quality and vigor and yield and plant breeding from 2022 to 2022	Google Scholar	620
✓ soy* and seed production or seed quality and vigor and yield and rnai or interference rna from 2017 to 2021	Google Scholar	848
✓ soy* and seed production or seed quality and vigor and yield and rnai or interference rna from 2022 to 2022	Google Scholar	50
✓ soy* and seed production or seed quality and vigor and yield and transgenic from 2017 to 2021	Google Scholar	996
✓ soy* and seed production or seed quality and vigor and yield and transgenic from 2022 to 2022	Google Scholar	309

Figure 1. Keywords used to search for published academic materials between 2017 and 2022, and number of academic materials retrieved per search. Image obtained from Harzing's Publish or Perish automation software on Google Scholar platform (Harzing, 2010).

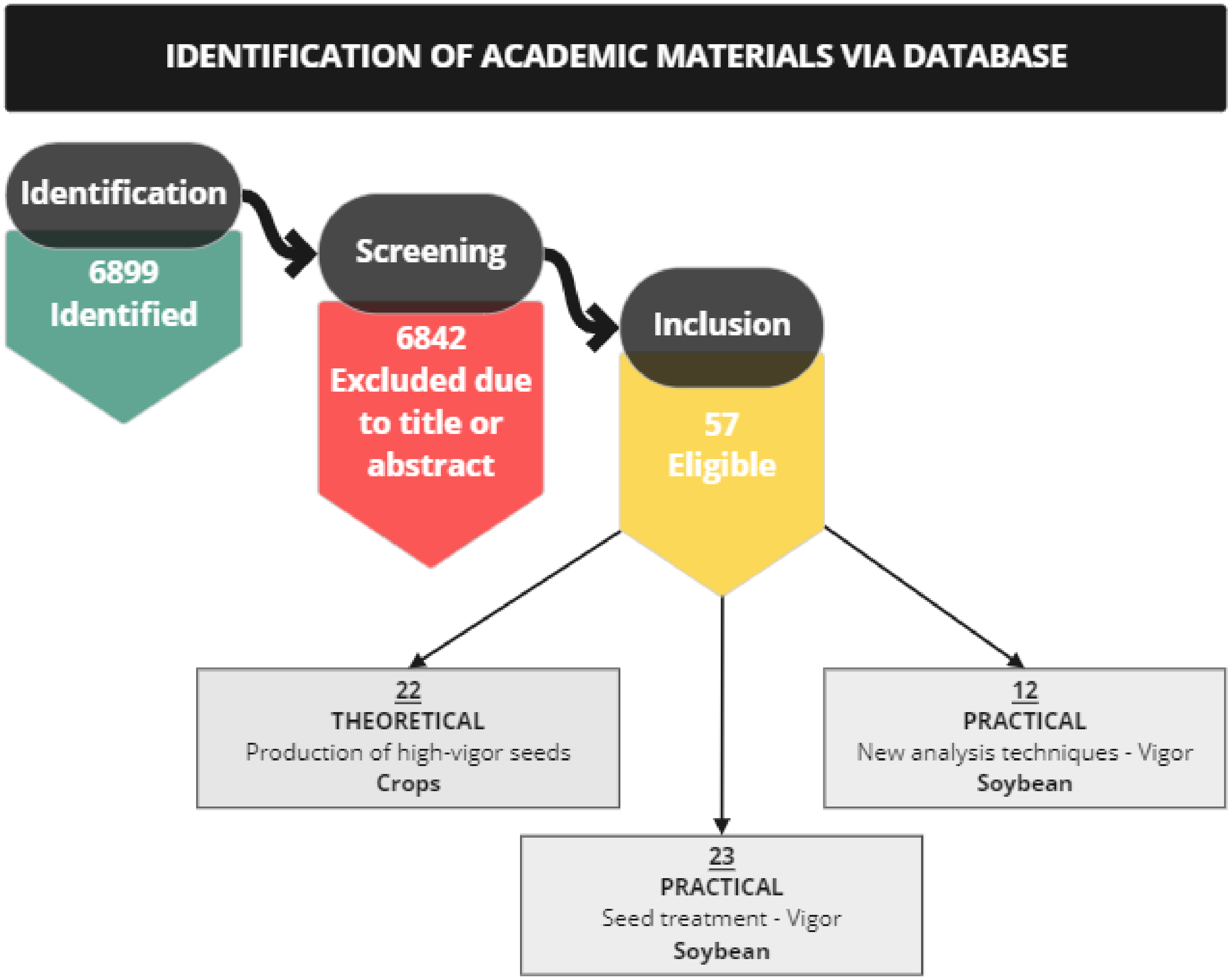


Figure 2. Flow of information to obtain data according to the guidelines of Page et al. (2021).

expression profiles highly correlated with seed longevity (Arif et al., 2022), enzymes such as SOD, CAT, GR and tocopherol isomers (Sooganna et al., 2021), have already been identified in the species. In addition, soybean seeds with high physiological quality have a high level of expression of genes that encode enzymes involved in respiration, such as alcohol dehydrogenase (ADH), malate dehydrogenase (MDH) and phosphoglucoisomerase (PGI), which can be quantified by the real-time PCR (qRT-PCR) technique (Baldoni et al., 2019).

Other factors intrinsic to the plant, such as sulfur and cysteine contents, are positively related to the physiological quality of seeds (Mondal et al., 2022). It was also discovered that some long mRNAs accumulated in mature seeds seem to be related to the maintenance of longevity and germination of seeds. However, the type of mRNA that accumulates in seeds is affected by the plant hormone abscisic acid (ABA) and environmental factors, and most of them accumulate in seeds in the form of monosomes (Sano et al., 2020).

Many studies involving the understanding of the molecular mechanisms underlying the physiology of crop yield and the processes that limit yield under field conditions have been trying to elucidate which combinations of favorable alleles are necessary to improve the yield and vigor of soybean seeds (Vogel et al., 2021). Some address mechanisms of seed deterioration during transport and storage, which indicates that such studies can enable the identification of key points involved in improving seed viability and vigor (Chhabra and Singh, 2019; Ebone et al., 2019). Mechanisms like these involve loss of seed quality, such as loss of membrane protection and permeability, lipid peroxidation and

ROS production; increased acidity of fats; increased enzymatic activity, consumption of reserves and damage to genetic material (Chhabra and Singh, 2019; Ebone et al., 2019; Ratajczak et al., 2019). Additionally, these studies make it possible to identify new markers of seed vigor, such as the AOX gene, which has been shown in several species to be an excellent marker of vigor, being a candidate for prebreeding and estimation of seed vigor (Mohanapriya et al., 2019).

The studies found in the literature demonstrated many possibilities that can be explored in order to identify new strategies to improve seed vigor (Figure 3).

Technologies used in the treatment of soybean seeds

The technologies aimed at the production of more vigorous seeds can be divided into methods of: a) **pre-harvest** - which refer to managements carried out in the parent plant still in the field; b) **post-harvest** (Seed enhancements), performed directly in the seed, through the application of chemical or biological phytosanitary products, in addition to different forms of treatment; c) **post-sowing** - carried out at the time of planting and, after it, to increase the viability and vigor of the seeds, for production in a greenhouse and in the field (Taylor et al., 2021).

In this review, the focus was on technologies applied directly to the seed (post-harvest and post-sowing) to increase vigor, which were grouped as Biological (Bio), Physical (Phys), Nutritional (Nut), Chemical (Chem) and Nanotechnological (Nano), according to Table 1.

The survey indicates a trend of studies related to the application of biological products in the seed, microorganisms isolated from the soil with rooting and nutrient-fixing action, promoting plant growth (PGPR) and their respective effects on germination, emergence, and seedling performance under ideal conditions or some type of environmental stress (Gregorio et al., 2017; Nazari et al., 2019; Khomari et al., 2018; Queiroz Rego et al., 2018; Tavanti et al., 2020; Abati et al., 2020; Paul and Rakshit, 2021; Sheteiwy et al., 2021).

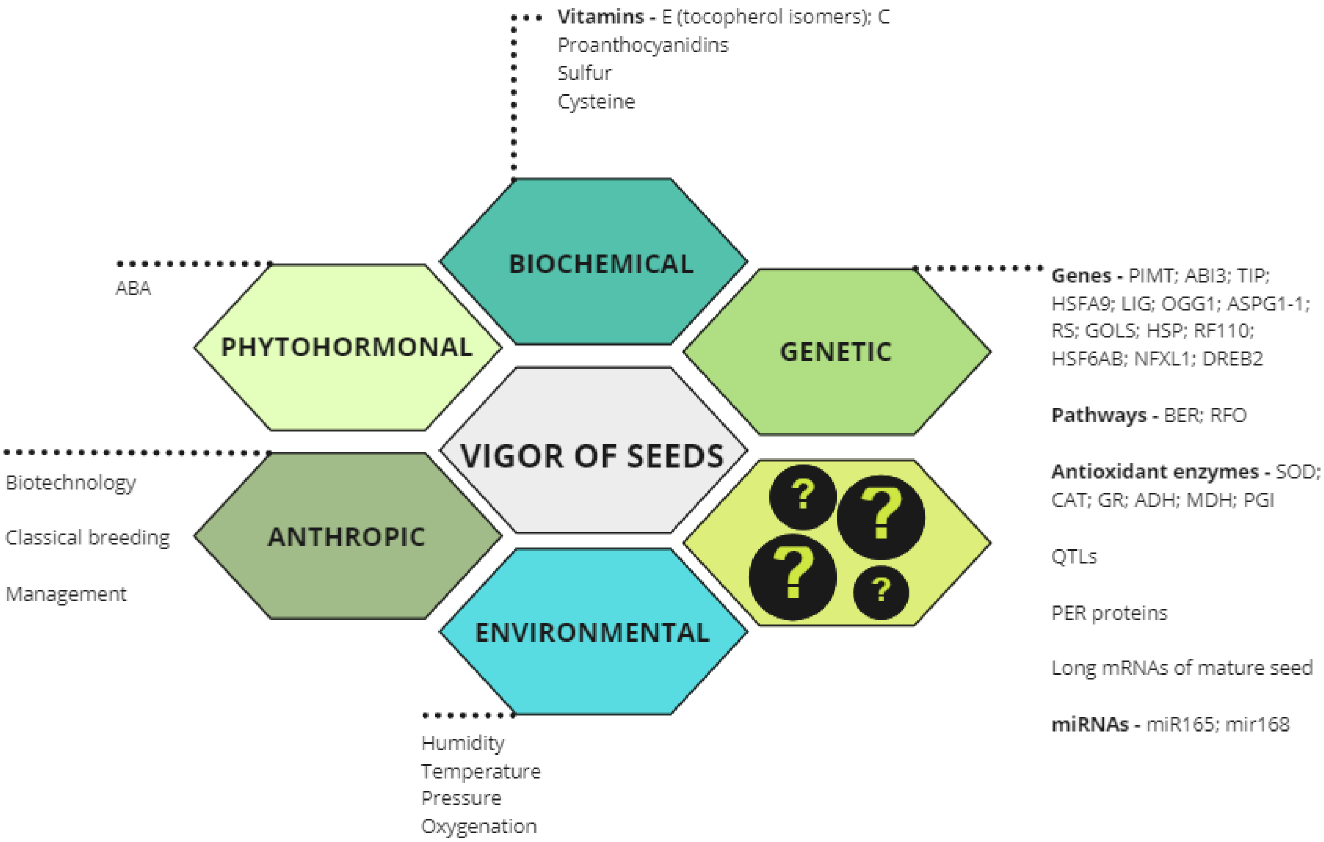


Figure 3. Biological, biochemical, genetic, phytohormonal, anthropic, environmental and future characteristics related to the vigor of soybean seeds.

Table 1. Summary of practical articles on soybean seed treatments.

Group – Seed treatment					Treatments	Increased		Reference
Bio	Phys	Nut	Chem	Nano		YC	SQ	Reference
INOC					*Bradyrhizobium japonicum* / *Azospirillum brasilense*	X	X	Queiroz Rego et al. (2018)
INOC					*Bacillus amyloliquefaciens* / Arbuscular Mycorrhizal Fungi (AMF)	X		Sheteiwy et al. (2021)
				NP	Metal sulphide nanoparticles		X	Afsheen t al. (2020)
INOC					*Bacillus subtilis*		X	Tavanti et al. (2020)
INOC		BION			Biopriming with *Trichoderma harzianum* (under NaCl stress)			Khomari et al. (2018)
INOC					*Trichoderma viride* BHU-2953 to increased phosphorus absorption			Paul and Rakshit (2021)
				NP	Zinc-functionalized thymol nanoemulsion	X		Kumari et al. (2019)
	EF				Electrostatic field (different values of direct current)		X	Mamlic et al. (2021)
INOC		BIOIN	RC		(carbendazim + thiram)/ (imidacloprid + thiodicarb) / (abamectin) / (cobalt, molybdenum and zinc) /polymer (peridiam) / (kinetin + gibberellic acid) and inoculant (*Bradyrhizobium japonicum*).	-		Abati et al. (2020)
	MF	BIOIN			Alterning magnetic field in combination with an algal extract		X	Michalak et al. (2019)
INOC		BIOIN			(chitosan + alginate/PEG) and commercial inoculant (HiStick® Soy) with Bradyrhizobium japonicum	X		Jarecki (2021)
	UV				Different doses of UV-C and UV-B radiation		X	Foroughbakhch et al. (2019)
	MF				Magnetopriming /Mancozeb / Neem leaf powder / Neem oil		X	Kataria et al. (2017)
		BIOIN			Vitamin E - priming			Alsamadany and Ahmed (2022)
			RC		Fipronil / Strobilurin / Pyraclostrobin / Benzimidazole / Thiophanate-methyl / Carboxamide / Fluxapyroxad		-	Khomari et al. (2017)
		PRIM			Salicylic acid		X	Nazari et al. (2019)
INOC				PVANF	Rhizobacteria (Pantoea agglomerans ISIB55 and Burkholderia caribensis ISIB40) immobilized in nanofibers		X	Gregorio et al. (2017)
	NTP				Time / 50Hz / Atmosphere (carrier gases) / Barrier		X	Pérez-Pizá et al. (2019)
	PAW + US				Plasma-activated water and ultrasound technologies		X	Porto et al. (2018)
	MF + LSR				TS laser (HeNe wavelength of 632 nm and density power of 1 mW/cm2) and magnetic field	X	X	Asghar et al. (2017)
	CAPP				Cold atmospheric pressure plasma		X	Švubová et al. (2021)

LEGEND: Bio – Biological / Phys – Physical / Nut – Nutritional / Chem – Chemical / Nano – nanotechnological.
INOC – Inoculation / ST – Seed treatment / PRIM – Priming / NTP – Non-thermal plasma / EF – Electric field / PAW – Plasma-activated water (PAW) / US - ultrasound / UV – Ultraviolet/ LSR – Laser / CAPP – Cold atmospheric pressure plasma / MF – Magnetic field / BIOIN – Bioinputs / RC – Commercial registered chemicals / PVANF - Polyvinyl alcohol nanofibers / NP – Nanoparticles/ YC – Yield components / SQ – Seed quality.

The studies grouped by physical treatments revealed the potential of new technologies such as electromagnetic field, laser, and the use of plasma to treat the seed or treat the water in which the seed will be soaked (Asghar et al., 2017; Kataria et al., 2017; Porto et al., 2018; Michalak et al.,2019; Foroughbakhch et al., 2019; Pérez-Pizá et al.,2019; Švubová et al., 2021; Mamlic et al., 2021).

Chemical treatments are mostly the traditional ones, with fungicides, insecticides, nematicides and commercial polymers, with trademark and wide use, evaluating how process adjustments during treatment can impact the quality of soybean seeds (Khomari et al., 2018; Abati et al., 2020; Alsamadany and Ahmed, 2022).

Another highlight is related to the increase of studies with nanotechnology in the treatment of soybean seeds (Kumari et al., 2019; Afsheen et al., 2020), either as a vehicle or polymer for bacterial immobilization (Gregorio et al., 2017).

New technologies in the evaluation of soybean seed vigor

The automation of seed quality evaluation is a trend in the seed sector (Pereira et al., 2020; Larios et al., 2020a), with potential for evaluating the physical, physiological and sanitary quality of seeds of various crops (Mortensen et al., 2021).

Although there are different techniques of image analysis, there is a standard process among them that can be organized in steps, as shown in Figure 4. The automation process can be divided into the following basic steps: sample preparation, image system calibration, image acquisition, segmentation of regions of interest, extraction of characteristics from the images of regions of interest and analysis of extracted characteristics (Mortensen et al., 2021).

The name of the analysis technique is usually related to the type of light used to illuminate the sample, depending on the wavelength and interaction with matter. The camera can be understood as the form of capturing the image; in this context, other factors can interfere, such as the lens, type of camera, sensor and environment in which the image was captured. If the light interacts with the sample and there is a sensor to capture this emitted radiation, chemometric analyses can be performed (chemical composition related to seed quality and spatial distribution of components); otherwise, only biometric analyses can be performed (size, shape, color, texture, visual damage). After obtaining the image, it is necessary to process the data, using statistical analysis software tools developed specifically for seeds and seedlings or more generic ones, using several algorithms for classification (categorization / clustering) calibrated to be used for machine learning, called Artificial Intelligence. The improvement in the machine learning process can be supervised and active, increasing the accuracy of the classification with the aid of human intervention during calibration (Pereira et al., 2020).

Several efforts have been made to automate the analysis process in order to reduce its intrinsic problems. Nondestructive analysis techniques (Figure 4) may vary depending on the method for image generation.

Another open-source software tool available is *IJCropSeed*, which can be used to assess tissue integrity and seed morphometry; in addition, seed viability and vigor can be evaluated indirectly through automated seed radiography analysis (Medeiros et al., 2020a).

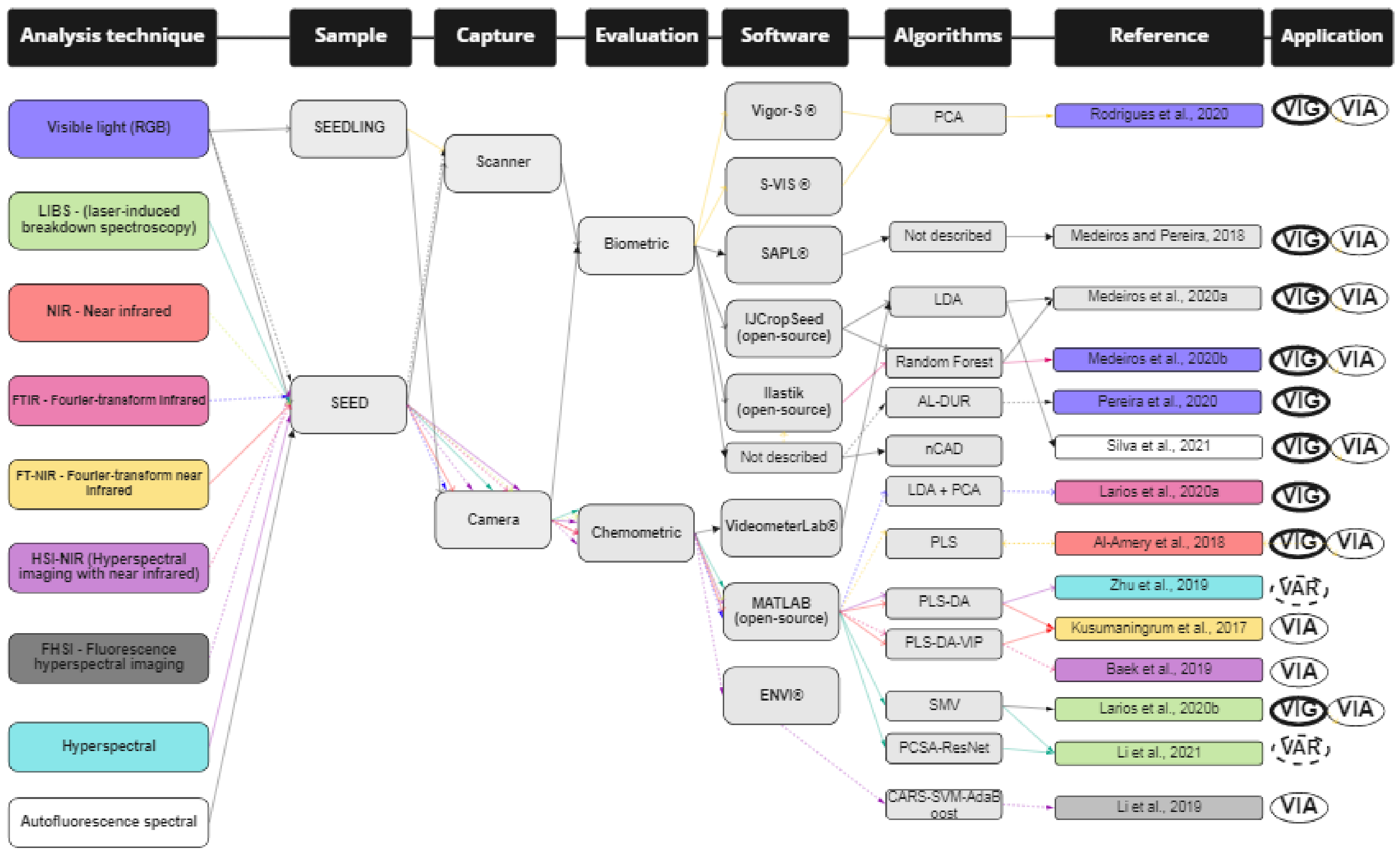
LIBS (laser-induced breakdown spectroscopy) is capable of evaluating the nutrient contents of seeds (Larios et al., 2020b).

Autofluorescence spectral image is used to classify soybean seeds with different vigor levels, presenting a strong correlation between autofluorescence spectral data and several quality indicators, such as early germination and seed tolerance to stressful conditions, in addition to a perfect correlation between fluorescence emission and total phenols in the embryo (Silva et al., 2021b).

The FTIR (Fourier transform infrared spectroscopy) technique, associated with chemometric and machine learning methods, is capable of pointing out which proteins, fatty acids and starch were the main molecules responsible for discriminating the vigor of the lots, discriminating them with high precision, close to 100% (Larios et al., 2020a).

NIR is a technique that helps to evaluate various characteristics of seeds in a nondestructive way, such as quality, health, deterioration, viability, vigor, including protein, starch and fatty acid composition, as well as biotic and abiotic damage, in addition to requiring short time for sample preparation and having high performance (Kusumaningrum et al., 2018).

For data processing and classification of samples from the images, it is necessary to use classifier algorithms, which



PCA – (Principal Component Analysis); LDA (Linear Discriminant Analysis); RF (Random Forest) and SVM (Support Vector Machine); AL-DUR (Active Learning through Diversity, Uncertainty and Representativeness); AdaBoost (adaptive boosting algorithm); PLS-DA-VIP (Partial Least Squares - Discriminant Analysis - Variable importance in projection); PCSA-ResNet (Propagation Coefficient Synchronous Adaptive Resnet). VIA – Viability; VIG – Vigor; VAR – Varieties. Sistema de Análise de Plântulas - SAPL®.

Figure 4. Flowchart of image analysis techniques tested on soybean seeds, from 2017 to 2022.

can be of various types depending on the objective of the classification; those with high performance for image analysis in the classification of soybean seeds and seedlings, assisted by machine learning, are described in Figure 4.

Other innovative techniques surveyed, but without the use of image analysis, that have been studied for the evaluation of soybean seed quality are: the profile of COVs (low-molecular-weight carbonyl compounds) (gases and volatiles) and water-soluble organic substances (enzymes and polysaccharides), released during imbibition and used as biomarkers (fingerprint). Silva et al. (2021a) developed an instrument to evaluate seed vigor based on seed respiration, measuring the concentration of CO_2 released when they are soaked in water.

There are other techniques surveyed in this review that have not yet been tested on seeds of other crops besides soybean, such as SeedGerm for image analysis with hardware and software (Matlab) (Colmer et al., 2020), X-ray technique, Thermal imaging, Electronic nose (Rhaman and Cho, 2016; Umarani et al., 2020), Raman spectroscopy and Infrared thermography (Xia et al., 2019).

FUTURE PROSPECTS AND CONCLUSION

Several traits have been studied and related to seed vigor, which can be used in biotechnological strategies, such as genes and proteins that are candidates for improvement and evaluated in seed production studies to optimize the responses of research on seed quality.

Seed treatment techniques with biological products and physical treatments are the most studied; however, there is a lack of studies validating the results and benefits in the field.

Other software programs and programming languages for data analysis and processing, not mentioned in the studies for soybean, such as R, ImageJ and Analysis in JAVA, can be used.

Spectroscopy instruments still have high value. What are the limitations of new seed analysis technologies? Do they have good reproducibility?

The devices need to be validated for several species and different cultivars, before being used in the field.

With the new technologies for automating the analysis, what are the necessary skills of the new seed quality control professional?

As future perspectives, seed technology research should try to aggregate these factors to have a broad view of seed quality. In addition, in the production of seeds in the field, it is necessary to survey the entire set of production data to generate new ideas that add quality, management, ranking of cultivars and breeding programs.

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