

A Systematic Review of the Literature on Machine Learning Methods Applied to High Throughput Phenotyping in Agricultural Production

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Abstract—The amount of images that can be extracted from crops such as soybean, corn, sorghum, etc., has increased exponentially due to the proliferation of remote sensing technologies such as Unmanned Aerial Vehicles (UAV). When processed and analyzed, images can provide valuable information and knowledge about High Throughput Phenotyping (HTP). Advances in HTP technology are essential to ensure that crop genetic improvement meets future global demands for food and fuel. In addition to UAVs, Digital Image Processing (DIP) and Machine Learning (ML) methods have shown to be promising tools in HTP to minimize the time and cost of analyzing entire crops. However, the performance and quality of the results obtained in HTP depend on the techniques used throughout the process. With this limitation in mind, the objective of this article is to present a Systematic Literature Review (SLR) on image capture techniques, DIP and ML applied to HTP. This review focuses on four sources of scientific searches, which initially returned 161 articles to be analyzed, of which 46 were excluded due to the Exclusion Criteria (EC), and 43 were duplicates, leaving only 72 for full reading. Of the 72 articles read, 27 were excluded due to the Exclusion and Quality Criteria (QC). Finally, 45 studies remained, resulting in a useful base on the cameras/sensors used in capturing the images, the most analyzed agronomic traits in the crops, in addition to a survey on the main DIP, ML techniques used in HTP.

Index Terms—High Throughput Phenotyping, Agricultural Production, Digital Image Processing, Machine Learning, Systematic Literature Review and Unmanned Aerial Vehicles

I. INTRODUCTION

According to data from the United Nations, the world population is expected to reach nine billion people by 2050 [1]. Because of this, the demand for food production grows to match the projected population growth. In order to prevent food insecurity, plant phenotyping is now at the forefront of plant breeding when compared to [2] genotyping. Plant phenotyping is defined as the assessment of complex agronomic traits, such as water stress [3, 4], growth habit [5],

disease resistance [6, 7], yield [8, 9] and [10], in addition to the basic measurement of individual quantitative parameters [2].

Traditional phenotyping techniques required destructive measurements, whereby crops were harvested at specific growth stages in order for genetic testing and mapping of agronomic traits to be performed [11]. Since breeding programs require repeated experimental trials to determine which traits are of interest, the process becomes expensive and significantly time-consuming [12]. Or, computational tools were used to speed up the phenotyping process, but the number of analyzed plants was relatively small [13, 14, 15, 16].

However, due to the high number of data to be analyzed for effective phenotyping, it was necessary to expand the research area for High-Throughput Phenotyping (HTP) [17]. HTP sets to reduce the time, cost and labor of the analysis of these traits. HTP uses non-invasive image capturing and processing techniques, as it allows the visualization of plant structures on a broader scale [18].

For these reasons, HTP has been widely adopted for several crops, including: Lettuce [19]; *Arabidopsis thaliana* [20]; Rice [21]; Grass [22, 23]; Conifers [24]; Bean [25] and [26], Corn [27, 28, 29, 30], Okra [31], Soy [32, 33], Sorghum [34, 35], [36, 37] Wheat [38] and Grape [39].

Another key point for HTP is the recent advances in sensor technology, which offer great opportunities for the use of Unmanned Aerial Vehicles (UAV) as a low-cost alternative to collect a large set of HTP data [40]. As these imaging technologies develop, it becomes possible to analyze more and more useful information, even investigating the biological growth of plants [41, 42].

These capture techniques include thermal imaging [43], fluorescence imaging [44, 45], digital imaging [5, 46, 47], infrared imaging [48, 49], and imaging spectroscopy [50]. These techniques make it possible to acquire data in laboratory and/or field environments. It is also possible to perform the 3D reconstruction of plants [51, 52].

Considering the important impact of Machine Learning (ML) algorithms on Digital Image Processing (DIP) and Computer Vision (CV) [53], several studies have emerged, such as [54], [55] and [56], which used DIP techniques combined with ML methods to aid in the assessment of agronomic traits. With this in mind, Nabwire et al. (2021)[57] analyzed articles published between 2010 and 2020 on artificial intelligence applied to HTP. This study provided an overview of current phenotyping technologies and the ongoing integration of arti-

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ficial intelligence into plant phenotyping. On the other hand, Zheng et al. (2021)[58] carried out a literature review on HTP techniques applied to strawberry crops.

The studies by Fernandes et al. (2020)[59] and Bresolin and Dórea (2020)[60] focused on the various computational acquisition and DIP techniques for HTP applied to livestock. In Furbank et al. (2019)[61], the authors carried out a literature review of HTP techniques supported DIP and imaging techniques used on wheat and sorghum. However, the work proposed in the present article is the first known to follow a systematic and reproducible approach of searching, analyzing and synthesizing studies on HTP supported by ML techniques. For the Systematic Literature Review (SLR) method used in this work, it is extremely important to have well-written, systematic and auditable documentation. For this reason, the objective of this work is to carry out an SLR aimed at identifying and analyzing the existing DIP and ML methods and techniques currently applied to HTP. This review demonstrates how these computational techniques tend to minimize the time and cost spent during the analysis of essential agronomic traits for the HTP. As well as the main technologies used for data acquisition and processing. Finally, a crossing of the obtained data was carried out, with this it was possible to see a pattern of behavior. An example of this was image segmentation, the most used DIP, when authors intended to estimate crop production.

The present article is organized as follows: Section II presents a comparative analysis of related works; Section III details the methods adopted in planning and carrying out the SLR protocol; Section IV reports results obtained from the SLR and their analysis; Section V summarizes the results of the SLR, and Section VI describes threats to the validity of the SLR; finally, Section VII presents the final considerations and future works.

II. RELATED WORKS

This section presents a number of literature reviews that address similar topics as proposed in the present work. Furbank et al. (2019)[61] presents a review of how genomics and HTP can provide for crop improvements in the next generations. For this, the study discusses DIP and ML applied to Wheat and Sorghum HTP as case studies. With these studies, these authors reached the conclusion that, by exploring allelic diversity, with the genome sequencing and HTP, it is possible to predict the commercial value of agronomic traits, as in the case of corn, for example.

Taberkit et al. (2021) [62] carried out a secondary study comparing several techniques that use UAV in Algeria. Nabwire et al. (2021) [57] analyzed over one hundred articles on artificial intelligence applied to HTP. It provides an overview of current phenotyping technologies and the ongoing integration of artificial intelligence into plant phenotyping. As a result of the analysis, these authors highlighted some studies based on ML applied to phenotyping tasks. It is the case of Scale-Invariant Feature Transform (SIFT), Support Vector Machine (SVM) together with K-nearest neighbors (kNN), decision tree and Multilayer Perceptron applied to wheat, rice,

corn and wheat, respectively. Another interesting approach was deep learning architecture applied to plant phenotyping using transfer learning. From the analysis carried out by the authors, it was possible to observe that each architecture in the literature was used for a specific objective, for example: GoogLeNet was used to classify plant diseases, VGG-16 to list the most frequent Image Processing Techniques. The authors concluded that recent advances in HTP technologies have led to significant advances in plant phenomics.

In review [58], the authors focused on recent approaches to phenomics in strawberry farming, particularly ones that employed remote sensing and ML. The study was categorized according to each strawberry trait analyzed. As a result of this review, the authors listed the articles according to some information extracted from strawberry crops, such as: fruit/flower detection, maturity, yield forecast and pest detection. For fruit/flower detection, automated fruit and flower counting from images is a critical step in automatic harvesting and yield forecasting. Initially, studies tried to perform this detection using morphological operations, but in recent articles (from 2016 onwards) some deep-learning artificial neural networks were used, such as VGGNet, YoloFaster, RCNN and Mask R-CNN, reaching accuracy of 95.78%.

Many contributions were identified from the articles discussed in this section. However, the studies by Nabwire et al. (2021)[57] and Zheng et al. (2021)[58] analyze specific crops, and not all crops using computational techniques applied to HTP. Another point is that Nabwire et al. (2021)[57] and Zheng et al. (2021)[58] are not a SLR¹, for this reason, they do not allow auditing or simply redoing searches with new information. The proposed work, as far as we know, is the first SLR focused on HTP that uses computational ML methods. For this reason, the steps taken in the proposed work can be reproduced at any time. In addition to the fact that much of the information was correlated, for example, all available crops, along with DIP and ML techniques, along with cameras/sensors and ways of capturing images.

III. METHODS

The Systematic Literature Review (SLR) is a well-known method that is widely used to identify, assess, and interpret studies that are relevant to a specific topic, area, or phenomena of interest [63]. A SLT is a secondary study, which aims to carry out a survey of studies within the same scope, critically assessing them regarding their methodologies and bringing them together in a statistical analysis or meta-analysis, when possible. For the implementation of a SLR, the methodology proposed in the work by Kitchenham (2004)[63] was used.

A. Literature Review Planning Protocol

This study was carried out in four well-known literature search databases with a scientific scope: Scopus [64], IEEE Xplore [65], ACM Library [66] and Engineering Village[67].

¹A SLR uses explicit and systematic methods selected to minimize discrimination, providing reliable results from which conclusions and decisions can be reached [63].

Kitchenham (2004)[63] considers the following planning protocol for literature reviews:

- **Research Question (RQ)**

RQ: How are machine learning methods used in high-throughput phenotyping?

- **Exclusion criteria (EC)**

EC₀: Studies on high-throughput phenotyping, but not aimed at agricultural production.

EC₁: Phenotyping methods or techniques that do not use imaging or other spectra.

EC₂: Papers other than primary studies or conference papers or articles.

EC₃: Studies that do not focus on HTP.

EC₄: Studies that do not employ machine learning.

EC₅: Articles that are not available for free.

EC₆: Articles not written in English.

- **Inclusion Criteria (IC)**

IC₀: Existing HTP methods and techniques, employing imaging techniques or other spectra.

- **Quality Criteria (QC)**

QC₀: Definition of the research problem.

QC₁: Environment of the study.

QC₂: Limitations of the study.

QC₃: Database composition.

QC₄: Integrated use of DIP techniques with the agronomic traits.

QC₅: Empirical investigation of the use of machine learning on HTP.

Each grade for the QC ranged from 0 to 2, where: (0) There is no description; (1): There is a brief description; (2): There is explicit information. QC₃, QC₄ and QC₅ had a weight of 2, as they are key points for quality analysis. With this, the Weighted Average QC (WAQC) was calculated for each article read as follows $WAQC = (QC_0 + QC_1 + QC_2 + QC_3 * 2 + QC_4 * 2 + QC_5 * 2)$. Then a rule of three was calculated, for the values are in the range of 0 to 10.

- **Fields in the Data Extraction Form (DEF)**

DE₀: Year.

DE₁: Analyzed crop.

DE₂: Agronomic traits.

DE₃: Tools used to capture images and other spectra.

DE₄: Image processing techniques.

DE₅: Machine learning techniques.

DE₆: Database composition.

B. Execution

The choice of keywords to build the search strings was based on terms commonly found in the literature and terms related to this review (i.e., DIP techniques and ML methods applied to HTP employing images and other spectra). To carry out the SLR, specific search strings were formulated

for each search source (Scopus, IEEE Digital Library, ACM Digital Library and Engineering Village), as described below:

- **Scopus:** TITLE-ABS-KEY (("high throughput phenotype" OR "high throughput plant phenotyping" OR "high throughput phenotyping") AND ("deep learning" OR "machine learning" OR "computer vision") AND ("image-based phenotyping" OR "RGB" OR "infra-red" OR "Fluorescence" OR "Thermography" OR "Tomography" OR "Spectroscopy" OR "hyperspectral" OR "multispectral")).

- **IEEE Digital Library:** (("high throughput phenotype" OR "high throughput phenotyping" OR "high throughput phenotyping") AND ("deep learning" OR "machine learning" OR "computer vision") AND ("image-based phenotyping" OR "RGB" OR "infra-red" OR "Fluorescence" OR "Thermography" OR "Tomography" OR "Spectroscopy" OR "hyperspectral" OR "multispectral")).

- **ACM Digital Library:** [[All: "high throughput phenotype"] OR [All: "high throughput phenotyping"] OR [All: "high throughput phenotyping"]] AND [[All: "deep learning"] OR [All: "machine learning"] OR [All: "computer vision"]] AND [[All: "image-based phenotyping"] OR [All: "rgb"] OR [All: "infra-red"] OR [All: "fluorescence"] OR [All: "thermography"] OR [All: "tomography"] OR [All: "spectroscopy"] OR [All: "hyperspectral"] OR [All: "multispectral"]].

- **Engineering Village:** (((("high throughput phenotype" OR "high throughput phenotyping" OR "high throughput phenotyping") AND ("deep learning" OR "machine learning" OR "computer vision") AND ("image-based phenotyping" OR "RGB" OR "infra-red" OR "Fluorescence" OR "Thermography" OR "Tomography" OR "Spectroscopy" OR "hyperspectral" OR "multispectral")))) WN ALL).

These strings were searched on September 30, 2022, in the respective databases, automatically. In the Scopus database, 112 studies were identified; 5 studies were found in the IEEE Digital Library; in the ACM Digital Library, 6 articles were returned; and in Engineering Village, 38 studies were found. The next section will present the results gathered from these searches and how they can contribute to the research area under analysis.

IV. RESULTS

This section was divided into two parts. The first is the **Article Analysis**, in which each article found in the searches was analyzed. The second step is the **Synthesis of the Data** obtained in the analysis phase.

A. Article Analysis

In total, 161 articles were found in the four data sources. Figure 1 presents the results in numbers for the articles obtained after the initial selection and those selected for full

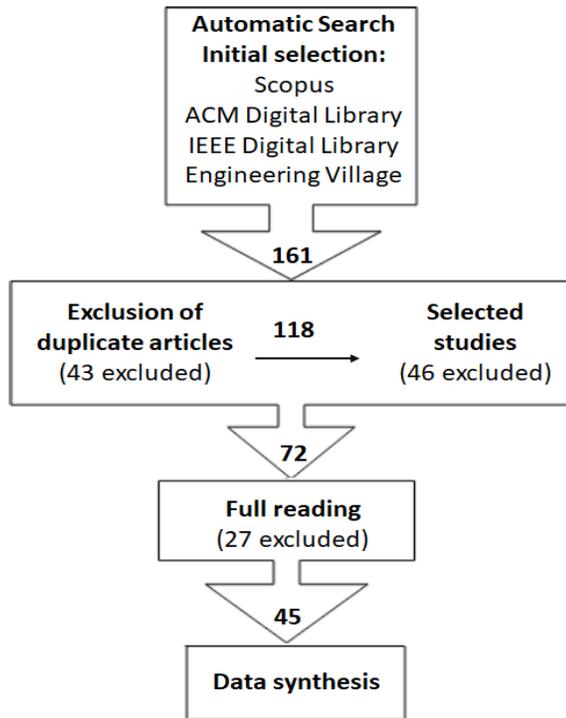


Fig. 1. A detailed look at the Article Analysis stage: automatic search, exclusion of duplicate articles, selected studies, data extraction and data synthesis.

reading. Both will be detailed below.

- **Initial selection:** The Parsif [68] tool was used to organize the articles to be read in full. This made it possible to quickly detect works that appeared in more than one database. In addition, the tool assisted in the initial selection and data extraction. Of the 161 articles found with the automatic search, 43 were removed for being duplicated, 46 were rejected due to EC_0 , EC_1 , EC_2 , EC_3 , EC_4 and EC_6 . At the end of the initial selection, 72 articles remained.

- **In-full reading:** Each of the 72 articles was read in full, however, not all of them could move forward to the data extraction stage. Although they were accepted in the initial selection based on their keywords, 8 of them were rejected due to EC_1 , EC_2 , EC_4 and EC_5 . In addition, another 19 articles were rejected due to $WAQC < 5.0$. These 19 studies lacked details on how the techniques were employed or how the database was built. At the end, 45 articles were selected for data extraction in this review.

- **Data extraction:** Figure 2 summarizes the number of articles identified in each database using search strings, as well as the number of articles from each source that underwent data extraction. For each of the 45 articles, DE_0 to DE_6 were extracted, and these data are discussed in subsection IV-B. These 45 articles are listed in Table I.

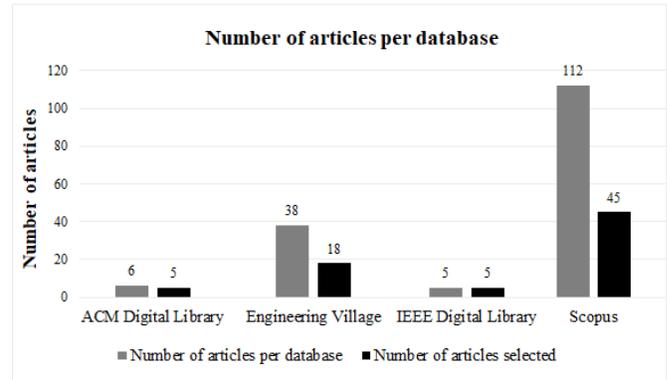


Fig. 2. Number of articles found and used in the data extraction stage.

TABLE I
SELECTED PRIMARY STUDIES

ID	TITLE	REF.
S1	A fully automated and fast approach for canopy cover estimation using super high-resolution remote sensing imagery	[69]
S2	A new image-based tool for the high throughput phenotyping of pollen viability: Evaluation of inter- and intra-cultivar diversity in grapevine	[70]
S3	A novel NIR-image segmentation method for the precise estimation of above-ground biomass in rice crops	[71]
S4	A two-step registration-classification approach to automated segmentation of multimodal images for high-throughput greenhouse plant phenotyping	[72]
S5	Assessment of mixed sward using context sensitive convolutional neural networks	[73]
S6	Assessment of plant density for barley and wheat using UAV multispectral imagery for high-throughput field phenotyping	[46]
S7	Combining UAV-RGB high-throughput field phenotyping and genome-wide association study to reveal genetic variation of rice germplasm in dynamic response to drought stress	[74]
S8	Comparing machine learning methods for classifying plant drought stress from leaf reflectance spectra in arabidopsis thaliana	[50]
S9	Computer vision and machine learning enabled soybean root phenotyping pipeline	[75]
S10	Convolutional neural networks to estimate dry matter yield in a guineagrass breeding program using uav remote sensing	[76]
S11	Development of methods to improve soybean yield estimation and predict plant maturity with an unmanned aerial vehicle based platform	[9]
S12	Ear density estimation from high resolution RGB imagery using deep learning technique	[77]
S13	Estimates of plant density of wheat crops at emergence from very low altitude UAV imagery	[78]
S14	Evaluation of the performance of machine learning methods in soybean segmentation for image-based high-throughput phenotyping in greenhouse	[10]
S15	LSTM-based cotton yield prediction system using UAV imagery	[79]
S16	Maize-IAS: a maize image analysis software using deep learning for high-throughput plant phenotyping	[47]
S17	Multi-resolution outlier pooling for sorghum classification	[80]
S18	Qualification of soybean responses to flooding stress using UAV-based imagery and deep learning	[43]
S19	Sorghum panicle detection and counting using unmanned aerial system images and deep learning	[81]
S20	Soybean yield prediction from UAV using multimodal data fusion and deep learning	[8]
S21	SpikeSegNet-a deep learning approach utilizing encoder-decoder network with hourglass for spike segmentation and counting in wheat plant from visual imaging	[82]
S22	UAV based remote sensing for tassel detection and growth stage estimation of maize crop using multispectral images	[5]
S23	UAV-based high throughput phenotyping in citrus utilizing multispectral imaging and artificial intelligence	[83]
S24	UAV-based high throughput phenotyping in specialty crops utilizing artificial intelligence	[84]
S25	Understanding growth dynamics and yield prediction of sorghum using high temporal resolution UAV imagery time series and machine learning	[85]
S26	Yield prediction by machine learning from UAS-based multi-sensor data fusion in soybean	[86]
S27	A Comparison of UAV RGB and Multispectral Imaging in Phenotyping for Stay Green of Wheat Population	[87]
S28	Above-Ground Biomass Estimation in Oats Using UAV Remote Sensing and Machine Learning	[88]
S29	ChronoRoot: High-throughput phenotyping by deep segmentation networks reveals novel temporal parameters of plant root system architecture	[49]
S30	Classification of rice yield using UAV-based hyperspectral imagery and lodging feature	[89]
S31	Estimating leaf area index using unmanned aerial vehicle data: Shallow vs. Deep machine learning algorithms	[90]
S32	Exploiting High-Throughput Indoor Phenotyping to Characterize the Founders of a Structured B. napus Breeding Population	[44]
S33	Estimation of Maize Yield and Flowering Time Using Multi-Temporal UAV-Based Hyperspectral Data	[91]
S34	High-Throughput Phenotyping and Random Regression Models Reveal Temporal Genetic Control of Soybean Biomass Production	[92]
S35	Hyperspectral imaging combined with machine learning for the detection of fusiform rust disease incidence in loblolly pine seedlings	[6]
S36	Hyperspectral leaf reflectance as proxy for photosynthetic capacities: An ensemble approach based on multiple machine learning algorithms	[93]
S37	Maize yield prediction at an early developmental stage using multispectral images and genotype data for preliminary hybrid selection	[94]
S38	Multi-feature data repository development and analytics for image cosegmentation in high-throughput plant phenotyping	[45]
S39	Multi-temporal predictive modelling of sorghum biomass using uav-based hyperspectral and lidar data	[95]
S40	Estimation of soybean grain yield from multispectral high-resolution UAV data with machine learning models in West Africa	[96]
S41	Hyperspectral Technique Combined With Deep Learning Algorithm for Prediction of Phenotyping Traits in Lettuce	[97]
S42	Identification and Comprehensive Evaluation of Resistant Weeds Using Unmanned Aerial Vehicle-Based Multispectral Imagery	[98]
S43	Rice bacterial blight resistant cultivar selection based on visible/near-infrared spectrum and deep learning	[7]
S44	UAV-based multi-sensor data fusion and machine learning algorithm for yield prediction in wheat	[99]
S45	Sugarcane yield prediction and genotype selection using unmanned aerial vehicle-based hyperspectral imaging and machine learning	[100]

B. Data Synthesis

This section and section V present several analyses of the data obtained from the previous data extraction. However, the databases differ for each of the 45 studies, in addition to crops, capture tools and computational algorithms. For this reason, it was not possible to compare the articles on all HTP processes and the results from each study. Comparisons were limited to the information contained in the DEF, as well as cross-referencing some information from the Data Extraction Form (DEF).

1) *Distribution of publications over the years:* Figure 3 shows the number of articles published between 2016 and 2022. Considering that the analysis includes 2022 articles published until the month of September, because that was when the searches were carried out in the databases, it is possible to notice that there is a growing curve of articles being published in the last 2 years, demonstrating scientific community is showing interest in the subject.

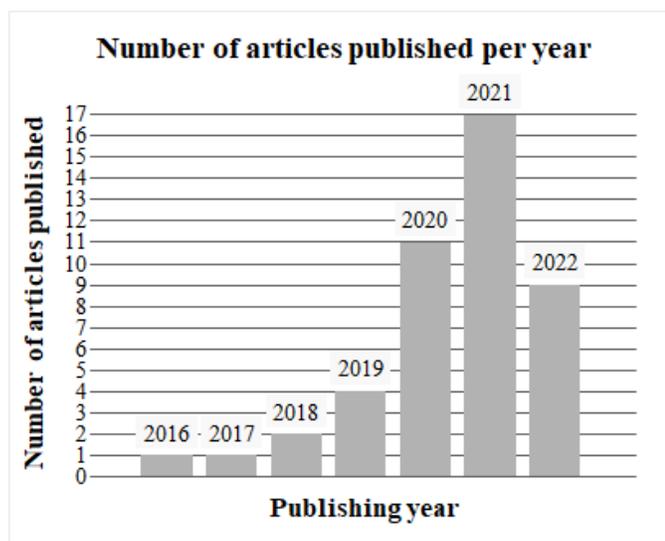


Fig. 3. Number of articles per year, showing an exponential growth of studies using computational techniques applied to HTP.

2) *Analyzed crop:* During the data analysis, it was possible to observe that wheat, soybean, sorghum and corn were the most often analyzed crops, present in 28 of the 45 articles, as observed in Figure 4. This is due to the fact that these crops are easier to access for trials, as they are used in the food industry. Another consideration is that the studies that have the highest WAQC = 10 (S26) and WAQC = 9.4 (S1, S13, S19 and S21) are precisely soybean, wheat and sorghum. This shows that these crops are already widespread in the literature, with very detailed studies on them.

3) *Agronomic traits:* Regarding agronomic traits, that is, what is most often analyzed/observed in each crop, yield is by far the most studied and tested agronomic trait. This information can be seen in Figure 5. Regarding the WAQC of the 18 articles that analyzed yield (S3, S4, S5, S11, S14, S15, S20, S24, S25, S26, S30, S33, S37, S38, S39, S40, S44 and S45), only S26, S14 and S5 reached WAQC > 8.0. That is, despite being present in a large portion of the studies, research

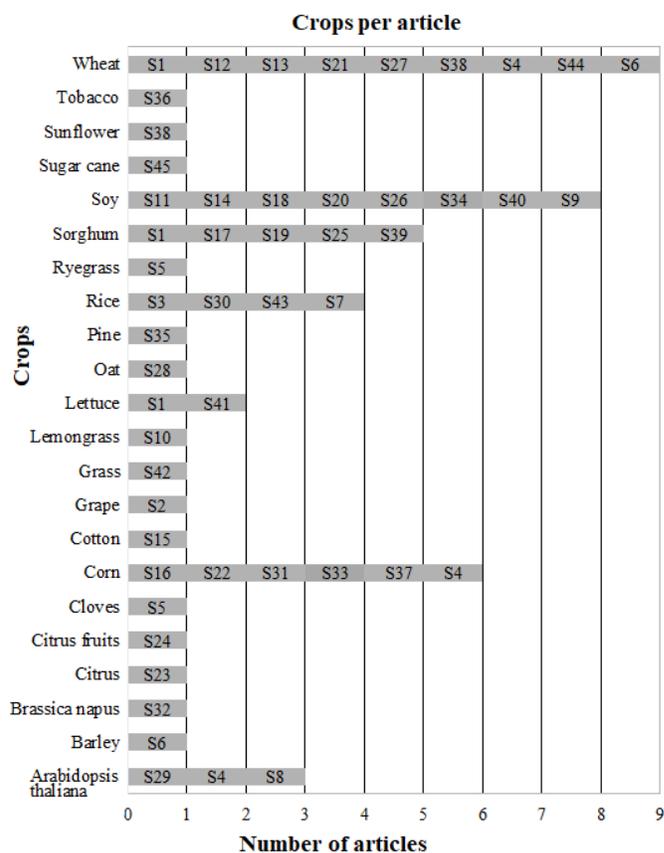


Fig. 4. Crops per articles.

is still not at an ideal maturity. This shows that the analysis of this trait is a recent trend and that there is still room for improvement.

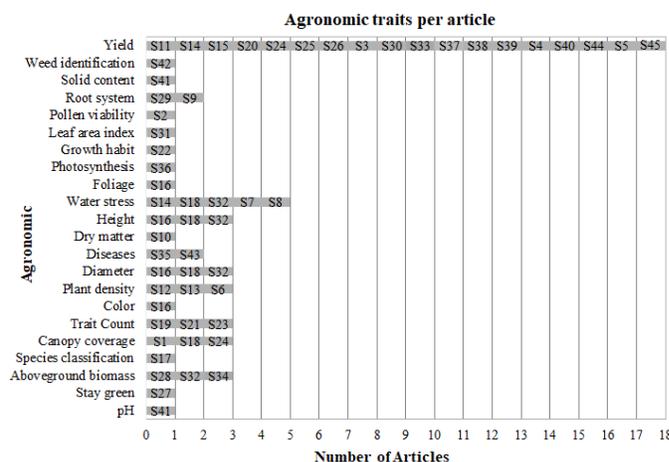


Fig. 5. Agronomic traits per article.

4) *Tools used to capture images and/or other spectra:* Digital images are obtained by means of RGB cameras/sensors and provide information about the size and color of the plants, which allows the assessment of the deterioration of the plant due to nutrient deficiencies or infections by pathogens, being used in several articles (S2, S5, S6,S7, S9, S10, S12, S13, S14, S15, S16, S17, S19, S20, S21, S22, S24, S26, S27, S31, S32,

S34, S39, S42, and S44). Multispectral images were captured to monitor plant photosynthetic pigment composition, assess water status, and detect abiotic or biotic plant stresses (S1, S6, S11, S18, S20, S22, S23, S25, S26, S27, S28, S31, S34, S37, S40, S42, and S44).

In addition, other image capturing tools were used, such as fluorescence and tomography. The sensor/camera model most often used among the works under analysis was a multispectral RedEdge-M camera, which collects five different bands (Red, Green, Blue, NIR and Red Edge) and a side GPS ². As for the WAQC calculated for these articles (S6, S18, S22, S23, S24, S25, S28, S31, S35 and S37), it was observed that of the 10 articles, 7 of them reached $WAQC \leq 7.0$ and are relatively new publications, mainly from 2021. That is, there are still many tests and studies to be done to reach a greater understanding of which cameras/sensors are ideal for each scenario.

These cameras/sensors are coupled to some form of capture tool, such as UAVs (as seen in articles: S1, S3, S6, S7, S10, S11, S13, S15, S18, S19, S20, S22, S23, S24, S25, S26, S27, S28, S30, S31, S33, S34, S36, S37, S39, S40, S42, S43 and S45). Proper flight planning is necessary to ensure that the tool produces high quality images, optimizing existing resources and minimizing capture time. Or, the cameras can be attached to other devices, as in the case of the sliding rail (S14 and S43); cameras fixed on rods (S12, S17); tripods (S9, S31 and S35); and rotation models (S4). In these cases, tests were performed in greenhouses where the images were captured at a fixed height and width. Of the 45 articles analyzed, 29 use cameras/sensors coupled to UAVs. In terms of the quality of the works that use UAVs, they have been carried out mainly since 2016, and 60% of them have $WAQC \geq 7.0$. That is, when the number of works that use UAVs and the WAQC of these articles are considered, it is possible to notice a great interest of the scientific community in this technology. This is due to the possibility of capturing a large area in a short period of time, a characteristic of UAVs that is essential for HTP.

5) *Digital Image Processing Techniques*: After capturing the images, the authors start to pre-process them. Some works made only basic adjustments to the images with application of filters, rotations, morphological operations, lighting adjustment, creation of orthomosaics, among others, always with the aim of facilitating data processing stage. But the most widely used technique was image segmentation, aiming to separate the plants from the soil. This technique was used in 23 studies (S3, S4, S6, S7, S9, S14, S16, S19, S21, S22, S23, S24, S27, S29, S30, S31, S32, S34, S35, S38, S42, S43 and S44). Of these 23 studies, 45% scored $WAQC > 7.0$. Although the contextualization and problems are well described in these studies, more information is still needed for QC_3 , QC_4 or QC_5 . This is due to the fact that these techniques are still experimental, and there is a lack of comparative studies on which DIP technique is ideal for each crop and agronomic trait. Figure 6 presents the three most frequent techniques.

²RedEdge-M is a MicaSense product.

The other techniques were not mentioned, as they only occur once or twice.

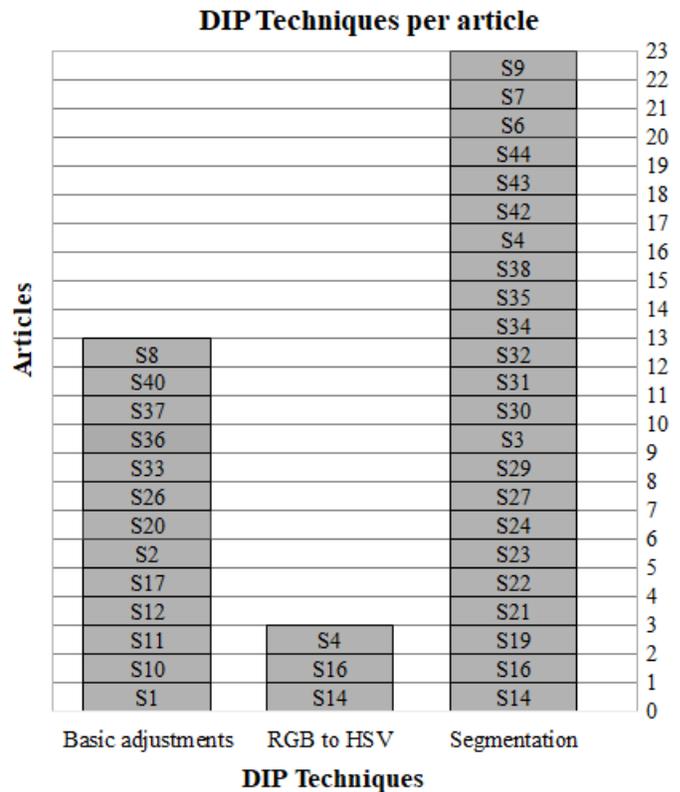


Fig. 6. Most common image processing techniques.

6) *Machine Learning techniques*: In general, processing agronomic traits for a large number of plants is a demand that has been around for many years but has only recently been met due to new image capturing tools. But this review has found that, in addition to quality images, the techniques used throughout the process are essential to achieve the expected goals. The ML techniques most often used in the articles are shown in Figure 7. The analysis showed that Convolutional Neural Network (CNN), Random Forest (RF) and Support Vector Machine (SVM) were used in 36 (S1, S4, S5, S7, S8, S10, S11, S12, S13, S14, S16, S17, S19, S20, S22, S23, S24, S25, S26, S27, S28, S29, S30, S31, S32, S33, S35, S36, S37, S39, S40, S41, S42, S43, S44 and S45) of the 45 studies used in data extraction. In some cases, the authors obtained satisfactory results when combining techniques, such as, for example, in S1, which combines RF and SVM. Because of the details presented by the authors, they reached $WAQC = 9.4$.

7) *Databases*: Of the 45 articles used in data extraction, 41 chose to build their own databases. As, for example, in S7, S8, S14 and S18, which investigated the impact of the climate conditions of the location under study on the analyzed crops. In S3, S4, S5, S11, S14, S15, S20, S24, S25, S26, S30, S33, S37, S38, S39, S40, S44 and S45, the authors needed to know how productive the cultivated crop would be. Only four works (S16, S17, S32 and S38) used databases that were ready previously.

In this sense, although there are some databases focused on

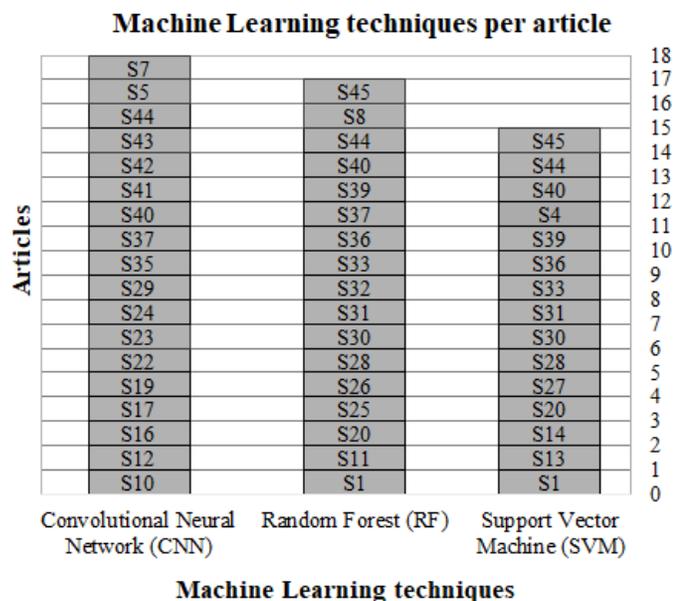


Fig. 7. The three most frequent machine learning techniques used in the articles.

HTP available in the literature, as in the case of [47, 80, 101, 102, 103], these databases are for specific crops, locations and climate conditions. Thus, most articles choose to build their own databases to analyze their crops and agronomic traits.

The following section answers the RQ, in addition to presenting the quality analysis of the articles.

V. DISCUSSION

Initially, for each of the 45 articles, information was extracted to fill out each of the DEF fields (Year, Agronomic traits, Analyzed crop, Tools used to capture images and other spectra, Image processing techniques, Machine Learning techniques, and Database composition). Each of these fields will be used to respond to the RQ. Finally, the quality analysis of the full articles will be presented.

A. How are Machine Learning Methods used in High-throughput Phenotyping?

To answer this question, the data obtained from the DEF will be divided into three stages described below.

1) *Stage 1 – Image capturing:* Digital images are obtained through cameras/sensors coupled to some capture tool, such as UAVs (as described in section IV-B4). Image capturing can be carried out for crops such as wheat (S1, S4, S6, S12, S13, S21, S27, S38 and S44), soy (S9, S11, S14, S18, S20, S26, S34 and S40), sorghum (S1, S17, S19, S25 and S39), etc. (crops were described in section IV-B2). The type of capture may vary according to the needs of each study or according to the agronomic trait to be analyzed (described in section IV-B3). By changing only the camera/sensor (RGB, multispectral, etc.) used, or the capture equipment (UAV, 4-wheel platform, slide rail in greenhouse, etc.), there is a possibility of obtaining considerably different images. These images will be used to start the construction of a database.

2) *Stage 2 – Pre-processing:* After capturing the images comes the time for pre-processing them (described in section IV-B5). This process may vary from basic adjustments to the images, such as morphological operations (opening S13, dilation and erosion S2), detection and extraction of areas of interest (S7, S16 and S41), as well as trait extraction (S13 and S25) and segmentation (S3, S4, S6, S7, S9, S14, S16, S19, S21, S22, S23, S24, S27, S29, S30, S31, S32, S34, S35, S38, S42, S43 and S44). Each of these techniques varies according to the needs of each study. If the authors are considering yield (the agronomic trait most often analyzed among the articles), a segmentation is usually carried out to highlight the planted area and reduce the importance of the non-planted area in the next phase. These pre-processed images are also added to the database.

3) *Stage 3 – Processing:* The pre-processed images will be used as inputs for the ML algorithms (described in section IV-B6), such as CNN (S5, S7, S10, S12, S16, S17, S19, S22, S23, S24 S29, S35, S37, S40, S41, S42, S43 and S44), RF (S1, S8, S11, S20, S25, S26, S28, S30, S31, S32, S33, S36, S37, S39, S40, S44 and S45), and SVM (S1, S4, S13, S14, S20, S27, S28, S30, S31, S36, S39, S40, S44 and S45). CNNs are a subset of ML tools, which are rapidly expanding and have caused a paradigm shift in image-based HTP. Because they are efficient in discovering complex structures in high-dimensional data, they are, therefore, applicable to a wide variety of images. However, no algorithm is ideal for all tasks. It is still a challenge to determine the best algorithm for each given problem [104].

After the ML technique has been chosen, image processing is performed. Training images (manually labeled) are used so that the algorithm learns to identify the traits presented to it. With the training finished, it is possible to carry out the validation/test. In this case, an image that was not used in training is presented to the algorithm. Ideally, the algorithm will have learned from training and will be able to identify the traits correctly. During the readings carried out, it was possible to verify that, for ML algorithms to obtain significant accuracy in HTP, properly choosing the technologies involved in all stages of the process is essential.

B. Study Quality Analysis

Another important point in the summary of the results is the relationship between each QC and the RQ, both defined in Section III.

1) The QC₀ and QC₁ were key for the exclusion of the 19 articles that passed the initial selection, but were eliminated during full reading, precisely because they did not detail the problem and the environment under study. This is something that directly impacted the whole work, as well as the way in which problems were solved, from image capture strategies and database composition to ML techniques.

2) With QC₂ it was possible to assess whether the limitations of each project were documented. This can help similar or complementary projects developed in the future. However, several works describe with little detail (S1, S2, S3, S5, S6, S8, S11, S12, S13, S16, S18, S19, S20, S21, S23, S25, S27,

S29, S31, S32, S34, S35, S36, S37, S38, S39, S42, S43 and S45) or did not describe (S4, S9, S15, S22, S24, S30, S33, S40, S41 and S44) their limitations. These three $QC_{0.3}$ did not alter the results of the WAQC significantly, since their weight was not doubled. However, it was possible to observe that when the three criteria were met, both the form of capture and the computational techniques were also described in detail.

3) QC_3 also assessed the description of the composition of each database, in order to subsequently perform comparisons between the 45 works. However, each study adopted a different strategy to create its database. For example, some works, such as S3, S11, S15, S20, S24, S25, S26, S30, S33, S37, S39, S40, S44 and S45, used UAVs to assess yield. However, it is extremely complicated to compare the results from these works, as the camera/sensor used in these 14 studies are not the same in all cases. If only works that use similar cameras/sensors are compared, there would be S3, S26 and S40, which opted for a Parrot Sequoia multispectral camera/sensor³, which provides a RGB sensor and a multispectral sensor (R, G, NIR and Red Edge); and S24, S25, S37 and S44, which used a RedEdge-M camera/sensor. And even in those cases where the studies share a similar camera/sensor, the image capturing tools, the agronomic traits, and the DIP and/or ML techniques are different. These were just a few issues that made it impossible to compare results between the 45 studies analyzed. But the main point was precisely the database, as each author performed the tests on a different database.

As for the composition of the databases, some works described it in more detail (such as S1, S2, S3, S5, S7, S12, S13, S19, S21, S23, S24 and S26), scoring $QC_3 = 2$. The rest of the studies describe this criterion in little detail, obtaining $QC_3 = 1$. Of the works that reached a score of $QC_3 = 1$ for database composition, 85.71% of them have $WAQC < 8.0$. As for the score $QC_3 = 2$ for database composition, 75% of these studies have $WAQC \geq 8.0$. That is, the details provided for the composition of the databases directly impacted the WAQC. With this detailed QC, it was possible to provide a better basis for the capturing steps (section V-A1) that made up Stage 1 of the answer to the RQ.

4) With QC_4 it was possible to assess how the DIP techniques used to process the agronomic traits were described. When describing the DIP techniques applied, some studies gave more details (such as S1, S2, S4, S6, S13, S14, S16, S18, S19, S21, S22, S23, S26, S28 and S32), scoring $QC_4 = 2$; others provided less details (S3, S5, S7, S8, S9, S10, S11, S12, S15, S17, S20, S24, S25, S27, S29, S30, S31, S33, S34, S35, S36, S37, S38, S39, S40, S41, S42, S43, S44 and S45), obtaining a score of $QC_4 = 1$. Of the works that obtained a score of $QC_4 = 1$, 88% of them have $WAQC < 8.0$. As for the studies that obtained $QC_4 = 2$, 66.67% of them have $WAQC \geq 8.0$. That is, these descriptions directly impacted the WAQC. With this very detailed QC, a better basis for the pre-processing stage (section V-A2) that made up Stage 2 of the answer to the RQ was possible.

5) QC_5 assessed the description of the use of ML applied to HTP. When describing the ML algorithms used, some studies

provided more details (S1, S5, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, S20, S21, S23, S25, S26, S28, S29, S31, S37, S36, S40 and S41), achieving a score of $QC_5 = 2$; other provided less details (S2, S3, S4, S6, S22, S24, S27, S30, S32, S33, S34, S35, S39, S38, S42, S43, S44 and S45), reaching a score of $QC_5 = 1$. Of the studies that obtained a score of $QC_5 = 1$, 100% of them had $WAQC < 8.0$, which shows that ML was essential in the composition of the WAQC. As for those that score $QC_5 = 2$, 80% of them had $WAQC \geq 7.0$, while 52% had $WAQC \geq 8.0$. With this very detailed QC, it was possible to provide a better foundation for the processing step (section V-A3) that made up Stage 3 of the answer to the RQ. Another key point in the analysis of the DEF fields was obtained by cross-referencing information, described in the next two subsections.

C. Crops Cross-referenced with Agronomic Traits

An interesting fact in cross-referencing the crops with the agronomic traits is that, if wheat (the most often analyzed crop among the articles, Figure 4) is cross-referenced with the 22 characters analyzed (Figure 5), this shows that this crop was tested for 5 different agronomic traits, namely: Canopy coverage S1; Plant density S6, S12 and S13; Measurement of a trait S21; Stay green S27; and Yield S4, S38 and S44. It can also be seen that, in some cases, the same article performed several types of analyses and experiments, as is the case of article S1, which studies the same agronomic trait for different crops (Lettuce, Sorghum and Wheat). In this case, the authors used different strategies for each test.

Figure 8 cross-references three frequently assessed agronomic traits with their respective crops. From this crossing of information, it was possible to observe that yield is a recurring concern for several crops. Of the 22 crops analyzed, 12 of them were assessed for yield (Cotton S15, Arabidopsis S4, Rice S3, Rice S30, Rye S5, Citrus fruits S24, Sunflower S38, Corn S33, Corn S37, Corn S4, Soy S11, Soy S14, Soy S20, Soy S26, Soy S40, Sorghum S25, Sorghum S39, Clove S5, Wheat S38, Wheat S4, Wheat S44, and Sugar cane S45). However, according to the types of data, each work outlined a different strategy. As a result, the average WAQC from these studies was 6.96. There is much to improve; most of these studies are recent and so far there is no agreed-upon methodology in the literature on sensors/cameras, image capture methods, and even DIP and ML techniques.

Another interesting result was obtained by cross-referencing agronomic traits and image capture method, described in the following subsection.

D. Agronomic Traits cross-referenced with Image Capture Method

Figure 9 cross-references the 3 most recurrent agronomic traits and the image capture method used for each one. Of the 18 articles that address crop yield, 14 employed UAV (S3, S11, S15, S20, S24, S25, S26, S30, S33, S37, S39, S40, S44 and S45). This might have to do with what is stated by [40] about the advantages of UAVs: they are a platform to capture a large number of images in high spatial, temporal and spectral

³produced by Parrot, a French company.

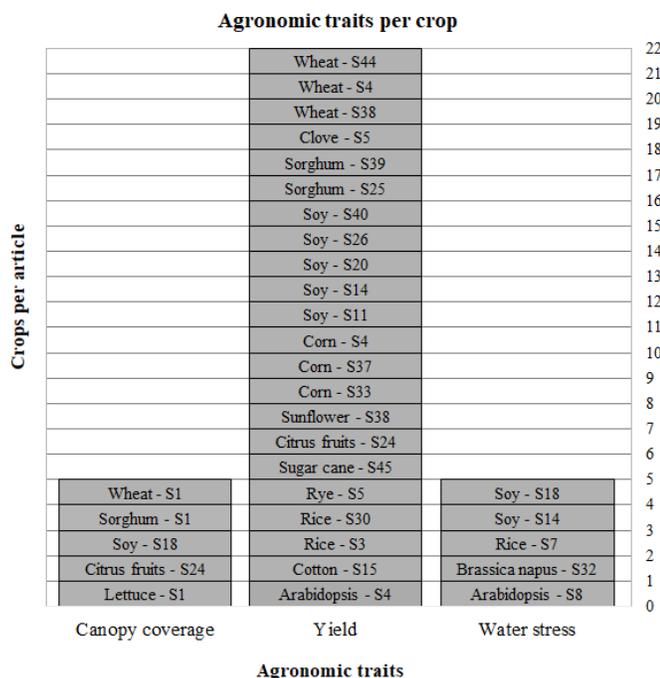


Fig. 8. Crops cross-referenced with agronomic traits.

resolution, storing precise information ideal for HTP in a short time and at low cost. With the UAVs it is possible to have a yield estimate consistent with the crop as a whole, because for that it is necessary to analyze as many plants as possible from that crop. Of the 13 works that investigated yield and used UAVs, only S26 had WAQC > 8.0, and the vast majority are works from 2020 and 2021. That is, they are recent studies and there is still much to research and discuss about this topic.

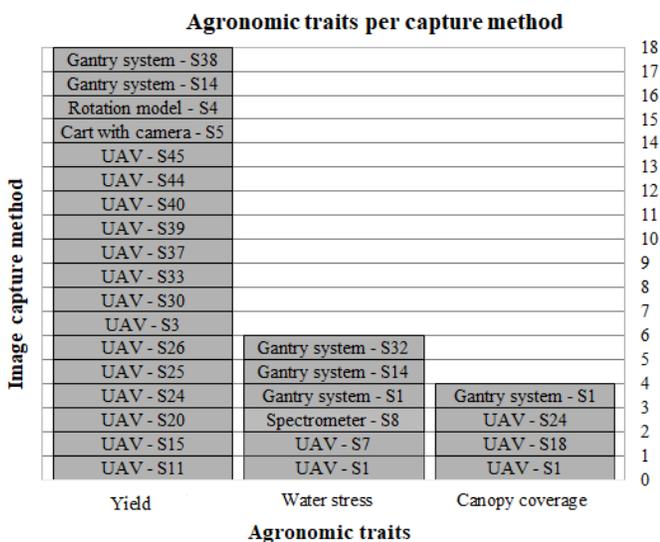


Fig. 9. Agronomic traits cross-referenced with Image Capture Method.

VI. THREATS TO VALIDITY

This section presents the threats to the validity of this SLR, and what strategies were adopted to mitigate these threats:

- 1) Regarding the definition of search strings:
 - Terms commonly used in primary studies related to this SLR were applied. For this, a survey and analysis of some related works was carried out, and the terms present in these works were used to make up the search strings.
 - The study followed terminologies and vocabulary related to the topic, with the help of a specialist in the area of DIP and ML, in addition to a specialist in HTP.
 - Search string sensitivity tests were performed to achieve a balance between retrieval and accuracy of search results. For this, the search strings were added to the databases, the results obtained were ordered by relevance, and the IC and EC were applied to the first ten articles from each source. This string adjustment was only finalized when at least 80% of these ten articles were really relevant.
- 2) Regarding the scope of the search strategy:
 - The databases were chosen with the help of experts; because of this, the articles found in the searches were related to the researched topic.
 - The searches were synchronous, that is, all searches in the data sources were carried out on the same day.
- 3) Regarding the quality assessment of the primary studies:
 - To formulate the QCs and weights used in this work, it was based on the methodology proposed by [105] since it is an article that is often cited on the subject.

- 4) About the congruence between RQ, IC, EC and DEF:
 - As much information as possible was related, for example, to answer the RQ each field of the DEF had to be filled out, and to assess the quality of the works, again the fields existing in the DEF were used. As for the IC and EC, if an article did not address fundamental terms existing in the DEF, it would be excluded; otherwise, it was included.
- 5) Possible biased assessment:
 - This study had two specialists, one focused on computing and its techniques, and the other focused on agronomy.
 - A pilot test of the selection criteria was carried out to improve the whole initial selection process.

VII. FINAL CONSIDERATIONS

This work developed a SLR covering the main studies on High-Throughput Phenotyping in agricultural production that use Image Processing and Machine Learning techniques and answering the research question (RQ) described in the literature review planning protocol.

When comparing the extracted data, it was possible to identify that each study built its own database and that most works did not make these bases available to the community, thus making it difficult to compare their results. In addition, it should be underlined that one of the advantages of using ML approaches in HTP is the ability to search and process a large dataset and discover patterns, simultaneously looking at a combination of information, something that is not possible

when each piece of information is analyzed separately. The possibility of analyzing large amounts of information simultaneously, and at a relatively low cost, is what has significantly enhanced the use of ML techniques in HTP.

The results obtained with this review show the potential for applying DIP and Machine Learning techniques, and therefore, can guide future research on the subject. For example, the recent use of DIP techniques to analyze a specific agronomic trait, cameras/sensors most used for a certain crop, or even which techniques to combine depending on the scenario to be analyzed, and so on. Finally, it is also noteworthy that this study includes a wide range of works dated until 2022, and therefore can provide a framework for new systematic reviews of the literature on the subject.

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