

Poplar Leaf Morphology Visualization Research based on Gene Expression

Jinliang Zhang¹, Yongjian Huai¹, Rongling Wu²

¹School of Information Science and Technology, Beijing Forestry University, Beijing, 100083, China

²Department of Statistics, University of Florida, Gainesville, 32611, USA

Abstract: The rapid development of computer hardware and software promotes the progress of research on plant visualization and improves the techniques. At the same time, the further research of biological information technology makes the correlation research of phenotypic information and genetic information become one of the current hot topics. In this article, we analyzed the main methods and techniques of plant visualization, analyzed the impact of gene expression for phenotypic traits. Molecular marker technologies have provided a powerful means for unraveling the genetic architecture of a quantitative trait. We construct a framework of simulation model based on gene information and put forward the idea of further study.

Keywords: Visualization; Gene expression; Mixture model; Poplar leaf

1. Introduction

The development and application of information technology has improved productivity greatly. At the same time, provides a wider platform for scientific research. At present, the systematic study of interdisciplinary and multi field rely on modern information technology has been a focus of modern scientific research and will be our future research direction. On the other hand, it can also open the rational and wisdom door for us to systematically understand the world and improve the living environment.

The growth and morphological structure of plants are affected by environmental factors (light, moisture, soil nutrients, etc.) and are controlled by genes. In genomic information, genes that affect and control plant morphology (outline, size, color, skeleton, etc.) are scattered in different loci of the genome and are extremely complex. The plant morphology belongs to plant phenotypic information. Different gene expressions control the formation of different shapes. It is one of the most important and most challenging problems in modern biology research to predict biological phenotype with the genotype. In the past 20-30 years, the rapid development of genotype sequencing technology triggered by DNA recombination technology, has enabled people to decompose phenotypic variation of complex traits into a variety of genetic components and their interaction. Compared with the progress of gene technology, the phenotypic technology development is relatively lagging behind, but in recent years the academic circles have taken it more and more seriously. In the long run, the ultimate goal of research that penetrate all agricultural, biological and medical, is to solve phenotypic problems.

Plant phenotype, in the field of information technology, is usually reflected or simulated by plant morphology

visualization which also called virtual plant. It focus on individuals or groups of plants, and simulate plant growth and development process in three dimensional space on the computer by using virtual reality technique. Virtual plant research is becoming the focus and direction of agricultural and forestry science and technology development at home and abroad. One of the key techniques of the virtual plant is the construction of mathematical model to describe the geometric shape and deformation of a plant, and then draw out the three dimensional morphology of plants with sense of reality on the computer, display the dynamic growth process. However, current researches on plant visualization algorithms and models are mostly based on plant shape itself. The morphological study with gene information on plant visualization is very little. As for complex biological trait, although is controlled by environmental factors, but more and more research indicates that gene also plays a crucial role in the process of variation in trait.

2. Plant Morphology Visualization Research Methods Analysis

The main methods of plant morphology visualization: Fractal method, L-system, mathematical methods based on stochastic process such as reference axis technique, AMAP software, etc. At present most of these computer models used to construct plant morphology mainly are morphogenetic models. Some typical plant visualization models are analyzed as follows.

2.1. Fractal method

Fractal method uses mathematical tools that describe the self-similarity to express the topology and morphological structure of plant growth. The method includes IFS (Iter-

ated Function System), Ramification matrix, Partial System, Formal Languages, A-system, etc. IFS is a typical fractal rendering method which through a number of affine transformation to transform the overall morphological to part. In 1991, Prusinkiewicz developed a method known as Language-restricted IFS which can generalize different kinds of IFS methods generally by adding constraint conditions to the transform sequence. Ramification matrix is to use a matrix to describe a plant ramification number, and the fractal structure of plant is produced by iteration. Partial System is proposed by Reeves in 1983 which fits for simulating macro scenes such as forests or grasslands. Formal Languages method can be used for tree modeling which developed by Smith based on parallel rewriting algorithm of formal language. A-system is for constructing plant morphology proposed by Aono.

All of these Fractal methods are focused on realistic visual effect.

2.2. L-System

L-System proposed by American biologist Lindenmayer in 1968 is a formal language method. Growth rules of plant organs and tissues are established through experience generalization. And then based on the rules developmental sequences of words are generated. According to the geometric interpretation given by the plant and continuous plant fractal generator, three dimensional effect of plant morphology can be generated. The mathematical description of L-System is as follows: L system can be denoted by $L = (V, W, \text{and } P)$, where V is the alphabet; W is the initial string of symbols, called axiom, $W \in V^+$, V^+ is defined in the V on a non empty words; P is a finite generating set of rules. The procedure can be explained as follows.

$W: A$

$P1: A \rightarrow B [A] [A]$

$P2: B \rightarrow B B$

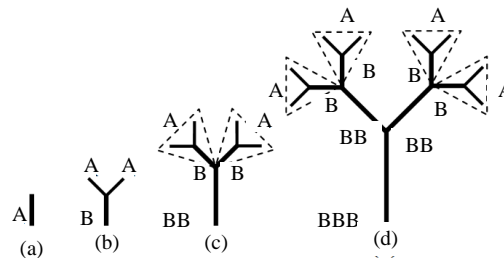


Figure 1. Schematic diagram based on L-system

That is L-System, a tree plant simulation method. According to the code programming, simulation can realize plant graphics on a computer. The L-system has a high degree of abstraction, the production rules while allowing the parameter values and the growth law of easy to change. But the botanical significance is not intuitive, practical simulation process is very complex.

2.3. Mathematical methods of stochastic process

Method of virtual plant stochastic process mainly refers to the finite automaton proposed by de Reffye et al., also known as "reference axis technique". The main principle of this method is to treat plant growth as a procedure with certain probability distribution (such as whether a bud will produce branch, the type of a branch, the time of emergence, etc.), to analysis evolution law of plant topological structure using the Markov chain theory and state transition graph (bud growth, bud dormancy, bud death, bud branching and so on), to extract growth rule through the pattern recognition method. On this basis, the process of plant growth can be simulated.

Virtual plant model established with reference axis technique can accurately reflect growth rules of plants, and can be verified according to the data that measured ac-

ording to the plant. Compared to L-systems, the physical significance of this modeling method is clear, simple data input, process analysis is very intuitive. So the scope of the application expands rapidly.

AMAP system is a virtual plant system based on the reference axis technique. The AMAP system is actually a set of software that contains multiple subsystems with different functions.

Among these plant visualization methods, either based on the mathematical model or based on plant body itself, they are mainly focused on the structure of the physical plant, and are short of the causal relationship of its inner development. So it makes the research of plant visualization slightly monotonous.

3. The Theoretical Framework of Gene Expression Analysis

3.1. Overview of the current research

Based on the hybrid model, Lander & Botstein (1989) created a statistical method which can locate and estimate quantitative trait loci (QTL) that lead to quantitative trait variation (QTV). At present, this method has become an important means of genetic studies of quantitative traits

and initiated an revolutionary change in quantitative genetics from the classic phenotypic variance analysis (Wu 1996) to genetic mechanism analysis based on molecular level. Using QTL positioning method, people have found ways to precise positioning and map based cloning of a gene, and have cloned maize branching habit, fruit size of tomato, tiller number and other important trait gene, QTL positioning method has promoted effectively biology developing forward and the animal breeding and plant breeding based on quantitative genetics.

With the function positioning, we can study comprehensively the phenotype forming stage influenced by gene, analysis gene effects in phenotype formation process and more comprehensively outline the genetic structure map of phenotype. By using the function positioning technique, for example, people have analyzed QTL of poplar trunk and roots growth curve, and have detected the effect of different types of QTL in regulating the growth process of these two traits: some QTL express in all growth process, and some QTL express in the developmental stage; and some QTL exhibit a strong epistatic interaction effect on growth and the development pattern.

3.2. Model construction

Consider a mapping population, such as the F2 group, there are three kinds of different genotypes at each lo-

cus. A QTL genotype that influences organ shape can be expressed as QQ, Qq, qq. QTL genotype cannot be observed directly, but can be deduced by molecular markers (M) that associated with it. The basic statistical models of QTL mapping use hybrid mapping models, each observed value Y is assumed to be determined by one of the three genotypes, each genotype can be modeled by density function (usually assumed to be normal distribution). We use finite mixture model to estimate the results of the influence of genotype:

$$y_i \sim L(y) = \prod_{i=1}^n [\pi_{2i} f_2(y_i) + \pi_{1i} f_1(y_i) + \pi_{0i} f_0(y_i)] \quad (1)$$

Table 1. QTL genotype (j)

QTL geno- type (j)	QQ	Qq	qq
Code	2	1	0

$f_j(y_i)$ is normal distribution density where mean for μ_j , variance for σ^2
 $\mu = (\mu_2, \mu_1, \mu_0)$
 $\pi =$ The conditional probability given flanking marker
 The model of genotype effect is shown in Figure2:

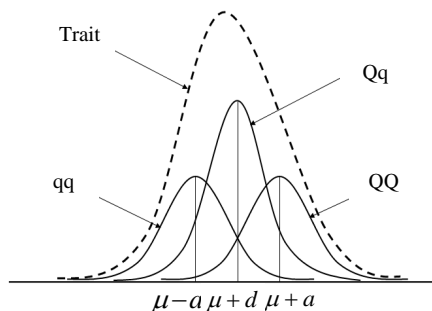


Figure 2. A finite mixture model which is used for estimating genotypic effects

In order to estimate the correlation between genotype and phenotype of a plant we should measure the phenotype data of the plant and get the corresponding markers in-

formation through experiments. And then establish data structure with the corresponding data form as Table 2.

Table 2. Data structure for finite mixture mode

Subject	Marker (M)	Phenotype (y)	Conditional prob. of QTL genotype		
	M1 M2 ... Mm		QQ(2)	Qq(1)	qq(0)
1	AA(2) BB(2) ...	y1	π_{21}	π_{11}	π_{01}
2	AA(2) BB(2) ...	y2	π_{22}	π_{12}	π_{02}
3	Aa(1) Bb(1) ...	y3	π_{23}	π_{13}	π_{03}
4	Aa(1) Bb(1) ...	y4	π_{24}	π_{14}	π_{04}
5	Aa(1) Bb(1) ...	y5	π_{25}	π_{15}	π_{05}
6	Aa(1) bb(0) ...	y6	π_{26}	π_{16}	π_{06}
7	aa(0) Bb(1) ...	y7	π_{27}	π_{17}	π_{07}
8	aa(0) bb(0) ...	y8	π_{28}	π_{18}	π_{08}

prob. means probability. y_i stands for phenotypic data of sample.

A QTL statistical model assumes that the QTL genotypes can be observed in a mapping population. This is not possible in practice. What we can do is to use observable markers to predict such unobservable QTLs through the linkage between markers and QTLs. Thus, by performing the association analysis between phenotype values and the markers, we can infer the effects of QTLs on phenotypic variation.

We use data like in table 1 to solve normal distributions of phenotypic values for each QTL genotype group, as

$$f_2(y_i) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left\{-\frac{(y_i - \mu_2)^2}{2\sigma^2}\right\}$$

$$\mu_2 = \mu + a$$

$$f_1(y_i) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left\{-\frac{(y_i - \mu_1)^2}{2\sigma^2}\right\}$$

$$\mu_1 = \mu + a$$

$$f_0(y_i) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left\{-\frac{(y_i - \mu_0)^2}{2\sigma^2}\right\}$$

$$\mu_0 = \mu + a$$

Study on the correlation analysis of plant genotype and phenotypic using this model has been paid more and more attention, and there are some achievements.

3.3. Three-dimensional structure and shape outline of poplar leaves

Different plant species have different shape characteristics with other species; on the other hand, the same species are not completely the same with the shape of its leaves and its three-dimensional structures. There is no tree with two leaves which are the same. There are differences with vein and size of leaves, petiole length, curve of leaf margin, etc.

There is a direct relationship between leaf shapes of plants and the environment, the leaf shape of a plant is the long-term result of natural selection and possesses heredity. That is, the shape characteristics of poplar leaves under different environment are different; the excellent results of long-term natural selection will be directly transmitted to its offspring. Under insufficient light circumstance, the leaves need to expand leaf area for lighting; many plants have special pollinators, and different leaf shapes give recognition signals, and all these have been solidified into genes. So, locating three-dimensional structure and shape outline of poplar leaves, we can strengthen the relationship with QTL gene information from the technical level of poplar leaf visualization.

At present, the QTL localization of shapes are mostly based on the method of multi variable morphology measurement such as organ length and width, height, ratio,

angle, etc. Because these methods in accordance with the simple geometric analysis cannot make deep analyze the complex structure of organ shapes, they are inadequate to get accurate QTL mapping of shapes. Measurement of geometric morphometric technology which developed about thirty years ago is a revolutionary breakthrough of morphological analysis. As the traditional multi variable morphology measurement method can only be used to study the larger variation phenotypes, such as floral symmetry. GM technology which may precisely measure organ shape can be used to study the more tiny variation phenotype that was difficult to measure before. Through the Descartes coordinates analysis of feature points, GM technology can not only retain the geometry information of digital data but also associate the abstract multivariate results with physical structures of sample. In recent years, image and digital technology has been greatly improved, and has been successfully applied to the description of biological traits. Using this technique, people can shoot pictures of biological geometry from different angles, and then represent with encoded image blocks. It has greatly promoted the accurate description of biological shapes with shape recognition and shape registration techniques.

Here, we will extend to the combination of 3D GM technology and the QTL mapping model, and derive the overall three-dimensional structure and the microstructure gene mapping method. We will embed the trait dynamic principle into QTL mapping framework, design statistical procedures to test the way of QTL mapping impacting on the shape development.

3.3.1. Three-dimensional structure

As for an individual i in a mapping population, we get the stereo image by micro CT method. The physical surface $v_i(\theta, \phi)$ can be described by the spherical harmonic expansion which can be used to reflect the three dimensional coordinate points x, y, z :

$$v_i(\theta, \phi) = (x_i(\theta, \phi), y_i(\theta, \phi), z_i(\theta, \phi))^T$$

$$= \sum_{l=0}^{\infty} \sum_{m=-l}^l c_{il}^m Y_{il}^m \tag{2}$$

The coefficient $c_{il}^m = (c_{ilx}^m, c_{ily}^m, c_{ilz}^m)^T$ in the formula is a 3D vector; it is usually made up of complex number. The surface of an object can be remodeled with these coefficients remodeling, the more coefficients the more detailed structure can be remodeled. These coefficients can be obtained by the least square method for solving linear equations. We give the likelihood function of QTL mapping three-dimensional object surface is

$$L(\theta | Y, X) = \prod_{i=1}^n \left[\sum_{j=1}^{2^m} P_{ij} \varphi(y_i, \mu_{ij}, \delta^2) \right] \tag{3}$$

Where y is variable, a is the mean value, b is the variance and $\varphi(y, a, b)$ is the normal density function.

In order to solve function (3), complete the following shape analysis steps: Rotation, Correspondence and Splicing. Rotation is to segregate the surface parameters space into three different spherical x, y, z . after rotate of Euclidean angle (α, β, γ) in the sphere, the newly formed 3D coefficient of individual i is expressed as

$$c_{it}^m(\alpha_i, \beta_i, \gamma_i) = \sum_{m=-l}^l c_{it}^{m'} D_{imm'}^{m'}(\alpha_i, \beta_i, \gamma_i) \quad (4)$$

Where $D_{imm'}^{m'}(\alpha_i, \beta_i, \gamma_i)$ is the rotation matrix, the calculation is $e^{-l m' \alpha_i} d_{imm'}^l(\beta_i) e^{-l m \gamma_i}$, include

$$d_{imm'}^l(\beta_i) = \sum_{t=\max(0, m-m')}^{\min(l+m, l-m')} (-1)^t f(t) g(\beta_i, t)$$

Where

$$f(t) = \frac{\sqrt{(l+m)!(l-m)!(l+m')!(l-m')!}}{(l+m-t)!(l+m'-t)!(t+m'-m)!t!}$$

$$g(\beta_i, t) = \left(\cos \frac{\beta_i}{2}\right)^{(2l+m-m'-2t)} \left(\sin \frac{\beta_i}{2}\right)^{(2l-m+m')}$$

Correspondence analysis is to make the Euclidean distance of two surfaces the shortest. For example, there are two surface described by $v_1(s)$ and $v_2(s)$, the Euclidean distance is $D(v_1, v_2)$:

$$D(v_1, v_2) = \left(\oint \|\mathbf{v}_1(s) - \mathbf{v}_2(s)\|^2 ds \right)^{\frac{1}{2}} \quad (5)$$

$$= \left(\sum_{f \in (x, y, z)} \sum_{l=0}^L \sum_{m=-l}^l (c_{if_1}^m - c_{if_2}^m)^2 \right)^{\frac{1}{2}}$$

On the condition of the shortest distance of two surfaces we can obtain the parameter estimation. Splicing is generally using the search method based on repeated sampling. Put a parameter fixed in the mesh point, let the other parameters rotate, proceed troubleshooting search, until obtain the shortest surface distance defined by the equation (5), and now have completed the splicing task of surface correspondence analysis.

The aforementioned 3D image analysis is based on the feature point model. In order to capture the biological organ shape of complex structure, we should get the high dimensional pixel of shape edge and interior detail features. Vector description of organ shape denoted by coordinates $(x(s), y(s))$ ($s = 0, 1, \dots, m-1$), m is the number of coordinates (points), determines the accuracy of organ shape description. Organ shape analysis using digital image need calibration for the shape, the aim of calibration is to minimize the discrepancy generated by position. Organ shape calibration is the process to establish a reference coordinate for organ shape status (the position, size and rotation, etc. of a shape).

Based on the above reconstruction method, we remodeled a piece of poplar leaf using 3D GM technology with 17 feature points and semi-feature points (Figure 3). We scanned a poplar leaf with micro CT scanning, and ana-

lyzed the feature points. (a) is a real leaf image. (b) is the 3D remodeled image with feature points. The selection of feature points must reflect the structure and shape of a leaf. In (b), altogether 10 such homogeneous feature points, including organ endpoints, base points etc. and 7 semi-feature points are also selected, on the midpoint between the feature points, especially on the feature points of curved surface. We found that the remodeling poplar leaf analyzed with 3D GM has a high similarity with the original piece and reached more than 95%. It shows the effectiveness of this technology.

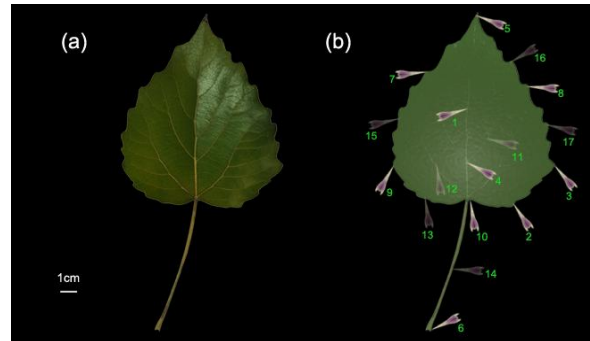


Figure 3. Analysis of the characteristics of a poplar leaf

The basic principle of feature point's selection is that they must totally reflect the shape and structure of the leaf. 1 feature point on each of the two petiole ends, 1 point on top of the leaf, 1 on the main vein midpoint (not counting the petiole), 1 on the center of gravity position of the leaf (including petiole), the other 5 are distributed on the leaf margin at the major inflection point position, these feature points determines the main profile structure of a leaf. The semi-feature points are usually used to further refine the structure and help depict the details.

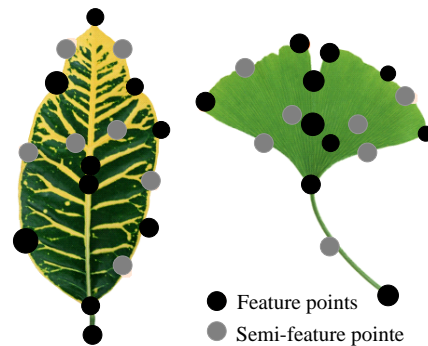


Figure 4. Feature points and secondary feature points

For example points can be on the middle of the petiole, and on the leaf margin between feature points, as well as on the detailed part of the prominent vein. As shown in figure 4:

The selection of feature points will influence the final leaf remodeled results. Generally feature points will affect the whole structure of a leaf, and the semi-feature points will affect part details.

3.3.2. Shape outline

In order to obtain the poplar leaves outline, we established the leaf shape correlation among the different points based on optimized nonlinear mapping model. Using KL divergence (Kullback-Leibler divergence) we developed a model for curve matching. It provides a method to find the correlation between curves by constructing a basic curve and make corresponding analysis between the basic curve and other curves within the group. The model will have inherent symmetry and transitivity features, the basic idea is as follows:

Assume that there are two different two-dimensional organ shape, and each expressed with a different smooth curve C_i ($i = 1, 2$). Every smooth curve C_i can be expressed with the angle function $Q_i(s)$ which used in several feature points (S_i) and arc length $s \in I_i$ ($I_i = [0, S_i]$), and consists of a velocity vector and the positive x-axis. For calibration of the two curves, we find a basic curve of $C_0: \theta_0(s), s \in I_0 = [0, S_0]$ which provides an optimal relationship between the C_i and C_0 , by using nonlinear correspondence analysis. To achieve this objective, we try to search for the optimal shape of $g_0(s)$,

$g_1(s), g_2(s)$ ($s \in I = [0, S]$) so as to make an optimal correlation between $\theta_0(g_0(s)), \theta_1(g_1(s)), \theta_2(g_2(s))$ and C_0, C_1 and C_2 . We get from I to IL ($L = 1, 2$) all possible shape expressed as

$$G_l := \{g_l \mid g_l : I \mid I_l, g_l'(s) > 0, g_l(s) = s_l, g_l(m) = m_l\} \quad (6)$$

In order to make C_0 and C_1 perfectly matched to each other, we establish the Bregman energy function based on divergence and make it the minimum:

$$\min_{g_1, g_2, g_0, c_0} \{D(g_1, g_0, c_0) + D(g_2, g_0, c_0)\}$$

According to the Bregman divergence properties, for all $s \in I$, optimal shapes are given by Bregman distance properties as $g_0(s) = (g_1(s) + g_2(s))/2$; optimal curves are expressed as $\theta_0(g_0(s)) = (\theta_1(g_1(s)) + \theta_2(g_2(s)))/2$.

In Figure 5 we have compared the existing methods with what we have proposed, and obtained the correlation analysis results. It displays that our method can obtain better results by researching on a pair of closed or unclosed organ shape. We have proved in theory that the new method is not dependent on the parameters, further confirmed the existence of solutions in the ideal set $G1XG2$. In this study we have confirmed the validity and robustness of the new method through numerical investigation, and further integrate the organ shape correlation analysis model into QTL mapping to detect and control poplar leaves shape QTL.

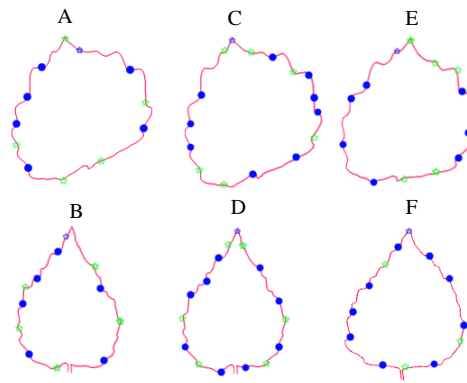


Figure 5. Comparative analysis method of poplar leaves

A and B are the traditional analysis results; C and D are our model results; E and F are of non closed leaf-shapes corresponding analysis results using our model. Our model can detect more subtle shape differences. Five pointed stars and dots are feature points.

3.4. Imagine

We now know, gene is the main material of genetic variation, and is a DNA fragment with genetic effects. Genes in the chromosome are linearly arranged in chromosomes. A Gene sequence is composed of 5 kinds of bases: CYTOSINE (C), GUANINE (G), ADENINE (A),

THYMINE (T, DNA only) and URACIL (U, RNA only). Gene sequence form as follows:

Gene sequence: tttgaaagca aaatggatga agagtatgct...

Using L-system modeling method we know that L-system is a visualization method based on string rewriting technique. With different initial values and axioms it can generate a string of characters through iterative algorithm, the string form as follows:

[+pL][- pL]I[-I][iL][iL]iiF[+I][iL][iL]iiF}...

We can make an assumption that there exists some relationship between gene sequence and L system strings, for example, t is equivalent to [pL], g is equivalent to [-I],

and so on. It will be possible to establish L system models based on gene sequence information. In reference axis technique, we analyze the evolution law of plant topological structure using Mark off chain theory and state transition graph. And a series of plant-state strings will be generated along the axis. As shown in

Figure 6, we generated the branch distribution topology of an apple tree trunk using the reference axis technique. Still suppose that there is some correlation between gene sequence and status strings, we can generate more reasonable models with reference axis technique.

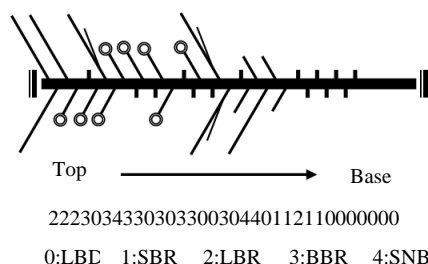


Figure 6. Model based on reference axis technique

LBD (Latent Bud), SBR (Short Branch), BBR (Bearing Branch), SNB (Secondary Branch)

If we can establish some rational and stable relationship between plant visualization models and gene information or gene expression information, the plant visualization models and techniques will have more vitality and will get leap-forward development.

4. Conclusions

Based on the statistical analysis of plant gene expression information, and combining the method of function mapping of QTL, correlation analysis of plant phenotypic characteristics and the genetic information makes the study of plant visualization has basis of life and biological significance. Although the research is still in its early stages of development, the effective combination of visualization technique and the genetic information still in the exploratory stage, I believe that one day in the future we will realize the rapid development of correlation research between genetic information and plant visualization, then, using a leaf or a piece of root of a plant, we will be able to simulate the morphological structure of the plant on the computer.

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