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Image Analysis: The New Bottleneck in Plant Phenotyping

Plant phenotyping is the identification of effects on the phenotype $\mathbf{P} = \mathbf{G} \times \mathbf{E}$ (i.e., the plant appearance and performance) as a result of genotype differences (i.e., differences in the genetic code) and the environmental conditions to which a plant has been exposed [1]–[3]. According to the Food and Agriculture Organization of the United Nations, large-scale experiments in plant phenotyping are a key factor in meeting the agricultural needs of the future to feed the world and provide biomass for energy, while using less water, land, and fertilizer under a constantly evolving environment due to climate change. Working on model plants (such as Arabidopsis), combined with remarkable advances in genotyping, has revolutionized our understanding of biology but has accelerated the need for precision and automation in phenotyping, favoring approaches that provide quantifiable phenotypic information that could be better used to link and find associations in the genotype [4]. While early on, the collection of phenotypes was manual, currently noninvasive, imaging-based methods are increasingly being utilized [5], [6]. However, the rate at which phenotypes are extracted in the field or in the lab is not matching the speed of genotyping and is creating a bottleneck [1].

While the bottleneck was previously the equipment (the hardware), it is now the analysis (the software). There is a need to develop accurate, robust, and automated analysis algorithms that can extract phenotypic information from experiments on the small (cell) or large scale (field), in two or three dimensions, in the lab but more importantly in the field on real crops. These algorithms should be coupled with affordable platforms and should deal with an immense amount of data produced in these experiments. Experts (from biology as well as data analysis) now agree that the analysis of imaging data is currently the weakest, or even the missing, link due to the major challenges in computer vision and image processing we are currently facing.

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COMPUTER VISION AND IMAGE PROCESSING CHALLENGES

Noninvasive plant investigations are done on different scales and modalities using a variety of sensors [2], [5]. This includes optical imaging, hyperspectral imaging to reveal rich pixel information on plant properties, and even magnetic resonance imaging (MRI) and positron emission tomography (PET). Spatial scales vary from the microscopic subcellular level to large outdoor fields. Typical problems in measuring a plant's visible properties comprise measuring size, shape, and other structural traits of whole plants, their organs, or plant populations.

Plants are not static, but self-changing systems with complexity in shape and appearance increasing over time. They emerge below image resolution and grow exponentially in time until, for a single leaf, growth levels off typically at several cm^2 size—i.e., several orders of magnitude change. Relevant timescales for cellular processes may be seconds or minutes, for growing leaves in the range of hours, and the status of whole plants changes over days or even months, in which the surrounding environmental (as well as measurement) conditions may also change.

Algorithms must deal with the aforementioned complexity, and the following sections describe unique challenges by illustrating typical applications. Clearly, the list of applications can never be complete, but we present some of the major themes.

CELLS AND ORGANS: DETECTION, TRACKING, AND STRUCTURAL BREAKS

One of the earliest forms of phenotyping where imaging-based setups were used is in the context of microscopy [2]. Plant tissue samples are excised and imaged under a microscope to reveal the cellular structure [cf. Figure 1(a)]. From an image processing perspective, the automated delineation of cell walls to establish cell morphology and cell count is typically needed.

However, more interesting problems arise from the use of recent techniques such as confocal microscopy, optical projection tomography, and optical coherence microscopy, which permit the noninvasive quantification of cellular morphometry at a variety of scales and depths. These techniques enable the observation of plant tissue dynamics on a short (and long) timescale, therefore tracking problems arise. These become particularly challenging when cell genesis needs to be observed and quantified, since cell division and expansion impose high spatiotemporal fidelity requirements. From a computer vision perspective, this problem, which also occurs in other biomedical applications, entails the inference of time and location of when and where such events occur within the scene, a task radically different from the typical tracking of objects entering or leaving the scene.

Over the last decade, several controlled setups [see Figure 1(c)–(e)] have emerged

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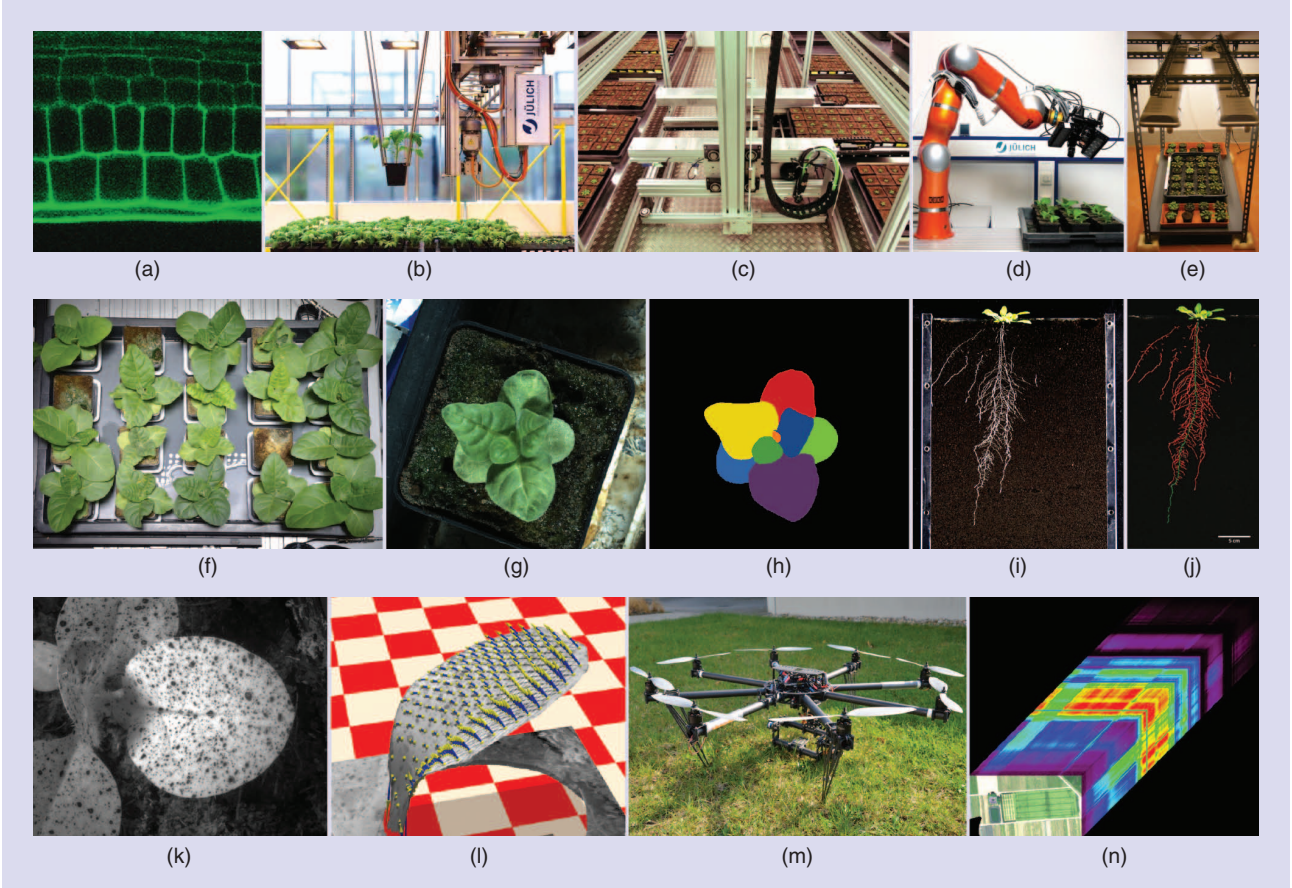
that image top-down views of small rosette plants, e.g., Arabidopsis or young tobacco, acquiring either one plant per image or several plants at once [see Figure 1(f)–(g)], housed in so-called growth chambers, where environmental conditions are controlled. Even in this very restricted imaging scenario, fully automatic segmentation of single plants can be a challenge due to, e.g., background clutter from moss growing on the soil, plant-to-plant overlap, heavy contrast changes due to (self-)shadowing, leaf color changes due to stress (e.g., drought), different light conditions and pathogen infections, and plant shape or size variation due to genotypic differences (cultivars or mutants) and treatments.

Segmenting single leaves is a typical multi-instance segmentation task [see Figure 1(g) and (h)]; however, even though all the objects share a wide range of features (e.g., they are mostly green with similar brightness distributions), they show rich variations. Leaves differ in size over several orders of magnitude, introducing a structural break due to resolution limitations, and algorithms need to deal with leaves emerging in the scene.

In addition, leaves vary in shape, and while they do share a certain basic shape, they overlap, bend, and vary in pose. Even for the same species, leaves may differ substantially, as leaf shape, size, color, and overall appearance of a plant depend on the genotype (e.g., there are thousands of mutants available for Arabidopsis alone), environmental factors (drought, low or high light, and temperature), and the age of each leaf. Readily apparent approaches based on learning shape from a labeled data set reveal their limitations when having to deal with such shape diversity and different acquisition conditions. While counting and segmenting leaves from such images can be simple for a human, no automated algorithmic solution is yet available that comes close to human performance.

WHOLE PLANTS: ANATOMICALLY CORRECT 3-D GEOMETRIC MODELING

For larger plants, reconstruction from a single image and viewpoint is not sufficient. Most approaches aim at obtaining an as complete three-dimensional (3-D) shape reconstruction as possible, geometrically



[FIG1] An example of plant phenotyping setups and images. (a) At the smallest scale, microscopy can image cells. (b)–(d) Movable imaging setups [15] or a (e) single overview camera setup can be used to image (f) many plants or (g) single plants, where (h) leaf segmentation is a sought-after outcome yielding growth measurements. Roots can be imaged in (i) rhizotrons [15], requiring (j) delineation. (k)–(l) Optical flow tracking [15] can measure finer leaf level growth. Airborne vehicles, e.g., (m) drones, can provide information on fields, e.g., (n) hyperspectral images [15]. [(a) is adapted from [7] and reproduced by permission of Elsevier. (b), (c), and (m) are courtesy of Alexander Putz, (i) and (j) are courtesy of Kerstin Nagel, and (n) is courtesy of Uwe Rascher.]

modeling the overall above-ground part of a plant, i.e., the shoot. However, details of parts are also investigated, such as grains on an ear (e.g., of corn), berries on wine grapes, flower development, etc. Imaging becomes more and more automated using conveyor-belt or robotized systems [see Figure 1(b)–(d)], allowing high throughput with thousands of plants. Automation of image analysis is then a must.

A variety of 3-D measuring strategies is currently being investigated, e.g., correspondence-based triangulation methods, silhouette-based carving, time-of-flight cameras, or light detection and ranging laser scanning (see [8] for a comprehensive overview). Setups are usually tailored to a particular species and conditions. This is, for example, due to size and image resolution constraints, or self-occlusion and self-similarity hampering triangulation.

A major challenge for all 3-D measuring methods is plant motion during acquisition. Time delays due to scanning or sequential image acquisition lead to notable geometric distortions, especially for outdoor measurements with wind. The data then cannot be described by a static model and all current approaches doing so fail one way or another.

From the 3-D data, quantitative information about plant traits need to be extracted. Simple summary traits, such as covered volume or plant height, could be estimated from images alone without 3-D reconstruction. But organ-wise traits, e.g., accurate leaf size or branching angle, require interpretation of 3-D data and plant part models. Simple models are used today (e.g., fitting two-dimensional surfaces to patches and merging them), but for most species new anatomically correct models are required.

WHOLE PLANTS BELOW GROUND: CLUTTERED IMAGES OF ROOTS

It is not possible to look through soil with the naked eye. Thus, classical root system analysis is invasive, meaning that plants are dug out and the roots washed and imaged. Usual image analysis then applies threshold-based segmentation, connected component labeling, and skeletonization, followed by estimation of traits such as overall graph length, branching angles, and others. All solutions available to date have only limited effectiveness when root systems are heavily entangled. Obviously, no time-series analysis can be performed when plants are dug out.

In soil, roots can be imaged noninvasively using so-called rhizotrons [9], i.e., flat pots with large vertical windows, such that parts of the roots visibly grow along the window [see Figure 1(i) and (j)]. In dark soil and at high spatial resolution, segmentation of bright roots may be done with solutions developed, e.g., for angiograms in medicine; but under realistic conditions this is difficult: even with high-resolution cameras (in the 30 megapixel range) fine roots may be only few pixels wide, blurred and with poor contrast to the surrounding soil. Many current segmentation solutions are slow or even break down when applied to such large images. Thus, computational efficiency is an issue. In addition, windows can get scratched by frequent use and soil contains all sorts of clutter. To date, reliable segmentation of such images can only be done semiautomatically, requiring user assistance. Even learning-based methodologies yield unimpressive results, which point to the need for finding (or learning) better feature representations.

Using penetrating radiation or modalities such as MRI, PET, and X-ray computed tomography, roots can be imaged in soil in 3-D, where different imaging techniques yield complementary contrast information and metabolic function (e.g., with PET). Challenges are similar to medical applications including proper (co)registration of time series of deforming objects of potentially different modalities, disentangling objects, measuring geometric traits, etc. However, artifacts and structures are different.

ADDING DYNAMICS: TRACKING, FLOW, AND GROWTH ESTIMATION

For many plant traits, temporal dynamics are of high relevance. Growth analyses on the local tissue level are typically performed on image sequences with frame rates in the range of one per minute. A long-established technique restricts the leaf of interest to a plane by pulling it flat and images it using a single camera. Growth is then calculated as divergence of an estimated optical flow field. Unfortunately, with this simple engineering solution, gene expression analyses have shown that "tension-stress genes" are turned on during such experiments, and thus the observed growth may be influenced on the molecular level. For nonfixed leaves moving in 3-D, calculating scene flow from multicamera "light-field" image sequences has been investigated [see Figure 1(k) and (l)]. This allows precise translation and rotation field estimation. Local growth can also be estimated from divergence, however, signal-to-noise-ratio is relatively poor. To date, no reliable local growth measuring technique without fixating leaves is available.

When aiming for growth analysis (in terms of summary growth over an organ), segmentation or reconstruction techniques as described earlier are needed. For simple plant architectures, e.g., young tobacco with up to eight leaves, leaf-wise tracking in temporally sufficiently high-resolved data sets has been demonstrated [10]. No reliable method for leaf-wise tracking has been reported in the literature so far for when time intervals become larger, or plant complexity is higher.

THE GREENHOUSE, FIELD, AND FARM: MORE VARIABILITY

While experiments in the laboratory do advance our knowledge of biological systems and their functioning, ultimately phenotyping must translate the knowledge to the society and stakeholders, such as breeders and farmers [3]. Phenotyping investigations must then be conducted under "real" (or realistic) conditions in the greenhouse or field, on crops that carry agricultural importance, such as corn, wheat, rice, barley, etc. [11].

Starting with the greenhouse, automated systems that are able to water and image plants, either move the plant to the imaging station or move the imaging apparatuses to the plants. Independent of setting, any positioning differences, either of the camera or the plant, radically complicate the process of establishing temporal correspondences between consecutive measurements. Taking the imaging apparatus

outside and in the field introduces additional challenges. Several approaches exist that mount sensors on specialized carriers: human-controlled tractors or other ground vehicles, or in the air with unmanned aerial vehicles [Figure 1(m)] operated either remotely or in an automated fashion. Image data differ tremendously in resolution, detail, motion blur, or clutter, severely affecting subsequent analysis tasks, thus, more robust algorithms are necessary. Computational efficiency is an issue, as the amount of imaging data produced is enormous [cf. Figure 1(n)], and analysis tasks can be significantly complex. Efforts in directly using analysis results for cultivation practices are the central theme in precision agriculture [3], which aims at tailoring treatment at the individual plant level. Thus, computer vision becomes crucial in supporting the whole process and evidently there is now the additional challenge of identifying low-complexity approaches to robust vision.

AFFORDABILITY: COPING WITH RESTRICTIONS

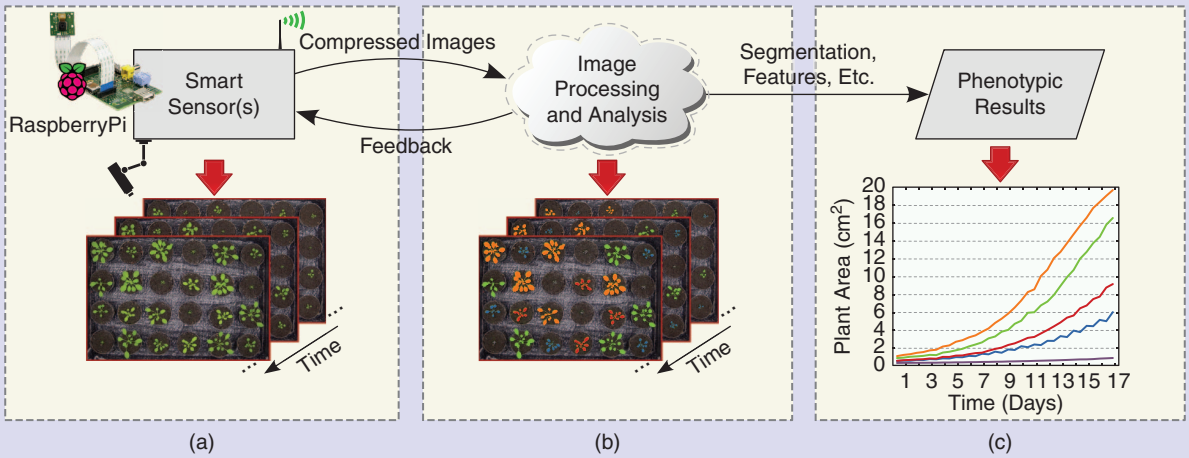
Currently, most versatile solutions are too expensive, and many labs instead develop highly customized (hardware and image analysis) solutions tailored to their experimental setting that are capable of addressing only specific phenotyping problems. Even when they are affordable, this variability in methods and setups creates standardization problems.

The use of off-the-shelf commercial equipment (such as commercial cameras [12] or the Kinect [5]) could facilitate standardization across experiments, lower the entry barrier, offer affordable solutions, and help many labs adopt the image-based approach to plant phenotyping.

Our recent project [16] aims to provide a universal turnkey and modular platform based on a distributed sensing and analysis framework [13], as shown in Figure 2. This distributed approach presents several key advantages. Affordable and easy-to-install sensors can be deployed in laboratories (growth chambers), the greenhouse, or the field to cover wide areas, before resorting to more costly and complex solutions based on robotics and automation. It is easy to become accustomed to a cloud-based storage and analysis application that is always up to date. It relieves users from maintaining a computing infrastructure and, importantly, it also permits consistency in experiments among different labs by standardizing equipment and analysis.

This centralized design, particularly when combined with an open architecture, can benefit the entire community, providing a modular and expandable architecture (by changing or adding new camera sensors), favoring software reuse (e.g., user-contributed algorithms can be adopted by other labs), and knowledge sharing (e.g., a common repository of acquired data and meta-data, and also the analysis application itself learning on the user's feedback).

Affordability and remote processing, however, pose technical challenges. The choice of optics and the fixed field of view restrict the quality (in resolution and sharpness) of the acquired images and the plants this setup can image (e.g., it may not be suitable for not coplanar plants). An affordable sensor will have limited computational power and knowledge access, thus, it requires low-complexity algorithms to perform some of the tasks outlined in previous sections, and as such remote processing is necessary. Then the transmission of (possibly) large volumes of image data necessitates compression to meet bandwidth constraints. While this loss of information will affect the accuracy of the analysis algorithm, recent advances in application-aware compression can tune compression parameters to meet analysis accuracy needs [13], [14]. From a software engineering perspective, backward compatibility of the analysis framework and of the computational backbone has to be ensured, such that experimental protocols and results obtained previously remain valid.



[FIG2] (a) Affordable camera sensors (e.g., based on the Raspberry Pi [17]) acquire time-lapse sequences of the scene, including one or multiple plants. (b) Images are compressed and transmitted to the cloud, where high computational power and a broad knowledge base enable sophisticated computer vision tasks (e.g., leaf segmentation and tracking, optical flow analysis). Additionally, information is fed back to the sensor. Relying on Web-based graphical user interfaces, (c) phenotyping results are presented to the user for interpretation.

A TIMELY AND UNIQUE CHALLENGE

A quantitative description of plant phenotypes is a key ingredient for a knowledge-based bioeconomy, and this not only literally helps in the efforts to feed the world but is also essential for fiber and fuel production, the so-called Green Revolution 2.0. In fact, comparing the "Top 10 list of Emerging Technologies" in 2012 according to the world economic forum, the top 1, 2, 3, and 5 technologies are directly addressed by plant phenotyping research [18]. Recently, we have even witnessed direct investments in helping the translation of agricultural technology in farming. For example, Farm2050 [19] includes the information extraction powerhouse Google and drone company 3-D Robotics among its partners.

There is not only growing interest from the application side, both scientifically and commercially, but exciting computer vision and image processing problems exist that differ from other biomedical applications. While medicine focuses on the status of a single species (i.e., humans) in a diagnostic capacity, plant phenotyping addresses a large number of different plant species with hundreds to thousands of genotypes (cultivars) per species, usually in group-wise experiments. It addresses the development over time in addition to static snapshots and under a wide range of environmental conditions, using various imaging setups (as opposed to medical imaging where predefined protocols are in place and equipment variability is relatively limited). Thus, even within a single application, diverse conditions need to be addressed, to ascertain a robust image-based measurement of the phenotypic trait. Plant phenotyping at a high throughput requires reliable image processing algorithms that could batch process many data accurately, and an integration with genetic databases and other frameworks.

The previous sections outlined a series of challenges (e.g., dealing with structural breaks in tracking/detection), for which our community can get involved. In this article, although we focus on extracting information from images, data mining and combing the information from genotyping, environmental, and phenotyping sources are by themselves a big undertaking as well. Jointly, we must make the effort to solve these problems and push the envelope further, and by including the resources in Table 1, we hope to help facilitate this. We need to cooperate with different disciplines to integrate expertise across the spectrum and provide biologically or agronomically meaningful and technically robust solutions [3], [7] to help resolve this bottleneck.

[TABLE 1] GET INVOLVED: A COLLECTION OF ONLINE RESOURCES.

NAME	DESCRIPTION
SPECIALIZED SCIENTIFIC EVENTS	
CVPPP2014 [20] AND CVPPP2015 [21]	THE FIRST AND SECOND WORKSHOP FOR COMPUTER VISION PROBLEMS IN PLANT PHENOTYPING, ALSO HOSTING CHALLENGES SUCH AS THE LEAF SEGMENTATION CHALLENGE (LSC)
IAMPS [22]	INTERNATIONAL WORKSHOP ON IMAGE ANALYSIS METHODS FOR THE PLANT SCIENCES
PHENODAYS [23]	INTERNATIONAL SYMPOSIUM INVOLVING SEED INDUSTRY, BREEDING INSTITUTES, AND ACADEMIC BREEDING GROUPS
IPPS [24]	INTERNATIONAL PLANT PHENOTYPING SYMPOSIUM
ICPA [25]	INTERNATIONAL CONFERENCE ON PRECISION AGRICULTURE
IMAGE DATABASES	
LSC CHALLENGE [26]	IMAGES AND LEAF-BASED SEGMENTATION MASKS AS PART OF THE FIRST LSC CHALLENGE
MAIZEGDB [27]	IMAGES OF MAIZE
CWFID [28]	THE CROP/WEED FIELD IMAGE DATA SET (CWFID) CONTAINS IMAGES WITH CROP/WEED DELINEATIONS FOR A CLASSIFICATION TASK IN PRECISION AGRICULTURE
PHENOPSIS DB [29]	ARABIDOPSIS THALIANA PHENOTYPING DATABASE
CONSORTIA AND ORGANIZATIONS	
IPLANT COLLABORATIVE [30]	CONNECT SCIENTISTS TO PUBLIC DATA SETS, MANAGE AND STORE THEIR DATA AND EXPERIMENTS, ACCESS HIGH-PERFORMANCE COMPUTING, ETC.
IPPN [31]	INTERNATIONAL PLANT PHENOTYPING NETWORK
EPPN [32]	EUROPEAN PLANT PHENOTYPING NETWORK
EPSO [33]	EUROPEAN PLANT SCIENCE ORGANISATION
FESPB [34]	FEDERATION OF EUROPEAN SOCIETIES OF PLANT BIOLOGY
IEEE RAS [35]	AGRICULTURAL ROBOTICS AND AUTOMATION
ISPA [36]	INTERNATIONAL SOCIETY OF PRECISION AGRICULTURE
E-AGRICULTURE [37]	ICT FOR SUSTAINABLE AGRICULTURE
BSA [38]	BOTANICAL SOCIETY OF AMERICA, LISTING FURTHER PLANT SOCIETIES AND ORGANIZATIONS [39]
SOFTWARE DATABASES	
PLANT IMAGE ANALYSIS [40]	THIS DATABASE CURRENTLY PROVIDES A COLLECTION OF APPROXIMATELY 120 ANALYSIS TOOLS

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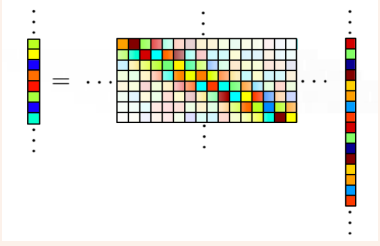

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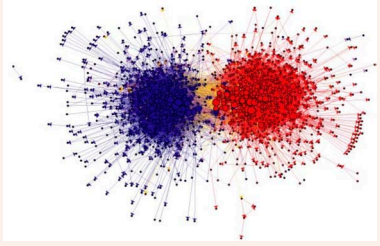

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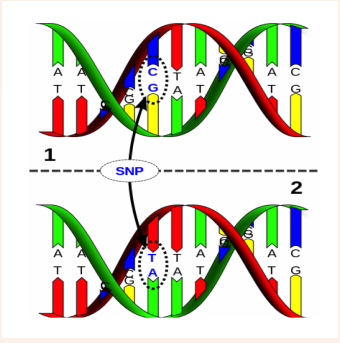

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