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Visual Growth Tracking for Automated Leaf Stage Monitoring based on Image Sequence Analysis

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Abstract: Rigid-body visual tracking is an active research field with many practical applications including visual surveillance and intelligent transport system. In this paper, we define a new problem domain, called *visual growth tracking*, to track different parts of an object that grow non-uniformly over space and time for application in image-based plant phenotyping. The paper introduces a novel method to detect and track each leaf of a plant for automated leaf stage monitoring. The method has four phases: optimal view selection, plant architecture determination, leaf tracking and generation of a leaf status report. The proposed method uses a graph theoretic approach to reliably detect and track individual leaves by overcoming the challenge of leaf-losses based on temporal image sequence analysis for automatically generating the leaf status report containing the following phenotypes, i.e., the emergence timing of each leaf, total number of leaves present at any time, the day on which a particular leaf stopped growing, and the length and relative growth rate of individual leaves. The proposed method demonstrates high accuracy in detecting leaves and tracking them through the early vegetative stages of maize plants based on experimental evaluation on a publicly available benchmark dataset.

Keywords: Plant architecture determination; graph theoretic approach; leaf detection; leaf tracking; leaf status report.

1. Introduction

Visual tracking is an emerging research field which deals with the problem of localizing a pre-specified object in a video sequence. It is a challenging problem with many practical applications, e.g., player detection and tracking in sports video [1], tracking of pedestrians in video sequences for visual surveillance and scene awareness [2], and moving vehicle detection and tracking for traffic surveillance [3,4]. More recently, tracking has been applied in a completely different domain, i.e., image-based plant phenotyping analysis, for leaf growth monitoring of Arabidopsis [5,6]. Different plants exhibit different architectures, complexity of which gradually increases with time. This results in automated growth monitoring of a plant challenging, as a whole and its parts (e.g., leaves, flowers, roots), based on image sequence analysis. Hence, this research area requires focused and long-term attention from the computer vision community. The role of plants is critical in the context of food security, and the well being of humans and animals. The application of visual tracking in automated growth stage determination of economically important crops, e.g., maize and sorghum, for plant phenotyping is yet to be explored despite their role as the source of staple food in most areas of the world.

Image-based plant phenotyping facilitates the extraction of advanced biophysical traits by analyzing large number of plants non-destructively in a short period of time with limited manual intervention. Understanding genetic diversity and the impacts of abiotic and biotic stresses on plant performance and yield is of critical importance to address current and emerging issues related to food security and climate variability. For example, in maize, the vegetative growth stage which is important for yield predictions, is determined by the emergence of number of leaves before flowering. Maize is the one of three grain crops along with rice and wheat, which directly or indirectly provide half of total world calorie consumption each year. Hence, the study of its growth stages influenced by various stress conditions, e.g., drought, salinity and heat, is of critical importance [7,8]. However, after the natural loss of lower leaves, the growth stage determination requires manual splitting of the lower part of the stalk to inspect for the internode elongation. To the best of our knowledge, there is no previous study for the image-based automated leaf growth stage monitoring to replace this manual time-consuming process. Thus, the paper introduces a novel method for automated monitoring of leaf growth stages of the maize plants, i.e., to accurately detect the emergence timing of individual leaves and track them over the vegetative stage life cycle. The method is applicable to other economically important grain crops that share similar architecture and growth pattern like maize, e.g., sorghum, for all existing high-throughput plant phenotyping systems in the controlled greenhouse environment (such as LemnaTec Sanalyzer 3D [1], PlantScreenTM modular system [2] and Phenomix automated greenhouse system [3]), where plants are placed on metallic carriers upon a conveyor belt that moves the plants from the greenhouse to the imaging cabinet one at a time for proximal sensing.

Unlike visual tracking of rigid bodies, e.g., vehicles, pedestrians, we define a new problem *visual growth tracking* (i.e., tracking of different parts of an object that grow at different rates over time) using plant image sequences with a different set of computer vision challenges. The plants are not static but living organisms with constantly increasing complexity in terms of shape, structure and appearance. While rapid displacement of the entire body takes place for the vehicles and pedestrians in motion, plants remain fixed at the soil but their different parts grow at different rates at different times. Plants alter leaf positioning (i.e., phyllotaxy) in response to light signals perceived through the phytochrome in order to optimize light interception. In addition to variation in phyllotaxy, growth of individual leaves over time leading to self-occlusions and leaf crossovers also pose additional challenges to automated leaf growth monitoring.

The proposed method is divided into four phases: (a) optimal view selection, (b) plant architecture determination, (c) leaf tracking and (d) computation of leaf status report. The high-throughput plant phenotyping proximal sensing systems are constrained by fitted with a single camera in the imaging cabinets. Thus, each cabinet has a pneumatic lifter fitted with an electric motor rotator that rotates the plant at a desired angle in the range [0° 360°] to capture images from multiple view angles. We first identify the view of the plant that provides the most detailed structure of the plant from all available views. We represent each single plant image in the sequence as a graph to detect the plant components, i.e., leaves and stem, using a graph theoretic approach. The leaves in the plant images in the sequence are relabeled by their emergence order to track them over time. Here, we exploit an important growth characteristic feature of a maize plant, i.e., the leaves in maize emerge using bottom-up approach in alternate-opposite orientation. The algorithm addresses the challenge of leaf losses and emergence of a new leaf for efficient growth stage monitoring. The growth pattern in the early stage of life cycle provides the most crucial phenotypic information related to yield, and hence, is of interest to the plant scientists. The challenge of leaf intersections are uncommon in the early growth stages, but an usual occurrence in late vegetative stages. Hence, we introduce a novel curve tracing technique based on angular consistency check to address the challenge of leaf crossovers to achieve robustness.

[1] https://www.lemnatec.com/plant-phenotyping/

[2] https://www.qubitphenomics.com/plantscreen-modular-systems/

[3] http://phenomix.fr/en/home

The emergence timing, total number of leaves present at any point of time, total number of leaves emerged, the day on which a particular leaf stopped growing or was lost, and the length and relative growth rate of individual leaves are the significant phenotypes (i.e., observable morphological and biophysical traits of the plants regulated by genotype and the environment) that best assess the health of the plants. Automated growth stage monitoring by leaf tracking will enable us to develop a novel system which will accept the plant image sequence as the input and will automatically produce a leaf status report containing the above-mentioned phenotypic information for monitoring leaf-growth, and thus, overall growth of the plant. The proposed method is evaluated on the benchmark dataset called University of Nebraska-Lincoln Component Plant Phenotyping dataset (UNL-CPPD) [9].

The rest of the paper is organized as follows. Section 2 discusses related research in this emerging field and Section 3 presents the proposed method. Section 4 provides discussion on the benchmark dataset UNL-CPPD used to evaluate our method. Section 5 presents the experimental results and Section 7 concludes the paper.

2. Related work

Multiple object tracking is a challenging task, yet of fundamental importance for many real-life practical applications [1]. The method in [1] uses a progressive observation model followed by a dual-mode two-way Bayesian inference based tracking strategy to track multiple highly interactive players with abrupt view and pose variations in different kinds of sport videos, e.g., football, basketball, as well as hockey. The method in [2] uses an interacting multiple model to simultaneously track multiple pedestrians in monocular video sequences. Computer vision based vehicle detection and tracking plays an important role in the intelligent transport system [4]. The method in [4] presents an improved ViBe for accurate detection of vehicles, and uses two classifiers, i.e., support vector machine and convolutional neural network, to track vehicles in presence of occlusions.

The emergence of a new leaf, the growth of the individual leaves over time and growth cessation followed by senescence leading to increased complexity with variations in shape and appearance of the plant, pose a different set of challenges compared to visual tracking of vehicles or humans. Although few attempts have been made to count and track individual leaves of plants, these are only conducted on top view images of rosette plants at their early growth stages, e.g., Arabidopsis (Arabidopsis thaliana) and tobacco (Nicotiana tabacum), which are commonly used as the model plants for the image-based plant phenotyping research [10–12]. The method in [10] combines the local leaf features extracted in the log-polar domain to form a global descriptor which is then fed to a support vector regression framework to estimate the number of leaves of rosette plants. A probabilistic parametric active contours model is applied in [10] for leaf segmentation and tracking to automatically measure the average temperature of leaves by analyzing infra-red image sequences. However, this method does not address the challenge of overlapping leaves. The method in [5] proposed a joint framework for multi-leaf segmentation, alignment and tracking of the rosette leaves by analyzing fluorescent image sequences to account for leaf-level photosynthetic capability of the plants. The method uses Chamfer matching followed by forward and backward warping for multi-leaf alignment, and overcomes the challenge of overlapping leaves. In addition to the leaf counting and tracking, rosette plants have been used for the study of leaf segmentation using 3-dimensional histogram cubes and superpixels [11], plant growth and chlorophyll fluorescence analysis exposed to abiotic stress conditions [12], automated plant segmentation using active contour model [13] and the rate of leaf growth monitoring following leaf tracking using infrared stereo image sequences [14].

Compared to the rosette plants, computer-vision based research for automated plant phenotyping analysis of the three most important cereal crops, e.g., rice, wheat and maize, is only in the budding stage due to their more complex architecture. The method in [15] uses a graph theoretic approach for the determination of stem angle to account for stem's susceptibility to lodging by analyzing visible light image sequences of the maize plants. A time series clustering followed by genotypic purity analysis on a public dataset called Panicoid Phenomap-1 established that the temporal variation of the stem angles is likely to be regulated by genetic variation under similar environmental conditions. The method in [9] introduces a set of new component phenotypes, e.g., junction-tip distance, leaf curvature and integral-leaf skeleton area, with a discussion on their significance in the context of plant science. In this method, the leaves are tracked manually over the image sequence to demonstrate the temporal variation of these phenotypes regulated by genotypes.

Motivated by the unavailability of any previous study on the automated growth stage determination of the cereal crops, we introduce in this paper a novel algorithm to accurately detect the emergence timing of individual leaves and track them over the vegetative stage life cycle of the plant, based on plant architecture determination using a graph theoretic approach. The algorithm accepts a temporal image sequence of maize plant as the input and automatically generates a leaf status report which contains information on entire life history of each leaf. Most importantly, this defines a pioneering study in the field of visual tracking which tracks parts (i.e., leaves) of a growing living object in an image sequence.

3. Materials and Methods

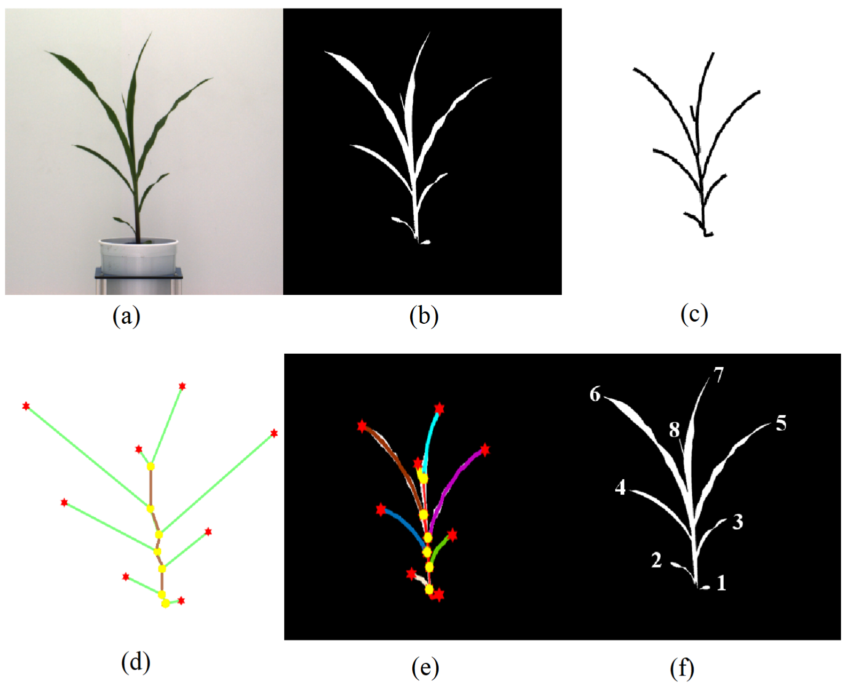


Figure 1. Image based plant phenotyping computation pipeline: (a) original image; (b) binary image; (c) plant skeleton; (d) graphical representation of the plant with tips and collars identified; and (e) plant skeleton with leaves marked with different colors; and (f) leaf labeling in order of emergence.

Figure 1 shows an overall image processing pipeline for the proposed method. Figure 1(a) shows the original image and Figure 1(b) shows the corresponding binary image. The binary image is then skeletonized (see Figure 1(c)) to determine the graph representation of the plant as shown in Figure 1(d). Figure 1(e) shows each detected leaf marked with a distinct color. Finally, Figure 1(f) shows each leaf numbered in order of emergence. The proposed method accepts a sequence of plant images captured at regular intervals over the vegetative stage life cycle of a plant as the input, and generates a leaf

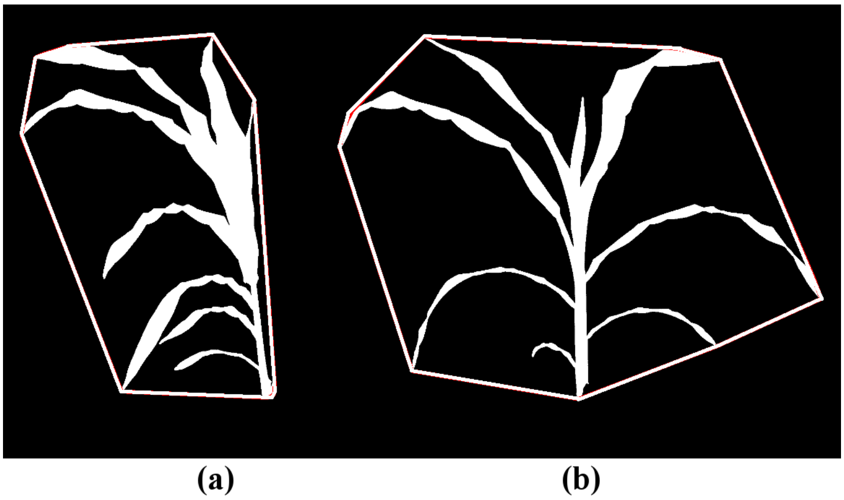


Figure 2. Illustration of view selection: (a) binary image of a maize plant enclosed by convex-hull at side view 0°; and (b) binary image of the same maize plant enclosed by convex-hull at side view 90°.

status report along with a visual representation that encode the dynamic properties of all leaves that emerged during this period. The embedded phenotypic information is useful to the plant scientists to provide greater understanding of the underlying physiological processes. This novel objective is achieved in four phases:

- *View selection*: Each plant is captured from multiple viewpoints to get a more accurate representation. We select the view at which the leaves most distinct.
- *Plant architecture determination*: For each image in the sequence, we determine the architecture of the plant using a graph theoretic approach.
- *Leaf tracking*: The plant architectures are reconciled to determine the correspondences between the leaves over time to track them over vegetative stage life cycle and demonstrate the temporal variation of the leaf-based phenotypes.
- *Leaf status report*: A leaf status report is produced as an output of the algorithm containing phenotypic information related to entire life history of each leaf that best contribute to assess plant vigor.

3.1. View selection

Many plants alter leaf positioning (i.e., phyllotaxy) in response to light signals to optimize light interception [16]. To determine a plant's architecture, accurate location of junctions (or collars, i.e., the points of contacts of the leaves to the stem) and the tips (free endpoints of the leaves) is critical. Therefore, each plant is imaged from multiple viewpoints. The best view of the junctions are obtained in a view of the plant at which the line of sight of the camera is perpendicular to the axis of the leaves as evident from Figure 2. In this view, the plant has the largest projection in the image. To determine this view, we first compute the area of the convex-hull of the plant for the available number of m views for day the plant is imaged. The view at which the area of the convex-hull of the plant is the maximum, is selected for subsequent analysis. Given that a plant is imaged at m viewing angles each day, the optimal view ($OView_i$) for day i is given by:

$$OView_i = view_{i,j} : \; CH(view_{i,j}) < CH(view_{i,k}), \quad \forall j \neq k, \quad 1 \leq j,k \leq m. \tag{1}$$

for i = 1,...,n, where n denotes the total number of imaging days and $view_{i,j}$ is the j'th view of the plant on day i and the function CH returns the area of the convex-hull of the plant in the image.

3.2. Plant architecture determination

The steps for plant architecture determination are described below.

3.2.1. Segmentation

In a high-throughput phenotyping system, the plants are grown in a controlled environment like a green house and imaged in a closed chamber. Thus, the imaging environment also is consistent in both camera and plant locations. Therefore, a frame-differencing approach using background subtraction gives a good approximation of the segmented plant [15]. This is followed by color-based thresholding to extract the foreground, i.e., the plant. The simple erosion removes noisy pixels and a dilation step is used fill up any small holes inside the plant image. At the end, the largest connected component in the image is deemed to be the plant.

3.2.2. Skeletonization

Skeletonization, i.e., the process of reducing a shape into one-pixel wide connected lines, is widely used in object representation and recognition, character recognition, image retrieval, biomedical image processing and computer graphics. Since many plants, including grasses such as corn and sugarcane, have elongated primary structures (stem, leaves, etc.), the skeleton provides the basis for the plant's architecture.

Skeletonization algorithms are mainly based on morphological operations, discrete domain analysis using Voronoi diagram, and fast marching distance transform. The morphological thinning based methods iteratively peel off the boundary layer by layer, identifying the points whose removal does not affect the topology. Although straight-forward, it requires extensive use of heuristics to ensure the skeletal connectivity, and hence does not perform well in the case of complex dynamic structures like plants. The geometric methods compute Voronoi diagram to produce an accurate connected skeleton from the connected component. However, their performance largely depends on the robustness of the boundary discretization, and is computationally expensive. We propose the use of fast marching distance transform to skeletonize the binary image[17] of the plants due to its robustness to noisy boundaries, low computational complexity and accuracy in preserving skeleton connectivity structures.

The skeletonization process often results in the formation of unwanted spurious branches or spurs, which, in our application, can be erroneously identified as leaves [18]. The proposed method uses a thresholding based skeleton pruning technique to remove spurs, i.e., if the length of the edge is less than the threshold value, it is considered as a spur, and hence discarded. The threshold can be determined through experimentation or using a supervised learning approach. Based on experimental analysis on our dataset, we set the threshold value as 10 pixels, as this value removes spurs from all images of the dataset. Irrespective of the method chosen, in rare cases, this process will eliminate true leaves, when they are very small, right after emergence. However, leaves are dynamic structures, they will grow and be identified accurately in the image at the next time point.

Graph representations of skeletons have been investigated in the literature in many object recognition problems [19]. The method in [19] uses a skeletal graph to model a shape in order to use graph matching algorithms to determine similarity between objects. In this paper, we propose a graph representation for a plant. Plant structure lends itself naturally to such a representation since it consists of branches emerge from the main trunk and sub-branches emerge from branches and so on. Thus, the points where branches connect (and their ends) can be represented as nodes in a graph and the branches (and leaves) and the internode segments in the stem can be represented as edges. The skeleton for the plant already is a good starting point to develop the graph representation. Furthermore, use of graphs make it efficient to decode the underlying structures (e.g. leaves and branches) and hence easier to track the dynamic properties of plants at a high level.

Before we formally introduce the algorithm for plant architecture determination, we define a few basic terms and show them graphically in Figure 1(d).

- *Base*: The base of the plant is the point from where the stem of the plant emerges from the soil and is the lowest point of the skeleton.
- *Collar/Junction*: The point at which a leaf is connected to the stem. The junctions, i.e., collars, are nodes of degree 3 or more in the graph.
- *Tip*: The free end of the leaf that is not connected to the stem.
- *Leaf*: The segments of the plant that connect the leaf tips and collars on the stem.
- *Inter-junction*: The segments of the plant connecting two collars are called inter-junctions.

A number of important properties of a plant can be directly identified from the graph representation. For example, the leaf tips and the base are nodes with a degree of 1 and the collars are nodes of degree 3. There are two types of edges in the graph: (a) leaves and (b) inter-junctions. Similarly, the stem of the plant can be formed by iteratively traversing the graph from the base along a connected path of collars.

Formally, we represent the plant by a graph $G =< V, E >$, where V and E denote the set of vertices and the set of edges, respectively. The set of vertices is defined as $V = \{B\} \cup T \cup J$, where B is the base of the plant, T is set of tips of leaves, and J is the set of collars. The set of edges is defined as $E = L \cup I$, where, L and I represent the set of leaves and inter-junctions, respectively.

Algorithm 1 outlines the steps used for the determination of a plant's architecture. We begin with a sequence of images of a plant P. Without loss of generality we assume that the plant is imaged at regular daily interval starting with Day 1. Thus, $P = \{p_1, p_2, \ldots, p_n\}$, where p_i is the image of the plant in day i and n is the number of days the plant was imaged. After view selection, each image is segmented to generate a sequence of segmented images $P^s = \{p_i^s, p_2^s, \ldots, p_n^s\}$.

Each segmented image is then skeletonized. The skeleton is transformed into a graph representation after the removal of spurious branches. The vertices and edges of the graph are directly determined from the skeleton. As described before, the vertices of the graph with a degree of 1 represent either the tip of a leaf or the base of the plant. Since the base of a plant holds a unique landmark in a plant, we first identify it. The base is determined by examining the degree one nodes (the base must have one of the lowest y-coordinates) and the edge that connects to the plant (it must be a straight-line segment that is close to vertical). These special conditions are needed since a leaf may droop in such a way that its tip may fall below the base.

Once the base is determined, the next step is to determine the stem of the plant since all leaves emerge from it. We again leverage the structure of the stem, i.e., it is straight and consists of inter-node segments. Thus, starting from the base and following the edges neither of whose nodes has a degree one (collar), generates the stem of the plant. This is summarized in Algorithm 2. After the stem is identified, we determine the orientation of each leaf. In the maize plant, the leaves emerge in alternate-opposite orientation. Without loss of generality, we assign the leaves emerging to the left as 0 and those emerging to the right as 1.

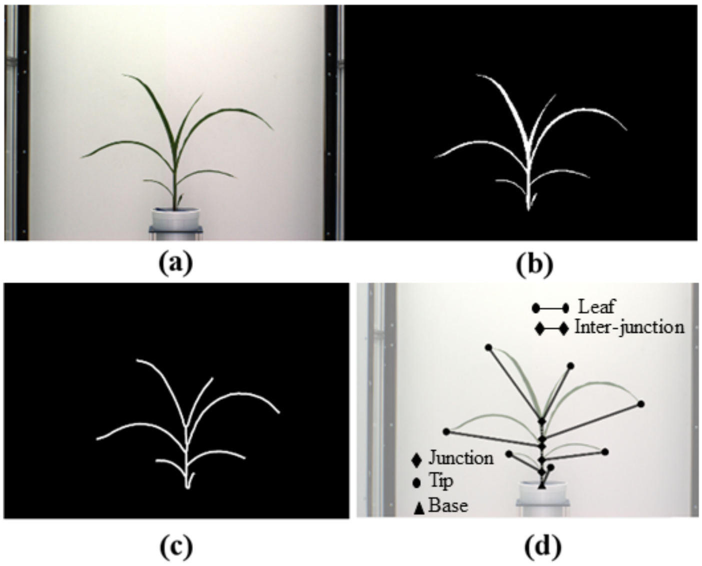


Figure 3. Illustration of graph representation of a plant: (a) original image; (b) binary image; (c) skeleton; and (d) graph representation.

Algorithm 1 Plant architecture determination: Produces graphical representation of all the images of a plant sequence to detect the leaves and stem.

Input: The image sequence of a plant, i.e., $P = \{p_1, p_2, \ldots, p_n\}$
Output: $G = \{G_1, G_2, ..., G_n\}$, where G_i is the graphical representation of the i-th image $\forall\ i = 1,...,n$.

for $i = 1 : n$ **do** {
  $p_i^s = segmentation(p_i)$ // segment the image for day i
  $w_i = skeleton(p_i^s)$ // compute the skeleton w_i of the segmented image p_i^s.
  $z_i = removeSpur(w_i, \alpha)$ // remove spurious edges with a threshold of α pixels.
  $G_i = determineGraph(z_i)$ // represent the plant skeleton as a graph

  $< V_i, E_i >= G_i$;// vertices and edges of the graph
  $D1_i = \{v_{i,j} \in V_i : degree(v_{i,j}) = 1\}$ // degree 1 vertices
  $base_i = determineBase(G_i, D1_i)$; // find the base
  $tips_i = D1_i - \{base_i\}$; // tips of the leaves
  $collars_i = V_i - D1_i$;// collars or junctions
  $stem_i = findStem(G_i, base_i)$;
  $computeLeafOrientation(G_i)$; // Assign orientation to each leaf

  count = 1; // starting label for leaves
  for $e_{i,j} \in stem_i$ **do** { // follow junctions starting from base
    $[c_{i,j}, v_{i,j}]$=$e_{i,j}$; // extract the vertices from edge
    if ($c_{i,j} = base_i$) continue;
    $tips_{i,j} = \{v_{i,j} \in V_i : ([c_{i,j}, v_{i,j}] \in E_i) \wedge (degree(v_{i,j}) = 1)\}$
    if ($|tips_{i,j}| = 1$) { // there is only one leaf at the junction
      $[c_{i,j}, v_{i,j}]$.label = count;
      count ++;
    } else { // there are multiple leaves at the junction
      orient = $getOrientation(G_i$, count $-1)$ //previous leaf-orientation
      orient = ¬ orient; // next leaf-orientation
      leaves = $tips_{i,j}$// initialize with the leaves at the node
      while ($leaves \neq \phi$) **do** {
        $nextLeaf = tipMax : \forall_{tip \in tips_{i,j} \wedge tip \neq tipMax}$
          $length([c_{i,j}, tip]) < length([c_{i,j}, tipMax])$
          $\wedge\ [c_{i,j}, tip].orientation = orient$;
        $orient = \neg orient$; // orientation of the next leaf
        $leaves = leaves - \{nextLeaf\}$ // remove the leaf
        count ++;
      end while
  end for
end for

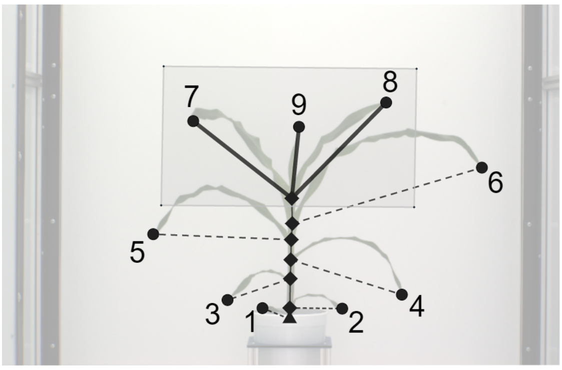


Figure 4. Illustration of leaf labeling in case of a junction containing more than two leaves.

The final step in the plant architecture determination is the identification of the leaves and labeling them in emergence order. We use two properties of the plant growth in this process: (a) the order of the emergence of leaves in the plant is bottom to top, (b) a new leaf emerges on opposite side of the last leaf in the plant and (c) older leaves are typically longer than newer leaves. Thus, the oldest leaf is closest to the base of the plant and the newest the farthest. Hence, our algorithm follows the stem from the base and at each collar (c) determines the leaves present by identifying the edges with one vertex as the collar and a leaf tip as the other vertex (degree 1 vertex). A counter (label) is used to keep track of the label for the next leaf in the emergence order. If there is only leaf present at the collar, then it is labeled with the value of the counter and the counter is incremented. It is possible, however, that in some cases, typically the last collar, multiple leaves may be connected to a single collar (see Figure 4). In such a case, we use the constraint that the next leaf to be the longest leaf in the set that has the orientation opposite to the previous leaf. This process is repeated until all the leaves in the set are labeled. Figure 3 show the process of graph representation of a plant from the original image.

Algorithm 2 $findStem$: Determine the stem in a graph.

Input: A graph G and its base B.
Output: A list of edges in G that constitute the stem (S).

```
v = B;
done = FALSE;
S = [];
while ( ¬ done) {
    v' = getNextCollar(G, v); // find the non-visited adjacent vertex of degree ≠ 1
    if (v' = NULL) // there is no adjacent vertex with degree ≠1
        done = TRUE;
    else{
        S.append(< v, v' >) // add the inter-stem segment to the stem
        v = v';
    }
}
```

3.3. Leaf tracking

Each leaf in a plant has a unique time of emergence, pattern of growth and senescence. We, therefore, assign each leaf a label that determines its emergence order. Thus, the leaf that emerged first, will be a labeled 1 throughout the life of the plant, even if the leaf may die. Thus leaf tracking problem is equivalent to the determination of the correct label for each leaf in the plant in a sequence of plant images. The correspondence between the leaves in any two images (or any sequence of images) can be directly determined from the labels.

Our leaf tracking algorithm is based on the following set of properties that hold for a large class of plants including grasses like maize.

- A new leaf emerges above the last leaf in opposite alternate orientation, i.e., if the previous leaf emerged from the left side, the next leaf will emerge from the right side and will originate from a collar situated above the collar of the immediate previous leaf.
- In the event of a loss of a leaf, its height of the collar decreases and the length of the corresponding inter-junction increases compared to the previous image.

In addition, we make the following assumptions which hold in most high throughput phenotyping systems, where each plant is imaged on a daily scale.

- No more than one leaf may die in two consecutive images in a sequence.
- No more than one new leaf may emerge in two consecutive images in a sequence.

Based on these properties, only four scenarios as possible when examining an image in a sequence with respect to the previous image (illustrated in Figure 5).

1. **Leaf emergence:** A new leaf emerged, but no leaf was lost (Figure 5(a)).
2. **No change:** No new leaf emerged and no leaf was lost (Figure 5(b)). In this case, we transfer the labels from the previous graph to the next graph.
3. **Leaf loss:** A leaf was lost but no new leaf emerged (Figure 5(c)).
4. **Loss and emergence:** A new leaf emerged and a leaf was lost (Figure 5(d)).

Algorithm 3 summarizes the leaf tracking process for the plant image sequence using graphs generated by Algorithm 1. Leaf tracking algorithm begins with a sequence of labeled graphs $\{G_1, G_2, \ldots, G_n\}$, where G_i is the graph for day i for a plant and n is the number of days the plant is imaged. The leaves for each plant in the graph are labeled starting with 1, as each plant was labeled independently. The problem of tracking reduces to finding the correspondence between the leaves of two consecutive plants, i.e., graphs G_i and G_{i+1}. We assume that G_i has been properly labeled and we must label G_{i+1}. As stated before, since the plants are imaged frequently, the change in $G_{i,j}$, if any, can come in the form of either a new leaf or a dead leaf or both.

New leaf: Since new leaves always emerge from the last collar, the newest leaf in a plant will have the highest label in its corresponding graph. Given graphs G_i and $G_{i,j}$, if the leaves with the highest labels in the two do not match in the image, then a new leaf has emerged in $G_{i,j}$. Matching can be done by simply matching their orientations. Thus, G_{i+1} has new leaf with respect to G_i, iff

$$\text{lastLeaf}(G_i).\text{orientation} \neq \text{lastLeaf}(G_{i+1}).\text{orientation}, \tag{2}$$

where lastLeaf returns the leaf (edge in a graph) whose label is the highest. In this case, the each label of the leaves in G_{i+1} is incremented by the label of the first leaf in G_i.

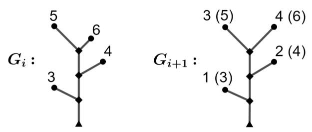
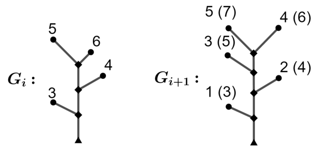
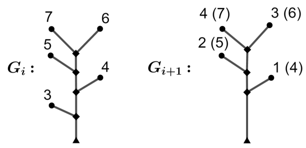
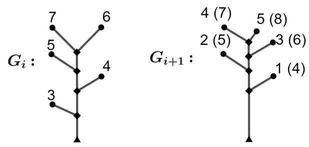
Dead leaf: Similarly, the oldest visible leaf in a plant will have the lowest label, in its corresponding graph. Thus, if the leaves with the smallest labels do not align in graphs G_i and $G_{i,+1}$, then a leaf in G_i has been lost in G_{i+1}. In such a case, the first leaf G_i will not align with first leaf (Leaf 1) G_{i+1}. Again, the alignment can be done by simply matching their orientations. Thus, G_{i+1} has lost a leaf with respect to G_i, iff

$$\text{firstLeaf}(G_i).\text{orientation} \neq \text{firstLeaf}(G_{i+1}).\text{orientation} \tag{3}$$

where firstLeaf returns the leaf (edge in a graph) whose label is the smallest. In this case, the labels for the rest of the leaves in G_{i+1} are transferred from G_i.

Table 1 summarizes the four possible scenarios when tracking the leaves from G_i to G_{i+1}. The leaf tracking process is summarized in Algorithm 3. We assume that $_i$ is correctly labeled. We then update the labels for G_{i+1} from G_i starting with $i = 2$. At each step, we first examine if a leaf has been lost or if a new leaf has emerged. If no leaf has

Table 1: Possible scenarios and corresponding actions for leaf tracking for two consecutive images.

Lost Leaf	New Leaf	Action
No	No	Transfer labels from G_i to G_{i+1} 
No	Yes	Transfer labels from G_i to G_{i+1} and increment other labels Δ 
Yes	No	Transfer labels from G_i to G_{i+1} $\forall$ G_i $\in$ G_{i+1} 
Yes	Yes	Transfer labels from G_i to G_{i+1} $\forall$ G_i $\in$ G_{i+1} and increment other labels Δ 

been lost, we simply update the labels of the leaves in $Gi + 1$, by incrementing them by the label of the first leaf of G_i (Δ). If however, a leaf is lost, the increment term (Δ) is the label of the second leaf in G_i.

Algorithm 3 Leaf tracking algorithm.

Input: $G = \{G_1, G_2, \ldots, G_i, \ldots, G_n\}$, where G_i is the graphical representation of the i-th image $\forall\ i = 1,..., n$, obtained from Algorithm 1.
Output: $G' = \{G'_1, G'_2, \ldots, G'_i, \ldots, G'_n\}$, where G'_i is the graphical representation of the i-th image $\forall\ i = 1,..., n$, with the leaves correctly tracked and labeled.

$G'_1 = G_1$
for $i = 2 : n$ **do** {
    lostLeaf = checkLostLeaf(G_i, G_{i+1}); // See Eq. 3
    if ($\neg$ lostLeaf)
        Δ = firstLabel(G'_{i-1});
    else
        Δ = firstLabel(G'_{i-1}) + 1;
    G'_{i+1} = updateGraph(G_{i+1}, Δ);
end for

3.4. Leaf status report

Once all the leaves are tracked from their emergence over the life cycle of the plant, a leaf status report can be generated to provide significant phenotypic information based on property of each leaf. For this paper, we report the length of the leaves which may be replaced or augmented with other phenotypes (e.g., curvature) seamlessly. The steps to compute the length of a leaf are as follows:

Leaf Length

Leaf length can be computed by counting the number of pixels for an edge in the graph in the corresponding skeleton segment. A more accurate approach may use a curve fitting approach as follows. Let the n-th order polynomial curve p for each leaf is given by

$$y = p(x) = p_1 x^n + p_2 x^{n-1} + p_3 x^{n-2} + ... + p_n x + p_{n+1}, \tag{4}$$

where, p_1, p_2, ..., p_{n+1} are the coefficients of the best fit polynomial for the leaf skeleton optimizing the least square error. The leaf length is measured by

$$\int_{x_c}^{x_t} \sqrt{1 + (dy/dx)^2}, \tag{5}$$

where, x_c and x_t denote the x-co-ordinates of the collar and tip for the leaf, respectively.

The leaf status report displays the phenotypic information of each leaf as a function of time throughout its life. It explicitly provides the following phenotypic information that are of significance in the context of plant sciences: (a) the total number of leaves emerged during the life cycle, (b) the day on which a particular leaf emerged, (c) the number of leaves present at any point of time, (d) the length of each leaf at any point of time, (e) the day on which a particular leaf died and (f) the rate of growth of each leaf.

4. UNL-CPPD

UNL-CPPD is introduced to stimulate research in the development and comparison of algorithms for leaf detection and tracking, leaf segmentation and leaf alignment of cereal crops, e.g., maize and sorghum [9]. UNL-CPPD is freely available from http://plantvision.unl.edu/datasets. The dataset is created using Lemnatec Scanalyzer 3D high-throughput plant phenotyping system at the center for plant science innovation in the University of Nebraska-Lincoln (UNL), USA.

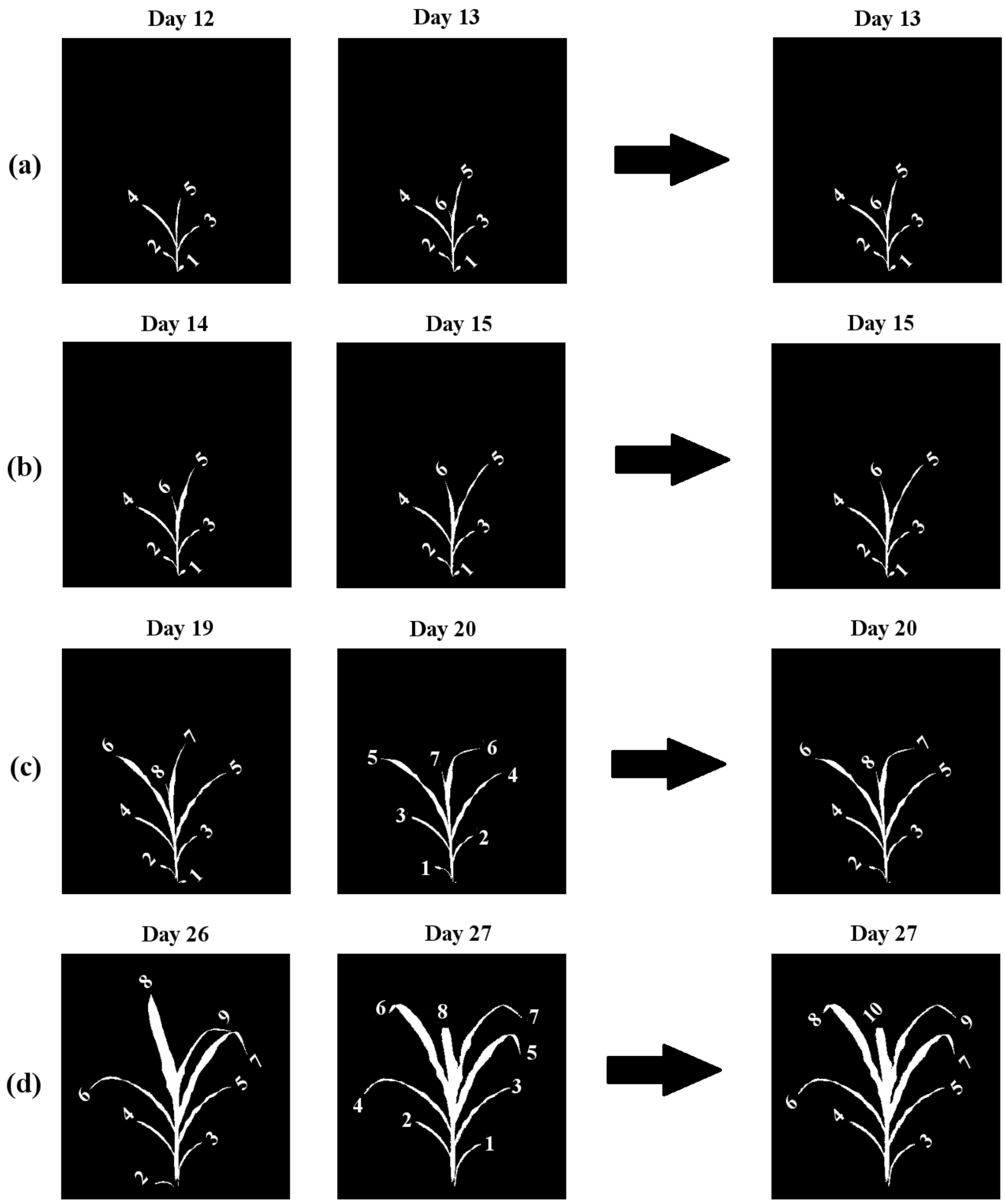


Figure 5. Four scenarios for leaf emergence ordering for tracking: (a) case 1- leaf emergence; (b) case 2-no change; (c) case 3-leaf loss; and (d) case 4-loss and emergence.

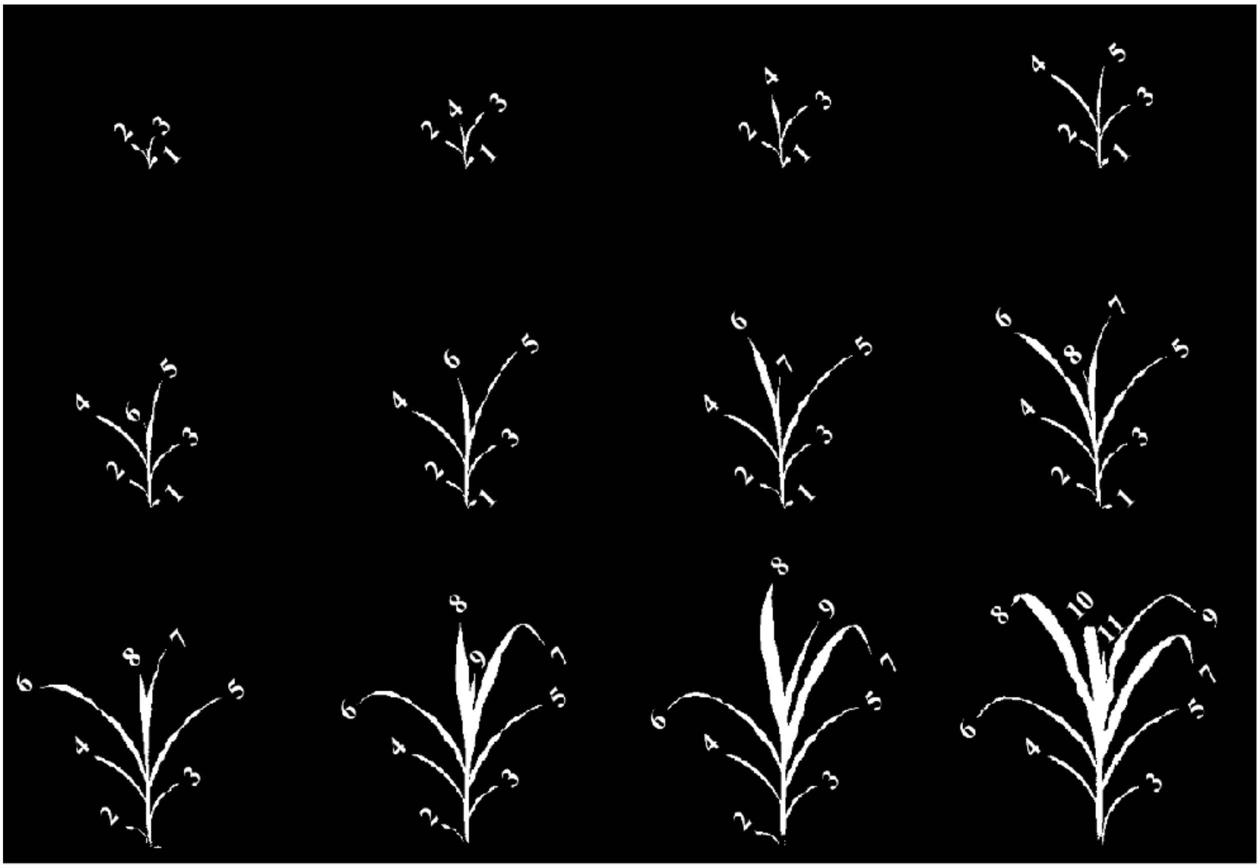


Figure 6. Illustration of leaf tracking (with each leaf numbered in order of emergence) using a growing plant image sequence consisting of images captured on alternate days starting from Day 5 (top-left) until Day 27 (bottom-right).

UNL-CPPD has two versions: UNL-CPPD-I (small) and UNL-CPPD-II (large). UNL-CPPD-I consists of images of 13 maize plants for 2 side-views, i.e., 0° and 90°, captured by the visible light camera once daily for 27 days, starting from two days after germination that merely exclude self-occlusions due to crossovers. UNL-CPPD-II comprises images of the same 13 plants for the same two views, but for longer duration, i.e., 32 days, that include images of plants with leaf crossovers and self-occlusions [9]. Each image of the UNL-CPPD dataset is accompanied by the ground truth in the form of (a) an XML document that embeds the information about the plant id, the coordinates of the base of the plant, the information about the leaves including its leaf number (in order of emergence), the coordinates of the collars and the tips and if the leaf is alive or dead and (b) an annotated image with each leaf numbered in order of emergence [9].

5. Results

We evaluated the performance of the proposed method using UNL-CPPD-I and provided improvement directions to handling the leaf tracking challenges due to the presence of intersecting leaves using UNL-CPPD-II. We examine the accuracy of leaf tracking, demonstrate the benefit of leaf status reports and present the run-time analysis of the algorithm.

5.1. Leaf tracking accuracy

The success of leaf tracking algorithm depends on how accurately the leaves are detected. Thus, the performance of the proposed method is evaluated using two criteria.

- Leaf-detection accuracy (LDA): The leaf-detection accuracy and leaf-tracking accuracy are respectively given by

$$\mathrm{LDA} = \frac{1}{n}\sum_{i=1}^{n}\frac{N_i^d - N_i^f}{N_i^g}, \tag{6}$$

  where N_i^d, N_i^f and N_i^g are the number of detected leaves, number of false leaves and the actual number of leaves (as noted in the ground truth) for the i-th day for a given plant. This is computed for each plant separately.
- Leaf-tracking accuracy (LTA): This measures the accuracy of our leaf tracking algorithm and is given by:

$$\mathrm{LTA} = \frac{1}{n}\sum_{i=1}^{n}\frac{N_i^t - N_i^w}{N_i^g}, \tag{7}$$

  where N_i^t, N_i^w and N_i^g are the number of correctly tracked leaves, number of wrongly tracked leaves and the actual number of leaves (as noted in the ground truth) for the i-th day for a given plant, respectively. This is also computed for each plant separately.

Table 2 shows the results of experimental analyses of the proposed method on UNL-CPPD-I. In the case of 7 out of 13 plant sequences, all leaves are tracked correctly, showing 100% LTA. However, the poor performance of $Plant\_001-9$ and $Plant\_016-20$ in terms of LTA is attributed to the fact, that a failure in detection of a leaf in the early stage has rendered the tracking of leaves wrong throughout the life cycle. The proposed method achieves promising LTA for the remaining 4 sequences. The table shows that the average LDA is 92%, whereas the average LTA is 88%. Figure 6 shows the results of tracking using a plant sequence ($Plant\_191-28$) from UNL-CPPD-I.

5.2. Leaf status report

Figure 7 shows the leaf status report generated for a plant sequence (i.e., $Plant\_104-24$) in the dataset. Each leaf in the plant is represented by a graph. The axes of the graphs are time (in days) and a phenotype (leaf length in Figure 7). The report shows the dates

Table 2: Performance summary on UNL-CPPD-I dataset. Keys-N^g: number of leaves in the groundtruth; N^d: number of detected leaves; N^f: number of false leaves; N^w: number of incorrectly tracked leaves; 'LDA': leaf detection accuracy; 'LTA': leaf tracking accuracy.

Plant-ID	N^g	N^d	N^f	N^w	LDA	LTA
$Plant\_001-9$	116	93	1	78	0.79	0.16*
$Plant\_006-25$	138	136	0	2	0.98	0.98
$Plant\_008-19$	142	140	0	8	0.98	0.94
$Plant\_016-20$	103	86	0	47	0.83	0.45*
$Plant\_023-1$	113	101	0	0	0.89	1.00
$Plant\_045-1$	122	120	3	2	0.96	0.98
$Plant\_047-25$	148	142	2	0	0.94	1.00
$Plant\_063-32$	149	138	0	0	0.93	1.00
$Plant\_070-11$	125	111	0	0	0.89	1.00
$Plant\_071-8$	141	131	0	0	0.93	1.00
$Plant\_076-24$	135	126	2	0	0.92	1.00
$Plant\_104-24$	144	140	0	0	0.97	1.00
$Plant\_191-28$	137	111	0	2	0.96	0.98
Average	132	123	<1	10.69*	0.92	0.88

of emergence of the leaves, e.g., Leaf-1 emerged on Day 4, whereas Leaf-5 emerged on Day 10. Furthermore, we can get information on the length of each leaf on any given day, e.g., the length of Leaf-4 on Day 10 is 180 pixels. The report shows that senescence (death) for Leaf-1 occurred on 22 and Leaf-2 on Day 26. It is evident from the report, that growth of the leaves that emerged later in the plant's life are significantly higher compared to the leaves that emerged during the early phase of the plant. One possible explanation for this pattern is the reduction in the amount of sunlight received by the lower leaves as they grow under the upper leaves. Note that for some days, the length of a leaf decreases from the previous day, e.g., Leaf-4 on Day 10. Some factors that influence this include plant rotation, occlusion and the fact that that the measurements are made from the 2D projection of the 3D leaves.

5.3. Limitation Handling

The growth pattern in the early plant stages provides critical phenotypic information related to yield, and hence, is of most interest to the plant scientists and agronomists. The early growth stages are characterized by the absence of self-occlusions and leaf crossovers, and the proposed method achieves high proficiency in tracking the leaves in that scenario. However, the architectural complexity of plants increases with time due to the development of new organs resulting in more frequent occlusions and crossovers. With a limited number of views, the determination of plant architecture based on skeleton-graph transformation becomes increasingly challenging in the late vegetative stages.

When two leaves in a plant intersect, their representations in the skeleton-graph share one of more nodes. Furthermore, the skeleton-graph is no longer a tree since it contains one or more loops due to the intersections. Based on the nature of contact between the leaves, the intersections are classified into three types: (a) tip-contact, (b) tangential-contact, and (c) crossover. Figure 8 shows examples of these cases where the proposed algorithm fails to track the leaves accurately. The proposed method can be extended to address the above three failure cases by leveraging the growth characteristics of the leaves, i.e., the leaves represented as the edges in the skeleton-graph must demonstrate angular consistency.

The proposed algorithm tracks each leaf starting from its junction, using a bottom-up approach, by following the edge until it reaches a tip (degree 1 node). When there is no

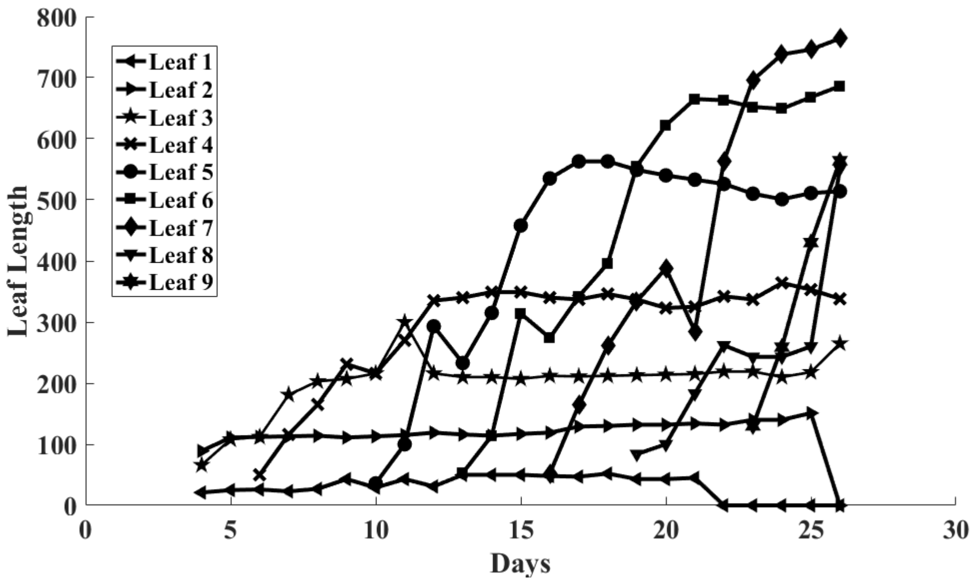


Figure 7. Temporal variation of the length of each leaf starting from emergence.

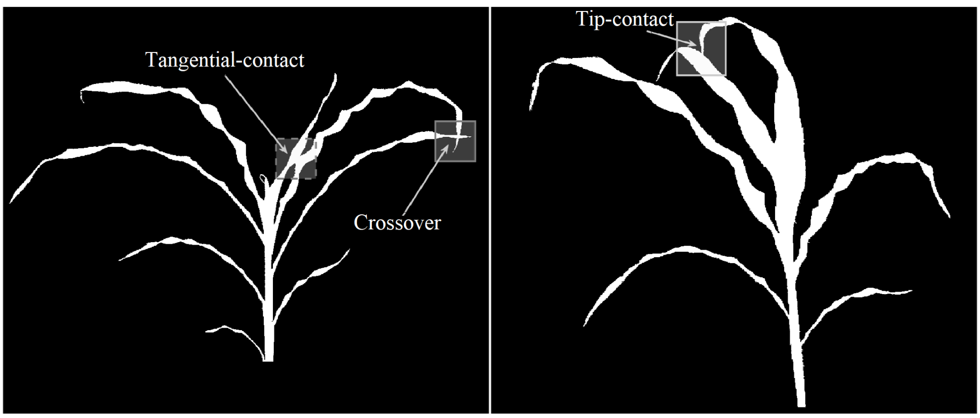


Figure 8. Illustration of types of leaf intersections: tip-contact, tangential-contact and crossover.

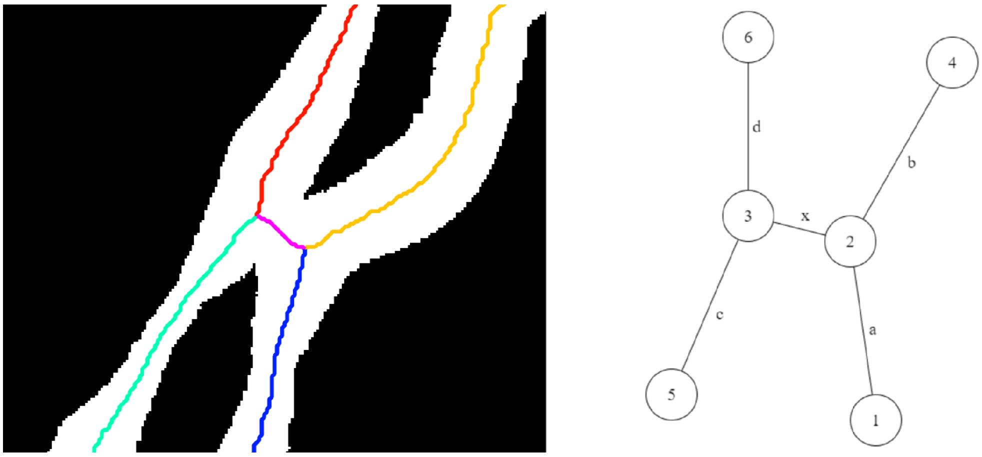


Figure 9. Illustration of tangential-contact: (left) skeleton; (right) skeleton-graph representation.

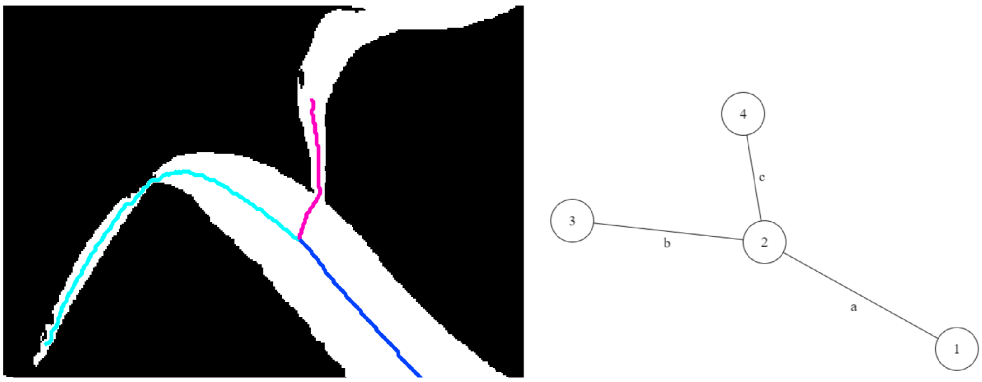


Figure 10. Illustration of tip-contact: (left) skeleton; (right) skeleton-graph representation.

leaf intersection, only degree two nodes are encountered along the way. In the presence of leaf intersections, the algorithm encounters higher degree nodes and must select the edge that represents the continuation of the current leaf. A look-ahead approach is used to determine the next node in the path, i.e., we select the node that provides the highest continuity, measured by angular consistency, with the leaf segment traced so far. Figures 9, 10, and 11 illustrate this process for three common scenarios of leaf intersections, i.e., tangential-contact, tip-contact and crossover, respectively. In every case, the algorithm starts with node 1, follows edge a, and reaches node 2, a degree-3 node. The algorithm must now choose between two nodes: node 3 and node 4 as the continuation of the current leaf. Depending on the degrees of these two nodes, the following two scenarios can arise:

1. Case A: The degrees of both the nodes are less than 3. This case corresponds to scenarios shown in Figure 9. In this case, the node with the most angular consistency with the edge a is chosen. In Figure 10, node 3 is selected and the edge b is marked with the current leaf number. When node 2 is reached via edge c, the algorithm stops tracking since there are no unseen edges to follow and labels it as the tip for that leaf.
2. Case B: One node has a degree 3, and the other has a degree 2 or less: This case may correspond to the scenarios in either Figure 10 or Figure 11. In this case, a two-node look-ahead (from the new degree-3 node) is performed to identify all combinations of edges that form a path from node 1 to the resulting nodes from the second look-ahead. The path with the highest angular consistency is chosen to continue the current leaf.
   Depending on the type of intersection, the edge x is either shared (in the case of a crossover) or ignored (in the case of a tangential contact) for detecting leaves

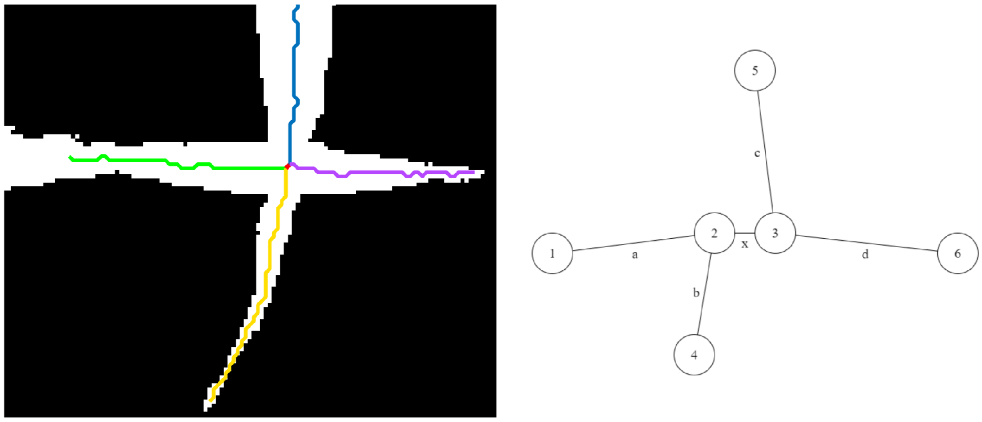


Figure 11. Illustration of crossover: (left) skeleton; (right) skeleton-graph representation.

accurately. In Figure 9, the possible leaf segments are $\{ab, axd, axc\}$, and the path ab is selected for the highest angular consistency. When the algorithm reaches node 5 from node 3 following edge b, a similar analysis will continue the leaf to node 6 using edge d, and the edge x will remain unused and eventually ignored by the algorithm. For the scenario in Figure 11, however, with the same set of possible leaf segments, i.e., $\{ab, axd, axc\}$, the path axd chosen for the highest angular consistency. When the algorithm reaches node 2 from node 4, the same analysis will select bxc as the best path for leaf continuation, in essence, sharing the edge x. Thus, the leaves are detected accurately in the presence of different types of intersections.

6. Discussion

Leaves are one of the primary organs of plants which transform solar energy into chemical energy in the form of carbohydrate through photosynthesis, releasing oxygen as a byproduct. The total number, emergence timing and size of leaves are therefore related to plant photosynthetic light efficiency and net primary productivity. Leaf stage monitoring of cereal crops plays a crucial role in the understanding of plant's vigor and yield prediction modeling. The paper introduces a new concept of visual growth tracking to solve a previously unexplored topic of automated leaf stage monitoring of maize plants.

The proposed method is applicable for plants with distinct stems that are above-ground, not highly branched, and characterized by distinct nodes and internodes. In this work, the experimental evaluations are conducted using image sequences of maize plants only, however, in future work, we will consider creation of a new dataset consisting of a larger number plants sharing similar architecture of maize, i.e., sorghum, to demonstrate the efficacy of the proposed method. A plant's overall growth is significantly impacted by environmental stress factors. The proposed method has the potential to investigate the effect of drought or thermal stress on leaf growth stages regulated by genotypes.

The proposed method is implemented using Matlab R2016a on an Intel(R)Core(TM) i7 processor with 16 GB RAM working at 2.60-GHz using 64 bit Windows 7 operating system. The average execution time of a single plant sequence consisting of $27 \times 2 = 54$ images is 15.38 minutes. The time includes view selection, determination of individual plant architecture, leaf tracking and leaf status report generation.

7. Conclusions

The paper introduces a novel method for automated tracking of individual leaves that change in size, shape and structure over time, using multi-view image sequences of a plant for application in phenotyping. This is a pioneering study that replaces the manual and destructive process of growth stage determination of economically important corp like maize. The method has four phases: (a) optimal view selection; (b) plant architecture determination based on a graph theoretic approach; (c) leaf tracking to assign labels to each leaf based on the order of emergence; and (d) generation of leaf status report. The method starts with an image sequence of a plant captured by a visible light camera as the input and produces a leaf status report containing phenotypic information useful to assess the plant vigor, i.e., timing of emergence and senescence of each leaf, length of each leaf on a particular day, and the relative growth rates of individual leaves. The paper introduces a curve tracing technique based on angular consistency check in an attempt to augment the proposed algorithm to address the challenge of intersecting leaves for robust leaf tracking.

Author Contributions: SB and SDC developed and implemented the algorithm, and led experimental analysis, and the manuscript writing. AS supervised the research and provided constructive feedback throughout the algorithm development process. AS, TA contributed to the manuscript writing. All authors contributed to the article and approved the submitted version.

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Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: A short video of the result is available at https://plantvision.unl.edu/projects-0.

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